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A novel strategy to obtain quantitative data for modelling: Combined enrichment and real-time PCR for enumeration of salmonellae from pig carcasses

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PROCEEDINGS BOOK

9th International Conference on
the Epidemiology and Control of biological,
chemical and physical hazards
in pigs and pork

PROCEEDINGS BOOK

Dear colleagues,

Welcome at Safepork; the 9th international conference on the epidemiology and control of biological, chemical and physical hazards in pigs and pork.

Pork is the most import meat for many consumers; e.g. from the 86 kg meat eaten by Dutch consumers, 42 kgs are pork, and pig farming has intensified in the past decennia to meet the growing global demand for pork in an efficient way. This has resulted in highly competitive farming practices associated with a decrease of the number of pig farms, but a strong increase in the number of pigs per farm. A similar intensification of production has taken place in the meat industry. Moreover, changing balances in supply and demand in countries have resulted in changing trade patterns.

Among the many other challenges pig and pork production is facing, such as increasing feed prices, animal welfare and environmental issues and shortage of qualified personnel, food safety remains a very important one. Although pork has never been safer than it is today, the risk consumers are willing to take is also much lower than it was in the past. As a consequence, methods to further increase food safety and communicating the safe production systems in a transparent way to consumers is vital.

Combining all these trends Safepork IX has three key food safety topics. The first one is modernisation of meat inspection, as today's meat inspection is a system that was developed to detect the old zoonotic diseases, like tuberculosis, but its value for zoonotic agents like Salmonella and Toxoplasma is highly questionable. Several initiatives have been taken in recent years, which will be presented during this conference.

A second important topic of today is antimicrobial resistance. Human medicine is looking at the use of antimicrobials in animals as one of the sources of antimicrobial resistance of pathogens in man and, consequently, also the pig industry needs to respond to this concern. Several studies will be presented showing not only the development and prevalence of resistant bacterial strains, especially MRSA, but also possibilities to reduce the use of antimicrobials in pig husbandry. We hope the conference will contribute to successful strategies to allow pig production with limited amounts of antibiotics.

Former Safepork conferences focussed on epidemiology and control Salmonella. In this conference control of Salmonella and other bacterial pathogens, pre- and post harvest, still receives a lot of attention. The EU base line study showed that Salmonella is wide spread within Europe, with big differences between slaughterhouses. This large variation implies that improvement is possible if we implement the right tools.

Thanks to the many contributions from all over the world and excellent key note speakers, you will experience a programme that contains state of the art science on a large variety of subjects related to the safe production of pigs and pork. Sessions and breaks will give you ample opportunities to discuss with you colleagues and, moreover, we are convinced that our attractive social programme will help you expand and strengthen your network. Safe production of pigs and pork can only be achieved by a tight cooperation between Science, Industry and Legislators. Hopefully Safepork 2011 will stimulate this cooperation.

We thank the scientific committee for reviewing the abstracts and their help in designing the programme. Moreover, we thank our sponsors for making this conference possible. Most of all we thank you, participants of Safepork, for your contributions and your presence at SafePork 2011. We wish you all an inspiring conference.

Arjan Stegeman   Lourens Heres   Manon Swanenburg   Peter van der Wolf

Scientific

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Modernization of meat inspection

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Abstract

Current pig meat inspection still has significant value in detecting and controlling hazards related to animal welfare, animal health and meat quality, but public health-relevant hazards detected largely include those that are transmitted to humans primarily via routes other than eating pork or lack evidence of causing human disease via pork consumption. On the other hand, the main pork safety hazards presently causing the majority of human foodborne illness (e.g. enteric pathogens Salmonella, Campylobacter, Yersinia), or causing serious concerns (e.g. protozoan parasite Toxoplasma gondii) do not cause any lesions observable by the current meat inspection. Furthermore, manual meat inspection techniques mediate cross-contamination with microbial pathogens. The enteric bacterial pathogens are faecally excreted by asymptomatic pigs and cross-contaminate other pigs, the abattoir environment and carcass meat; therefore, they are largely a process hygiene problem. When considering how to make meat inspection truly risk-based and target the most relevant hazards, firstly, the hazards need to be identified and, secondly, they need to be risk-ranked. To control the most relevant pork-safety hazards, the risk-based approach would logically include differentiation between, and risk ranking of, both incoming batches of pigs (based on food chain information, epidemiological intelligence) and abattoirs (process hygiene assessment, performance). For both, appropriate targets/criteria would be needed. The control system for those hazards could include balancing between risk categories of the pig batches and risk categories of the abattoirs conducting slaughter, as well as process- and technology-based controls for higher-risk situations (e.g. surface decontamination/freezing/cooking treatments) where achieving the final targets is uncertain otherwise. In terms of the underlining rationale/philosophy and its nature, such a system would represent more a pork safety assurance rather than meat inspection.

Evaluation of the usefulness of carcass-weight, meat-percentage or identity of pig-producer in future-risk-based meat inspection

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Abstract

In the search for new and risk-based ways of conducting meat inspection, a pilot study was conducted with the aim of investigating whether carcass weight in combination with meat percentage, or producer-identity could be used as indicators for rejection of finisher pig carcasses. Data covering April 2010 to March 2011 were obtained from one Danish abattoir. Data about production type and herd size were obtained from the Danish Producer Registry. The total number of recordings included 4,665,812 pig carcasses delivered by 3,267 producers. Lesions leading to total rejection were found in 6,752 of the carcasses (0.1%). Among the rejected carcasses the most frequent lesions were: acute pleuritis (17.7%), rectal stricture (16.9%), osteomyelitis (15.3%), circulation disorders (14.6%), infected tails (14.5%), pyemia (10.7%) and emaciation (9.9%). Among the accepted carcasses the most frequent lesions were: chronic pleuritis (23.6%) and contusions/bursitis (2.3%). A logistic regression analysis indicated that rejection could be predicted by carcass weight, but the effect was too low in order to be suitable for meat inspection purposes. The effect of producer was, however, strongly significant. About 46.0% of the producers did not get any carcasses rejected because of lesions during the study period. Of the 1,659 (51.0%) of the producers that had 1.0% rejected carcasses in the study period, 454 had rejections two or more months in a row and large size deliveries. Among the 101 producers who on average got >1.0% of their carcasses rejected (high-risk), 43 producers seemed to have persistent problems because they experienced rejection at least 2 months in a row. When investigating herd size and production type for these herds in the Danish database for producers, the herd sizes were on average 1,133 pigs – and hence of a medium size in a Danish perspective. The production type was mainly integrated. Since some producers deliver to more than one abattoir and more frequently than once a month, a further exploration of data from all Danish abattoirs on data of delivery is needed before any conclusions might be drawn regarding the usefulness of producer as an indicator for meat inspection. An investigation of the most frequently reported lesions in carcasses originating from the low- and high-risk herds will also be conducted in order to reveal presence of persisting disease problems in those herds.

Introduction

The traditional meat inspection procedures were established 100 years ago, when the prevalence of animal and zoonotic diseases in Europe were very different from today. The implications of using outdated meat inspection principles might be that new emerging diseases are not in focus, and that resources are spent for limited food safety, animal health and welfare value. Hence, meat inspection is currently up for discussion. How can it be conducted in a more cost-effective way than at current where each carcass receives the same amount of attention?

It might be speculated that certain indicators might be used to allocate carcasses into two groups, where one group has a low probability of being rejected and the other group has a higher probability. And hence, the meat inspectors would be able to conduct a more extensive meat inspection of the latter group than of the first, which could undergo a more superficial inspection.

In Denmark, around 21 million finisher pigs are slaughtered and inspected annually. In the search for new and risk-based ways of conducting meat inspection, a pilot study was conducted with the aim of investigating whether carcass weight in combination with meat percentage, could be used as indicators for rejection of finisher pig carcasses. This was judged relevant to study, because meat percentage is measured by use of the AutoFom system prior to meat inspection at some of the large Danish abattoirs. It was also hypothesized that in some pig herds disease – or conditions leading to disease (and

rejections of carcasses at slaughter) – is persisting. In a statistical analysis, this would be seen as clustering on producer level. Again, it was judged as relevant to study the effect of producer on the probability of rejection, because it would be easy to sort pigs/carcasses by producer. Other factors like production size and production type were also investigated.

Material and Methods

Abattoir data describing findings at meat inspection of finisher pigs (weighing   109.9 kg) from one Danish abattoir from April 2010 to March 2011 were selected from the abattoir company's database.

During meat inspection, a carcass could get between one and four different remarks based on lesions observed on the carcass or in the organ systems caused by infectious or non-infectious diseases. Remarks indicating generalised infection would lead to rejection in agreement with the current Danish meat inspection circular which is based on the EU legislation on meat inspection.

The pigs with lesions that led to rejection were defined as cases, whereas the other pigs acted as controls. Controls could also have lesions such as chronic pleuritis or parasite-affected livers. Such disease codes would typically have led to local condemnation of the affected organ.

Observations that had a meat percentage <1 and a weight above 109.9 kg were excluded from the analyses. An unrealistic low meat percentage was considered as an artefact in the data. Only slaughter pigs (weight below 110 kg) were included in the analysis because an animal weighing more than 109.9 kg was not considered a finisher pig but a gilt/boar or a sow which might have another disease pattern than finisher pigs.

The descriptive statistical analyses and the logistic regression (GLIMMIX) were conducted in SAS. The Figure was made in Excel.

Information about herd production type and size were obtained from the Registry of producers (CHR-registry).

Results

Descriptive results

The total number of pig carcasses slaughtered and inspected during the study period included at the abattoir was 4,665,812. Among those, lesions that led to total rejection were found on 6,752 corresponding to a rejection rate of 0.1% (Table 1). The pigs were delivered by 3,267 producers. The monthly number of producers delivering carcasses as well as the monthly number of slaughtered pigs is given in Table 1.

Among the cases (rejected carcasses) the most frequent acute lesions consisted of pleuritis (17.7%), osteomyelitis (15.3%), circulation disorders (14.6%), infected tail (14.5%), pyemia (10.7%), emaciation (9.9%), gastric ulcers (8.2%), Erysipelothrix rhusiopathiae infection (7.7%), and peritonitis (3.9%). The most frequently recorded chronic lesions were: rectal stricture (16.9%) and chronic peritonitis (2.9%).

The most frequently recorded remark among the controls (accepted carcasses) was chronic pleuritis (23.6%) followed by contusions/bursitis (2.3%) and abscesses in the head (1.4%). The numbers of carcasses with lesions that indicated that the pig had suffered from acute diseases or chronic diseases were summed up per month in Table 1.

Multi-variable analysis

The results of the logistic regression analysis indicated that rejection could be predicted by carcass weight, but the effect was too low to be of practical relevance during meat inspection.

The effect of producer was, however, strongly significant. The history of lesions related to the deliveries for each producer was therefore further explored.

Carcass rejection rate

The average rejection rate was 0.13% (min.: 0, median: 0 and max.: 33%) (Table 1).

Table 1. Number of pig producers, carcasses, rejected carcasses and carcasses with lesions caused by acute or chronic disease, delivered per month to a Danish abattoir, April 2010-March 2011.

Month	No. of producers delivering	No. of delivered carcasses	No. of rejected (%)	No. of carcasses with lesions caused by**	
				acute disease	chronic disease
April*	1552	329,230	416 (0.13)	1,230	97,349
May*	1578	319,587	424 (0.13)	948	94,023
June*	1584	359,285	456 (0.13)	1,077	104,601
July	1592	365,947	587 (0.16)	2,365	103,715
August	1581	363,465	541 (0.15)	2,455	101,912
September	1687	404,443	528 (0.13)	2,729	111,175
October	1707	405,162	539 (0.13)	2,998	105,595
November	1761	420,350	675 (0.16)	3,489	111,582
December	1797	412,729	611 (0.15)	3,145	108,216
January	1785	447,840	720 (0.16)	3,231	116,325
February	1756	405,139	613 (0.15)	3,021	112,585
March	1795	432,635	642 (0.15)	3,309	125,299
Total	3,267	4,665,812	6,752 (0.14)	29,103	1,274,176

* A revision of codes was conducted in July 2010 – hence lower number of the acute disease lesions in April-June 2010.

**Carcasses that are accepted could have recorded lesions of acute or chronic character.

A total of 46.0% the producers did not get any carcasses rejected at all because of disease during the entire study period (Figure 1). These might be considered as zero-risk herds. Only 101 producers (3%) had 1% of their carcasses rejected (high-risk herds) and the remaining 1,659 producers (51%) had 1% percentage of their carcasses rejected (low-risk herds).
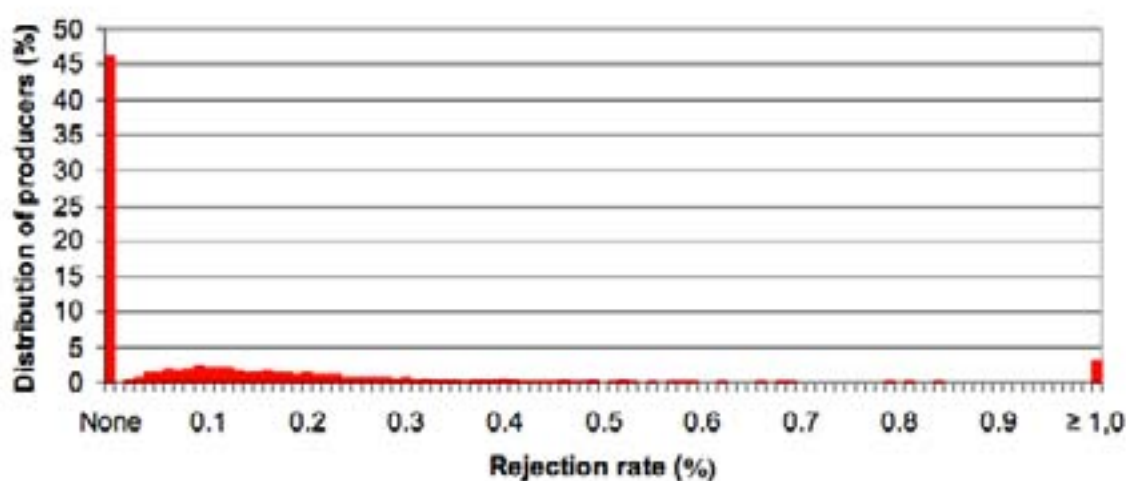
Figure 1. The distribution of rejection rates among the 3,267 Danish pig-producers delivering finisher pigs to one abattoir from April 2010 to March 2011. Further inspection of the low- and high-risk herds

An inspection of the 1,659 low-risk herds revealed that 454 had experienced rejection of carcasses in 2 months in a row. The main characteristics of those were that they had large size deliveries with a mean of 556 (min.: 113, median: 497, max.: 2060) implying that the herds were large. Of the 101 of the high-risk herds, 43 had carcasses rejected 2 months in a row and the main characteristics for them were small deliveries with a mean of 127 (min.: 21, median: 86, max.: 586). According to the Danish Producer Registry, the average herd size for the 43 herds was 1133 pigs (min.: 200, median: 1631, max.: 8000). All of the 43, except from two, were integrated herds.

Discussion

The first analyses showed that carcass weight was a statistically significant predictor for rejection. The effect was, however, too low for practical use when categorising a pig into one or another meat-inspection regime. The same analysis revealed that cases of rejection were clustered around the random variable herd-identity. This variable was therefore further explored as a possible candidate for categorising into differentiated meat-inspection regimes.

The number of rejected cases from each producer was a function of the number of delivered carcasses; large herds having more carcasses rejected (Table 2). The overall rejection rate was 0.1% but the variation was wide (min.: 0, max.: 50%). About 46.0% did not experience rejections due to disease during the study period while 51.0% experienced a low rejection rate. Only about 3.0% of the producers experienced a high rejection rate and carcasses that were rejected ≥ 2 months in a row. The findings that some producers were associated with increased risk of rejection of their pigs might be related to management – or it might have been that the delivery consisted of a pen or two with slow-growing pigs. Some of the producers deliver to several abattoirs. The average herd size for these herds was found to be medium in a Danish perspective and almost all of them were integrated herds. Hence, no particular factors were revealed that could characterise this group of producers so far except from individual management. Hence, before making any conclusions about the producer-effect as an indicator of rejection in meat inspection a more thorough investigation of the overall deliveries (on delivery date) for each producer must be made. The 46.0% of the producers that never got their pigs rejected would be of particular interest. In addition, the most frequent lesions recorded in carcasses originating from the low-risk herds and high risk herds that had experienced rejection of carcasses in ≥ 2 months in a row needs to be explored in order to investigate whether these herds have persisting disease problems.

New strategies in meat inspection need also to be fundamental in the practical feasibility. Analyses of whether a change in logistics in the meat inspection would be possible in practical life needs therefore also to be conducted.

Conclusion

Future meat-inspection strategies could be based on allocation of producers into two groups; carcasses from the high-risk group would be subjected to a more extensive meat control whereas the low-risk group could undergo a more superficial inspection. This study of pig-deliveries from one year from one abattoir revealed that 46.0% of the producers never had a single carcass rejected. Of the producers who had more than 1.0% of their carcasses rejected only 43 herds delivered and had rejected carcasses two months in a row or more.

Further studies of the effect of producer as an indicator for rejection will be conducted.

State of Art of meat inspection of pigs in the EU

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Abstract

The current meat inspection in the European Union (EU) is based on principles that are around 100 years old. However, the zoonotic hazards have shifted and the production systems for livestock are changing. This makes it necessary to look at whether the present way of conducting meat inspection is efficient or not. The elements of meat inspection in EU are specified in Regulation (EC) 854/2004 which also opens up for modifications of the existing practices if certain requirements are met. In essence, finisher pigs might be subjected to so-called integrated meat inspection, if they originate from integrated production systems where the animals are raised under controlled housing conditions since weaning. More over, Food Chain Information should be exchanged prior to slaughter. This paper evaluates the current state of meat inspection for finisher pigs in the EU. The evaluation is based primarily on 1) results of a workshop aiming at identifying the aims of different components of meat inspection, 2) information obtained through a questionnaire survey mailed to all EU member states, 3) Food and Veterinary Office (FVO) inspection reports.

The evaluation of the detailed aim of meat inspection revealed that there are several aims: food safety, notifiable diseases, animal health and welfare as well as international trade. The results of both the questionnaire and the FVO reports show that over-all speaking the member states are complying with the current regulation – but not necessarily in details. Only three member states have introduced integrated meat inspection in pigs. This implies omission of routine incision into the mandibular and/or mesenterial lymph nodes, and no routine opening of the heart. In some cases, the lungs are not palpated routinely. In member states where the pig population is considered free from Bovine Tuberculosis (TB) and Trichinella, only few elements of traditional meat inspection in pigs are related to food safety. Despite that Salmonella ascribed to pork is causing a much higher number of human cases than bovine TB and Trichinella, this and similar food borne agents are not dealt with effectively at meat inspection in many cases. The FVO reports also show that lack of compliance with current regulation in particular regarding ante mortem inspection as well as stunning and bleeding. This is of relevance for both animal welfare and the ability to identify notifiable diseases should these occur.

Further work is needed regarding how to make full use of risk-based principles and cost-effectiveness in meat inspection for the benefit of consumers, society and industry while ensuring international trade. Here, we should consider use of new diagnostic techniques, requirements for increased biosecurity practices, and identification and increased focus on high-risk pigs/herds.

Introduction

The current meat inspection regulation in the EU is based on principles that are around 100 years old. However, the zoonotic hazards and the livestock production systems are changing, making it necessary to look at whether the present way of conducting meat inspection is efficient. The concept of integrated production system has recently been introduced in the EU to describe pig herds with high biosecurity. The regulation has opened up for modifications to existing meat inspection in such herds. But what is the State of Art of meat inspection in the EU? What are the challenges? And where are we heading? This was studied in a project conducted by the Danish Agriculture & Food Council in 2010-2011.

Figure 1. Evaluation of aim(s) of specific component of post mortem meat inspection and most important lesions and if possible their causes, based partly on results from a workshop in Denmarka, partly on Jensen et al. (2006) – Pigs

PIGS	Aim					
Inspection (palpation/incision if listed) of organs/area	Food safety	Notifiable diseases	Animal Production diseases	Animal Welfare	Quality	Trade
Head, mouth, throat & tongue		Foot and mouth, SVD			Abscess	
Mandibular lymph nodes – incision	Avian/ bovine TB?	Bovine TB				Bovine TB
Lungs, trachea & oesophagus	Avian/ bovine TB?	Bovine TB	Pleuritis and pneumonia due to AP, Mycoplasma	Actinob. Pleurpneum.		
Lungs, bronchial & mediastinal lymph nodes – palpation	Pyemia /septicaemia	CSF	Pleuritis and pneumonia due to e.g. AP	Actinab. Pleurepneum.		
Trachea and large bronchia - Longitudinal incision[b]	Bovine TB?	Bovine TB	Lung worms		Lung worms	
Heart and pericardium – Incision	Pyemia /septicaemia	CSF	Erysipelothrix Strept. suis		Endo-/pericarditis	
Diaphragm	Cysticercus cellulosae	CSF				
Liver and portal lymph nodes – Palpation	Intoxication Avian/bovine TB?	Bovine TB,	Abscess		Abscess cirrhosis	Bovine TB
Gastro-intestinal tract & mesenterium	Salmonellosis		Enteritis e.g. due to Lawsonia	Gastric ulcer Chronic enteritis	Enteritis	
Gastric & mesenterial lymph nodes	Avian/bovine TB?	Bovine TB	Indicators for enteritis	Indicators for enteritis		Bovine TB
Spleen – palpation if necessary	Pyemia /Septicaemia	Classical & African swine fever				
Kidneys	Intoxication /septicaemia	Classical & African swine fever	Renal insufficiency		Appearance smell & texture	
Pleura and peritoneum	Bovine TB?	Bovine TB CSF	Pleuritis	Peritonitis	Pleuritis => Not edible	
Genitalia		Brucellosis	Reproductive disorders in sows	Late pregnancy/ recent calving torsion of testicle		
Udder and its lymph nodes[b]			Abscess			
Umbilical area and joints of young animal – palpation	Septicaemia		Many infections		General poor quality	

(a): Workshop held in Denmark in September 2010 with participation from different stakeholder groups. b: incisions not required (or question not relevant) if organs are not destined for human consumption. A question mark highlights that it is doubtful whether these agents are meat-borne.

Material and Methods

First, the detailed aims of meat inspection as described in Regulation 854/2004 were evaluated on a workshop in September 2010 in Denmark with participation of different stakeholders: is the purpose to ensure food safety, notifiable diseases, animal health or welfare, trade, or meat quality? The result of the workshop was supplemented with information from a text book on pathology (Jensen, 2006). Next, a questionnaire was sent out to EU member states to obtain detailed information about the way that meat inspection is conducted. A total of 22 questionnaires were received. Finally, FVO reports were studied to obtain information of level of implementation of the current regulations. The different reports were obtained from the homepage of FVO (http://ec.europa.eu/food/fvo/index_en.cfm). The full report of the study will become available mid-2011 and can be obtained upon contact to the authors.

Results

Figure 1 presents an evaluation of the aim of the individual component of meat inspection. The primary aim of meat inspection is usually recognised as food safety. However, meat inspection is also used to survey and identify notifiable animal diseases, animal health and welfare as well as to ensure trade and quality. It is in fact noted in Fig. 1 that only few components of meat inspection are related to food safety – in particular for member states that are free from TB.

Traditional meat inspection according to Regulation (EC) 854/2004 is conducted in most member states. Only Denmark, Germany and the Netherlands have modified inspection programmes in place for the part of their pig production that fulfils the requirement for integrated production systems (animals should be raised under controlled housing conditions since weaning). The modification related to omission of routine incisions into the mandibular and mesenterial lymph nodes, and no routine opening of the heart. In some cases, the lungs are not palpated routinely. Several member states expect to be introducing similar meat inspection practices for pigs within the coming years.

Both the questionnaire data and the summary of results of FVO inspections confirm that most member states in principle meet the requirements in Regulation (EC) 854/2004. However, the regulation is not necessarily fulfilled with respect to all details. In many member states incisions into the mandibular lymph nodes and palpation of the mesenterial lymph nodes (which are done to identify TB) are not necessarily done routinely. Neither is the heart of finisher pigs routinely opened in all abattoirs at all times. Zoonotic agents like Salmonella play a limited role in the current meat inspection. Moreover, improper ante and post mortem inspection occurs widely. Regarding animal welfare, the results in the FVO reports reveals incompliances regarding stunning and bleeding. Finally, the FVO reports reveal lack of compliance when the local and central competent authorities are conducting their work.

Discussion

Details in Fig. 1 might be discussed; e.g., the exact allocation of some diseases and conditions. The message in the figure is that there are several aims of meat inspection. Despite that the perception is that food safety is the most important reason for conducting meat inspection other aims are playing a role (Fig. 1). One of these aims is surveillance for notifiable diseases like foot and mouth disease and classical swine fever. An early diagnosis of such diseases might prevent an outbreak from turning into an epidemic. And here, the efficacy of ante and post mortem inspection should be studied further. Meat inspection data might be used to document freedom from notifiable diseases to trading partners. Moreover, systematic collection of certain meat inspection data might be valuable for use in herd health management and for monitoring of animal welfare. However, there are most likely individual needs for how individual countries would collect and use such data. Regarding international trade, meat inspection plays a role with respect to surveillance for both notifiable diseases and zoonotic infections. Thorough observations at meat inspection make it possible to give specific guarantees in relation to trade. Finally, inspection for quality could be performed by the slaughterhouse.

In member states that are considered free from Bovine TB and Trichinella in livestock, only few elements of traditional meat inspection in pigs are related to food safety. Moreover, in these member states the lack of full compliance with the current regulation - found by FVO - that requires incision/palpation into mandibular and mesenterial lymph nodes is judged as having limited if any impact on food safety. Interestingly, other food safety hazards like Salmonella - which is causing many more human cases than bovine TB, Brucella, or Trichinella - are not dealt with effectively in the current meat inspection in most member states. Incompliance found related to ante mortem inspection might jeopardize the member states with respect to notifiable livestock diseases, since such diseases, hence, might be diagnosed too late, making disease-control more difficult than necessary. Moreover, the lack of compliance with regards to stunning and bleeding might have a detrimental impact on animal welfare. The competent authority should here be seen as drivers aiming at ensuring the compliance with the current regulation. Unfortunately, the FVO reports contain many comments to the conduct of the local or central competent authority.

Further work is needed regarding how to make full use of risk-based principles and cost-effectiveness in meat inspection. Risk-based surveillance might imply inspection of high-risk animals/premises that are identified based on a specific list of risk factors / parameters. Such an approach is different from the current practice where attention is paid equally good or bad to all animals. However, which should be the requirements for such risk-based programmes? And where are the pitfalls? And does it make sense to operate with 27 different programmes in the EU instead of a generic programme for free states and non-free states, respectively? This needs to be discussed. Another approach is to replace some specific

components of current inspection with control actions in other parts of the food chain. An example is to prohibit the use of peat (sphagnum) as litter material for pigs unless heat-treated to avoid exposure to avian tuberculosis. This will make sense, if such control actions are more cost-effective than the current meat inspection that involves incision and palpation of selected lymph nodes. Meat inspection should here be seen as a kind of surveillance that can deal with infected/affected animals. For some hazards, meat inspection is the only way of identifying a positive animal. But for other hazards, pre-harvest intervention/inspection, biosecurity requirements can also be considered. In line, new tools such as multi-diagnostics might offer promising results; by use of meat-juice samples the status with respect to several agents might be revealed before the carcass leaves the cooling unit. However, issues like cross-contamination at abattoir, and low positive predictive value when testing for notifiable disease will need to be discussed.

Conclusion

Most member states in EU still conduct traditional meat inspection. However, meat inspection is up for debate in all parts of the world. Further work is needed regarding how to make full use of risk-based principles and cost-effectiveness in meat inspection for the benefit of consumers, society and industry while ensuring international trade. Many important issues remain to be discussed according to the current regulation before we can move on to a more risk-based meat inspection, among others:

1. Which hazards should be included in a surveillance system at the slaughterhouse?
2. How can surveillance and control for zoonotic parasites and notifiable diseases be conducted risk-based?
3. Which notifiable diseases should be surveyed at meat inspection?
4. Are data collected at meat inspection usable for the purpose?

Acknowledgement

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Proceedings - Orals

Risk-based meat inspection: Implementation experiences in Germany and integration of animal-oriented welfare criteria

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Abstract

The paper describes the experiences from seven pilot projects in Germany on implementing the risk-based meat inspection: analysing the status quo per slaughter house, defining the specific risks of the region of the supplying herds, creating the preconditions for recording and exchanging a meaningful set of data for the food chain information including animal health and welfare criteria, training of risk-oriented logistic slaughter and adding targeted inspection procedures in case of increased food safety risks indicated for entire herds or slaughter batches.

Introduction

Implementing the risk-based meat inspection with its three goals: improvement of food safety, animal health and animal welfare – understanding the obstacles

After the so-called "Hygiene Package" of the new EU food safety concept was issued in 2004 and put in force in 2006, the German federal risk assessment authorities predominantly thought of ONE national "prescription" of how to implement the risk-based meat inspection in all slaughterhouses in the country. Furthermore, according to the traditional understanding of the food safety responsibility, the general expectation was that the new approach has to be implemented predominantly by the official veterinary food safety services. This was mainly due to the difficulty of switching from the traditional prescription of "what to do" to "reaching food safety goals". Another difficulty was the disposal of the food safety responsibility to the food business operator, with the veterinary authority mainly controlling the self-control.

After these basic principles of the new food safety paradigm were better understood, it became obvious that there is not ONE way to implement the risk-based meat inspection in all slaughter facilities, but several ways to achieve the same goal. The reason for this flexibility is that slaughterhouses with their specific set of pig supplying farmers have very different supply chain conditions (number and size of herds, quality of cooperation between suppliers and slaughterhouse, quality of information exchange, etc.). Even the risk patterns vary between regions and slaughterhouses (outdoor holdings vs. confinement; small vs. large pig units, straw or wood chips litter vs. slatted floor, etc.).

However, apart from the intended improvement of food safety by targeted process optimisation measures in the pre-harvest stage and risk-based inspection methods at the slaughter line, the modernization of the meat inspection has the additional goals of:

a) improving herd health by a feedback system for slaughter check results, and
b) improving animal welfare by adding animal-oriented welfare criteria recorded during unloading the animals and at slaughter to the feedback system.

Material and Methods

Since the integration of the risk-based elements including the food chain information about the pre-harvest stage of meat production and the recordable animal-oriented welfare criteria is not yet a standardised routine in the current meat inspection systems in Germany, the objective of our research is to develop a system of assessing the health status of pig herds and a feedback system for animal-oriented welfare criteria as tool for farmers and veterinarians to improve the animal health and welfare status of pig supplying herds.

Animal-oriented welfare criteria (injuries, disease, pain, cachexia, etc.) indicate deficiencies in animal husbandry, genetics, and animal handling (both in the herd and during the transport). These "output" criteria are to be added to the

traditional used "input" criteria that evaluate and measure only the housing conditions for the animals (space per animal, slatted or plain f oor, etc.).

So far, the ante-mortem inspection of slaughter pigs conducted by the official veterinarian during unloading and lairage has been targeted to assure that only animals fit for slaughter were entering the slaughter line, and with this, the food chain. Although during the traditional meat inspection procedure cases of very severe violations of animal welfare rules and obvious cruelty were reported to the appropriate authority, less severe, but measurable deficiencies in animal welfare have not been routinely documented for each individual herd.

Assessments at unloading and lairage are to be included and criteria such as lameness, abscesses, body condition and cannibalism lesions as well as abrasions due to animal abuse are to be documented per herd. Furthermore all findings during slaughter with respect to animal welfare such as beating marks, multiple abscesses, chronic arthritis, and excessive disease-related slaughter check results per herd that indicate pain and suffering of the animals at farm level or during transport and lairage are to be cumulated for benchmarking the health and welfare status of all pig herds that are supplying animals to the slaughter plant in question.

Results

Our research team has started to consult seven large scale slaughterhouses as pilot projects. All operators of the seven enterprises including the corresponding official meat inspectors were at the beginning fully concentrated on changes in the meat inspection procedure at the slaughter line with the expectation to implementing the "visual" meat inspection – the food business operator targeting at reducing the meat inspection costs, and the official veterinarians being afraid of lowering the food safety assurance. Our research team succeeded over time to make understood that first the food business operator TOGETHER with his supplying farmers has to create the necessary preconditions for being able to provide the responsible veterinary authority with a meaningful set of data for the food chain information. Graph 1 shows the major elements for implementing the risk-based meat inspection.

Graph 1: Developed tools for measurement of food safety risks, animal health status and welfare level
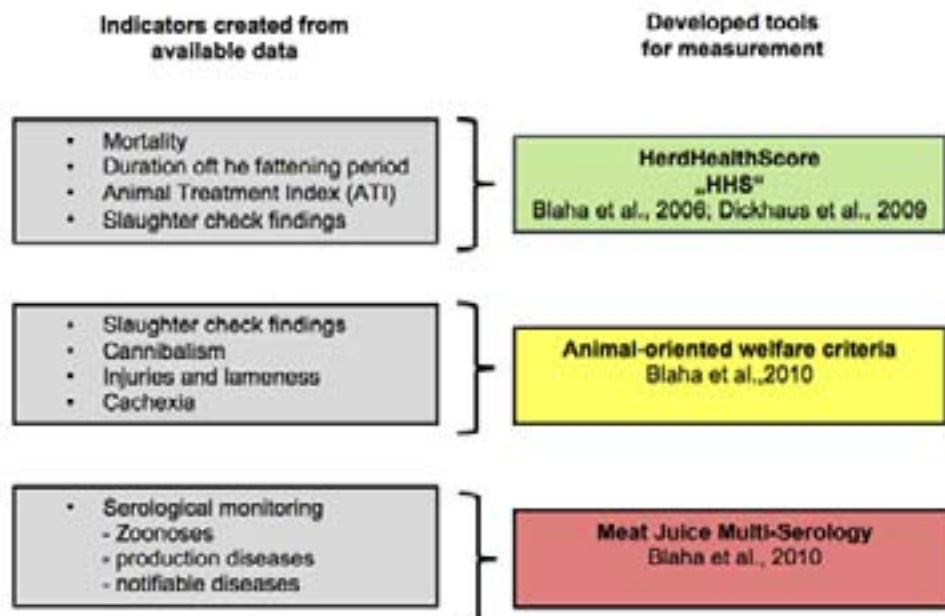
Discussion

The initial misunderstanding of the risk-based meat inspection as only "visual" inspection without incision and palpation lead to a prolonged implementation of the new European food safety concept in Germany. The major obstacle was that in the beginning all intended changes were thought to be only necessary at the slaughter line. It took some time to overcome the food processors' expectation of reducing the costs for the meat inspection and the fear of the official veterinarians that the food safety assurance level could be lower than the level guaranteed by the traditional meat inspection. In all pilot projects, after the three stakeholder groups (food business operators, farmers and veterinary authority) understand the need to first organise a functioning food chain information system, slaughterhouse-specific systems of the risk-based meat inspection start to develop, and will gradually improve over time. One major experience is that a good cooperation between the food business operator and the farmers is as crucial for the implementation of the new system as the permanent exchange of information about the developmental process with the veterinary authority from the very beginning.

Conclusions

Despite quite good steps forward in the pilot projects, there is still the traditional scepticism towards the food chain information parts that are based on the data given by the farmer, when no official controls are possible to verify them. Especially the development of the meat juice multi-serology, covering the major latent zoonoses (Salmonella, Yersinia, Toxoplasma, Trichinella and Mycobacteria) in pigs without the involvement of the farmer and/or the private veterinarian made the official veterinary authority more appreciative of the new food safety concept. The acceptance of the need to provide the food chain information from the farm by the farmers, which is also necessary for the success of the new system, can be remarkably improved, if the meat juice multi-serology is also providing useful information about the occurrence of production disease pathogens such as Mycoplasma hyopneumoniae, Influenza A, Actinobacillus pleuropneumoniae, and PRRSV. Adding to the multi-serology the testing against notifiable diseases such as Classical Swine Fever and Aujeszky's Disease would increase the value of such a serological monitoring system with the opportunity of sharing the costs by the three stakeholder groups: the state, the farmers and the food business operator.

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Experiences with a risk based meat inspection standard in pigs

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Abstract

The European Union legislation provides several possibilities to modernize meat inspection. Improvement of food safety by active contribution of food business operators in the supply chain being responsible for food safety is envisaged in these new standards.

In 2006 Dutch pork slaughterhouses were the first to implement a risk based meat inspection system for pigs. Food safety is ensured in this system by using controlled housing systems for pigs, integrated forward and backward data exchange of relevant food chain data and by surveillance of hazards with serology on blood. Slaughterhouse data and inspection results are used to inform farmers and for targeted monitoring of antibiotic residues. Incision of lymph nodes could be omitted because pig herds accepted for risk based meat inspection have a controlled risk regarding Mycobacterium avium and classical tuberculosis. Quality control systems (including HACCP) are an integrated part of this system with information exchange and obligatory corrective actions, thus increasing the safety of pork. With this risk based meat inspection system a frame work is build where food safety hazards are controlled in a targeted approach. This framework offers clear opportunities to develop the system further so new/other relevant hazards can be targeted easily. The most relevant hazard in this respect is Toxoplasma gondii.

Application of the DIVA principle to Salmonella Typhimurium vaccines in pigs avoids interference with serosurveillance programmes

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Abstract

Salmonellosis is one of the most important bacterial zoonotic diseases in humans and Salmonella infections are often linked with the consumption of contaminated pork. In order to reduce Salmonella Typhimurium infections in humans, minimization of the Salmonella intake into the food chain is important. Vaccination has been proposed to control Salmonella infections in pigs. However, pigs vaccinated with the current vaccines cannot be discriminated from infected pigs with the lipopolysaccharide (LPS) -based serological tests used in European serosurveillance programmes. We therefore examined which LPS encoding genes of Salmonella Typhimurium can be deleted to allow differentiation of infected and vaccinated pigs, without affecting the vaccine strain's protective capacity. For this purpose, deletion mutants in Salmonella strain 112910a, used as vaccine strain, were constructed in the LPS encoding genes: rfbA, rfaL, rfaJ, rfaI, rfaG and rfaF. Inoculation of BALB/c mice with the parent strain, rfaL, rfbA or rfaJ strains but not the rfaG, rfaF or rfaI strains protected significantly against subsequent infection with the virulent Salmonella Typhimurium strain NCTC12023. Immunization of piglets with the rfaJ or rfaL mutants resulted in the induction of a serological response lacking detectable antibodies against LPS. This allowed a differentiation between sera from pigs immunized with the rfaJ or rfaL strains and sera from pigs infected with their isogenic wild type strain.

Introduction

Salmonella infections in humans are often linked with the consumption of contaminated pork [1] [2]. Vaccination has been proposed to control Salmonella infections in pigs [1] [3] [4] and has already proven to be efficient in laying hens, reducing faecal shedding and internal egg contamination [5] [6]. Currently, one licensed Salmonella Typhimurium live vaccine for pigs is commercially available in Europe [7]. The use of this vaccine is limited due to interference with European Salmonella serosurveillance programmes based on the detection of antibodies against the lipopolysaccharides (LPS) of Salmonella [8]. It was therefore the aim of this study to develop a DIVA-vaccine strain (Differentiation of Infected and Vaccinated Animals), without attenuating the vaccine strain, which would not interfere with current LPS-ELISA based serosurveillance programmes.

Material and Methods

Salmonella Typhimurium strain 112910a, phage type 120/ad, isolated from a pig stool sample and characterized previously [3], was used as the wild type background to construct several isogenic LPS knock-out mutants: rfbA, rfaL, rfaJ, rfaI, rfaG and rfaF. A commercially available enzyme-linked immunosorbent assay (ELISA) (HerdChek Salmonella; IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, The Netherlands) for the detection of porcine antibodies against the LPS of Salmonella was used as a reference according to the manufacturer's instructions. Besides, an in-house Salmonella Typhimurium strain 112910a whole cell ELISA to detect porcine anti Salmonella Typhimurium antibodies, was prepared as described before [9]. In a mouse model, we tested whether the LPS mutants affect the protective capacity of Salmonella Typhimurium strain 112910a against a subsequent challenge with a highly virulent strain. For that purpose, seven groups of ten mice were inoculated first via the orogastric route with 2×10^7 CFU/ml of one of the LPS mutant strains (either: rfbA, rfaL, rfaJ, rfaI, rfaG or rfaF) or with the wild type Salmonella Typhimurium strain 112910a. Four weeks after primary inoculation, all mice were challenged with 10^8 CFU of the virulent Salmonella Typhimurium strain NCTC-12023Nal20 by the orogastric route. In a second in vivo study, we examined whether it was possible to discriminate between the serological response induced after immuneization of pigs with either Salmonella Typhimurium strain 112910a or one of its isogenic strains (rfaL rfaJ) on the one hand and after infection of pigs with Salmonella Typhimurium strain

112910a on the other hand. Therefore, 14 piglets were randomly allocated to three vaccinated groups (n = 12) and one sham-vaccinated control group (n = 2). Vaccinated animals were intramuscularly immunized (2x) with one of the formalin-inactivated Salmonella strains (either: Salmonella Typhimurium strain 112910a, rfaJ or rfaL) in Freund's incomplete adjuvant. To obtain sera from Salmonella Typhimurium infected piglets, one experimental group (n = 3) was orally inoculated with approximately 2ˣ107 CFU of Salmonella Typhimurium strain 112910aNal20.

Results

Vaccination of mice with rfbA, rfaL and rfaJ but not rfaI, rfaG and rfaF protects mice against a Salmonella Typhimurium infection:

Oral immunization of mice with Salmonella Typhimurium strain 112910a, rfbA, rfaL or rfaJ induced a significant (P < 0.05) protection against subsequent challenge with NCTC12023Nal20 in both spleen and liver compared to non immunized control animals. Results are shown in figure 1.
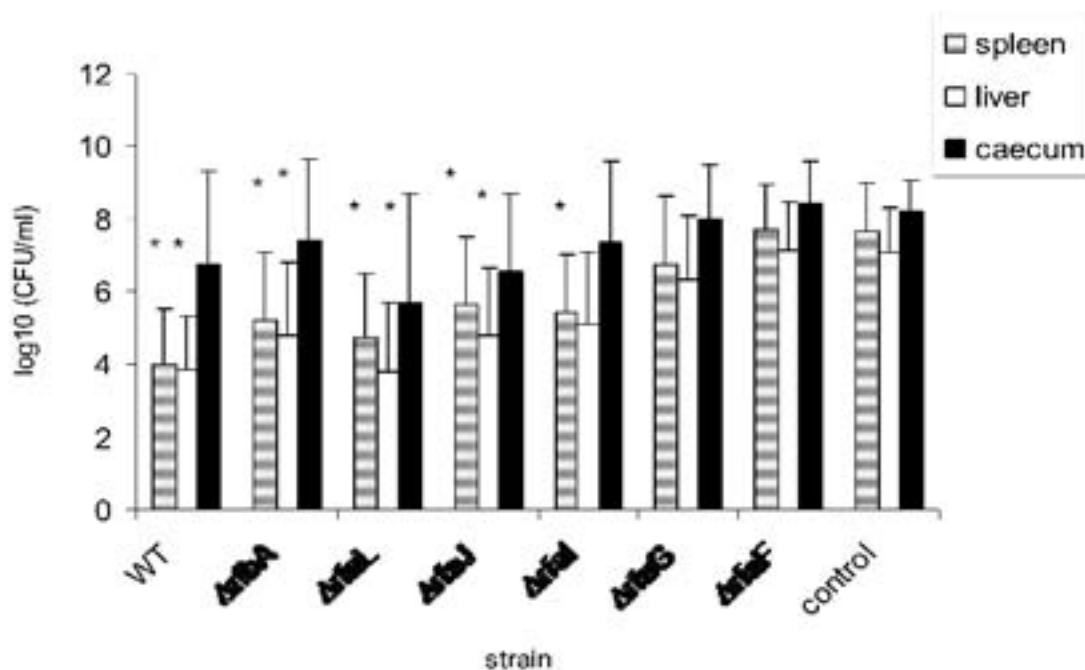
Figure 1: Recovery of Salmonella bacteria from various organs of mice immunized with either Salmonella Typhimurium, one of its isogenic LPS mutants or non immunized control animals and subsequently challenged with Salmonella Typhimurium strain NCTC12023Nal20. The log10 value of the ratio of CFU per gram sample and standard deviations are given. An asterisk refers to a significant difference with the control group (P < 0.05).

Pigs, immunized with the rfaL or rfaJ mutant, can be serologically differentiated from Salmonella infected animals:

Results showed no significant seroconversion (P > 0.05) in animals immunized with inactivated rfaJ or rfaL strains and in sham-vaccinated control animals (non immunized and non infected animals), when using the commercial IDEXX ELISA. Conversely, marked seroconversion occurred in pigs immunized with the inactivated Salmonella Typhimurium strain 112910a. Results also illustrate a clear differentiation between sera from piglets immunized with the rfaJ strain or rfaL strain and sera of pigs infected with their isogenic wild type strain. Anti-Salmonella-antibody titers were detected in the serum of all immunized and infected animals, when using the in-house whole cell ELISA. Results are illustrated in figure 2.
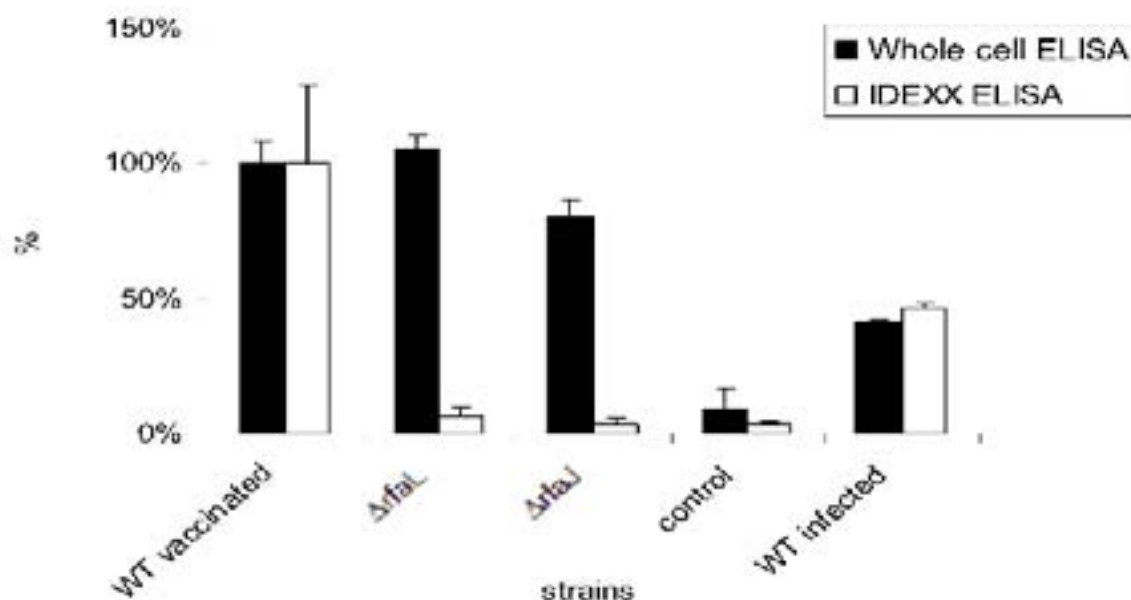
Figure 2: Serological results of pigs immunized with ΔrfaL, ΔrfaJ or Salmonella Typhimurium strain 112910a, control pigs (animals that were not immunized and not infected) and pigs infected with Salmonella Typhimurium strain 112910a Nal20. Values are represented as a percentage compared to the wild type vaccinated group. We emphasize that these results are based on a small sample size.

Discussion

DIVA vaccines are a recent advance in vaccinology enabling distinction between an animal that is seropositive to a particular infectious agent because it has been vaccinated, and one that is seropositive because it has been infected with virulent field organisms [10]. Because current Salmonella serosurveillance programmes are generally based on detection of antibodies against LPS antigens, we selected six LPS genes that might be suitable markers to develop a LPS based DIVA-vaccine. In a mouse in vivo experiment we showed that the rfaG and rfaF mutant strains were not able to protect BALB/c mice against a subsequent infection with Salmonella Typhimurium NCT12023Nal20 and that the rfaI strain was only able to significantly reduce bacterial counts in the spleen of mice. Conversely, rfbA, rfaL and rfaJ strains, with less truncated LPS, were able to successfully protect BALB/c mice against a Salmonella Typhimurium infection and their protective capacity was not impaired compared to their isogenic wild type strain. These results strongly suggest that a confined truncation of LPS is essential to maintain protection against challenge with the virulent strain Salmonella Typhimurium NCTC12023Nal20 in mice. The ultimate goal of this study was to verify whether LPS mutant strains were able to elicit a DIVA humoral immune response in pigs. Our results illustrate that both the rfaL and the rfaJ strain gave no seroconversion when using a LPS based ELISA, while a clear-cut seroconversion was observed when using an in-house Salmonella Typhimurium strain112910a whole cell ELISA. Besides, immunization of piglets with the rfaJ or rfaL mutants resulted in the induction of a serological response allowing clear differentiation between sera from piglets immunized with the rfaJ or rfaL strains and sera of pigs infected with their isogenic wild type strain when using a LPS based ELISA.

Conclusion

In conclusion, applying deletions in the rfaJ or the rfaL gene in Salmonella Typhimurium strain 112910a allows differentiation of infected and vaccinated pigs in an LPS based ELISA without reducing the strain's protective capacities in mice.

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Transmission study of Salmonella in pigs with 3 intervention strategies

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Abstract

In this study, the effect of 3 different intervention strategies on the transmission of Salmonella in pigs was evaluated: feed supplementation with coated calcium-butyrate salt, vaccination and acidified drinking water. Strategies were evaluated serologically and bacteriologically using an experimental in vivo seeder setup. Significantly higher antibody titers were detected in the groups with acidified drinking water and vaccination. In the group with calcium-butyrate supplemented feed and in the group with oral vaccination, significantly less infected animals were observed during the transmission experiment. The overall level of Salmonella-specific antibodies in meat juice was significantly lower compared to serum. Our results indicate that vaccination with Salmoporc® and feed supplemented with coated calcium-butyrate limit Salmonella Typhimurium transmission in swine. The detection of Salmonella-specific antibodies in vaccinated pigs however, does not allow differentiation of vaccinated and infected animals and can therefore interfere with the Belgian monitoring and surveillance programme. Salmonella-specific antibody levels in meat juice are lower than those in serum, which might have consequences in surveillance programmes based on meat juice analysis.

Introduction

Despite current control measures, Salmonella in pigs still remains a major public health problem and causes – even the more common subclinical infections in swine - large economic losses (Kranker et al., 2003). The exact proportion of human Salmonella cases in Belgium that are due to the consumption of pork/meat products is unknown. However, it is widely assumed that pork/meat products are an important source of Salmonella infections in humans (Griffith et al., 2006); the serovar Salmonella enterica subspecies enterica serovar Typhimurium representing 64% of the porcine Salmonella isolates in the period between 2005 and 2009 in Belgium (CODA-CERVA, 2009). Because a reduction in preslaughter infection rates should result in increased pork safety (Hurd et al., 2002) and better pig health, we investigated 3 control strategies against S. Typhimurium. The detection of Salmonella in persistently infected pigs continues to be problematic as well. Hence, we compared the suitability of serology on diaphragm fuid, easy to sample at slaughter, with bacteriological isolation and serum serology to determine the Salmonella-status at animal level.

Material and Methods

Sixty-nine Salmonella-negative, 20-21 day old weaned piglets of mixed sexes were moved to the animal facility of the VAR and randomly assigned into 5 groups: The first group (n=8) received feed supplemented with (0.3%) coated calcium-butyrate salt (Green-Cab-70®, Sanluc International), the second group (n=8) was orally vaccinated at 3 and 6 weeks of age with a commercial Salmonella Typhimurium vaccine (Salmoporc®, IDT), and a third group (n=8) received drinking water adjusted to a pH 3.5-3.8 using a mixture of formic acid (50%), propionic acid (10%), acetic acid (10%) and lactic acid (5%) (Agrocid Super®, AgroLogic). A positive control group (infected/untreated; n=8) and a negative control group (uninfected/untreated; n=5) were included as well. Treatments were applied from weaning (3 weeks of age) until the end of the experiment (14 weeks of age). Each intervention group was duplicated.

At 8 weeks of age, 2 pigs of every pen - except the negative control group - were moved to a separate pen and were orally challenged (Day -1) with 108 cfu of the nalidixin-resistant Salmonella serovar Typhimurium strain 112910a, phage type 120/ad (Boyen et al., 2008). After 24h, these 'seeder' pigs were placed back in their original pens (Day 0).

From 3 weeks of age until the euthanasia at 14 weeks of age, blood samples were collected from all 69 pigs once a week and Salmonella-specific antibodies were detected with a commercial ELISA test kit (HerdChek Swine Salmonella®, IDEXX Laboratories) following the manufacturer's instructions. Before the challenge at 8 weeks of age, slurry samples were taken once a week and after challenge individual fecal samples were collected twice a week; Salmonella was isolated using the ISO 6579 annex D method. Salmonella counts were made on 3, 7 and 24 days post inoculation (DPI) using

standard enumeration protocols. At necropsy, samples from ileocecal lymph nodes, ileum, ileum contents, caecum, caecum contents, rectal feces and tonsils were analyzed for the presence of Salmonella. The diaphragm samples were frozen at -20°C, and then thawed again to collect exudates for detection of Salmonella-specific antibodies using the above mentioned ELISA.

The transmission of Salmonella Typhimurium in every group was estimated on the basis of the stochastic 'SI' infection model (Susceptible-Infectious) using an adjusted reproduction ratio 'RT' ('Transmission') that expresses the mean number of secondary infected animals caused by 2 infectious animals (I0=2) in a population of 6 susceptibles (S0=6) during a period of 6 weeks. This RT value was estimated via the Maximum Likelihood Estimation (MLE), and this for all groups (except the negative control) and for the following parameters: i) total amount of positive feces samples, ii) number of positive samples of ileum and/or ileum contents and/or caecum and/or caecum contents, iii) number of positive lymph nodes and/or tonsils, iv) the number of all positive organs and/or feces samples (table 1). Differences in the serologic response between the intervention groups were analyzed through the module PROC MIXED in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

All pigs were seronegative before the start of the study. The evolution of serum titers of the different treatment groups is shown in figure 1. The group receiving acidified water had a significantly higher mean antibody level compared to the positive control group and the Ca-butyrate supplemented feed group.

Results on Salmonella isolation from feces are shown in figure 2; significantly fewer pigs of the vaccination and the Ca-butyrate supplemented feed group excreted the challenge strain, compared to the acidified water and the positive control group (fewest in vaccination group, most in acidified water group). The quantitative determination of Salmonella spp. in feces 3, 7 and 24 DPI confirmed these isolation results. The same significant differences were found in the organs. A significantly lower antibody level was detected in the diaphragm fluid compared to serum.

The calculated adjusted reproduction ratio RT indicated that transmission was less successful in the vaccination group (RT=2.6) and in the group with coated butyric acid in the feed (RT=1.76) compared to the positive control and the group with acidified water (RT=+   with lower limit 1.88), looking at all organs and/or all collected faeces samples (table 1).

Discussion

In this study we evaluated and compared 3 intervention strategies on the transmission of Salmonella Typhimurium in pigs. Previous studies have demonstrated the possible utility of organic acids in feed or drinking water, which may reduce the number of Salmonella-positive pigs in a farm, especially coated butyric acid (Boyen et al., 2008). In the present study we demonstrated that coated calcium-butyrate (Green-Cab-70®, Sanluc International NV) was also capable of reducing Salmonella transmission between animals: in this group the fewest animals got infected and the lowest number of infected organs was seen. However, the antibody level of this group did not differ significantly from the positive control.

Vaccination with the commercial vaccine Salmoporc® (IDT) has also been shown to reduce both fecal shedding and colonization of the porcine intestinal tract (Springer et al., 2001).Though in the present study, this group had the lowest number of shedding pigs, it was the group with the highest S/P ratio and the number of colonized organs was comparable with that of the positive control group. Thus, given that neither the antibody level nor the organ contamination matched the infectiousness of the concerned vaccination group, this might compromise the ambition to change the current surveillance site in Belgium from farm to slaughterhouse.

Interestingly, the third intervention, the addition of a mixture of formic acid, propionic acid, acetic acid and lactic acid (Agrocid Super®, AgroLogic) to the drinking water, increased the transmission rate: in comparison with the positive control group, more pigs got infected, the S/P ratio was higher and more organ samples turned out positive for the infected strain. The higher S/P ratio we found in serum than in meat juice, shows that it is essential to recalculate the percent optical density (OD%) data obtained from meat juice by the ELISA (IDEXX HerdChek swine Salmonella), although the instructions to the kit for the detection of Salmonella antibodies state that its product is directly suitable for antibody detection in meat juice and sera (Wilhelm et al., 2007).

Conclusion

The results obtained demonstrate that vaccination with Salmoporc® and coated Ca-butyrate supplemented feed decrease the transmission of Salmonella Typhimurium in swine.

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The detection of Salmonella-specific antibodies against the vaccine strain however, poses a new challenge for the assignment of Salmonella positive herds in the Belgian Salmonella monitoring and surveillance programme. Further investigations on new interventions that do not interfere with interpretation of serological data, or investigations on new diagnostics that are not infuenced by vaccination, are needed.
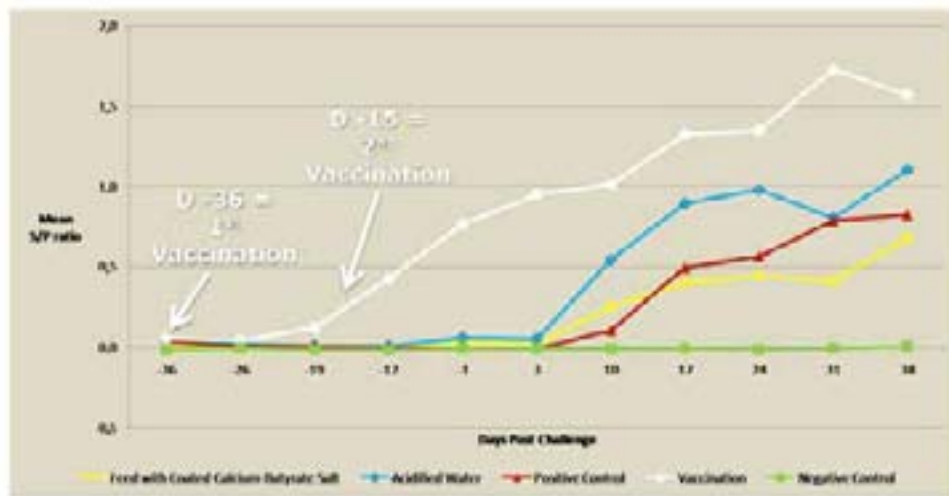
Figure 1: Serology results: Salmonella-specific antibody detection in serum

Figure 2: Bacteriology results: Isolation of inoculated Salmonella Typhimurium strain in feces.

Table 1: Calculated RT-values with the 95% confidence intervals

R_T	All feces	Ileum/contents/ cecum/contents	Lymph node/ tonsil	All organs/feces
Acidified feed	1.6 [0.85; 8.43]	0.8 [0.35; 3.80]	0.3 [0.12; 2.94]	1.76 [1.02; 9.01]
Acidified Water	3.5 [1.88; 11.70]	2.5 [1.67; 24.87]	3.5 [1.88; 11.65]	$+\infty$ [1.88; $+\infty$]
Vaccination	1.2 [0.56; 3.95]	1.0 [0.56; 3.95]	1.2 [0.70; 7.18]	2.6 [1.21; 9.45]
Pos control	3.5 [1.88; 11.70]	1.9 [1.22; 24.78]	0.9 [0.47; 4.23]	$+\infty$ [1.88; $+\infty$]

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A farm transmission model for Salmonella in pigs for individual EU Member States

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Abstract

The burden of Salmonella entering pig slaughterhouses across the European Union (EU) is considered to be of public health significance. Therefore, targets will be set for each EU Member State (MS) to reduce the prevalence of Salmonella in pigs at slaughter. As part of the evidence base for the development of National Control Plans (NCPs), a Quantitative Microbiological Risk Assessment (QMRA) was funded to support the scientific opinion required by the EC from the European Food Safety Authority, and subsequently adopted by the BIOHAZ panel. We present the farm transmission model component of the QMRA, which was used to model the transmission of infection between pigs and investigate the effect of on-farm interventions in reducing human salmonellosis attributable to EU pig meat consumption.

The magnitude of Salmonella shedding by the sow was a good predictor of eventual batch prevalence in slaughter pigs, as the subsequently infected piglets became a large source of Salmonella once mixed during weaning. As a direct result of this effect, it was concluded that MSs with high breeding herd prevalence (i.e. > 10-15% of breeding herds are infected with Salmonella) must tackle the breeding herd as part of any NCP in order to achieve a significant reduction in national slaughter pig prevalence. Conversely, it was predicted that MSs with low breeding herd prevalence would benefit most from controls on feed contamination, as this becomes relatively more important as a route of transmission of Salmonella when the sow is rarely a source of infection.

Introduction

The burden of Salmonella entering pig slaughterhouses across the EU is considered a primary food safety concern. Therefore, through EU legislation, targets are being set for each MS to reduce the prevalence of Salmonella infection in pigs at slaughter (probably as measured by infection of the ileo-caecal lymph node). These targets will be based on scientific studies commissioned by the EC/EFSA, including a QMRA. The primary aim of the QMRA was to assess the effectiveness of on-farm and abattoir interventions in reducing Salmonella levels in pigs and/or humans. In this paper we discuss in detail the Farm model, which describes the transmission of Salmonella within pig herds, and which can be used to investigate interventions that may reduce prevalence in pigs at slaughter.

Infectious disease transmission models have been developed for a variety of animal diseases, including Salmonella in pigs (Hill et al. 2008; Lurette et al. 2008) and are now often used within a full farm-to-consumption QMRA in order to predict the impact of on-farm interventions on public health (e.g. Simons et al. In Preparation). Typically these models have become more detailed over time and in the case of a recent study the traditional use of "general" transmission parameters was replaced by specifically modelling the environmental transfer of Salmonella via the faecal-oral route (Lurette et al. 2008). A transmission parameter is essentially a "black-box", which encompasses many different factors, including the resistance of the pig to infection and the level of contamination in the environment. However, in order to investigate interventions (such as cleaning and disinfection, vaccination etc…) it is necessary to differentiate between those factors that increase/reduce the level of contamination in the environment and those factors that affect the resistance of the pig to infection. The farm model was designed to be generic and can be parameterised (given relevant and available data) to represent any EU MS. In this paper the results from two case study MSs are described (one "low-prevalence" MS (MS1) and one "high-prevalence" MS (MS2)).

Material and Methods

The farm model is an individual-based stochastic Susceptible-Infected-Susceptible (SIS) model, therefore considering the infection status of every individual pig on a farm. The model is implemented using Monte-Carlo simulation and run for 1000 iterations, where each iteration represents production from one farm over a 500 day period, incorporating farrowing, weaning, and grower and finisher production. Management factors (for example flooring, feed type used) were used to define farm types. Farm types were allocated proportionally to the 1000 farms to represent the national structure of the pig herd within a particular MS, based on data from the EFSA breeding pig survey (EFSA, 2009). Over the 500-day cycle of production batches of pigs are sent to slaughter on a weekly basis. Two outputs are generated for each batch of pigs sent to slaughter: the prevalence of lymph-node infection and a distribution for the concentration of Salmonella shed within the faeces of infected pigs.

For each iteration there are a large number of spatial and temporal events that can occur at random, including the seeding of infection into the farm, the response to exposure (in terms of whether or not infection occurs given exposure to a particular dose) and subsequently the shedding rate. All farms are set to be Salmonella-negative at the start of an iteration (day 1). There are four assumed sources of infection: sows, feed, wildlife and the introduction of new infected stock. Following initial infection of the herd, which can occur at any time, transmission is described by an individual-based environmental infection model, which tracks i) the shedding and inactivation/movement of Salmonella in the environment and ii) the dose-response of pigs exposed to environmental contamination (see Figure 1). The schematic is appropriate for all rearing stages: farrowing, weaning, growing and finishing.

More detail on the methods and data used to parameterise the model can be found in the full QMRA report (EFSA, 2010).

Results

The average within-batch prevalence of lymph-node positive pigs at slaughter age was estimated to be 0.007 (5th percentile 0, 95th percentile 0.031) for MS1, and 0.176 (0, 0.813) for MS2. The percentage of positive batches for MS1 and MS2 were estimated to be 38.0% and 62.9% respectively. Comparing the percentage of positive batches with the average within-batch prevalence shows that the majority of batches are infected at low levels, even in "high"-prevalence MSs such as MS2.

Figure 2 summarises the impact of each source of infection (sow, feed, external contamination including wildlife) in determining the slaughter pig prevalence within the two case study MSs. Within MS2 reducing breeding herd prevalence to zero (i.e. pbreedingherd = 0) removes the vast majority of infections at depopulation; conversely, removing feed or external contamination as sources does little to change the national pig prevalence. This result suggests that the sow is a major source of infection; only when sow infection is rare (as in MS1), does feed play an important role in determining slaughter pig prevalence.
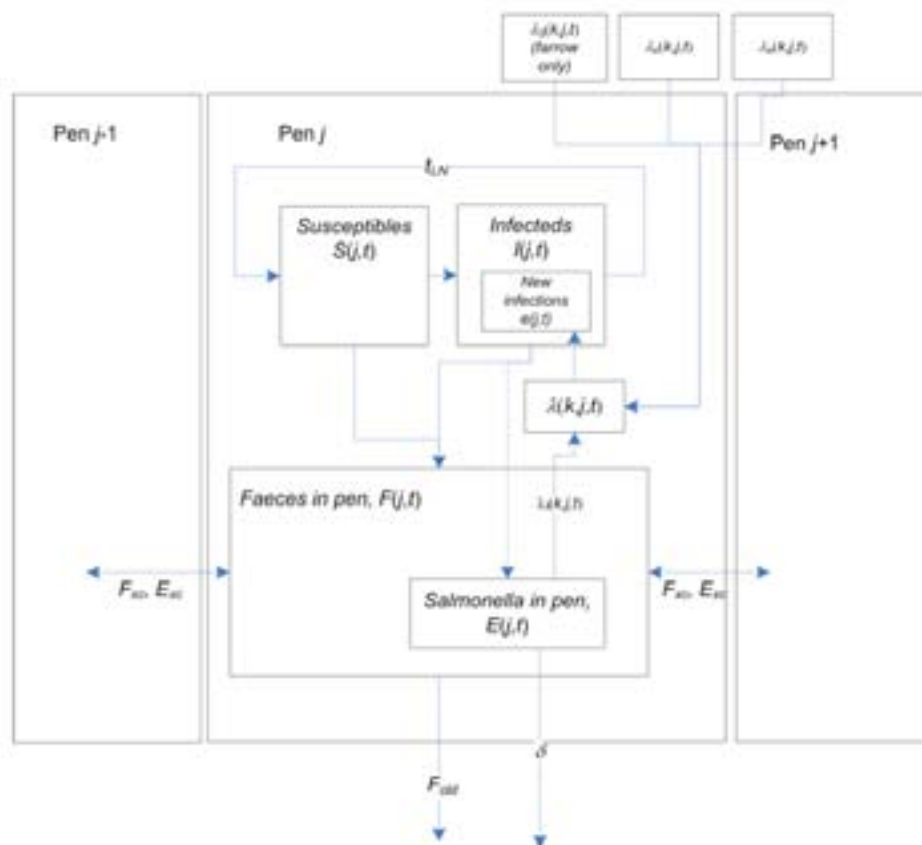
Figure 1: Schematic diagram of transmission model. Only the interactions associated with pen(j) are shown. The total faecal material in the pen, F(j,t), is added to each day by Susceptibles (S(j,t)), Infecteds (I(j,t)) and cross-contamination from other pens (Fxc) while it is simultaneously reduced each day via cross-contamination (Fxc) or removal (Fold). This faecal material contains E(j,t) salmonellas, which are added to each day from the infected group via shedding in their faeces and reduced each day as a result of decay, λ, and cross-contamination Exc. Each pig k ingests λ_i organisms per day via the amount in the faeces, λ_f via feed and λ_e via the environment and λ_s organisms from sow faeces (λ_s= 0 unless pig k is a piglet in farrowing). This process results in e(j,t) new infections according to the dose ingested and the dose-response relationship applied.

Discussion

Analysis of the model pointed to one overwhelming conclusion: the level of infection within a MS's breeding herd largely determines the slaughter pig prevalence for that MS. However, in low prevalence MSs of which MS1 is typical, infection of the sow is relatively rare and the proportion of initial infections of a piglet, weaner etc. via either feed or external contamination are relatively much higher. This result of breeding herd prevalence determining slaughter pig prevalence is supported by data from the EFSA Salmonella in pig surveys; breeding herd prevalence was correlated with slaughter pig prevalence (EFSA 2008, 2009).

Conclusion

In summary, breeding herd prevalence is likely to be a strong predictor of national pig prevalence and feed only becomes an important source of infection once contamination of the environment by sows or other slaughter pigs is reduced to low levels.
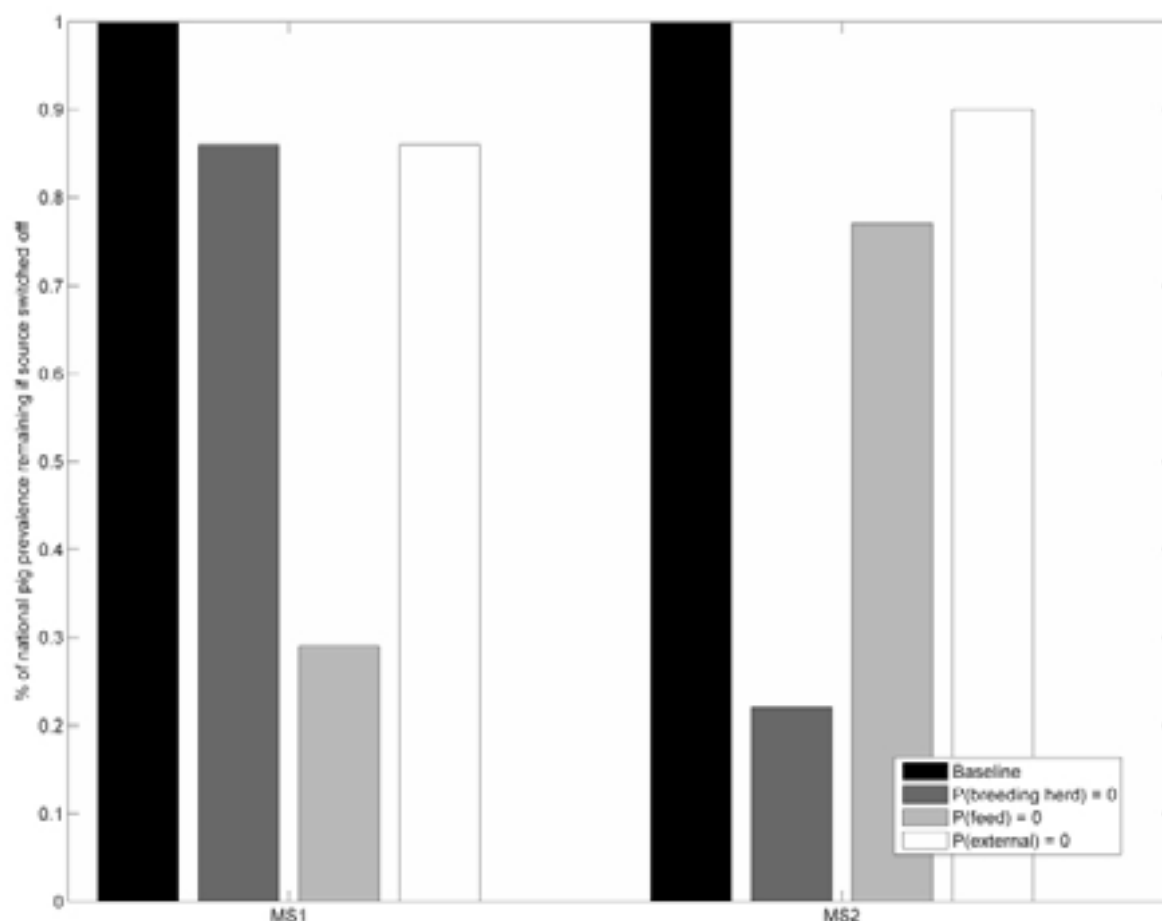
Figure 2: Relative impact on national pig prevalence for MS1 and MS2 if each source of infection is set to zero. Baseline (black), breeding herds all negative (dark grey), feed all negative (light grey), no external contamination events (white).

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Risk-mitigation for antimicrobial resistance in Danish swine herds at a national level

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Abstract

In Denmark, actions to mitigate the risk related to antimicrobial resistance have been put in place continuously. Due to an increase in the consumption of antimicrobials in the Danish pig production further actions were implemented in July 2010. These were: a voluntary ban on use of cephalosporin in Danish swine herds for a 2-year period and a so-called "Yellow card" scheme from the Danish Veterinary and Food Administration (DVFA). Farmers with the highest use of antibiotics receive a Yellow Card. Approximately 10% of Danish herds are above the yellow card threshold value. The consumption in pigs is evaluated as animal daily doses (ADD) per 100 animals seen over the last 9 months (by age group). Current permit limits for a yellow card in ADD/100 animal days are 5.2 (sows and piglets), 28 (weaners), and 8 (finishers). In July 2010, farmers with an antimicrobial use close to these limits were warned by the DVFA, that unless actions were taken to reduce their antimicrobial use, they would receive a Yellow card in December. The means are, for instance, restrictions on oral medication usage and supervision from the authorities to which most expenses are to be covered by the farmer. The warning resulted in a decrease in the national consumption to pigs of 12.5 % during the last half-year of 2010 compared to the same half-year in 2009. This decrease has continued into 2011 were the consumption in January-February was 24.5% lower than for January-February 2010.

Introduction

In Denmark, actions to mitigate the risk related to antimicrobial resistance are put in place continuously. Actions have for instance been implementation of the monitoring system called VETSTAT based on recording of drug usage at herd level (Stege et al., 2003), herd health agreements, restrictions on the use of furoquinolones and supervision of all veterinary practitioners in large animal practices by the DFVA. As veterinary medicine in Denmark is prescription only, the focus of risk management by DVFA, in order to reduce antimicrobial consumption, has been on the veterinary practitioner. Despite of this, the consumption of antimicrobials in the Danish pig production increased from to 3.4 g per pig in 2008 to 3.8 g per pig in 2009. The amounts used per pig are low compared to other countries with a similar pig production, and so is the prevalence of resistant zoonotic bacteria in both human and animal isolates (Danmap 2009). Hence, the current food safety risk related to antimicrobial resistance in Denmark occurs to be low.

However, among politicians and stakeholders there was a growing concern due to both the increased use of antimicrobials and due to the occurrence specifically of MRSA and ESBL in the Danish swine production. Therefore, further actions were required. The interventions applied were: 1) a voluntary ban on the use of cephalosporin in Danish swine herds for a 2-year period and 2) a so-called "Yellow card" initiative from the Danish Veterinary and Food Administration (DVFA). Thus, for the first time focus is on risk communication to the herd owner regarding the use of antimicrobials in his herd. This paper describes the interventions applied, and their effect on the total consumption of antibiotics in the Danish swine production.

Material and Methods

In Denmark, the monitoring system VETSTAT makes it possible to monitor the antimicrobial consumption closely both at the herd- and national level, and at the level of the individual antimicrobial compound. The herd's consumption can be followed closely by the farmer and herd-consultants and compared to the national average. This makes it possible to implement interventions based on the level of antimicrobial consumption in the individual herd.

In July 2010, initiatives were taken to decrease the use of antibiotics (Initiative 1) and prevent a further development of ESBL (Initiative 2).

Initiative 1. The course of events in DVFA's implementation of the "Yellow card" scheme was following:
In July 2010, permit limits for the Yellow Card scheme were defined for each of the three age groups: sows, weaners and finishers. For this purpose, the consumption was given as animal daily doses (ADD) per 100 animals, calculated as a simple 9-month moving average (by age group). Approximately the upper 10 % of the swine herds were affected, depending of the age group. The current permit limits, given for the years 2010 and 2011 are 5.2 (sows and piglets), 28 (weaners), and 8 (finishers) ADD/100 animal days.

In July 2010, 1249 farmers with an antimicrobial consumption close to or above these permit limits were warned by the DVFA, that unless actions were taken to reduce antimicrobial use, they would receive a Yellow Card scheme in December. In the future, Yellow Cards will be given monthly to new herds whose use of antimicrobials exceeds the defined permit limits. Yearly, permit limits will be regulated towards new lower values, as the consumption is reduced.

For farmers receiving a Yellow Card scheme, the following course of events may occur (Figure 1):
a. A 14-day examination of party, during which the farmer can object to the decision
b. If the objection is not accepted by the DVFA, a 9-month period of surveillance is started, during which the farmer must bring his herd's consumption below the permit limit. During this period there will be restrictions on oral medica tion usage and supervision from the authorities. All expenses are to be covered by the farmer.
c. If the consumption is not below the limit following the first 9-month period, then further restrictions are implemented. These include a tightened supervision, 2nd opinion visits by external consultants who elaborate a plan of actions to be followed to decrease the consumption below the permit limits. Such a plan could include changes in the management or purchasing of animals. The farmer is charged for the expenses related to supervision, enforcement orders and bans, and 2nd opinion visits.

Figure 1. Course of events following the pig producer's receipt of a Yellow Card warning

Initiative 2. With effect from July 2010, a voluntary ban on the use of cephalosporin in Danish swine herds was implemented for a 2-year period. Exemptions are only granted to farmers, who can document that cephalosporin is the only drug with effect towards a given disease causing problems within the herd.

Results

Until the Yellow Card warning in July 2010, the total consumption of antimicrobials had increased from 2008 to 2009. However, after the warning there was a significant decrease in the total consumption of antimicrobials (Figure 2). Thus, for the period July-December 2010 the total consumption was 12.5% lower than during the same half-year in 2009. This decrease is continuing into 2011 were the consumption in January-February was 24.5% lower than for January-February 2010. It is especially the use of tetracycline, macrolide, sulfa-TMP and lincomycin, which has decreased. The use of simple penicillins has increased slightly.

In December 2010, 1100 Danish farmers received a Yellow Card scheme. However, in 600 of these cases, the apparent high use was due to mistakes in the database (e.g. to low a number of animals registered to be present on the farm). The remaining 500 herds are now required to lower their antimicrobial use to below the permit limit within the next 9 months.

Figure 2. Total consumption of antimicrobials in the Danish pig production (January 2008 - February 2011). Dark line: 9-month moving average.
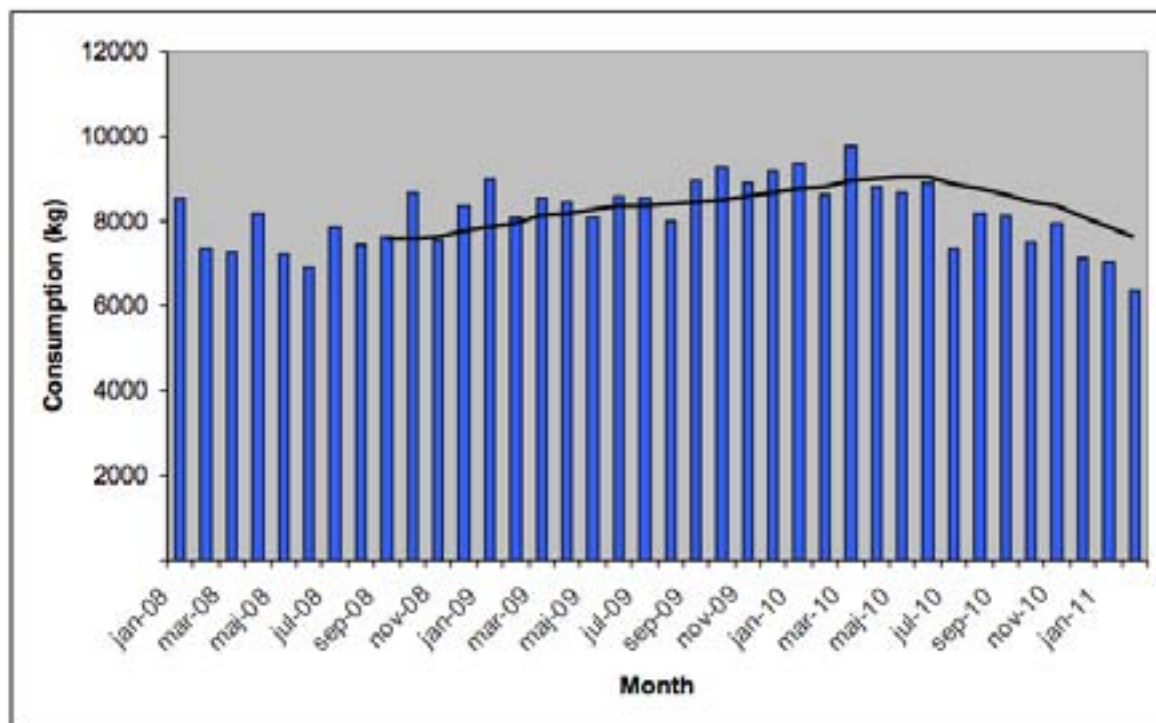
Exemptions from initiative 2, the cephalosporin ban in Danish pigs, have only been given to one herd. Therefore, the use of cephalosporin in Danish swine production is now negligible.

Discussion

The implementation of the Yellow Card Scheme has had a significant effect on the national consumption of antibiotics in the Danish swine production. Presumably some of the main motivators have been the fear of intensified supervision, risk of being publicly exposed and economically strained by charges and restrictions. However, the individual farmer may not necessarily see him or herself as contributing to the national consumption unless directly paid responsible. The decreased use of tetracyclines and macrolides indicates a declined oral medication of weaners; while the declined use of sulfa-TMP and lincomycin, could be due to less medication of sows.

The implementation of the Yellow Card scheme was possible because of the Danish monitoring programme VETSTAT. This system is very unique for Denmark, and therefore the Yellow Card intervention cannot easily be imitated in other countries. However, the Yellow Card scheme also has its drawbacks. These include f aws in the registration of the number of animals

presenting a herd. Thus, if a herd is registered incorrectly as having a low number of animals, the ADD/100 animal days will be falsely high, and the herd may incorrectly receive a Yellow Card. Also, outbreaks of diseases can periodically cause a significant increase above the permit limits.

It still needs to be explored if this significant decrease in antimicrobial consumption has caused an under-treatment of animals and therefore is jeopardizing animal welfare. Although the driver behind the Yellow Card scheme is to improve or ensure food safety, no specific follow-up on the occurrence of resistance has been planned. The national surveillance on resistance in Denmark, DANMAP, may, in time, give an indication of the effect of the decreased consumption on the prevalence of resistant bacteria. It should here be born in mind that there are several other – presumably more cost-effective ways of mitigating the exposure of consumers to resistant bacteria through food, e.g. by having focus on hygiene and decontamination procedures in relation to slaughter of pigs.

Conclusion

The recently introduced Yellow Card scheme has a significant effect on the total consumption of antimicrobials in the Danish swine production. This might, in part be due to the fact that the risk management focus have been on the herd owner for the first time.

Already after the warning in July 2010 there was a significant decrease in the total consumption of antimicrobials. Thus, for the period July-December 2010 the total consumption was 12.5% lower than during the same half-year in 2009. This decrease is continuing into 2011 were the consumption in January-February was 24.5% lower than for January-February 2010.

Also, the voluntary ban on the use of cephalosporin in pigs has lead to a negligible use of this class of antimicrobial, preventing the development of ESBL.

It still needs to be explored if this significant decrease in antimicrobial consumption has caused an under-treatment of animals and therefore jeopardized animal welfare.

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Association between Salmonella sp. and Yersinia enterocolitica infection in swine

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Abstract

Swine are known reservoirs for both Salmonella and Yersinia enterocolitica. Both are foodborne pathogens and can result in zoonotic disease if contamination of pork products occurs during harvest. The epidemiology of Y. enterocolitica and Salmonella in swine is not well understood. Previous reports from experimental studies in mice suggest that, via quorum-sensing, Salmonella detects Y. enterocolitica signals, increasing Salmonella colonization. The objective of this study is to determine if there was an association between fecal shedding of Salmonella and Y. enterocolitica in naturally infected swine. DNA was extracted from 1232 fecal samples collected from finishing pigs at commercial farms. The Salmonella status of the samples was known from previous culture results. All positive samples and a random selection of negative samples were included in the study. High throughput duplex real-time PCR reactions were conducted to detect the presence or absence of Y. enterocolitica. TaqMan® assays targeted the Y. enterocolitica ail gene and a Yersinia specific region of the 16S rRNA gene. The prevalence rate of Y. enterocolitica in Salmonella positive versus Salmonella negative fecal samples was 3.9% and 7.5%, respectively. Based on cross-sectional sampling, and the status of an individual fecal sample, Salmonella positive pigs were less likely to be Y. enterocolitica positive. At the pig level, there was no significant association between Salmonella and Yersinia enterocolitica status.

Introduction

Salmonella and Yersinia enterocolitica are both important foodborne pathogens of which swine are a reservoir. There are limited epidemiological investigations regarding co-infections with foodborne pathogens in swine. Yet, there are suggestions that that Y. enterocolitica infection may be associated with Salmonella infection.

Quorum sensing has been established as a form of communication between cells and a method by which bacteria can regulate their expression and colonization factors. Bacteria use LuxR-type transcription factors to detect self-produced N-acylhomoserine lactones (AHLs) in order to gain information about their own population density (Asad et al., 2008). Salmonella cannot synthesize its own AHLs, but does encode a LuxR-type AHL receptor, SdiA. It has been demonstrated that Salmonella can detect AHLs from other bacteria species, including Y. enteroclitica in a mouse model (Dyszel et al., 2009). Furthermore, Salmonella was seen to colonize the small intestines and Peyer's patches in higher number when the mice were co-infected with Y. enterocolitica. Given this prior research, our study was designed to explore a possible association between Salmonella sp. and Y. enterocolitica in the fecal shedding of swine. We hypothesized that swine shedding Salmonella in their feces would be more likely to be Y. enterocolitica positive as compared to swine from which Salmonella was not cultured from their feces.

Material and Methods

Sampling: Fecal samples were collected from various commercial finishing-stage swine operations in the state of Michigan between June 2008 and April 2010. The fecal samples were collected as a component of an on-going project investigating risk factors for Salmonella shedding in swine. Pigs were sampled 8 times at 2 week intervals from 10-26 weeks of age. All Salmonella culture positive fecal samples (n=383) and a random sample of culture negative fecal samples (n=849) were selected for inclusion in this study. These samples originated from 535 individual pigs. The fecal samples (250 mg) were stored at -80°C prior to inclusion in this study. In addition, since the Salmonella status of the pig during the entire finishing phase was known, pigs that were represented by the 1,232 samples (n= were categorized as "ever culture positive" meaning they had at least one fecal positive sample during finishing or "always culture negative" during the finishing phase.

DNA Extraction: DNA extraction was performed using the QIAamp DNA Stool Mini Kit (Qiagen, Inc.) following the manufacturer's protocol under "Stool Pathogen Detection" with slight modification. A vacuum manifold was utilized to remove the lysate and wash buffers from the spin columns instead of the centrifugation method as stated in the instructional booklet. In addition, only 75μl of AE buffer was used during the elution step.

Real-time Quantitative PCR: We targeted both the 16S rRNA gene (Sen , 2000) and ail gene (Mäde et. al 2008). Probes were purchased from Applied Biosystems and primers were constructed by the Macromolecular Core of the Research Technology Support Facility at Michigan State University. The forward primer for the 16S rRNA had the sequence 5'CG-GCAGCGGGAAGTAGTTT3' and the reverse primer 5'GCCATTACCCCACCTACTAGCTAA3'. These primers amplified a segment of the 16S rRNA gene spanning nucleotides 47 to 247. The TaqMan fuorescent probe had the sequence 5'VIC-AAGGTCCCCCACTTTGGTCCGAAG-TAMRA3'. VIC is the reporter dye and TAMRA (6-carboxytetramethylrhoda-mine) is the quencher dye. Primers targeting the ail gene had the following sequences. Forward primer 5'GGTTATG-CACAAAGCCATGTAAA3' and reverse primer 5'AAACGAACCTATTCCCCAGTT3' with the probe sequence of 5'FAM-AACCTGAAGTACCGTTATGAACTCGATGA-DQ3'. FAM (6-carboxyfuorescein) is the reporter dye and DQ is the quencher dye. TaqMan® assays were run on the AB 7900HT Sequence Detection System. Preliminary tests were run with the primers/probes on Y. enterocolitica strains 8081V and ATCC9610 (which were also utilized as positive controls for all runs). The results garnered showed that this particular set of primers and probes would be efficient and adequate at detecting Y. enterocolitica.

Statistical Analysis: To compare the association between Salmonella and Y. enterocolitica status of a fecal sample, an odds ratio was calculated and p-value determined using the $\chi 2$ statistic. The same method was used to compare the association between Salmonella status of the pig during the finishing phase (ever/never Salmonella positive) and Y. enterocolitica status based on the selected samples.

Results

A total of 76 samples (6.2%) were positive for Y. enterocolitica by PCR detection of the 16S rRNA target. Of these 73 samples (96.1%) were PCR positive for the ail gene. Six of the samples that tested positive for Y. enterocolitica amplified only one of the genes. (3 detected only ail; 3 detected only 16S rRNA). We considered a sample Y. enterocolitica positive if either the 16SrRNA or ail targets were detected (n=79).

Of Salmonella positive fecal samples, 3.9% were Y. enterocolitica positive (Table 1). Of Salmonella negative samples, 7.5% were Y. enterocolitica positive. Salmonella positive fecal samples were 0.5 times as likely to be Y. enterocolitica positive. (OR 0.5; 95% CI 0.27, 0.87; p<0.01)

Because the Salmonella status at the pig level during the finishing phase was known as a result of the on-going study from which the samples originated, we evaluated the odds for a sample to be Y. enterocolitica positive if the pig had ever been detected as Salmonella positive during the finishing period. Of the 535 total pigs that contributed to the study 22.6% (121/535) had at least one Salmonella positive fecal sample detected during the finishing period, and 11.8% (63/535) had at least one positive Y. enterocolitica sample detected within this study. The proportion of pigs that were ever detected as Salmonella positive that had a positive Y. enterocolitica sample detected in this study was 14.1% and for pigs from which Salmonella was never detected the proportion of pigs with a positive Y. enterocolitica sample was 11.1%. The odds of pig that was Salmonella positive during the finishing period had a Y. enterocolitica positive sample in this study was 1.3 (95% CI 0.67-2.44, p>0.05).

Discussion

The results of this study do not support our original hypothesis that Salmonella positive swine are more likely to be colonized with Y. enterocolitica.

There are several limitations to this study, not the least of which is that the sampling strategy was a convenience sample of a subset of fecal samples that were selected for another purpose. Therefore, the sampling strategy did not allow longitudinal within pig assessment of Y. enterocolitica status (which was available for Salmonella). Therefore, we have an incomplete picture of the Y. enterocolitica status of these pigs over the same period for which Salmonella status is known. Future evaluations of samples targeted at the specific research question of this investigation are planned.

One of the effects this study could have on food safety is the potential for an alternative way to help decrease the prevalence of Salmonella and other foodborne pathogens on farms. If mechanisms that impact pig susceptibility to infection with foodborne pathogens, such as fomented co-infection via quorum sensing, are demonstrated to be important, it may provide an additional tool for control of foodborne pathogens on farms.

Conclusion

In this study, there was no evidence that pigs that were Salmonella positive on fecal culture were more likely to be Y. enteroclitica positive. Future studies with appropriate study design are necessary to evaluate this association.

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Aerial dissemination of Clostridium difficile spores inside and outside a pig farm

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Abstract

In both human and veterinary medicine Clostridium difficile infections are increasingly reported. The observation that aerial dissemination occurs in a hospital environment and can play a role in the transmission of human C. difficile infection, resulted in the present study to the occurrence of airborne C. difficile in, and nearby a pig farm with a high prevalence of C. difficile. Airborne C. difficile was detected in all farrowing and weaned piglets' wards, and up to 20 m distant from the farm. Personnel activity in the farrowing pens was significantly associated with peaks in airborne C. difficile colony counts.

Introduction

Clostridium difficile has been described as a pathogen for piglets since the beginning of the 21st century (Songer, 2004). In North America, C. difficile infection (CDI) is now considered the most important cause of neonatal diarrhea in piglets (Songer & Uzal, 2005). In humans, the bacterium is known since decades as a major cause of nosocomial infections. Recently, CDI is increasingly reported as a community acquired infection (Bauer et al, 2009, Indra et al., 2009). The majority of the community acquired infections are caused by C. difficile PCR Ribotype 078 (Kuijper et al., 2006; Wilcox et al., 2008; Bauer et al., 2009). The 078 strain is also predominantly present in piglets with CDI (Keessen et al., 2010). The genetical similarity of the human and pig strains gave rise to the concern that zoonotic transmission of this strain is likely to occur (Debast et al., 2009; Rupnik & Songer, 2010; Weese, 2010). Nonetheless, until now there is no evidence of a zoonotic transmission (Weese 2010) and there is still little knowledge on possible transmission routes from animals to humans (Jhung et al., 2008).

Aerial dissemination of C. difficile was suggested to play a role in the transmission of human CDI in hospitals (Best et al., 2010). This prompted us to study whether aerial dissemination of C. difficile also occurs in pig farms and whether personnel activity is related to increases in spore counts. An additional goal of this study was to determine whether C. difficile could be detected in the air in the close vicinity of the farm.

Material and Methods

2.1. Farm

Air sampling was done at a pig-breeding farm with a known high prevalence of Clostridium difficile in the pigs. The ventilation in the pens is based on a negative pressure system. Fresh air enters the pens from the hallway through slotted air inlets in the doors. The air leaves the pens through a fan, at a height of four meter, which directs the air into an airshaft or directly into the outside environment.

2.2. Sampling procedure

A MB1 MICROBIO Air Sampler (Parrett Technical Developments) was used for collection of airborne Clostridium difficile. The air was directed on commercially prepared C. difficile agar plates (CLO-agar, BioMérieux). Following sampling the agar plates were kept in a refrigerated box, until the laboratory was reached.

2.3. Sampling strategy

2.3.1. Inside air sampling

Sampling of the farrowing wards was performed in the ventilation shaft of the building. The numbers of pigs and piglets of each farrowing ward and pen were registered at the beginning of the experiments. Subsequent parturitions and

changing number of piglets in the farrowing ward were registered as well. The air of two farrowing wards was continuously sampled combined with the registration of personnel activity. Comparison between the activity data and the colony count was possible as both were taken as a function of time.

2.3.2. Continuous sampling during movement of weaned piglets
On three occasions sampling was performed prior, during and after movement of weaned piglets from their farrowing pen to the weaned piglets ward. Air coming from these farrowing pens was sampled continuously with a sampling time of 5 minutes.

2.3.3. Outside air sampling
Outside air sampling was performed above roof exhausts and at distances 20, 40, 80 and 140 meter downwind from these exhausts at a height of 1.5 m. Sampling time was set on 5 minutes. Data of the Dutch Meteorological Institute was used to determine wind speed and temperature. Control sampling was performed at an upwind point 20 meter distant from the nearest exhaust to exclude any other sources of airborne Clostridium difficile.

2.4. Analysis procedure
Samples were incubated on the CLO-agar plates at 37 °C for 48 h under anaerobic conditions. Using Gram staining the isolates with morphology typical of C. difficile were identified. Per ward and experiment two isolates were randomly chosen, both to be ribotyped according to the method described by Paltansing (2007). All Isolates from the outside samples were ribotyped as well. Colony counts were calculated per m3.

2.5. Statistical analysis
Data from the continuous sampling experiments were analyzed using the t-test to investigate the correlation between personnel activity and colony count.

Results

3.1. Inside air sampling
Personnel activity was significantly associated with an increase in airborne colony count in both farrowing wards (P = 0.043, P = 0.034). Highest colony counts up to 575 colonies per m3 were found during or shortly after feeding, ear tagging and entrance of the farmer.
Most peaks in colony counts corresponded with activity prior to sampling. One of the two highest peaks (575 colonies per m3) was not preceded by registered activity. On the other hand, activity was not always related to a peak; e.g medical care by students did not result in an increasing number of airborne colonies.
Movement of the weaned piglets from their farrowing to the weaned piglets' ward correlated significantly to an increase in colony count (P = 0.028).
The numbers of colonies found during movement increased on average 7.7 times compared to the numbers of colonies found prior to the movement. The numbers of colonies of the three pens show a fast decline once the piglets have been moved. The air of one pen continued to have a high concentration of colonies, with the highest concentration found 20 minutes after movement of the pigs.

3.2. Outside air sampling
Air from all four exhausts on the top of the building (consisting of air coming from farrowing, boar and young sow ward) tested positive for C. difficile, the numbers ranged from 6 colonies/m3 to 120 colonies/m3. Outside air tested positive 2 out of 4 times at a distance of 20 m downwind from the building. No colonies were found 40, 80 and 140 meter distant of the building. Outside temperature ranged from 2 °C to 8 °C, airspeed ranged from 0.83 m/s to 5.3 m/s. Positive air samples were obtained with the highest airspeeds (5.3 and 3.2 m/s). All upwind air samples were negative for C. difficile.

3.3 PCR Ribotyping
In most air samples within the farm and at 20 m distance from the farm C. difficile was detected. A collection of this share was ribotyped. All 20 C. difficile isolates were identified as PCR ribotype 078.

Discussion

The aim of this study was to detect C. difficile in the air of a pig farm and to relate colony counts of C. difficile in air samples to personnel activity. The results demonstrate that personnel activity correlates significantly to an increase in colony count. One of the highest peaks was not linked to personnel activity. An explanation for this peak might be that this increase in colony count was caused by animal activity, though this was not registered.

In outside air, colonies were detected up to 20 m distant from the farm. The large decrease in colony count immediately outside the building is a logical consequence of the dilution by outside air, and generally applies to the total bacteria concentration (Homes et al., 1996). Limited dispersal of airborne Clostridium difficile to the outside environment could implicate a low risk of human exposure to airborne Clostridium difficile. We could not find previous studies on the potential and mechanisms of infection by airborne spread of C. difficile in animals. Other gastro-intestinal pathogens such as Salmonella species, Campylobacter species and Clostridium botulism have been proven to be able to infect by airborne transmission (Sugiyama et al., 1986; Pillai & Ricke, 2002; Oliveira et al., 2006).

Conclusion

This study demonstrates a significant correlation between personnel activity and airborne C. difficile colony counts. The widespread aerial dissemination of Clostridium difficile in the farrowing wards may have implications for aerial transmission of Clostridium difficile between piglets. The finding of C. difficile in limited numbers at a 20m distance in the air needs further research to the spread of C. difficile in the environment of pig farms, to determine its significance for human health.

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Stress induced Salmonella Typhimurium re-excretion by pigs is associated with cortisol induced increased intracellular proliferation in porcine macrophages

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Abstract

Infections of pigs with Salmonella enterica subspecies enterica serovar Typhimurium (Salmonella Typhimurium) often result in the development of carriers that intermittently excrete Salmonella in very low numbers. During periods of stress, recrudescence of Salmonella may occur. The mechanism of stress related re-excretion of Salmonella by pigs is poorly understood and the aim of the presented study was to determine the role of the stress hormone cortisol on Salmonella re-excretion by pigs.

We showed that a 24 hour feed withdrawal increases the Salmonella Typhimurium load in pigs, which is correlated with increased cortisol blood levels. A second in vivo trial showed that the stress related re-excretion of Salmonella Typhimurium in pigs can be induced by intramuscular injection of dexamethasone. Furthermore we demonstrated that cortisol promotes intracellular proliferation of Salmonella Typhimurium in porcine alveolar macrophages, but not in intestinal epithelial cells, at a concentration (1 µM) that did not exert a notable effect on porcine cell viability and gene expression of Salmonella Typhimurium. This implies that the enhanced survival of Salmonella is probably caused by an indirect effect of cortisol on the cell.

Introduction

Pigs infected with Salmonella Typhimurium can carry this bacterium asymptomatically in their tonsils, gut and gut-associated lymphoid tissue for months resulting in so called Salmonella carriers. During periods of stress recrudescence of Salmonella may occur (Berends et al., 1996). Until now, the mechanism of stress related re-excretion of Salmonella in pigs is not well known. We hypothesized that cortisol plays a role in the stress related recrudescence of Salmonella Typhimurium in pigs.

Material and Methods

Salmonella strain: Salmonella Typhimurium strain 112910a, isolated from a pig stool sample and characterized previously by Boyen et al. (2008), was used.

In vivo trials: In a first in vivo trial, we investigated the effect of different types (feed withdrawal, isolation and overcrowding) of stress on the re-excretion of Salmonella Typhimurium by carrier pigs. In a second in vivo trial, we intramuscularly injected carrier pigs with 2 mg dexamethasone per kg body weight to test our hypothesis that cortisol plays a role in the recrudescence of Salmonella Typhimurium in pigs.

Cytotoxicity assays: The cytotoxic effect of cortisol on porcine alveolar macrophages and IPEC-J2 cells was determined using the lactate dehydrogenase cytotoxicity detection kit (Roche Applied Science, Bazel, Switzerland), in accordance to the manufacturer's instructions.

Effect of cortisol on the growth of Salmonella Typhimurium: The effect of cortisol on the growth of Salmonella Typhimurium in LB broth was examined during 24 hours.

Effect of cortisol on the gene expression of Salmonella Typhimurium: RNA was isolated from Salmonella Typhimurium using the SV Total RNA purification kit (Promega, Leiden, the Netherlands). Gene expression was measured using a Salmonella microarray constructed at the Institute of Food Research, Norwich, UK.

Invasion and intracellular survival assays: The ability of Salmonella Typhimurium to invade and proliferate in PAM and IPEC-J2 cells after exposure to cortisol was performed as described by Boyen et al., 2009.

Macrophage chemiluminiscence: The effect of cortisol was examined on the reactive oxygen species production of porcine alveolar macrophages, as described by Boyen et al., 2006.

Results

Feed withdrawal stress results in increased numbers of Salmonella Typhimurium bacteria in the gut of pigs and elevated cortisol levels

As illustrated in figure 1, carrier pigs subjected to feed withdrawal stress, 24 hours before euthanasia, showed elevated numbers of Salmonella Typhimurium in their bowel contents and organs in comparison to the control group that was not stressed. Furthermore, these pigs had significantly elevated serum cortisol levels (66.88 ± 6.72 nM) compared to the control group (48.65 ± 4.67 nM).
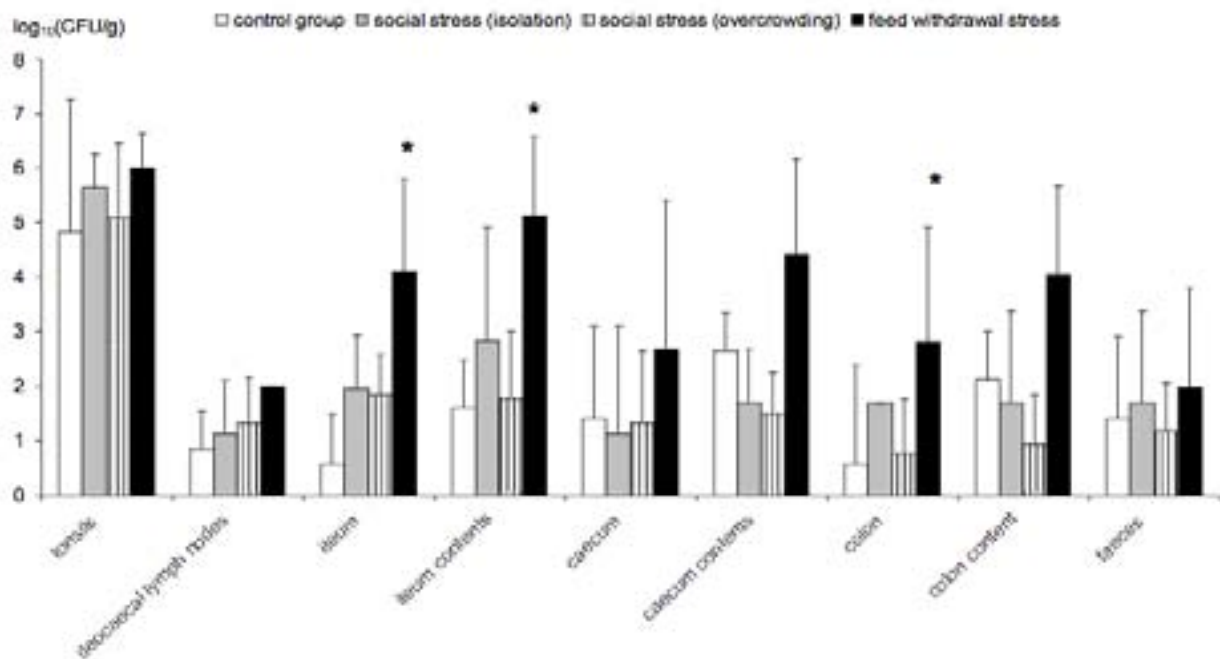
Figure 1: Recovery of Salmonella Typhimurium from pigs that were submitted to either feed withdrawal (n = 6) or social stress, isolation (n = 3) and overcrowding (n = 9), 24 hours before euthanasia. Six pigs were not stressed and served as a control group. The log10 value of the ratio of CFU per gram sample is given as the mean + standard deviation. Superscript (*) refers to a significant difference compared to the control group (p < 0.05).

Dexamethasone increases the number of Salmonella Typhimurium bacteria in the gut of pigs

As illustrated in figure 2, carrier pigs that were intramuscularly injected with 2 mg dexamethasone per kg body weight, 24 hours before euthanasia, showed elevated numbers of Salmonella Typhimurium in their gut tissues and contents in comparison to the control group.
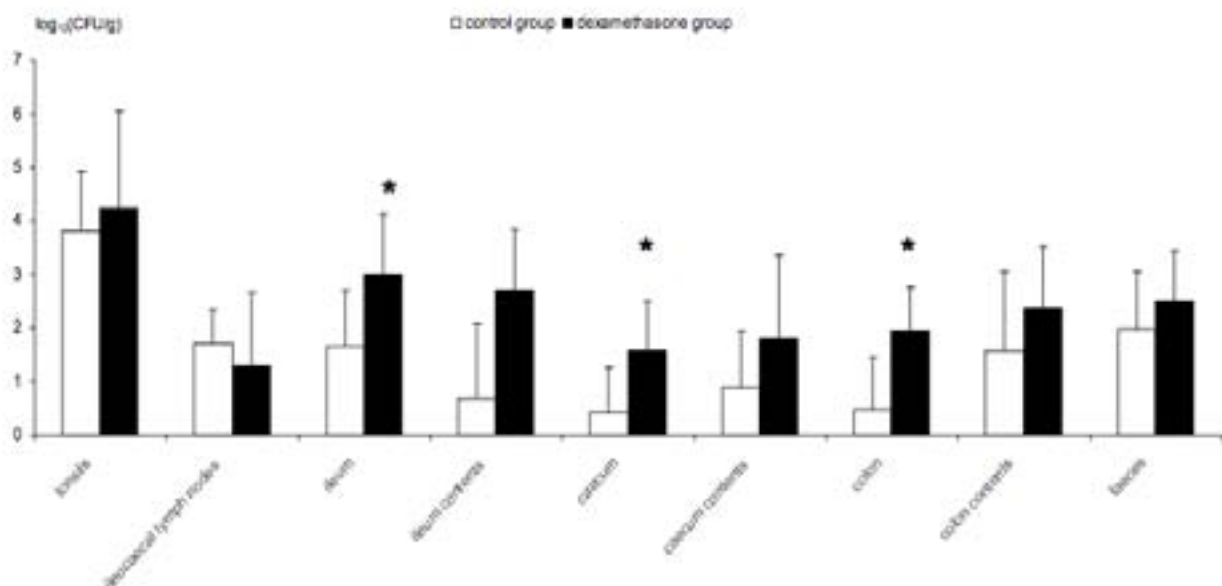
Figure 2: Recovery of Salmonella Typhimurium bacteria from pigs that were injected with either HBSS (control group, n = 9) or 2 mg dexamethasone per kg body weight (dexamethasone group, n = 9), 24 hours before euthanasia. The log10 value of the ratio of CFU per gram sample is given as the

mean + standard deviation. Superscript (*) refers to a significant difference compared to the control group (p < 0.05).

Cortisol does not affect Salmonella growth and gene expression, porcine intestinal epithelial cell viability and porcine macrophage viability and ROS production

Cortisol concentrations ranging from 0.001 to 100 µM did neither affect the growth of Salmonella Typhimurium, nor the viability of PAM and IPEC-J2 cells, during 24 hours. The exposure of Salmonella Typhimurium to 1 µM cortisol did not significantly affect gene expression levels. No significant differences were noticed in ROS production between Salmonella Typhimurium treated PAM in absence or presence of 1 µM cortisol.

Cortisol and dexamethasone promote the intracellular proliferation of Salmonella Typhimurium in porcine macrophages but not in porcine enterocytes

The intracellular proliferation of Salmonella Typhimurium was higher in cortisol and dexamethasone treated PAM in comparison to non-treated cells. Cortisol and dexamethasone did neither affect the intracellular proliferation of Salmonella Typhimurium in IPEC-J2 cells, nor the invasion in PAM and IPEC-J2 cells.

Discussion

Our results are in accordance with earlier studies conducted in pigs that showed that feed withdrawal is associated with increased shedding of Salmonella Typhimurium (Isaacson et al., 1999; Martín-Peláez et al., 2009; Morrow et al., 2002). Until now, the mechanism of stress related re-excretion of Salmonella in pigs remains unknown, but we showed that starvation stress results in elevated serum cortisol levels and that dexamethasone could induce recrudescence of Salmonella Typhimurium in pigs. This implies that stress induced release of cortisol in the bloodstream could alter the outcome of a Salmonella Typhimurium infection in pigs.

Earlier research in vitro has shown that norepinephrine in vitro promotes the growth and the motility of Salmonella enterica (Bearson and Bearson, 2008; Methner et al., 2008). We provided evidence that cortisol does not have similar effects on growth and does not influence gene expression of our Salmonella Typhimurium strain. Cortisol and dexamethasone nevertheless promote intracellular proliferation of Salmonella Typhimurium in porcine macrophages at concentrations that do not exert a notable effect on cell viability and ROS production by PAM.

These current results highlight the role of cortisol in the re-excretion of Salmonella Typhimurium by pigs and they provide new evidence for the role of microbial endocrinology in host-pathogen interactions.

Conclusion

In conclusion, we showed that the glucocorticoid cortisol is involved in a stress induced recrudescence of Salmonella Typhimurium in carrier pigs. In addition to this we pointed out that cortisol promotes the intracellular proliferation of Salmonella Typhimurium in pig macrophages, which is probably caused by an indirect effect through the cell.

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Genetic characterization of Yersinia enterocolitica collected from tonsils of slaughtered pigs

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Abstract

From January to March 2009, detection of pathogenic Yersinia enterocolitica was done from 900 tonsils swabs collected from 45 pig batches in one slaughterhouse. 316 Y. enterocolitica isolates were collected and confirmed as pathogenic biotype by biochemical tests. For this study, these strains were genetically characterized on the basis of their virulence genes and their PFGE profiles.

Real Time PCR was used to evaluate the presence of genes ail, myfA, and ystA on the genome and the gene yadA on the pYV plasmid. PFGE analysis using XbaI enzyme was also realised.

Most of the isolates belonged to biotype 4 (85.1 % of the isolates) and 14.9% of the isolates were from biotype 3. All the isolates carried the 3 chromosomal genes, ail, myfA and ystA genes, except one for which ystA was not detected. Among these strains, 278 isolates carried the pYV plasmid (88.0%); 233 from biotype 4 and 45 from biotype 3. All the 31 positive pig batches in pathogenic Y.enterocolitica have at least one isolate which exhibits the virulence plasmid.

The PFGE revealed 5 Xba1 genetic profiles coded G1 to G5. Forty-nine % of the isolates highlighted the same major PFGE patterns, pattern G4; followed by pattern G5 (16.7% of the isolates), pattern G2 (14.8% of the isolates), pattern G1 (12.3%) and pattern G3 (7.0%). Among the patterns, patterns G2 and G3 were only associated to biotype 4 while patterns G1, G4 and G5 were found for the biotype 4 and 3. On the 31 positive pig batches, one to three different PFGE patterns could be found in one batch, with several possible combinations.

The study on this collection of isolates showed that all the pathogenic isolates have virulence genes. This indicates that identification of pathogenic biotype can be realised by detection of the ail and myfA genes through PCR method instead of biochemical tests. PFGE analysis using XbaI enzyme showed that Y. enterocolitica is very clonal with a major PFGE patterns but this method do not allowed the differentiation of biotype 4 from biotype 3.

Introduction

In 2009, yersiniosis was, for the sixth consecutive year, the third most frequently reported human zoonosis in the Europe, with a total of 7,595 confirmed cases (EFSA, 2011). The species is divided into six biotypes. Y enterocolitica can be classified into biotype 1A, generally regarded as nonpathogenic, and the pathogenic biotypes 1B, 2, 3, 4, 5 (Wauters et al, 1987). In France and most other countries worldwide, biotype 4 is the most prevalent biotype isolated from humans (69%), followed by biotype 2 (30%) and biotype 3 (Savin and Carniel, 2008). Pigs are considered the principal reservoir for the types of Y. enterocolitica pathogenic to humans. Pigs do not develop clinical signs, but they do carry Y. enterocolitica in their oral cavity, on tongues and tonsils, and in lymph nodes, and excrete this bacterium in their feces (Thibodeau et al., 1999).

Identification of biotype lies on panel of biochemical tests as described in the ISO 10273-2003 method allowing differentiation of pathogenic biotypes from the non-pathogenic biotype. Moreover, strains of biotypes 1B and 2 - 5 possess chromosomal virulence genes: the ail gene product is a small outer membrane protein, which promotes bacterial adhesion to, and invasion of, cultured epithelial cells; ystA, which is responsible for the production of thermostable enterotoxin, which facilitates the invasion of the Y. enterocolitica into tissues by damaging the intestinal epithelium; and the myfA gene, which encodes the major subunit of antigen Myf. This fibrillar structure has been found to promote the colonization of the intestine (Revell and Miller 2001; Tennant et al., 2003). Virulent Yersinia strains carry an approximately 70 kb plasmid termed pYV. The outer membrane protein YadA (Yersinia adhesin A) encoded by genes on the pYV plasmid has been found to play multiple functions in pathogenesis. YadA protects yersiniae against the antibacterial activity of complement and defensin (Roggenkamp et al. 2003) and mediates specific binding of Y. enterocolitica to collagen, laminin and cellular fibronectin (Heesemann et al., 2006).

The aim of this study was the confirmation of the pathogenicity of Y. enterocolitica strains isolated from pigs by using Real-Time PCR assays for the detection of plasmid- and chromosome-borne virulence genes. Moreover, the analysis of the occurrence of virulence genes in different biotypes and PFGE Xba1-patterns was conducted.

Material and Methods

Origin of the strains
A total of 316 isolates of pathogenic Y. enterocolitica were collected from tonsils swabs of 31 pig batches (on the 45 studied) in one slaughterhouse between January and March 2009 (Fondrevez et al., 2010). Biotyping was realised by biochemical tests as described in the ISO 10273-2003 method with the following tests: esculin hydrolysis, indole production, and fermentation of xylose and trehalose. Strains of biotype 1A (IP124), biotype 4/0:3 (IP134), biotype 3/0:5,27 (IP29228) and biotype 2/0:9 (IP383), purchased from the Pasteur Institute (Paris, France), were used as controls.

DNA extraction and Real-Time PCR for detection of virulence genes
Real Time PCR was used to evaluate the presence of virulence genes ail, myfA, and ystA carried by the genome and the gene yadA carried by the pYV plasmid.
Strains were sub-cultured on PCA at 30°C for 24h. DNA was extracted from some colonies with QIAamp DNA mini kit (Qiagen, USA) following the manufacturer's instructions. The 4 PCRs were performed on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California), in a final volume of 25µl with the Sybr® Green JumpstartTM Taq ReadyMix TM (Sigma-Aldrich, Saint Louis, Missouri).) at 1X. The genes ail, myfA, yadA (Kot et al, 2010) and ystA (Ibrahim et al, 1997) were detected with specific primers as indicated in table 2. The final concentration of primers in the PCR reaction was 0.3 µM for ail and myfA, 0.2µM for yadA and ystA. The amplification conditions for each gene are detailed in table 1.

Table 1: Primer sequences and PCR conditions for detection of virulence genes by Real-Time PCR

Genes	Primer sequence	First step	Cycle of amplification	Melt Curve	Size in bp	Expected Tm
ail	F - TGG TTA TGC GCA AAG CCA TGT R - TGG AAG TGG GTT GAA TTG CA	94°C 2 min	35 cycles of Denaturation 91°C 30 sec Annealing 57°C 30 sec Extension 72°C 60 sec	55°C -> 89°C, increment of 0.5°C for 5 sec	356	82.5°C
myfA	F - CAG ATA CAC CTG CCT TCC ATC T R - CTC GAC ATA TTC CTC AAC ACG C	94°C 2 min	35 cycles of Denaturation 94°C 60 sec Annealing 58°C 90 sec Extension 72°C 90 sec	56°C -> 95°C, increment of 0.5°C for 5 sec	272	84.5°C
yadA	F - TAA GAT CAG TGT CTC TGC GGC A R - TAG TTA TTT GCG ATC CCT AGC AC	94°C 2 min	35 cycles of Denaturation 94°C 60sec Annealing 58°C 60sec Extension 72°C 60sec	55°C -> 95°C, increment of 0.5°C for 5 sec	747	86°C
ystA	F - AAT GCT GTC TTC ATT TGG AGC R - ATC CCA ATC ACT ACT GAC TTC	94°C 2 min	35 cycles of Denaturation 92°C 10sec Annealing 55°C 25 sec Elongation 72°C 20 sec	54°C -> 90°C, increment of 0.5°C for 5 sec	145	83.5°C

RFLP/PFGE for genotyping
Strains were sub-cultured on PCA at 30°C for 24h. Bacterial suspension in TN were adjusted to an optical density (600 nm) of 1.5 and mixed with 1% agarose for the plug preparation. Plugs were incubated for 24h at 50°C in lysis solution (Na2EDTA 0,5M, pH9, N-lauryl-Sarcosyl 1% (p/v), protéinase K 1 mg/ml). A total of six washes (0.01 M Tris-EDTA buffer, pH 8.0) were used to remove excess reagents and DNA was then digested with 40U of XbaI at 37°C for 4 hours. The electrophoresis conditions had an initial switch time of 1.5s, with final switch time of 18.0s, for 25h.
Gel images were captured on a gel doc 1000 imaging system (Bio-Rad, Hercules, California) and analysed with BioNumerics software (Applied Math, Sint-Martens-Latem, Belgium).

Results

Most of the isolates belonged to biotype 4 (85.1 % of the isolates) and 14.9% of the isolates were from biotype 3 (table 2).

Table 2: distribution of the isolates according their biotype, virulence genes and PFGE patterns

Biotype	Presence of virulence gene	PFGE patterns					
		G1	G2	G3	G4	G5	total
Biotype 4	ail+, myfA+, ystA+, yadA+	7	45	21	122	38	233
	ail+, myfA+, ystA+, yadA-	5	2	1	14	13	35
	ail+, myfA+, ystA-, yadA-	0	0	0	1	0	1
Biotype 3	ail+, myfA+, ystA+, yadA+	27	0	0	18	0	45
	ail+, myfA+, ystA+, yadA-	0	0	0	0	2	2
	Total	39	47	22	155	53	316

All the isolates carried the 3 chromosomal genes, ail, myfA and ystA genes, except one for which ystA was not detected. Among these strains, 278 isolates carried the pYV plasmid (88.0%); 233 from biotype 4 and 45 from biotype 3. Only one biotype 4 isolate didn't carry the ystA gene but has the ail and myfA genes. All the 31 positive pig batches in pathogenic Y. enterocolitica have at least one isolate carrying the virulence plasmid. In 12 batches among the 31, isolates without plasmide were detected.

The PFGE revealed 5 Xba1 PFGE patterns coded G1 to G5 (table 2). Forty-nine % of the isolates highlighted the same major PFGE patterns, pattern G4; followed by pattern G5 (16.7% of the isolates), pattern G2 (14.8% of the isolates), pattern G1 (12.3%) and pattern G3 (7.0%). Among the patterns, patterns G2 and G3 were only associated to biotype 4 while patterns G1, G4 and G5 were found for the biotype 4 and 3.

On the 31 positive pig batches, 11 batches have isolates which belong to one PFGE pattern and 20 batches present a combination of two to three different patterns. Several combinations were found between the five patterns. Patterns 1 and 3 were never found alone in a batch, they were always associated with another pattern.

Discussion

Genotype ail+ myfA+ystA+yadA was predominant in our strain collection whatever the biotype. This is consistent with the result of Kot et al., (2010) for which all the biotype 4;0:3 had all these virulence genes. Results confirming the presence of the ail and ystA genes in biotype 1B.,2, 3 and 4 were also collected by Thoerner et al. (2003). In our study, 12% of the isolates did not carry the pYV plasmid. It is known that strains subjected to long-term storage and repeated passaging at 37°C lost their virulence plasmid, but in our survey the DNA of isolates was extracted shortly after their isolation, and no step was made at 37°C after the swabbing. So it can be assumed that strains were without pYV plasmid on our pig tonsils. Therefore, the assessment of the indicators of Yersinia enterocolitica virulence need not to be restricted to the detection of plasmid-localized genes of virulence, but requires at least one chromosomal gene of virulence be present so that errors are avoided in the estimation of strain virulence.

All our isolates could be biotyped by biochemical tests and detection of the virulence genes confirmed their pathogenicity. However, authors (Kote et al., 2010) indicated that some strains are not typeable according to the scheme of Wauters et al., (1987). Consequently, detection of the virulence genes can be useful to separate pathogenic strains from non-pathogenic strains as it is known that the ystB gene is in nearly all the pig-derived strains of biotype 1A (Platt-Samoraj et al., 2006) and that the ail gene (Wannet et al., 2001; Kote et al ., 2010) and the myfA gene (Kote et al., 2010) were in nearly all the pig- and human-derived strains of pathogenic biotype.

The virulence profile obtained for the biotypes and serotypes of Y.enterocolitice, based upon the selected genes of virulence, can be applied as distinguishing markers and indicators of the potential virulencq of Y.enterocoliticq strains, excluding bio-serotyping tests.

Conclusion

The study on this collection of isolates showed that all the pathogenic isolates have virulence genes. This indicates that identification of pathogenic biotype can be realised by detection of the ail and myfA genes through PCR method instead of biochemical tests. PFGE analysis using XbaI enzyme showed that Y. enterocolitica is very clonal with a major PFGE patterns but this method do not allowed the differentiation of biotype 4 from biotype 3.

Acknowledgements

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The infection biology of pig-associated Salmonella

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Abstract

Through the use of an established line of porcine intestinal epithelial cells, known as IPEC-1, this in vitro work examines the initial adhesion, invasion and persistence abilities of different Salmonella serovars and phage types, including multiresistant and monophasic S. Typhimurium DT193 isolates. The resultant innate immune response of the porcine cells to the isolates is assessed through determination of interleukin (IL)-6 and IL-8 concentrations present in cell culture supernatants. The results are discussed in relation to preliminary work, including growth curve data and basic morphological description of the bacterial isolates. Results show that there is variation between the strains in invasiveness (ANOVA p=<0.001) and in the concentrations of IL-6 and IL-8 elicited (ANOVA p=0.001 and <0.001, respectively). Specifically, an S. Derby isolate investigated here exhibits greatly reduced ability to invade, and a biphasic DT193 isolate stimulates very high levels of IL-8 released from the IPEC-1 cells. The variation between strains shown here emphasises the need for a multivalent vaccination to be developed, as opposed to a monovalent one.

Introduction

The infection of pigs with Salmonella enterica subspecies enterica (hereafter Salmonella) is a continuing animal health problem that has high zoonotic potential. The UK pig industry, under the Zoonoses National Control Programme (ZNCP), is currently working towards its target of reducing the number of pig carcasses at the abattoir testing positive for the ELISA antibody test to below 10%. A missing component of many Salmonella control strategies is the use of vaccination, because only inactivated vaccines are available and the efficacy of these is uncertain. There is therefore a need to investigate the possibility of development of an effective multivalent vaccine for pigs to protect against Salmonella. A multivalent vaccine as opposed to a monovalent one is the most sensible option because it has been shown that pig herds can be exposed to several different phage types and serovars of Salmonella during their time on a particular farm (Wales et al., 2009). S. Typhimurium has consistently been the most frequently isolated serovar in the UK and in the EU as a whole, and S. Derby is often the second most common serovar (EFSA, 2008). The monophasic variants of the Typhimurium serovar are being isolated with increasing frequency, to the extent that they have been more common than S. Derby in the UK in the past year (Veterinary Laboratories Agency, 2010).

Studies investigating host-pathogen interactions of S. Typhimurium and S. Enteritidis in chickens have shown that even within the same phage type there can be marked differences in virulence (Humphrey et al., 1996; Humphrey et al., 1998; Williams et al., 1998). Until now there has been little comparable data for pigs. Existing pig models have looked at the host response to single strains of S. Typhimurium (Arce et al., 2008; Meurens et al., 2009; Collado-Romero et al., 2010) and compared the behaviour of different Salmonella serovars (Osterberg et al., 2009), but little focus has been directed towards populations of Salmonella. The aim of this study is to characterise pig-associated field isolates from the UK in a similar way to that of Bergeron et al. (2009) who examined isolates from the Canadian Salmonella population.

A key attribute of Salmonella pathogenesis following oral infection is the attachment to and invasion of intestinal epithelial cells, both of which are thought to be major steps related to the virulence of the bacterium (Boyen et al., 2006). Following this invasion, the host mounts an innate immunological response through production of several cytokines, including IL-1, IL-6, IL-8 and TNF-α (Volf et al., 2007) by enterocytes and macrophages. This response is elicited both by recognition of the pathogen, primarily fagella and lipopolysaccharide, through Toll-like receptors and by secreted virulence factors of the pathogen. For this study, the attachment and invasion abilities of several different Salmonella serovars and phage types were investigated using a porcine intestinal epithelial cell line and the consequent cytokine (IL-6) and chemokine (IL-8) production of these cells was measured.

Materials and Methods

Bacterial strains

Six field isolates of Salmonella kindly provided by the VLA were used in this preliminary study alongside one lab strain, known as S. Typhimurium 474 and which is associated with infection in cattle. Its behaviour in vitro is well characterised. The field isolates include three S. Typhimurium multi-resistant DT193 strains: one with the 4,12:i:- serotype, another with the 4,5,12:i:- serotype and a third with the normal serotype. The other field isolates were one of each of S. Typhimurium DT206 and U288 and one S. Derby. The isolates were grown in LB medium in a shaking incubator at 150rpm and on nutrient agar, both at 37°C. Prior to cell invasion experiments, growth curves were produced for each isolate by measuring optical density (OD600) of a growing culture every hour for 12 hours and then again at 24 hours. Gram staining of each isolate was also done.

Cell line and culture conditions

The IPEC-1 cell line is an intestinal epithelial cell line derived from the small intestine of a neonatal unsuckled piglet (Gonzalez-Vallina et al., 2006). The cell line was a generous gift from Dr. T. Cogan, University of Bristol, UK. The cells were maintained in 75cm3 plastic cell culture flasks at 37°C in a humidified incubator with a 5% CO2 atmosphere. They were cultured and maintained in Dulbecco's Modified Eagle Medium (DMEM/F-12; Invitrogen, UK) supplemented with 5% foetal calf serum, 100 IU/ml penicillin, 100µg/ml streptomycin, 5µg/ml insulin, 5µg/ml transferrin, 5ng/ml selenium (ITS Premix; BD Biosciences, UK) and 1ng/ml human recombinant epidermal growth factor (EGF; BD Biosciences, UK), hereafter referred to as IPEC-1 media. Continuous cultures of IPEC-1 cells were maintained by seeding culture flasks at 1:3 ratios at each passage. For the adhesion and invasion assays cells were grown in antibiotic-free IPEC-1 media in 24-well cell culture plates at 37°C and 5% CO2 atmosphere.

Invasion assay

The number of bacteria that successfully invade and persist in the IPEC-1 cells was determined for each isolate by a cell invasion assay. Bacterial strains were grown overnight in 10ml LB medium in an orbital shaking incubator at 150rpm and 37°C. After 16 hours of growth the optical density was monitored until it reached 1.2 at 600nm, indicative of late-log phase growth. Bacterial cells were then added at a multiplicity of infection of 10 (10 bacteria per cell) to IPEC-1 cells that had reached confluence in a 24-well plate and incubated at 37°C for 1 hour. The media was then removed, fresh IPEC-1 media containing 150µg/ml colistin sulphate was added, and the cells were incubated for 90 minutes to kill any extracellular bacteria. Colistin was chosen because two of the DT193 strains are resistant to the more commonly used gentamicin, and colistin has been shown to be effective at killing extracellular bacteria (Kusters et al., 1993; Bergeron et al., 2009). Cells were washed four times with 1× phosphate-buffered saline (PBS) and overlaid with 0.5% (w/v) Triton-X 100 (Sigma Aldrich, UK) in PBS to lyse the cells and release any internalised bacteria. The cell lysate was then diluted and plated on nutrient agar to determine viable bacterial counts. A second 24-well plate was infected at the same time, but the fresh IPEC-1 media that was added after the incubation period contained a reduced concentration of 30µg/ml colistin. This plate was then incubated for 48 hours before continuing the invasion assay to assess the number of bacteria that persisted within the IPEC-1 cells. Cell culture supernatants (150µl) were collected from each well of this plate at 24h and frozen at -20°C for IL-6 and IL-8 determination.

Adhesion assay

The number of bacteria that adhere to the IPEC-1 cells was determined for each isolate by a cell adhesion assay. Bacterial strains were grown in the same way as for the invasion assay and added to the IPEC-1 cells at the same multiplicity of infection. After the bacteria and cells were incubated together for one hour the media was removed, cells were washed six times with PBS before being lysed and the cell lysate diluted and plated out. The viable counts for this assay gave the total number of bacteria that adhered to and invaded the IPEC-1 cells, so the number of invading bacteria, as determined by the invasion assay was subtracted from this value to give the number of bacteria that adhered.

Enzyme-linked immunosorbance assay (ELISA)

IL-6 and IL-8 concentrations were determined using the porcine IL-6 and IL-8 Quantikine® Immunoassay kits (R&D Systems Europe, Ltd., UK) which employ the quantitative sandwich enzyme immunoassay technique. The assays were performed according to the manufacturer's specifications.

Statistical Analysis

All experiments were performed in triplicate. Mean values and standard deviation were calculated and individual isolates were compared using one-way ANOVA. The Kruskal-Wallis non-parametric test was used on data sets where the standard deviations were not similar enough to satisfy the assumptions of ANOVA. Differences were considered significant at p<0.05. MiniTab15 was used.

Results

The data indicate variation between strains in invasiveness (ANOVA F=5.53, p=<0.001), and in persistence (Kruskal-Wallis H=13.53; p=0.035) (Figure 1). The S. Derby isolate was significantly less invasive than the following strains: S. Typhimurium 474 (p=0.0052), DT208 (p=0.0081) and 4,5,12:i:- DT193 (p=0.006). The adhesion assays showed that the number of bacteria associated with the cells, which is the total number that adhered and/or invaded, was very similar for all strains: 5.18 ± 0.5 log colony-forming units (CFU).
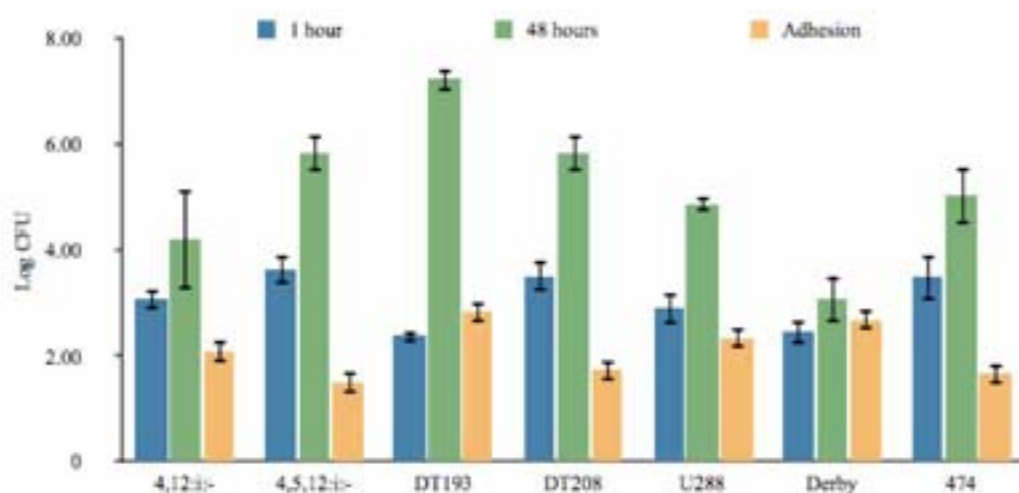
Figure 1 Mean and standard error of log number of intracellular Salmonella after 1 hour and 48 hour culture periods with IPEC-1 cells, and log number of Salmonella adhered to IPEC-1 cells after 1 hour. The number internalised after 1 hour indicates invasive ability and number internalised after 48 hours indicates ability to persist within the cells.
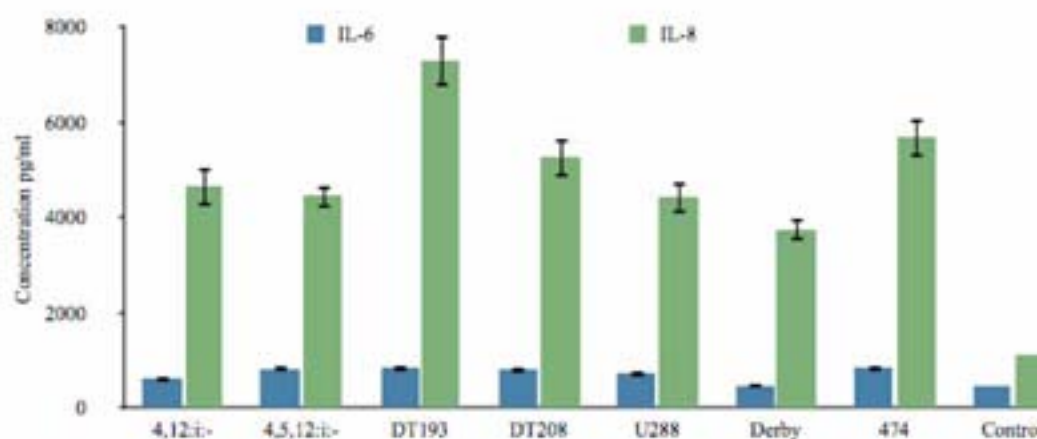
Figure 2 Mean and standard error of concentrations of porcine IL-6 and IL-8 detected by ELISA in IPEC-1 cell culture supernatants after 24 hour incubation with different Salmonella isolates. Uncontaminated LB broth served as a negative control.

After the same incubation time, the concentration of IL-8 released by the IPEC-1 cells was much higher than that of IL-6 (Figure 2). Variation between isolates in production levels of both IL-6 (F=4.64; p=0.001) and IL-8 (F=6.49; p=<0.001) was found. For example, the S. Derby isolate elicited significantly less IL-6 production than S. Typhimurium 474 (p=0.0027), DT193 (p=0.0204), DT208 (p=0.0465) and 4,5,12:i:- DT193 (p=0.0277). Similarly, infection with the multi-resistant DT193 isolate resulted in a significantly higher release of IL-8 by IPEC-1 cells than U288 (p=0.0006), S. Derby (p=0.0002), and both of the monophasic DT193 strains (p=0.0040 and 0.0027).

The growth curves produced showed that there is apparent variation in the growth patterns of the different isolates. The multi-resistant S. Typhimurium strains with normal serotype and 4,5,12:i:- serotype both appeared to exhibit reduced growth rates. Gram staining of these two isolates revealed that both have a different phenotype to the characteristic rod-shape of Salmonella, with smaller barrel-shaped morphology being evident.

Discussion

The results presented here indicate that there is variation between certain Salmonella isolates associated with infection in pigs in: phenotype, ability to invade and persist within intestinal epithelial cells and in the resultant chemokine and cytokine expression. Finding that the number of bacteria associated with the cells during incubation together was the same across all strains suggests that all have similar attachment abilities, and that variation in invasiveness is the key difference. This is an important finding because it indicates that for a potential vaccination to be multivalent, it would need to prevent the initial adhesion of the bacteria to the enterocytes. Of potential importance is the evidence from the invasion data and the cytokine data is that S. Derby might be of less concern to public health because it has significantly reduced invasive ability and fails to amount an IL-6 driven inflammatory response from the IPEC-1 cells. S. Derby, however, might be capable of causing systemic disease in pigs through this evasion of host immune responses. However the current study is limited to one isolate of the serovar and analysis with a greater number of isolates is needed before any conclusion can be reached. IL-8 is well described as a neutrophil chemoattractant that induces neutrophil degranulation and also promotes CD4+ and CD8+ T cell migration. The high concentration of IL-8 produced by IPEC-1 cells following infection with the biphasic S. Typhimurium DT193, and the consequent gut inflammation that would be expected to occur in vivo, suggests that this strain is likely to cause enteritis in pigs. It is interesting that the monophasic variants of this phage type do not elicit as strong chemokine response, and possibly suggests that they have different mechanisms of invasion.

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The use of quantitative Real-Time PCR to estimate Salmonella shed in fecal samples from naturally infected finishing pigs

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Abstract

The objective of this study was to describe the shedding pattern of Salmonella in finishing pigs, as well to quantify the Salmonella load. A longitudinal study was conducted in 12 cohorts of pigs in a multi-site farrow-to-finish production system. At the beginning of each cohort, 50 pigs (10 ± 2 weeks old) were randomly selected and individually identified. Individual pig fecal samples were collected and cultured every 2 weeks for 16 weeks (8 collections). Further, quantitative real-time PCR (q-PCR) targeting the invA gene was performed in a subset of the culture negative samples (555) and in all available culture positive samples. At the time of submission, Salmonella was cultured from 397 (8.74%) of 4540 individual fecal samples. Overall incidence of Salmonella was 24.8% (149/600 pigs). The proportion of positive samples decreased over the finishing period from 16.75% (10 weeks old) to 4.30% (24-26 weeks old). At the present, all Salmonella culture negative samples subjected to q-PCR were PCR negative. Of culture positive samples, 16% were detected by q-PCR, and only 3 % of the samples were within the quantifiable range of detection (>103 colony forming units per gram of feces). Of those samples within the quantifiable range, the bacterial concentration ranged from 1.05x103 to 1.73x106 invA gene copies/g feces. The results suggest that point estimates of Salmonella prevalence may not accurately describe the Salmonella status of finishing pigs. The majority of pigs shed Salmonella at low concentrations. These preliminary data can contribute to quantitative risk assessments of the association between concentrations of Salmonella shed by pigs during the finishing phase and contributions to carcass contamination at slaughter.

Introduction

Salmonella is one of the major causes of bacterial foodborne disease in United States (Henao et al., 2011). Salmonella is one the most important bacteriological zoonotic hazards transmissible from pork to consumers (Boyen et al., 2008). Reduction of the Salmonella contamination of pork and pork products requires interventions at pre-harvest, harvest and post-harvest (Lo Fo Wong et al., 2002). Longitudinal studies have shown high variability in Salmonella shedding over time at both the farm and individual level (Funk et al., 2001b; Rajic et al., 2005). Therefore, there is a need to better describe Salmonella shedding in order to understand Salmonella dynamics and implement control measures to reduce Salmonella pre-harvest. Limited research has been conducted to quantify Salmonella load in naturally infected finishing pigs. Enumeration of bacterial load can be used to identify contamination pressure and to implement effective control measures to reduce contamination (Fravalo et al., 2003; Boughton et al., 2007). In addition, quantitative data is needed for quantitative microbial risk assessments (QMRA) and modeling transmission patterns of Salmonella (Bollaerts et al., 2009; Lanzas et al., 2011). The objective of this study was to describe the shedding pattern of Salmonella in finishing pigs, as well to quantify the Salmonella load.

Materials and Methods

A longitudinal study was conducted in three sites of a multi-site farrow-to-finish production system. At each 4-barn site (A, B, C) one barn was selected for study inclusion. For each barn selected six (sites A & B) and two (site C) consecutive cohorts of pigs were included in the study. At the beginning of each cohort, 50 pigs (10 ± 2 week old) were randomly selected and individually identified. Individual pig fecal samples (10g) were collected and cultured every 2 weeks for 16 weeks (8 total sampling periods per cohort). Fecal samples were culture using standard enrichment adapted from Davies et al. (2000) for 10 gram fecal weights (TTB, RV, XLT-4). An additional fecal sample from each pig (200mg) was kept frozen (-80°C) for later analysis using quantitative real-time PCR (q-PCR). A random selection of culture negative samples (n=555) and all the available culture positive samples (n=381) were submitted for real-time q-PCR targeting the invA gene. The

DNA was extracted from 200 mg of feces using the Qiagen Qiamp Stool Mini Kit (Qiagen, Valencia, CA) according to manufacturer instructions. The q-PCR was performed in triplicate for each sample of DNA using PerfeCTa qPCR SuperMix, low Rox (Quanta Biosciences, MD). The PCR primers and probe used for amplification and detection were described by Hoorfar et al. (2000). The limit of detection of the q-PCR was determined using sterilized fecal samples spiked with serial 10 fold dilutions of Salmonella. The concentration of Salmonella at the endpoint of detection in triplicate was confirmed by culture and colony count to be 103 CFU per gram of feces. A pig was Salmonella-positive if at least one fecal sample tested culture positive. Descriptive statistics of bacteriologic culture were estimated at the sample and pig level. Salmonella apparent prevalence (proportion of positive samples) and respective 95% confidence intervals were determined for each site, season, pig age and overall. Pearson Chi-squared analysis with Bonferroni adjustment was used to compare apparent prevalence among sites and season. Chi-squared test for trend in proportions (Cochran-Armitage Test) was applied to test change over time. A significance level of 0.05 was considered for comparisons (SAS 9.2). Descriptive statistics of q-PCR results were presented as copy numbers of invA gene/gram of feces and a scoring system which combined results of culture and q-PCR.

Results

At the time of submission 12 cohorts are completed. All cohorts were Salmonella-positive in at least one fecal sampling. The range of positive samples per cohort was 1 to 156 samples. Salmonella was cultured from 8.74% (397 /4540; 95% C.I. 7.96-9.60%) individual fecal samples. Overall incidence of Salmonella was 24.8% (149/600 pigs; 95% C.I. 21.54-28.45%). Of positive pigs (149), 60 were positive once, 22 were positive twice, 26 pigs were positive three times, 16 four times and 25 pigs were positive five or more times during the sampling period. Among the positive pigs (89) that were detected positive in more than 2 sampling occasions, 42.69% (38/89) had consecutive positive culture samplings , 25.84% (23/89) had one culture negative fecal sample within the period of shedding and 31.46% (28/89) were culture negative in more than 2 occasions between two culture positive sample collection periods.

There was a significant difference between sites in the overall relative proportion of positive samples (p-value <0.05). Site B (12.71%; 95% C.I. 11.26 -14.33%) had higher proportion then site A (7.26%; 95% C.I. 6.18-8.47%) and site C (3.21%; 95% C.I. 2.14-4.62%). There was a significant difference in the overall relative proportion of positive samples between seasons. Fall (14%; 95% C.I. 12.04-16.23%) and winter (13.03%; 95% C.I. 11.33-14.92%) had a significantly higher proportion of positive samples when compared with spring (1.88%; 95% C.I. 1.09-3.16%) or summer (4.34%; 95% C.I. 3.39-5.55%). The proportion of positive samples decreased significantly over time (p-value <0.0001). The apparent prevalence decreased from 16.75% (95% C.I. 13.96-19.96%) at the beginning of the finishing period (10 weeks old) to 4.30% (95% C.I. 2.76-6.59%) at end (24-26 weeks old) (p-value <0.0001).

At the present, all of the Salmonella culture negative samples subject to q-PCR were PCR negative. Of culture positive samples, 16% (61/381) were detected by q-PCR, but only 3% (13/381) of the samples were detected in triplicate and were within the quantifiable range (>103). Individual samples were classified in 4 scores based on the detection limit of q-PCR, culture and q-PCR results. Those scores were combined in order to estimate a gradient of concentration. The four scores were: a) score 0: culture-negative and q-PCR-negative (555); b) score 1: culture-positive and q-PCR negative (321); c) score 2: culture and q-PCR positive, not within quantifiable range (48); d) score 3: culture positive and q-PCR positive within quantifiable range (13). The concentration gradient was assumed to increase from score 1 to score 3. In score group 3, the concentration ranged from 1.06x103 to 1.73x106 copies of the invA gene/gram feces. Fifty three percent (7/13) of the score 3 group samples belonged to the same pig. Fifty-seven percent (4/7) of the pigs in score 3 group were from the same cohort and pen.

Discussion

The overall prevalence reported in this study is higher than others that have targeted the finishing phase in the US (Wang et al., 2010; Rostagno et al., 2011). Several explanations can be in base such as farm factors, study design, sampling strategy and diagnostic test (Rajic et al., 2005). The intensive repeated sampling conducted in this longitudinal study might have increased the probability of detecting positive pigs.

There was high variability in the prevalence among cohorts and sites, identical findings were described by others researchers. Therefore, point estimates might misclassify Salmonella status of farms or pigs (Funk et al., 2001b; Kranker

et al., 2003; Rajic et al., 2005). There was a trend of decreasing prevalence as the pigs got older. The prevalence was higher during the fall and winter, and seasonality on shedding has been reported by other authors (Funk et al., 2001a).

There was intermittent detection of shedding in almost fifty percent of the pigs with multiple culture-positive collections. Intermittent shedding has been reported in experimental and epidemiologic studies. Salmonella carriers can shed intermittently and for long periods (Funk et al., 2001b; Kranker et al., 2003; Scherer et al., 2008). Intermittent shedding could be an intermittent detection of an on-going infection or a new infection after clearance of a previous infection. The diagnostic sensitivity of culture ranges from 10% to 80%, depending on the protocol (Rajic et al., 2005). The imperfect sensitivity of the culture methods may affect the detection of the active shedders, thus some those culture-negative samplings could be a false negative. Although a relative short sampling interval (15 days) was conducted in this study, new infections could occur between sampling occasions.

The majority of the pigs shed low concentrations, below the quantitative limit of q-PCR. In a quantitative study using mini-MSRV, 97% of swine fecal samples had less than 200 organisms per gram (Fravalo et al., 2003). Quantitative studies at the lairage have reported variable and relative low bacterial load, median pen concentration ranging from 1.8-11.5 organisms/100cm2 (Boughton et al., 2007) and 457-1071 organisms/ml (O'Connor et al., 2006) respectively. However, the findings between lairage and individual pigs are not directly comparable because of different sampling methodologies and the likely cumulative contamination of lairage. Naturally infected pigs tend to shed low numbers of Salmonella in feces. Only a small percentage of samples had higher concentrations and seemed to be clustered within cohort and pig.

Conclusion
This interim report of an ongoing study suggests that point estimates might underestimate prevalence, and at least in this study, the majority of culture positive pigs shed Salmonella in low concentrations.

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Salmonella Typhimurium interference with the humoral immune response of pigs

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Abstract

Foodborne salmonellosis is one of the most important bacterial zoonotic diseases worldwide. Salmonella Typhimurium is the serovar most frequently isolated from slaughter pigs in Europe. Circumvention of the host's immune system by Salmonella might contribute to persistent infection of pigs. We found that Salmonella Typhimurium strain 112910a, which is able to persist in pigs, was capable of downregulating the expression of major histocompatibility class II (MHC II) molecules on porcine alveolar macrophages (PAM) in a Salmonella pathogenicity island 2 (SPI-2) dependent way and that MHC II downregulation was Salmonella strain dependent. The MHC II downregulation capacity was abolished when bacteria were opsonized with Salmonella-specific antibodies. Furthermore, intracellular proliferation of Salmonella Typhimurium opsonized with Salmonella positive pig serum was significantly impaired compared to that of the bacteria opsonized with negative pig serum. In a subsequent in vivo experiment, Salmonella Typhimurium strain MB2216 that did not induce MHC II downregulation in vitro, was shed less and persisted less but induced earlier seroconversion in pigs than strain 112910a. From the in vitro data, it is proposed that Salmonella Typhimurium downregulates the humoral immune response to promote intracellular survival inside porcine macrophages, contributing to long-term Salmonella persistence in pigs. The fact that the less persistent strain MB2216 induced earlier seroconversion than strain 112910a is of major interest for Salmonella-monitoring programs primarily based on serology, since this indicates that more persisting strains are more likely to escape serological detection.

Introduction

In European countries, Salmonella enterica subspecies enterica serovar Typhimurium (Salmonella Typhimurium) is the serovar most frequently isolated from slaughter pigs (Anon., 2008). In most cases, the bacterium will asymptomatically colonize pigs, resulting in a so called 'carrier status' (Wood et al., 1989). In the past, Salmonella infections in pig herds have traditionally been diagnosed by culturing intestinal or faecal samples. LPS-based serological surveillance is perceived as a practical and cost-effective alternative for monitoring Salmonella infection in pig herds (Proux et al., 2000) and is currently widely applied. The success of many persisting pathogens relies on their ability to interfere with the host's immune responses, for example by interfering with major histocompatibility complex (MHC) molecule expression and antigen presentation. SPI-2 plays a role in Salmonella-mediated downregulation of MHC II in human Mel JuSo cells (Mitchell et al., 2004). No data are available on this phenomenon in porcine cells.

In the present study, we hypothesized that Salmonella Typhimurium strain 112910a induced Salmonella pathogenicity island 2 (SPI-2) mediated downregulation of MHC II expression on porcine macrophages as a possible mechanism to circumvent antibody production by the pig's immune system. We then examined the role of antibodies in intracellular survival and proliferation of Salmonella in macrophages and in the bacterium's ability to interfere with the MHC II presentation pathway, and if this MHC II downregulating capacity was Salmonella strain dependent. Finally, we examined whether the Salmonella induced MHC II downregulation in porcine macrophages coincides with Salmonella persistence in pigs.

Material and Methods

Bacterial strains and manipulations

Salmonella Typhimurium strain 112910a, a pig stool isolate (Boyen et al., 2009), and its isogenic deletion mutants sseA and ssrA/B, constructed as described by Datsenko and Wanner (2000), were used. Other strains used in this study are Salmonella Typhimurium pig isolates MB2150, MB2216, MB2222, MB2223, MB2233 and MB2498 and the pigeon isolate DAB69, Salmonella serovars Brandenburg, Derby and Infantis, all isolated from pigs and a chicken

isolate Salmonella serovar Enteritidis 76Sa88. For flowcytometric analysis, strains were transformed with a plasmid expressing green fluorescent protein (GFP; Valvidia and Falkow, 1996).

Pig antisera against Salmonella Typhimurium
Porcine serum containing Salmonella-specific antibodies was raised by injecting pigs twice intramuscularly with a bacterin consisting of formalin-inactivated Salmonella Typhimurium strain 112910a suspended in phosphate buffered saline (PBS) and Freund's incomplete adjuvant (positive serum). Negative serum was collected from pigs, injected twice with an emulsion of Freund's incomplete adjuvant and PBS.

The effect of Salmonella Typhimurium on MHC expression on the surface of porcine macrophages
Porcine alveolar macrophages (PAM) were isolated and inoculated with GFP-transformed Salmonella strains and serovars, as described by Boyen et al. (2006). Macrophages were incubated with a primary MHC class I or class II antibody to detect MHC I or II expression, respectively, and then incubated with a secondary Alexa Fluor 633 antibody. MHC expression of uninfected and infected PAM was measured using a FACScantoTM II cytometer and analysed with FACS Diva software.

Impact of opsonization with antibodies on intracellular survival of Salmonella Typhimurium in porcine macrophages
Intracellular survival of bacteria that were either non-opsonized or opsonized with positive or negative pig serum, was assessed a gentamicin protection assay (Boyen et al., 2006).

Comparison of the in vivo behaviour of Salmonella Typhimurium strains 112910a and MB2216 after experimental inoculation of piglets
Salmonella-free piglets were orally inoculated with Salmonella Typhimurium strain 112910a (n=19), inducing MHC II downregulation in vitro, or strain MB2216 (n=9), not inducing MHC II downregulation in vitro, respectively. Faeces were collected at different days post inoculation (pi) and bacteriologically analyzed. Six days before inoculation and 11, 18, 26, 33 and 40 days pi, blood was collected and serum was isolated. Serum was analysed using an LPS-based Elisa (IDEXX Labs), and an in-house whole-cell ELISA (Leyman et al., 2011). Forty days pi, all pigs were euthanized and tonsils, ileocecal lymph nodes, ileum, ileum contents, caecum, caecum contents, colon and colon contents were collected and bacteriologically analysed (Van Parys et al., 2010).

Results
Infection with Salmonella Typhimurium strain 112910a did not result in a decreased MHC I expression level on PAM. In contrast, the MHC II expression level on strain 112910a infected PAM was significantly decreased (Figure 1A). MHC II expression levels between PAM inoculated with strain 112910a, sseA or ssrA/B did not significantly differ from each other, nor from the respective ratios directly after inoculation, suggesting that MHC II expression was partly restored when SPI-2 was abolished (Figure 1A). Among the 7 different Salmonella Typhimurium isolates tested, strain MB2216 did not induce MHC II downregulation on PAM, while the other strains exhibited downregulation of MHC II expression similar to strain 112910a. Furthermore, Salmonella Typhimurium strain DAB69 and the Salmonella Derby and Infantis isolates showed no MHC II downregulation, in contrast to serovar Brandenburg and Enteritidis strains (Figure 1B). PAM inoculated with Salmonella Typhimurium opsonized with negative pig serum showed a decrease in MHC II expression level, while the MHC II expression on PAM inoculated with Salmonella Typhimurium opsonized with positive pig serum remained unaffected. Furthermore, Salmonella was able to significantly proliferate intracellularly in PAM when they were not opsonized or when they were opsonized with negative pig serum, while bacteria that were opsonized with positive pig serum proliferated less.
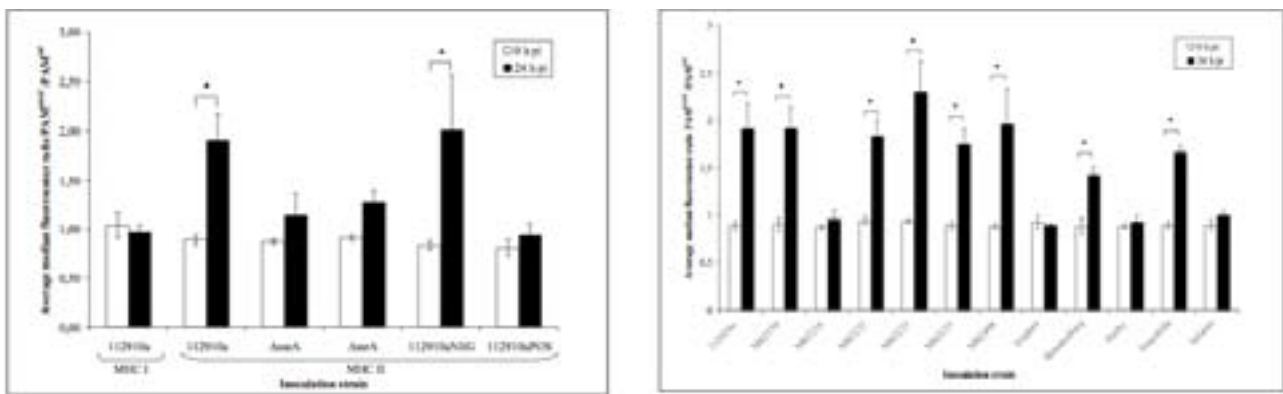
Figure 1. The capacity of Salmonella enterica to downregulate MHC expression on the surface of PAM. The average median FL-5 signal ratios between uninfected and infected porcine alveolar macrophages (PAM^{uninf} and PAM^{inf}, respectively) are presented with standard deviation, immediately (0 h) and 24 h post inoculation (pi) with Salmonella Typhimurium strain 112910a. The values were calculated from the ratios obtained from 3 independent experiments. A. Average median PAMuninf/PAMinf ratios 0 and 24 h post inoculation with Salmonella Typhimurium strain 112910a or its isogenic deletion mutants ΔsseA or ΔssrA. An asterisk refers to a significantly different average median fluorescence ratio between 0 and 24 h pi (p ≤ 0.05). 112910aNEG: Salmonella Typhimurium strain 112910 opsonized with negative pig serum; 112910aPOS: Salmonella Typhimurium strain 112910 opsonized with pig serum containing Salmonella Typhimurium-specific antibodies. B. Average median PAMuninf/PAMinf ratios 0 and 24 h post inoculation with different Salmonella Typhimurium strains and different Salmonella serovars. An asterisk (*) refers to a statistically significant higher ratio at 24h pi than 0h pi (p ≤ 0.05).

In a subsequent in vivo experiment, seroconversion and Salmonella faecal shedding and persistence were compared between pigs inoculated with either strain 112910a or MB2216, respectively inducing and not inducing MHC II downregulation, as assessed earlier in our study. The number of times that pigs tested positive for Salmonella in the faeces was significantly lower for strain MB2216 than for strain 112910a. Using the LPS-based ELISA, the proportion of positive piglets at 33 days pi was significantly higher in the MB2216 group. Using the whole-cell ELISA, the average antibody titre in the MB2216 group was higher than in the 112910a group at 33 and 40 days pi. At euthanasia, strain MB2216 was isolated in lower numbers from the tonsils than strain 112910a. The proportion of Salmonella positive lymph nodes was higher in the 112910a than in the MB2216 group.

Discussion

In this study, we found that Salmonella Typhimurium strain 112910a specifically interferes with the MHC II presentation pathway in porcine macrophages, leaving the MHC I pathway undisturbed. The fact that MHC II downregulation in sseA and ssrA/B infected PAM was partly restored, suggests a role for SPI-2 in downregulation of MHC II expression (Mitchell et al., 2004). The absence of MHC II downregulation and the decrease in survival in macrophages after opsonization of Salmonella might emphasize the importance for the bacterium to postpone seroconversion in pigs for successful persistence. The extent of MHC II downregulation differed considerably among various Salmonella Typhimurium strains and Salmonella serovars, suggesting that the MHC II downregulation capacity might have evolved independently. Oral inoculation of pigs demonstrated that Salmonella strain MB2216, which did not downregulate MHC II expression in vitro, induced earlier seroconversion than strain 112910a which did suppress MHC II expression. Tonsils and lymph nodes play a role in the host's immune response towards bacterial infections (Horter et al., 2003) and both organs are predominantly colonized by Salmonella Typhimurium (Wood et al., 1989). Since MB2216 was less able to persist in these organs in pigs, the earlier onset of Salmonella-specific antibody production may have led to a more efficient reduction of Salmonella, resulting in decreased persistence compared to the 112910a infected pigs. However, extrapolation of in vitro MHC II downregulation capacity to the observed in vivo results must be done with care.

Conclusion

We found that Salmonella Typhimurium strain 112910a was able to downregulate MHC II expression on PAM in a SPI-2 dependent way and that the MHC II downregulation capacity was strain dependent. We furthermore showed a correlation between early onset of seroconversion, reduced faecal shedding and reduced persistence capacity and vice versa, in an infection experiment with 2 different Salmonella Typhimurium strains. Circumvention of the pig's antibody response might therefore attribute to long-term Salmonella persistence in pigs.

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Molecular epidemiology of Giardia duodenalis and Cryptosporidium spp on swine farms in Ontario, Canada

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Abstract

A subset of swine farms in Ontario, Canada have been monitored for Cryptosporidium and Giardia. Fecal samples were collected from different stages of production as well as from manure pits. G. duodenalis cysts and Cryptosporidium spp. oocysts were detected in the manure samples using immunofuorescence microscopy. A nested PCR and sequencing method was performed to determine the genotypes. A mixed multivariable method was used to compare the prevalence of Cryptosporidium and Giardia in samples from different sources. Cryptosporidium oocysts and Giardia cysts were recovered from 51.0% and 44.3% of samples, respectively. However, using PCR, 66.4% of fecal samples were positive for Giardia and 55.7% for Cryptosporidium. Cryptosporidium was more likely detected in manure pits and weaners compared to finisher pigs but it was less frequent in sows than in finishers (P < 0.05). Prevalence of Giardia was less frequent among sows and weaners compared to finisher pigs (P < 0.05). In total, 92% of the Giardia isolates were Assemblage B and 8% were Assemblage E. The most prevalent Cryptosporidium genotypes were C. parvum (55%) and pig genotype II (38%). Only one (2%) of the Cryptosporidium spp. isolates was determined to be C. suis. These findings indicate that the occurrence of zoonotic G. duodenalis and Cryptosporidium are very high on swine farms in southern Ontario, and that there is a potential for transmission between swine and humans by means of cyst and oocyst contaminated water or foods.

Introduction

Gasterointestinal illnesses continue to be the important public health issue globally (Kosek et al. 2003) with a significant economic impact (Mead et al, 1999). In Canada, gastrointestinal diseases associated with Giardia and Cryptosporidium has been reported as one of the top three enteric illnesses in Canada (Demczuk et al., 2005). Giardia duodenalis (also is known as Giardia duodenalis and Giardia lamblia) and Cryptosboridium spp. are ubiquitous parasites and in addition to human can infect a wide range of mammalian species causing asymptomatic to severe intestinal infections. Although human, livestock, and wildlife have been shown as potential source of Giardia and Cryptosporidium in the environment (Heitman et al, 2002), a little is known about attribution of each source to the presence of those parasites in environment. The infection of Cryptosporidium parvum in human in developed countries is mostly attributed to animals (Xiao, 2010). The infective Cryptosporidium oocysts and Gairdia cysts are shed by infected animals and can survive for a long period in moist and cool environments (Olson et al, 2004). It is possible that those zoonotic agents get into ground water and contaminate the environment if spreading on land as fertilizer.

G. duodenalis and Cryptosporidium spp. infections in swine have generally been reported as being asymptomatic (Sanford, 1987), thereby the apparently healthy pigs may shed infective oocysts into environment. Exposure to infective oocysts/cysts through the contaminated water, food and produces is an important mode of infection in human. The aim of this paper is to describe the prevalence, as well as the genotypes and species of G. duodenalis and Cryptosporidium spp among pigs in different stage of production and in stored manure on a subset of Ontario swine farms in Canada.

Material and Methods

Ten swine farms were visited three times between September 2005 and May 2006. In each visit, fecal samples were collected from the stored manure pit and fresh samples obtained from finishers, sows, and weaners. In total, 122 fecal samples (31 manure pit, 43 finishers, 24 sows, and 24 weaners) were collected over the entire period of the study.

G. duodenalis cysts and Cryptosporidium spp. oocysts were detected in the manure samples using immunof uorescence microscopy. Also a nested PCR was used to determine the presence of Giardia and Cryptosporidium in feces. PCR products were sequenced to determine species and genotypes. A logistic regression modeling method with 'farm' variable as a random effect was used. The 'stage' variable was included as fixed effect into model in order to compare the presence of Giardia and Cryptosporidium among samples collected from manure pit, finishers, sows, and weaners samples.

Results

Cryptosporidium oocysts and Giardia cysts could be recovered at least from one sample on the all 10 farms which tested over the three visits of the study. Cryptosporidium oocysts and Giardia cysts were present in 62 (50.1%) and 54 (44.3%) of samples, respectively. However, using PCR, 81 (66.4%) and 68 (55.7%) of fecal samples were positive for Giardia and Cryptosporidium, respectively.

Prevalence of Cryptosporidium oocyst and Gairdia cysts in manure pits did not differ (P > 0.05) (Figure 1). However, finisher pigs appeared to shed Giardia cysts (65%) less than Cryptosporidium oocyst (37%) (P = 0.01). Sows deemed to be tested positive more frequently for Giardia cysts (29%) versus Cryptosporidium oocyst (4.2%) (P=0.02). Weaners shed more Cryptosporidium oocyst (67%) compared to Giardia cysts (42%) (P= 0.04).

Unlike the prevalence of Giardia that has changed over the period of study, no significant change was observed in the prevalence of Cryptosporidium over the three visits. Giardia was present in 64.3% of samples collected during the first visit but it was isolated from 42.5% and 45.0% of the samples in visit 2 and visit 3, respectively. Cryptosporidium was isolated from 52.4%, 37.50%, and 42.50% of the samples collected in visit 1, visit 2, and visit 3, respectively.

Figure 1
Prevalence of Giardia and Cryptosporidium on fecal samples collected from manure pits, weaners, finishers, and sows
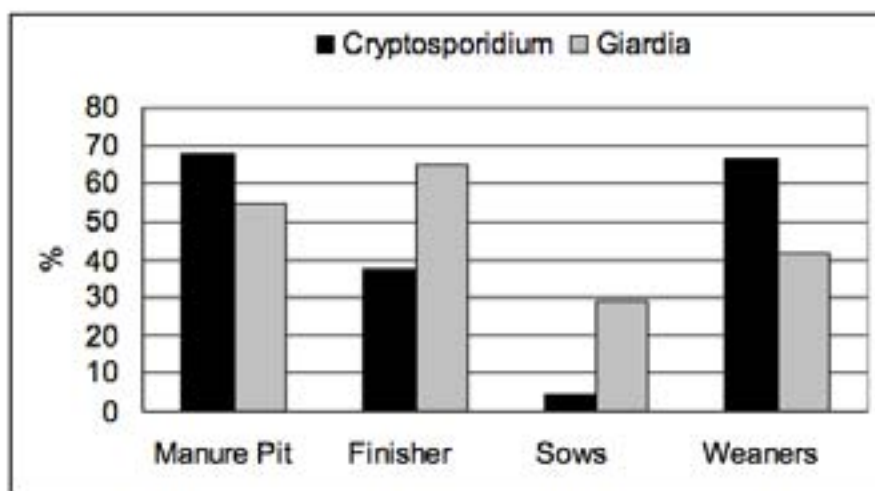
Cryptosporidium was more likely (OR=3.6) detected from manure pit samples and weaners (OR=3.3) compared to finisher pigs. However, it was less likely (OR=0.06) to be recovered from sows compared to finisher pigs. Prevalence of Giardia in samples collected from manure pits and finisher pigs did not differ (P > 0.05). However, Giardia had a decreased chance (OR=0.2) to be isolated from sows.

For Cryptosporidium spp., four different genotypes were determined; C. parvum (55.4%), pig genotype II (37.5%), C. muris (5.4%), and C. suis (1.8%) (Table 1). The two different Giardia genotypes were Assemblage B (92.1%) and Assemblage E (7.9%) (Table 2).

Table 1
Cryptosporidium genotypes in samples collected from finisher pigs, weaners, sows, and manure pits

	No (%) of isolates				
	Manure pit	Finisher	Sows	Weaners	Total
C. parvum	8 (62)	12 (50)	5 (100)	6 (43)	31 (55)
C. sp. pig genotype II	2 (15)	11 (46)	0 (0)	8 (57)	21 (38)
C. muris	3 (23)	0 (0)	0 (0)	0 (0)	3 (5)
C. suis	0 (0)	1 (4)	0 (0)	0 (0)	1 (2)
Total	13 (100)	24 (100)	5 (0)	14 (100)	56 (100)

Table 2
Giardia genotypes in samples collected from finisher pigs, weaners, sows, and manure pits

	No (%) of isolates				
	Manure pit	Finisher	Sows	Weaners	Total
Assemblage B	14 (87)	22 (92)	10 (100)	12 (92)	58 (92)
Assemblage E	2 (13)	2 (8)	0 (0)	1 (8)	5 (8)
Total	16 (100)	24 (100)	10 (100)	13 (100)	63 (100)

Discussion

A large proportion of the pooled swine manure samples tested in the present study showed the presence of G. duodenalis cysts and Cryptosporidium spp. oocysts. Similarly, high prevalences for both parasites have been previously reported in swine worldwide (Hamnes et al., 2007). However, the prevalence of both G. duodenalis and Cryptosporidium spp. infections in swine is believed to be age-specific. Giardia and Cryptosporidium have been reported commonly in different age groups of pig worldwide (Maddox-Hyttel et al., 2006; Xiao et al., 2004; Olson et al., 1997; Quìlez et al., 1996) with higher prevalence among weaners than other age group (Maddox-Hyttel et al., 2006; Xiao et al., 1994).

The molecular characterization of G. duodenalis and Cryptosporidium spp. isolates from livestock is very useful to investigate possible sources of infection in human. In the present study, Assemblage B predominated in all swine age groups. Since both G. duodenalis Assemblage A and B are commonly reported in humans, the predominance of Assemblage B in the present study suggests that there may be a greater zoonotic potential for G. duodenalis than previously thought. C. parvum that is generally recognized as the major zoonotic species and rarely reported in pigs (Kvac et al 2009a), was common to all age categories suggesting a zoonotic risk from swine. The presence of C. muris in pig manure samples suggests the presence of rodents on the farm and the possible transmission between rodents and pigs (Chen and Huang, 2007).

Presence of Cryptosporidium and Giardia in stored manure warrants serious attention. The zoonotic transmission of G. duodenalis and Cryptosporidium spp. infections from swine to humans may occur through the contamination of surface water and produce. The manure management procedures may not be effective in order to eliminate the zoonotic agents from hog manure and as such fails to prevent the environmental contamination (Ziemer et al., 2010). Therefore, it is possible that those zoonotic agents get into ground water (Thurston-Enriquez et al, 2005) and contaminate the environment if spreading on land as fertilizer.

Conclusion

The occurrence of both G. duodenalis and Cryptosporidium spp. was high in the swine manure samples in the present study and zoonotic genotypes and species were identified. Therefore, further study will be required to demonstrate the source of these infections in swine, and molecular characterization of human isolates in this region would be required to investigate the actual risk of transmission to humans.

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A novel strategy to obtain quantitative data for modelling: Combined enrichment and real-time PCR for enumeration of salmonellae from pig carcasses

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Abstract

The primary sources for the major zoonotic pathogen Salmonella are food-producing animals such as pigs and poultry. For risk assessment and hazard analysis and critical control point (HACCP) concepts, it is essential to produce large amounts of quantitative data, which is currently not achievable with the standard cultural based methods for enumeration of Salmonella. As part of the European research project BIOTRACER, this study presents the development of a novel strategy to enumerate low numbers of Salmonella in cork borer samples taken from pig carcasses as a first concept and proof of principle for a new sensitive and rapid quantification method based on combined enrichment and real-time PCR. The novelty of the approach is in the short pre-enrichment step, where for most bacteria, growth is in the log phase. The method consists of an 8-h pre-enrichment of the cork borer sample diluted 1:10 in non-selective buffered peptone water, followed by DNA extraction, and Salmonella detection and quantification by real-time PCR. The limit of quantification was 1.4 colony forming units (CFU)/20 cm2 (approximately 10 g) of artificially contaminated sample with 95% confidence interval of ± 0.7 log CFU/sample. The precision was similar to the standard reference most probable number (MPN) method. A screening of 200 potentially naturally contaminated cork borer samples obtained over seven weeks in a slaughterhouse resulted in 25 Salmonella-positive samples. The analysis of salmonellae within these samples showed that the PCR method had a higher sensitivity for samples with a low contamination level (< 6.7 CFU/sample), where 15 of the samples negative with the MPN method was detected with the PCR method and 5 were found to be negative by both methods. For the samples with a higher contamination level (6.7-310 CFU/sample) a good agreement between the results obtained with the PCR and MPN methods was obtained.

Improved risk-based strategies for disease management in the pig production chain

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Abstract

To minimize financial losses in times of crisis, it is necessary to prove methods of maintaining trade in a case of disease. This work shows that the identification of modules and clusters could be of high relevance if these clusters impede disease spread or if so-called Ad hoc-connector-points serving as routes of transmission between clusters could be identified. Furthermore the advantages of a risk-based selection of critical control points for surveillance or monitoring can be shown. This work provides new approaches to review and possibly optimize existing disease prevention and control strategies.

Introduction

One objective of animal disease control is to maintain the trade of live animals and animal products. Nevertheless, control measures itself cause financial losses within agricultural production chains. Therefore veterinary research has to give guidance to decision makers in order to limit the control measures to the necessary level. In this paper, results of research on risk-based strategies for disease management in the pig production chain are presented.

Movement of live animals between farms is a major risk factor for livestock disease spread. With access to data of the German animal movement database (Herkunftssicherungs- und Informationssystem für Tiere – HI-Tier) and by using methods of network analysis the German pig trade can be analyzed. The German pig trade network is very complex. It contains 119.858 pig premises or related enterprises (nodes) and 327.972 trade connections (edges). Lentz et al. (2011) showed that the German pig trade network is modular and can be divided into modules. A module is defined as subsets of nodes in which are significantly more edges (trade connections) than expected by chance [Newman, 2006, Lentz et al., 2011, 2009]. These modules are computed without any geographical information. By adding the geographical information, i.e. the location of premises, modules become to clusters. These clusters are regional delimited. Such structure can be of crucial importance in case of a crisis like an epidemic. The question is if the existence of modules and clusters can impede the spread of an infectious disease and in what way we can benefit from it regarding to optimize control strategies.
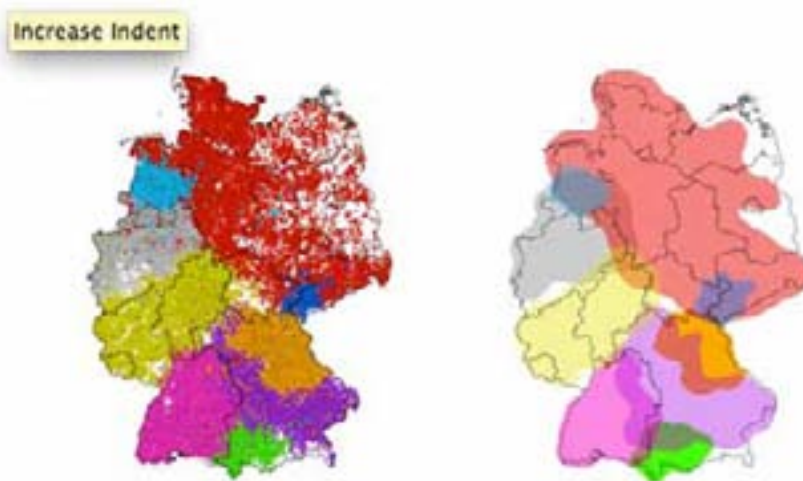
Figure 1: Spatial distribution and overlap of the trade communities. Left: dots represent premises at their approximate locations. The coloring indicates their community membership. Right: regions of spatial overlap.[Lentz et al.,2011]

The World Organisation for Animal Health (OIE) laid down in their Terrestrial Animal Health Code concepts to facilitate trade in animal products and products of animal origin. For implementing the trade in crisis situations the OIE applied the concept of compartmentalisation and zoning. The OIE definitions for these concepts are:

"Compartment means one or more establishments under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade.

Region/Zone means a clear defined part of a country containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and bio-security measures have been applied for the purpose of international trade." [OIE]. At first sight it seemed that modules and compartments as well as cluster and region/zone share common characteristics. In the case of a significant relevance of the modules and clusters with respect to the spread of diseases, it is to prove if the concept of compartments and zones could be transferred to the German national pig trade network. On the supposition that modules and clusters are important in a case of disease outbreak, the premises (nodes) which connect different modules are of high interest. To identify these connectors it is essential in order to hinder disease spread.

Considering closed trade networks the knowledge of relevant premises can be highly relevant in terms of disease control and gives a possibility to review current methods of monitoring and surveillance particularly with respect to the selection of control points. "Monitoring means the intermittent performance and analysis of routine measurements and observations, aimed at detecting changes in the environment or health status of a population. Surveillance means the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information to those who need to know so that action can be taken." [OIE] The correct selection of the control points is essential for successful monitoring and surveillance.

In this work we tested two different approaches for the suitability of premises for monitoring and surveillance and evaluate which one is most qualified to identify relevant control points: The identification of control points can be based on random selection or on a risk based selection. To use a risk based approach it is necessary to assess the risk of premises of the pig production chain. The risk of a premise to spread an infectious disease can be associated with easy to collect parameters like trade volume, trade frequency or number of trade partners. Furthermore data on bio-security and health status are assumed to mitigate the risk.

Material and Methods

Data
According to EU directive EC/2000/15 (EUR-Lex, 2000) collection of livestock trade data is compulsory. EU member states are obliged to establish and operate animal movement databases. According to the German Animal Movement Directive (Viehverkehrsverordnung), each pig premise or related enterprise (including stock farms, breeders, fatteners, slaughter houses, traders) must notify the purchase of pigs within a period of seven days. Notification includes the unique identification number of the purchasing and the selling premise, the number of purchased pigs and the date of trade. If pigs are moved from another EU member state to a German premise, the unique identification number of the selling premise is replaced by its country identification number. All data are stored in a database, the Herkunftssicherungs- und Informationssystem für Tiere, HI-Tier. This database is administered by the Bavarian State Ministry for Agriculture and Forestry on behalf of the German Federal states. Data on trade contacts that were recorded for German pig enterprises between 01 June 2006 and 31 December 2008 were used in this analysis. Two premises were considered linked if there was at least one trade contact between them during the study period.

Definitions
Modularity: Modules are subsets of nodes in which are significantly more edges than expected by chance [Newman, 2006, Lentz et al., 2011, 2009].

Loyalty: Values the propagation of infectious diseases between modules. If N is the number of secondary cases causes by an index case, Loyalty (L) relates the number of secondary cases (n) inside that cluster the index case belongs to, to the total number of cases: $L = n/N$. Loyalty figures range between 0 and 1. A figure of 0 indicates that all secondary infections do not belong to the cluster comprising the index case. In contrast, a figure of 1 indicates that all secondary infections belong to the same cluster as the index case. With respect to the risk of spreading the disease to other modules, so-called Ad hoc-connectors are of high interest.

Loyalty of farms is determined with the help of a computer simulation model. We simulate a disease with an endemic

character with a SIS-Model (susceptible-infectious-susceptible - this means that after a premise is infected it could be re-infected). First one premise in a selected cluster is infected at time t=0 and the chronology of contacts to other premises is followed. Cluster containing few premises and with a low spatial extent are chosen in this analysis. In the simulation it is assumed that a trade contact between the infectious farm and a susceptible farm immediately changes the status of the susceptible farm to infectious. All farms remain infectious for 20 simulation days. The simulation propagates the disease until there is no possibility to spread the infection further because there are no more trade contacts. This procedure is repeated for each premise in the chosen cluster. The simulation was done without any control measures preventing the spread of disease. Also notice that this simulation is a worst case approach, because the disease dynamics within farms is not considered here.

Ad hoc-connector: Connectors with a Loyalty of 0.

Risk-based selection: To use a risk based approach it is necessary to perform a risk assessment in order to find critical points in the production chain. In this work we assess the suitability of easy to determine network parameters like trade volume, trade frequency or number of trade partners. These parameters are used to perform a ranking of farms in order to identify premises which are of high relevance for risk based surveillance than others. The assessment is done by simulating an endemic disease using an SI-Model (susceptible-infectious – this means after a premise is infected it remains infectious). The principle of the infection follows the previously described simulation of a disease. But the focus here is on when (time period) the infection reaches one of the chosen control points. The results of the random-based and risk-based selection of critical points will be compared.

Results

Loyalty: First simulations have shown, that for all analyzed cluster secondary infections are mostly trapped within their initial modules, except one (module 53673) which shows the opposite (see figure 2). The reasons for this observation will be analyzed in further studies. In this case the high proportion of premises with a Loyalty of 0 is very interesting and these premises play an important role as Ad hoc-connectors between modules of the pig trade network.
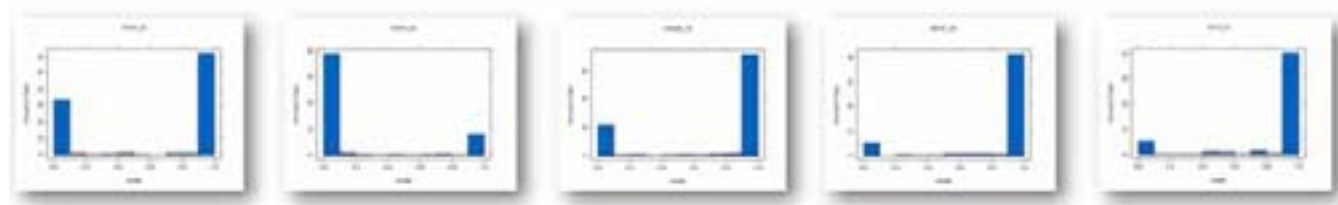
Figure 2: distribution of loyalty of the 5 chosen cluster. The number of farms within cluster decreaes from the left to the right figure. (from left to right: cluster ID 37424, 53673, 10456, 88797, 5574. The cluster Idsare arbitrarily chosen.)

Selection of critical control points: Furthermore the risk-based and random-based selections of control points were investigated. Here simulations have shown that surveillance on risk-based chosen points can detect a contamination or disease up to two months earlier than surveillance on randomly chosen control points. With the help of these methods useful tools for crisis intervention as well as prevention and surveillance can be developed. In terms of prevalence this can make the detection up to one hundred times faster.

Discussion

The first simulation provides information on the usefulness of the identified cluster and modules concerning the spread of disease. On the basis of the results, the modules may represent a starting point for the application of the principles of the OIE guidelines for compartmentalization for national trade.

Trade structure represents trade connections of premises in the pig production chain. These relationships are determined by management-decisions, which are made in respect to optimization of production processes. For this reason we presume that the creation of modules and clusters are determined by the influence of the production management. This assumption is supported by the results of the simulations to determine the Loyalty, because in most simulations the infections remained within the primary infected cluster. In order to implement the concept of compartmentalization, a module has to include the entire production chain. As the Loyalty is particularly high in small clusters, we assume the entire chain still exists in the modules and clusters. In addition to these finding, the detection of the Ad hoc-connectors is of a high relevance.

Nevertheless, it has to be noted that the Loyalty gives no information about the quantity of the secondary infections. Further analysis will be done in order to assess farms according to the number of secondary cases.

The second focus of this work is the assessment of a risk-based selection of control points. The simulation showed that with the use of this approach the detection time of a disease could decreased significantly in comparison to a random-based selection. Although it has been assumed that a perfect test is used, the simulation results enable to review existing control and prevention strategies.

Conclusion

Through simulations it could be shown that modular structures in the German trade network could be utilized to prevent a widespread propagation of a disease. The results of Loyalty support the thoughts of a combination of the OIE- compartmentalization and zoning with the module and cluster concept. In addition so-called Ad hoc-connectors with a Loyalty of 0 could be identified. The importance of these connectors has to be analyzed in further analysis. Furthermore, it was shown that a risk-based selection of control points in the trade network reduced the detection period of a disease significantly in comparison to a random-based selection. This work provides new approaches to review and possibly optimizing existing disease prevention and control strategies.

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A selective chromogenic plate, YECA, for the detection of pathogenic Yersinia enterocolitica: specificity, sensibility and capacity to detect pathogenic Y. enterocolitica from pig tonsils

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Abstract

A new selective chromogenic plate, YECA, was tested for its specificity, sensitivity and accuracy to detect pathogenic Y. enterocolitica from pig tonsils. We tested a panel of 26 bacterial strains on YECA and compared it to PCA, CIN and YeCM media. Detection of pathogenic Y. enterocolitica was carried out on 50 pig tonsils collected in one slaughterhouse. Enrichment was done in PSB and ITC broths. Streaking on YECA and CIN was done in direct, after 24H incubation of ITC, after 48H incubation of PSB and ITC. All the plates were incubated at 30°C during 24 hours. Presence of typical colonies on CIN and YECA was checked and isolates were biotyped. Pathogenic Y. enterocolitica strains showed an important growth on YECA with small and red fuchsia colonies while biotype 1A exhibited very few violet colonies. Enrichment in ITC during 48H gave the best performance for detecting positive samples in pathogenic Y. enterocolitica and YECA could detect directly pathogenic Y. enterocolitica strains (2, 3 and 4). Combination of ITC enrichment and YECA detection generates a time-saver by giving a positive test for pathogenic Y. enterocolitica in 72 hours.

Introduction

In 2009, yersiniosis was, for the sixth consecutive year, the third most frequently reported human zoonosis in the Europe, with a total of 8,354 confirmed cases (EFSA, 2010). In France and most other countries worldwide, biotype 4 is the most prevalent biotype isolated from humans (69%), followed by biotype 2 (30%) and biotype 3 (Savin and Carniel, 2008). Pigs are considered the principal reservoir for the types of Y. enterocolitica pathogenic to humans. Pigs do not develop clinical signs, but they do carry Y. enterocolitica in their oral cavity, on tongues and tonsils, and in lymph nodes, and excrete this bacterium in their feces (Thibodeau et al., 1999). Detection of Yersinia is carried out by using ISO 10273-2003 method. This method is recommended for both food and pig tonsil analyses (EFSA, 2007) but involves time-consuming enrichment steps in two broths, PSB and ITC, followed by plating on 2 selective media, SSDC and CIN (De Boer, 1992). Biochemical tests on isolates are also necessary to confirm Yersinia and to determine the biotypes.

In this work, we tested a new selective chromogenic plate, Yersinia enterocolitica agar (YECA), for its specificity, sensitivity, and accuracy to detect pathogenic Y. enterocolitica from pig tonsils.

Material and Methods

Specificity of YECA. The specificity of YECA (AES Chemunex, Combourg, France) was tested against 26 strains listed in table 1. Each culture was streaked on CIN agar plate, on YeCM medium (Weagant, 2008) and, on YECA plates. If growth of bacteria was observed on plate, importance of growth in a scale from 1 to 5 and characteristics of the colonies were noted.

Sensitivity of YECA. Y. enterocolitica strains from biotype 1A, 2, 3, and 4 were incubated in 5ml of BHI broth during 24h at 30°C. A 10-fold serial dilution of the cultures was done and 100µl of each dilution were spread on YECA and compared with PCA, CIN and YeCM media. Enumeration of the colonies was then performed after incubation of the plates at 30°C for 24hours.

Detection of pathogenic Yersinia enterocolitica from pig tonsils. 50 pig tonsils were collected from a slaughterhouse. From each tonsil, 10 g were cut in small pieces and put into a bag containing 90 ml of PSB broth. After stomaching, 10µl were streaked directly onto YECA and CIN plates; and 1 ml was transferred in 9 ml of ITC broth. PSB and ITC were incubated at 25°C for 48 hours, before a second streaking onto YECA and CIN. In addition, after 24 hours of enrichment in ITC broth, an extra streaking on YECA and CIN was performed. All the plates were incubated at 30°C for 24 hours. Presence of typical colonies on CIN and on YECA was checked. At least two typical colonies per plate were streaked on

YeCM and these plates were incubated at 30°C for 24 hours. This step on YeCM permitted to differentiate rapidly the pathogenic Y. enterocolitica (red bull's-eye-like colonies) from the non-pathogenic Y. enterocolitica (blue-purple colonies). Confirmation and biotyping was then done by biochemical assays as described in ISO 10273:2003 standard.

Results

The 3 pathogenic Y.enterocolitica showed an important growth with small and red fuchsia colonies on YECA. Growth of biotype 1A was much reduced with violet colonies and absence of growth or growth with non typical colonies was observed for the other strains (table 1). The other Yersinia-likes strains were able to growth on YECA but the number of colonies was very small. For the 14 non-Yersinia strains, we observed for CIN, YeCM and YECA an absence of growth or growth but as not characteristic colonies on these media. Numeration of pure culture of Y.enterocolitica strains on YECA was similar to those realised on PCA, CIN and YeCM, except for biotype 1A. For this biotype, colonies on YECA could be numerated only at the dilutions -1, -2, -3 while on PCA, CIN and YeCM, it was possible to count the colonies until the dilution -8.

Out of the 50 tonsils, pathogenic Y. enterocolitica were detected on CIN and YECA respectively from 17 and 15 tonsils after direct streaking, from 21 and 22 tonsils after ITC-24H, from 28 and 28 tonsils ITC-48H, and from 8 and 5 tonsils after PSB-48H. Enrichment in ITC for 48H gives the best performance for detecting positive samples and same number of positive samples was obtained from CIN and YECA. However, YECA compared to CIN could detects directly pathogenic Y. enterocolitica strains (2, 3 and 4) while CIN does not differentiate the biotype 1A from the pathogenic biotypes. A total of 141strains were collected on YECA and biotyped. Among the 141 strains, 135 were identified as biotype 4, two as biotype 3 and four as biotype 2. YECA is able to detect these 3 pathogenic biotypes from naturally contaminated pig tonsils. In addition, the ITC- YECA way generates a time-saver by giving a positive test in 72H.

Discussion

The ISO 10273-2003 standard is the reference method for isolating Y. enterocolitica from foods. This method is also recommended for pig tonsils analysis (EFSA, 2007) but involves time-consuming enrichment steps in two broths, PSB and ITC, followed by plating on 2 selective media, SSDC and CIN (De Boer, 1992). Van Damme et al. (2010) showed that the use of a two-day incubation period at 25°C, instead of five days, for the PSB broth resulted in a significantly higher recovery rate of Yersinia. Wauters et al. (1988) indicated that enrichment in PSB broth gave better results for non-pathogenic strains, whereas enrichment in ITC broth gave better results for pathogenic strains. The SSDC agar (Wauters et al. 1988) does not always allow differentiating Yersinia from interfering f ora such as Morganella, Proteus, Serratia and Aeromonas. The medium CIN (Schiemann et al., 1979) is highly selective but Citrobacter freundii, Enterobacter agglom-erans and the species of Aeromonas and Klebsiella produce colonies of similar morphology (Harmon et al., 1983). However, users recognized that detection on CIN agar is easier since Y. enterocolitica has relatively more characteristic colony morphology on this medium compared to SSDC (Fondrevez et al. 2010). This author recommends the use of CIN after the enrichment in ITC broth; tested on 900 pig tonsil swabs, this way recovered a larger number of positive samples (14.0% versus only 9.1% with the modified ISO method). These media, CIN and SSDC, moreover lack the ability to differentiate potentially virulent Y. enterocolitica from the non-pathogenic strains and other Yersinia. Only panel of biochemical tests (esculin hydrolysis, indole production, and fermentation of xylose and trehalose) as described in the ISO 10273:2003 method permits to identify the biotype. Recently, Weagant (2008) has developed a chromogenic medium (YeCM) for the specific detection of Y. enterocolitica. On this medium, pathogenic Y. enterocolitica strains grow as red bull's-eye-like colonies while non-pathogenic Y. enterocolitica grows as blue-purple colonies. Fondrevez et al. (2010) proposed its use after the CIN step to quickly discriminate the non pathogenic biotype from the pathogenic biotypes.

YECA showed a real capacity to favour the growth of the pathogenic Y. enterocolitica (Biotype 2, 3 and 4) with typical colonies, small and red fuchsia. Growth of biotype 1A was much reduced with violet colonies. Moreover, YECA exhibits a stronger inhibitor effect on the growth of the Yersinia-like strains while numerous colonies were observed on the chromo-genic media YeCM. Numeration of pure culture of Y. enterocolitica strains on YECA was similar to those carried out on PCA, CIN and YeCM, except for biotype 1A for which high inhibition was observed.

When tested from naturally contaminated pig tonsils, we observed a best performance for detecting positive samples after enrichment in ITC than in PSB, and we obtained similar percentage of positive samples between CIN and YECA after enrichment in ITC during 48 hours. This result is consistent with the work of Fondrevez et al. (2010). Because CIN does not differentiate biotype 1A from the pathogenic biotypes, isolates were confirmed as Yersinia and biotyped by biochemical assays as described in ISO method. All the isolates from YECA were however identified as pathogenic Y.

enterocolitica strains. It could be possible to isolate the three pathogenic biotypes 2, 3, and 4 on YECA after ITC enrichment; biotype 4 representing 95.7% of all isolates.

Conclusion

We have described a simplified method that efficiently detects pathogenic Y. enterocolitica in pig tonsils and that it is less time-consuming than the ISO 10273: 2003 standard. In three days, it was possible to detect pathogenic Y. enterocolitica strains from pig tonsils when using YECA after ITC. Combination of ITC enrichment and YECA detection generates a time-saver by giving a positive test for pathogenic Y. enterocolitica in 72 hours. Moreover use of YECA could decrease the need for biochemical tests for confirmation and biotyping.

Acknowledgements

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Table 1: Growth and color of colonies of strains used to test the specificity of YECA media.

Strains obtained from	Name of the strains	Growth* and color of colonies on CIN plate	Growth and color of colonies on YeCM plate	Growth and color of colonies on YECA plate
Yersinia RNC from Pasteur Institute (Paris, France)	Yersinia enterocolitica biotype 2 (IP383)	+++++ red with a translucent rim	+++++ red bull's-eye-like	+++++ small red fuchsia
	Yersinia enterocolitica biotype 3 (IP29228)	+++++ red with a translucent rim	+++++ red bull's-eye-like	+++++ small red fuchsia
	Yersinia enterocolitica biotype 4 (IP134)	+++++ red with a translucent rim	+++++ red bull's-eye-like	+++++ small red fuchsia
	Yersinia enterocolitica biotype 1A (IP124)	+++++ red with a translucent rim	+++++ blue-purple	+ violet colonies (5)
Collection of the Pasteur Institute (Paris, France)	Yersinia aldovae (CIP103162)	+++++ red with translucent rim	+++++ yellow/red with translucent rim	+ small red fuchsia (1)
	Yersinia bercovieri (CIP103323)	+++++ red with translucent rim	+++++ yellow/red with translucent rim	++ yellow/small red fuchsia
	Yersinia frederiksenii (CIP80.29)	+++++ red with translucent rim	+++++ blue to green	++ green/small red fuchsia
	Yersinia kristensenii (CIP80.30)	+++++ red with translucent rim	++++ red with translucent rim	++ pink//small red fuchsia
	Yersinia massilliensis (CIP109351)	+++++ red with translucent rim	+++++ green	++ green/small red fuchsia
	Yersinia mollaretii (CIP103324)	+++++ red with translucent rim	+++++ yellow/red with translucent rim	+ small red fuchsia (1)
	Yersinia rohdei (CIP103163)	+++++ red with translucent rim	+++++ yellow/red with translucent rim	+ pink (1)
	Yersinia ruckeri (CIP82.80)	No growth	No growth	No growth
Collection of the Pasteur Institute (Paris, France)	Salmonella Typhimurium (CIP55.43)	No growth	No growth	No growth
	Campylobacter jejuni (CIP70.2)	No growth	No growth	No growth
	Enterococcus feacalis (CIP55/42)	No growth	No growth	No growth
	Lactobacillus plantarum (CIP103151)	No growth	No growth	No growth
	Pseudomonas fluorescens (CIP525)	+++++ yellow	+++++ yellow	+ pink
	Brochothrix thermosphacta (CIP103251)	No growth	No growth	No growth
Field strains from Anses collection	Listeria monocytogenes	No growth	No growth	No growth
	Escherichia coli	No growth	No growth	No growth
	Staphylococccus aureus	No growth	No growth	No growth
	Klebsiella sp.	No growth	No growth	No growth
	Proteus mirabilis	No growth	No growth	No growth
Strains from Fondrevez et al., (2010)	Morganella morganii	+++++ yellow	+++++ yellow	++ yellow/pink
	Pseudomonas sp.	+++++ yellow	+++++ yellow	+ pink
	Serratia liquefaciens	+++++ pink with translucent rim	+++++ green	++++ green/blue/pink

*Growth was measured from no growth (absence of colonies) to +++++ (important culture with numerous colonies).

Yersinia enterocolitica strains were purchased from Dr. E. Carniel from the Yersinia RNC (Pasteur Institute, Paris, France)

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Comparison of DNA extraction methods to detect Salmonella spp. from pig faeces and pork

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Abstract

The quality of DNA extract significantly influences the outcome of PCR-based detection methods. Performances of four different DNA extraction methods were evaluated for their ability to recover Salmonella DNA from artificially contaminated specimens. Swine faecal and pork samples were spiked with known concentration of Salmonella Typhimurium, DNA was then extracted by each of the methods considered and finally tested by a commercial Salmonella Real Time kit. The QIA-gen and Adiafood kits were the most suitable methods to detect Salmonella in pig faeces. For pork samples the best performances were obtained using the Invitrogen kit. The use of appropriate DNA extraction methods is a critical issue for successful and valid PCR results and it is advisable that DNA extraction techniques are carefully selected with particular regard to the type of samples they should be used for.

Introduction

Salmonella is an important pathogen causing food-borne diseases and pork is recognised as one of the main sources of human infection (1,2). Salmonella Typhimurium, that is one of the main serovars involved in human illness, is frequently associated with pigs (3). Contamination of pig carcasses can occur in the slaughtering plants as a result inaccurate procedures or environmental contamination, and subsequently Salmonella may be present in pork.

Benefits of having rapid, sensitive, and specific tests for the detection of Salmonella in matrices such as faeces and food-stuffs are clear, for the possibility of timely identification of infection or contamination even at very low doses. However, some challenges associated with the usage of rapid methods based on PCR still remain and a crucial one is the removal of inhibitory compounds from target DNA (4). To date, only a limited number of studies comparing the efficacy of extraction methods to perform Real Time PCR, removing reaction inhibitors from different matrices, have been carried out. Thus, this study was aimed at identifying the most effective DNA extraction methods suitable for molecular protocols specifically for faecal and pork samples. Therefore different DNA isolation kits were tested to extract Salmonella DNA from artificially contaminated specimens and their performances were evaluated by applying a Real Time PCR kit. Preliminary results allow to identify the commercial kits yielding the best DNA products useful for further molecular analysis.

Material and methods

Spiked faecal and pork samples

Forty-four samples of pig faeces and 44 samples of pork were spiked with 3 different concentrations of S. Typhimurium (from 2 to 14 CFU/g). Each sample was prepared in triplicate. For all methods the first step was the pre-enrichment of 10 g of matrix (faeces or pork) into 90 ml Buffered Peptone Water (BPW).

DNA extraction

The following four extraction methods were used to extract DNA from faecal specimens, according to the manufacturers' instructions: - InviMag Stool DNA kit (Invitek, GmbH), combining the Invisorb® technology with the use of magnetic particles for isolation of nucleic acids at high purity level [1]; - QIAamp DNA Stool kit (Qiagen), based on an initial step with a fast spin-column, that specifically binds DNA to the silica-gel membrane, then a secondary step where the lysis using proteinase K ensures high yields of DNA in stool [2]; - Lysis reagent (iCheck-Bio-Rad), that uses a lysing solution and beads [3]; - Extraction DNA mix (AES Chemunex, AdiaFood), that uses a lysing solution combined with boiling method [4]. Similarly, DNA was extracted from pork samples using four different methods: Charge Switch gDNA mini Bacteria Kit (Invitrogen, Life technologies), that allows bacterial DNA purification by using the magnetic bead-based technology and cellular lysis with proteinase K and lysozyme [1], the boiling method [4], (5). Then two methods that were tested also

for faecal samples: Lysis reagent (iCheck-Bio-Rad) [2] and Extraction DNA mix (AES Chemunex, AdiaFood) [3].
In addition, each extraction method was tested both directly on the enriched samples and on the same ones pre-treated with magnetic beads named Dynalbeads Anti-Salmonella (Dynal, Invitrogen).

Real Time PCR analysis

Eventually, a Salmonella Real-Time PCR assay (AES Chemunex, AdiaFood) was used to compare the efficacy of the extraction methods to recover Salmonella DNA. The Real Time PCR analysis was performed on triplicate nucleic extracts prepared from spiked samples (faeces and pork) at different concentrations (Table 1 and 2);. To determine the extraction efficiency also the absorbance at 260 nm (A260) was taken into account since this parameter indicates the average nucleic acid extracted from each sample.

Statistical analysis

The Linear Mixed Model for repeated measures (LMM) was used to evaluate if a significant difference exists among the methods tested taking into account also the pre-treatment with Dynabeads.
Different models with fixed and random effect and structures of variance/covariance matrix were evaluated in order to identify the most appropriate; Maximum likelihood value, histogram, Q-Q plot and distribution of residuals, and Kolmogorov Smirnov test were used to verify the goodness of the model (SAS 9.1.3), (6).

Results

Comparison of extraction methods on faecal samples

DNA yields obtained for each extraction method carried out on faecal samples are summarized in table 1. Overall, the Lysis reagent [3] and the Extraction DNA mix [4] provided the greatest yield of DNA as calculated from A260 values. The InviMag Stool DNA kit [1] provided good performances in terms of DNA yielded, while the QIAamp DNA Stool kit [2] produced the least amount of DNA extracted.
The treatment with Dynalbeads did not result in relevant differences in the yield and purity of DNA extracted.

Comparison of extraction methods on pork samples

Table 2 shows the average DNA extracted from pork samples. Overall, the Lysis reagent [2] and the Extraction DNA mix [3] provided the greatest yield as calculated from A260 values. The Charge Switch gDNA mini Bacteria Kit [1] as well as the boiling method provided good performances in terms of DNA extracted.
Relevant differences were observed when the sample were pre-treated with Dynalbeads. In particular the DNA recovery from pork samples treated with Dynalbeads and extracted with methods 1 and 4 was lower than the one obtained from samples untreated with beads and extracted with the same methods.

	N	Mean	Median	Minimum	Maximum	SD
Test Herds						
DD/AY before	33	34.3	31.0	2.6	118.5	24.6
DD/AY after	33	24.4	23.4	1.5	84.8	19.3
Delta-DD/AY	33	-9.8	**-8.2**	-34.1	11.0	10.6
Control herds						
DD/AY before	29	41.8	28.0	2.9	210.8	42.8
DD/AY after	29	39.9	27.8	2.8	198.0	38.8
Delta-DD/AY	29	-1.9	**1.7**	-45.3	39.2	18.5

Table 1. Comparison of the four extraction methods used for the recovery (μg/ml) of DNA from faecal samples spiked with Salmonella Typhimurium (results are expressed as mean values determined for triplicate samples prepared at each concentration).
* samples without pre-treatment with Dynalbeads; ** samples with pre-treatment

Initial inoculum CFU/10gr	Method	1		2		3		4	
	Name	Charge Switch gDNA mini Bacteria		Lysis reagent		Extraction DNA mix		Boiling method	
	Pretreatment	*	**	*	**	*	**	*	**
40 CFU/10gr		173	15	200	200	200	200	113	55
84 CFU/10gr		143	48	200	200	200	200	165	77
119 CFU/10gr		155	12	200	200	200	200	77	54

Table 2. Comparison of the four extraction methods used for the recovery (µg/ml) of DNA from pork samples spiked with Salmonella Typhimurium (results are expressed as mean values determined for triplicate samples prepared at each concentration).
* samples without pre-treatment with Dynalbeads; ** samples with pre-treatment

Real Time PCR analysis of DNA extracted from spiked faecal samples

The best performance, calculated as the lowest Ct value, was obtained for samples extracted with QIAamp DNA Stool kit [2], followed by Extraction DNA mix [4]. Conversely, kit 1 and 3 failed to produce PCR products and this is probably due to the presence of inhibitors in the DNA extracted.

The best LMM for repeated measures data selected presents the significant fixed effects of the pre-treatment, the methods tested and the interaction among them (p<0.001). The residuals were normal (p>0.10) and without particular patterns. Dynalbeads pre-treatment clearly decreases the level of detection, and specifically for methods 2 and 4, a significant increase in the Ct values was observed. In particular, for method 4 the Ct values obtained were significantly higher than the ones provided by method 2 and these differences were evident more in samples pre-treated with Dynalbeads than in ones without pre-treatment (Figure 1).
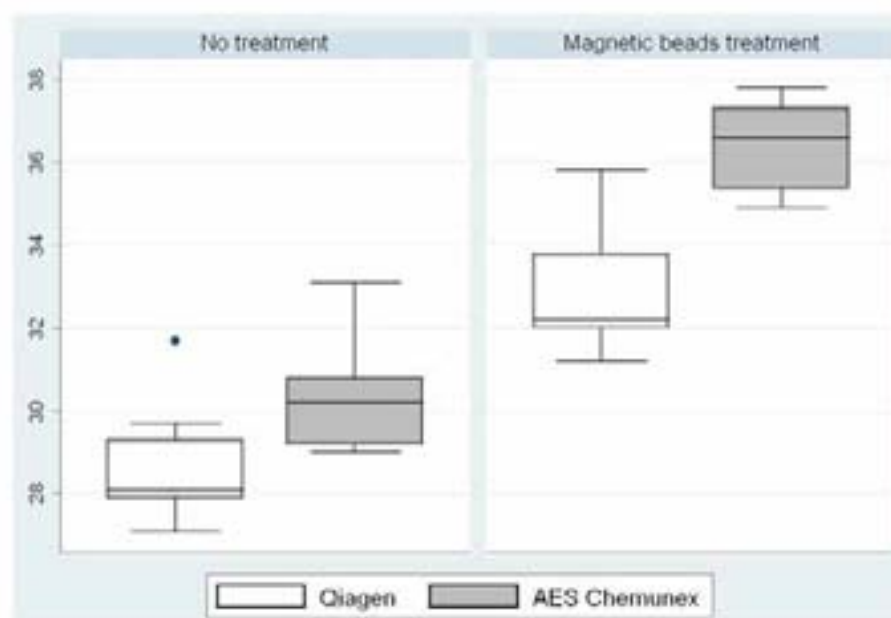
Figure 1: Box-and-whisker plots of distribution Ct values from different extraction methods on faecal samples pre-treated or not with Dynalbeads

Real Time PCR analysis of DNA extracted from spiked pork samples

The best method, identified as the one providing the lowest Ct values by Real Time PCR analysis, was the Charge Switch gDNA miniBacteria Kit [1], followed by the Extraction DNA mix [3] and the boiling method [4]. Conversely, method 2 failed to produce PCR products, probably due to the presence of inhibitors.

The best LMM presented the significant fixed effects of pre-treatment, methods of extraction and their interaction (p<0.001). The residuals are normal (p>0.10;) and without particular patterns.

For method 1 a significant change in terms of Ct values was not noted after the treatment with Dynalbead, on the contrary for methods 3 and 4 a certain difference was evidenced and more pronounced as far as method 4 is concerned. In particular the treatment with Dynalbeads resulted in an increase on the Ct values. (Figure 2).
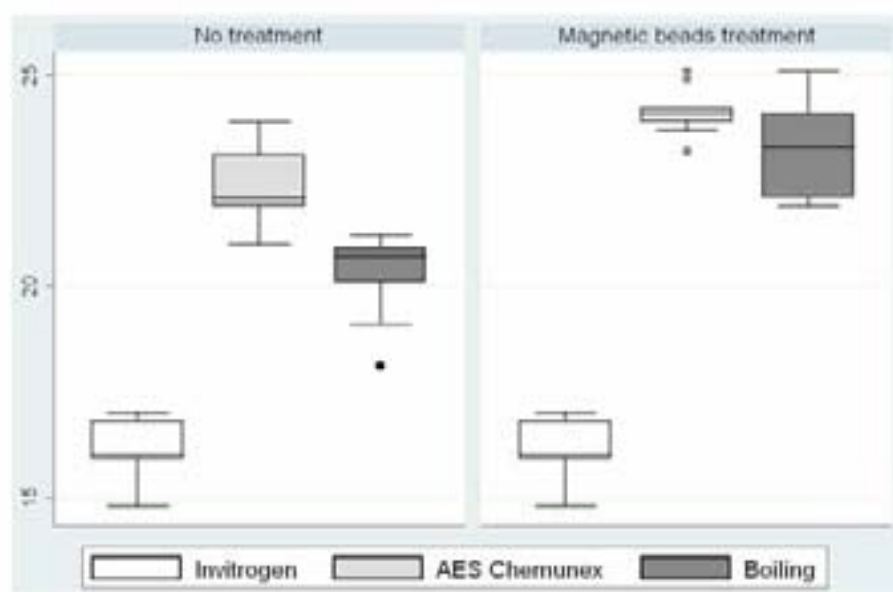
Figure 2: Box-and-whisker plots of distribution Ct values from different extraction methods on pork samples pre-treated or not with Dynalbeads

Discussions and conclusions

The efficacy of Real Time PCR analysis, as well as of other molecular methods can be enhanced by using suitable extraction methods for the matrix tested. On the other hand, many commercial DNA extraction kit are patented and this precludes the possibility of a comprehensive comparison of their technical features.

Although further and more extensive studies are needed, our results show that QIAamp DNA Stool kit [2], and Extraction DNA mix [4] are the most suitable methods to detect Salmonella in pig faeces. As far as pork samples are concerned, the best performances were obtained using the Charge Switch gDNA miniBacteria Kit [1]; however, the Extraction DNA mix [4] and the boiling method, possibly with some improvements, represent an inexpensive, handling and time-saving method to obtain Salmonella DNA from different pig matrices. Eventually, the pre-treatment of samples with Dynalbeads did not result in an improvement of the DNA recovery.

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Toxoplasma gondiiprevalence in confinement pig herds measured by meat juice serology at slaughter

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Abstract

The new European food safety legislative demands a risk-based improvement of the processes of meat production from farm to fork instead of just inspecting the carcasses of each slaughtered pig. One major tool to include the pre-harvest stage of meat production is profiling herds serologically especially for latent zoonotic infections. The paper describes the inclusion of continuously serological testing for Toxoplasma gondii antibodies using meat juice samples. This concept was applied at two abattoirs in the Northwest of Germany using meat juice according to the sampling for the German Salmonella monitoring programme. The results of testing 39 pig herds demonstrated an unexpected high frequency of Toxoplasma gondii antibody positive pigs (> 90% herd prevalence, up to 80% intra-herd-prevalence) although only confinement herds were included into the study.

Introduction

Human cases of Toxoplasmosis are caused by contact to Toxoplasma shedding cats, resp. to their excretions, transmission from pregnant women to their foetuses or by eating Toxoplasma burdened raw or undercooked meat. The latter is the major reason, why Toxoplasma gondii infections of pigs are of public health relevance, especially in countries like Germany with a high proportion of raw pork consumption.

Toxoplasma burdened carcasses cannot be detected by the tools of the traditional meat inspection (visual inspection, palpation, incision) and they enter the food chain, which potentially pose a risk to pregnant women and immune-deficient persons.

In contrast to the traditional meat inspection, a core element of the risk-based meat inspection is process control and process optimization on the basis of the so-called "food chain information" (mortality, morbidity, drug-use in the herd of origin) as required by the Reg. (EC) 853/2004. To add data on subclinical zoonotic infections of pig herds to this food chain information, it is necessary to detect subclinical diseases like Salmonellosis, Yersiniosis, and Toxoplasmosis by means of targeted, specific diagnostic tests (e.g. by serology). Serological monitoring results are valuable for deciding on the appropriate inspection method and selecting carcasses for e.g. the production of minced meat. The paper gives an overview on ways to integrate the Toxoplasma seroprevalence of pig herds into meat juice multi-serology programmes for implementing the risk-based meat inspection.

Material and Methods

Testing the comparability of blood serum and meat juice: For assessing the reliability of meat juice instead of blood serum as samples for the detection of antibodies against Toxoplasma gondii, 291 paired samples from pigs (i.e. 291 times serum and meat juice from exactly the same pig) out of six herds supplying slaughter pigs to one abattoir were tested together with six different ELISA-tests for detecting antibodies against Salmonella, Yersinia, Trichinella, Mycoplasma hyopneumoniae, Influenza A H1N1 and H3N2.

For detecting Toxoplasma gondii antibodies, the ANIMALTYPE Toxoplasma Ab ELISA (LDL, Leipzig, Germany) was used. This test is based on a recombinant antigen and contains a multispecies conjugate, which has been developed and validated for the use of blood serum and meat juice.

Since a relatively low seroprevalence for Toxoplasma gondii was expected, several assuredly positive Toxoplasma control sera and meat juices (produced via experimental infections) were provided by the Institute for Parasitology of the University of Veterinary Medicine Hannover, Foundation, Germany, were simultaneously tested with the field sera and meat juices.

To look into potential changes over time in the intra-herd prevalence of Toxoplasma antibodies, 160 meat juice samples from the same six herds were taken and tested in 2010.

Assessing a regional herd and intra-herd prevalence using meat juice: After confirming that this test produces highly comparable results for Toxoplasma gondii antibodies in blood serum and meat juice of the same animals, the ANIMALTYPE Toxoplasma Ab ELISA (LDL, Leipzig, Germany) was used also for a second study, this time using only meat juice, for estimating the seroprevalence of Toxoplasma gondii in 33 pig herds supplying also to only one abattoir, situated in the Northwest of Germany with a very high pig herd density in the region – all herds were completely confined without any outdoor facilities. Per herd 60 to 80 randomly collected meat juice samples were tested (n = 2359).

The epidemiological investigation of the farm characteristics for identifying risk factors is still going on, but 11 low-prevalence and 9 high-prevalence herds have been already visited and scrutinized for known and other possible risk factors.

Results

Testing the comparability of blood serum and meat juice:

The result of testing the paired pig samples (blood serum and meat juice) for Toxoplasma gondii antibodies in the framework of the meat juice multi-serology concept are shown in Tab. 1. In both the serum and the meat juice samples, the same six pigs were seropositive for Toxoplasma gondii.

Tab. 1: ELISA test results from blood serum and meat juice of 291 slaughter pigs in 2009 and the degree of agreement of the results

relevant for:	ELISA test for:	blood serum: proportion of positive samples (n/N)	meat juice: proportion of positive samples (n/N)	Sensitivity meat juice vs. serum	Specificity meat juice vs. serum
food safety (zoonotic diseases)	Toxoplasma gondii*	2% (6/291)	2% (6/291)	100%	100%
	Salmonella spp.	13% (38/291)	12% (36/291)	87%	99%
	Yersinia enterocolitica	69% (202/291)	72% (210/291)	100%	91%
	Trichinella spp.*	0% (0/291)	0% (0/291)	100%	100%
animal health (production diseases)	Mycoplasma hyopneumoniae	51% (149/291)	48% (141/291)	91%	96%
	Influenza A (H1N1)	32% (93/291)	20% (59/291)	61%	99%
	Influenza A (H3N2)	11% (31/291)	7% (19/291)	55%	99%

*all confirmed Trichinella and Toxoplasma positive control sera and meat juices were clearly identified as "positive"

The results of the repeated testing for Toxoplasma antibodies of the six herds in 2010 compared to the results 12 months earlier are demonstrated in Tab. 2.

Tab. 2: Changes in the Toxoplasma seroprevalence of six herds from 2009 to 2010

Herds	A	B	C	D	E	F	Total
Seroprevalence 2009	3% (3/108)	3% (1/31)	10% (2/20)	0% (0/28)	0% (0/63)	0% (0/41)	2% (6/291)
Seroprevalence 2010	9% (7/80)	0% (0/10)	20% (2/10)	0% (0/20	0% (0/20)	5% (1/20)	6% (10/160)

Assessing a regional herd and intra-herd prevalence using meat juice: Out of the 2359 pigs slaughtered from 33 herds of the area of Germany with the highest pig density, 229 (9.7%) were seropositive for Toxoplasma gondii at the animal level.

From 33 herds, 31 turned out to be Toxoplasma seropositive (1 and more positive sample per herd = positive herd), which results in a herd prevalence of 94%. However, the intra-herd prevalence was highly variable ranging from 0% to 84%, with the majority of herds showing intra-herd prevalence values below 10%. Except of the herd with 59 positive samples out of 70 (84%) the following intra-herd prevalence values above 10% were detected: 12%, 13%, 14%, 15% 2 x 16%, and 41%.

The 20 herds that so far were visited (eleven low prevalence herds including the two seronegative herds, and nine high prevalence herds including the two herds with the 84% and the 41% prevalence) did not show any plausible differences. Especially the presence of cats on the farms with and without access to the barns was evenly distributed in both groups.

Discussion

The results show that the use of meat juice instead of blood serum of pigs for detecting antibodies against Toxoplasma gondii as already done in other studies (McKean et al., 2009) is, especially for producing continuous serological herd profiles is highly justified. With this, the ANIMALTYPE Toxoplasma Ab ELISA (LDL, Leipzig, Germany) may be included into any meat juice multi-serology concept.

The results also show that the prevalence at pig level (9.7%) and at herd level (94%) of Toxoplasma antibodies in the tested herds of the German region with a very high pig density is remarkably higher than shown for Ontario (Canada) with 1.6% and 13.7%, respectively (Poljak et al., 2008).

The results of McKean et al. (2009) demonstrate a remarkably lower prevalence of Toxoplasma meat juice antibodies in large production systems compared to smaller production systems, which points to the role of biosecurity for the Toxoplasma prevalence of pig herds. The preliminary conclusions that can be drawn from the own study into the prevalence of completely confined pig herds support this assumption. The importance of the presence or absence of cats and of outdoor access for the pigs on a farm may be overestimated. General biosecurity deficiencies seem to determine the Toxoplasma prevalence of pig herds beyond cats, outdoor areas and rodents.

Conclusion

If Toxoplasma testing is included in a national zoonosis control programme which uses continuous testing of random samples of meat juice from all slaughter pig supplying herds, a tool for a) estimating the national toxoplasma prevalence, b) recognising herds with and without Toxoplasma infestation for epidemiological analyses and for intervention measures at farm level, c) providing food business operators and veterinary authorities with valuable information in the framework of the risk-oriented meat inspection.

It could be shown, that there are more Toxoplasma seropositive confinement herds in the Northwest of Germany than expected, and that including Toxoplasma serology into any multi-serological herd profiling is meaningful in terms of continuously improving the safety of pork.

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Development of a serological Luminex assay for Trichinella and Salmonella in swine

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Abstract

In order to develop veterinary serological multiplex assays, a singleplex bead-based array on the Luminex platform was developed, and with this experience the study was expanded by building a multiplex serological assay. First a serological Luminex assay was developed for Trichinella in swine. As the developed assay performed comparable to commercial ELISA's, work on this platform was continued by developing a serological multiplex assay for Salmonella in swine. This assay is based on five LPS variants of the most important serogroups occurring in pigs. The serological multiplex assay for Salmonella performed comparable to a commercial ELISA. The results from this study demonstrate the feasibility of the Luminex platform for multiplex serology. Ultimately, this type of assay can be used for routine screening of porcine serum samples for immune responses against multiple pathogens in one assay.

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Antimicrobial resistance in food-producing animals of public health concern. An overview of the current situation and options for control

DikMevius

Abstract

Since the detection of Livestock Associated MRSA (LA-MRSA) in pigs and other food-producing animals, the levels of concern about the consequences of antimicrobial resistant organisms in animals and foods thereof for public health have substantially increased. As a result, the topic of antimicrobial resistance in animal bacteria currently has a prominent position on the agenda of policy makers of national authorities and the EU. Moreover, EFSA has installed expert working groups to advise the European Commission on the risk associated with these organisms.

More recently the frequent detection of Extended Spectrum Beta-Lactamase (ESBL)-producing organisms in food-producing animals and their genetic association with isolates from human infection concerns has been described (Leverstein et al, CMI 2011). In The Netherlands in 19 % of the human clinical ESBL-producing E. coli the genes and plasmids were genetically indisguisable from ESBLs and plasmids from poultry sources. Almost all Dutch broiler chickens produced shed ESBL-E. coli in their faeces; 100% of the conventional poultry meat is contaminated and 84% of the organic poultry meat (Cohen Stuart et al, 2011). This strongly suggests that poultry products are the source for humans. Although currently poultry seems tobe the most important animal species in which these ESBL-producers occur so frequently, they have also been reported in pigs, including transfer to pig owners (Moodly et al, AAC. 2009; Cavaco et al, AAC, 2008; Jorgensen et al., JAC 2007). Moreover, Both in poultry and in pigs usage of cephalospoins is described to be a strong selecting agent (Dutil et al, EID 2010). Antibiotic usage is considered to be the most important determinant for the emergence and spread or these resistant organisms. Because of their multi-drug resistant nature, selection is not only the result of specific classes of antibiotics (eg. cephalosporins), but exposure to other frequently used antibiotic classes will have a positive selective effect as well. Therefore, control should not merely be aimed at usage of specific drug classes, but also at usage in general. Currently however, with the exception of a few countries, control of antibiotic usage including identification of high users or frequent prescribers is lacking.

During the presentation the current situation in food-producing animals will be presented including essential options for control.

Does nasal colonization with Methicillin-resistant Staphylococcus aureus (MRSA) in pig farmers persist after holidays from pig exposure?

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Abstract

Background: In Germany, it has been reported that up to 86% of pig farmers are colonized with Methicillin-resistant Staphylococcus aureus (MRSA) in the nares, at least intermittently. However, little is known about the long-term persistence of colonization, especially when the farmers do not have daily contact to pigs. Here, we analyzed whether an absence from work during the summer holidays had an impact on nasal MRSA colonization rates of pig farmers.

Method: Farmers with daily exposure to pigs during their work routine provided nasal swabs taken at the last three days before their summer leave 2010 and three additional swabs obtained at the first three days after return to work. Every first MRSA isolate was characterized using sequence-based typing of the S. aureus protein A gene (spa).

Results: Among 35 farmers screened, the length of the summer leave was <7d for two farmers, 7-14d for 22 and >14d for two farmers. MRSA was detected in at least one swab from 27 farmers (77%). From these, 16 (59%) were tested positive in all six swabs obtained before and after absence from work; three famers (9%) were tested positive before and negative in all three swabs obtained after the holidays; seven (20%) were tested negative in the swab obtained on the first day after return. One farmer (4%) was tested MRSA negative in all swabs before the leave and positive in all swabs after return from the holidays. The distribution of spa types was t011 (63%), t034 (22%), t108 (7%), t1197 and t1451 (each 4%).

Conclusion: We confirmed a high rate of intermittent MRSA carriage (77%) among German pig farmers. Mostly, holidays did not have an impact on colonization. Only 14% of the farmers lost MRSA during their leave and remained negative for three days after return.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) has been reported to colonize livestock (pigs, cattle, poultry) in the most European countries. In Germany, up to 70% of all pig farms [1, 2] are affected. Among the MRSA from livestock reservoirs, isolates belonging to one particular clonal complex (CC), CC398 as defined by multilocus sequence typing (MLST), are predominant and account for more than 90% of all isolated strains [1]. Moreover, it has been described that MRSA CC398 is frequently transmitted to persons with a direct contact to the animals leading to colonization rates of up to 86% among farmers from MRSA positive units [3]. However, a Dutch study has recently shown that persons with occupational exposure to the animals might be colonized only inpersistently. Field workers with a short-term exposure up to 3h daily were positive directly after their visit on a pig farm. But 94% appeared negative when a second nasal swab was collected 24 hours later [4]. Currently, little is known about the MRSA colonization dynamics of persons with a direct and regular contact to livestock. Therefore we invastigated whether an absence from the pig farm during the summer holidays had an impact on MRSA carrier rates among German pig farmers.

Material and Methods

Farmers in the German part of the Dutch-German border region (North Rhine-Westphalia, Lower Saxony) with daily exposure to pigs provided nasal swabs taken during the last three days before their summer leave 2010. Three additional swabs were obtained during the first three days after return to work. All swabs were obtained from the individual farmers in the morning before their first contact to the animals. All nasal swabs were streaked directly onto MRSA-ID (bioMérieux)

and were enriched using a selective medium (Phenolred mannitol broth + ceftizoxime/aztreonam). After 24h of incubation every enrichment culture was plated on MRSA-ID agar. Suspicious colonies were confirmed as MRSA using VITEK2 automated systems and mecA PCR. Every first MRSA isolate of each participant was characterized by sequence-based typing of the S. aureus protein A gene (spa) as described [5]. Cluster formation of spa types was done using the Based Upon Repeat Pattern (BURP) algorithm of the Ridom StaphType software (Ridom GmbH, Münster) with preset parameters as recommended [6].

Results

Among 35 farmers screened, the length of the summer leave was <7d for two farmers, 7-14d for 22 and >14d for two farmers (Table 1). MRSA was detected in at least one swab from 27 farmers (77%). 8 farmers were never tested positive (23%). From these, 16 farmers (59%) were tested positive in all six swabs obtained before and after vacation; three famers (9%) were tested positive before and negative in all three swabs obtained after the holidays; seven farmers (20%) were tested negative in the swab obtained on the first day after return, but positive in at least one consecutive sample. One farmer (4%) was tested MRSA negative in all swabs before the leave and positive in all swabs after return from the holidays. The distribution of spa types was t011 (63%), t034 (22%), t108 (7%), t1197 and t1451 (each 4%). According to the spa repeat pattern, BURP indicated that all spa types belonged to one group.

Discussion

We have found a high MRSA carrier rate among German pig farmers; 77% of which were colonized at least intermittently. All farmers were colonized with spa types indicative for the MRSA CC398 lineage. This confirms previous investigations where it was shown that the colonization rate in this group of persons varies between 23% and 86% [3, 7, 8]. This finding is important, since, especially for nosocomial infections, it has been demonstrated that the colonization with MRSA is a major risk factor for the development of subsequent illness [9]. Swabs were only taken from the anterior nares, as the predilective sites of MRSA carriage. This might lead to an underestimate of the colonization rates, because the farmers might have been colonized at other body sites.

In contrast to investigations among probe collectors suggesting a high rate of intermittent carriage, the majority of farmers in this study (59%) were still colonized on the day after return from the holidays. This might indicate that those persons with regular contact to the pigs are persistently colonized whereas persons with sporadical contact [4] might rather be "contaminated" (e.g. via dust inhalation) than "colonized". Interestingly, 29% of the farmers were tested positive before the leave and negative on all or at least the first day after return. This could be due to an intermittent carriage which was cleared during the holidays. However, 70% of these farmers were tested MRSA positive again on day two or three after their return which could either show a re-colonization after the first contact to the animals or could be explained by a lack of sensitivity of the nasal swabs used to detect carriage. Only 9% of the farmers remained MRSA negative in all three swabs after their holidays. From a practical point of view, these results are useful for recommendations regarding decolonization therapies. When elective surgical procedures are planned, farmers are often tested positive in an MRSA screening performed prior to the intervention. Our data indicate that in most cases, it is necessary to perform decolonization therapies (e.g. using mupirocin nasal ointment and antiseptic body washes), since even absenting from the stables for more than 7 days is unlikely to clear colonization.

Conclusion

In conclusion, absence from the pig holdings during the summer leave mostly did not have an impact on MRSA colonization of pig farmers. Only 9% of the farmers lost MRSA during their leave and remained negative for three days after return. This suggests that farmers are more likely to be nasally colonized with MRSA CC398 rather than "contaminated" via dust inhalation or hand contact.

Table 1 MRSA colonization of German pig farmers before and after a summer leave from the pig holdings.

Farmer No.	before holidays			Length of holidays	after holidays			*spa* type
	day 1	day 2	day 3		day 4	day 5	day 6	
1	-	-	-	<7 days	-	-	-	-
2	+	+	+	7-14 days	+	+	+	t011
3	-	-	-	7-14 days	+	+	+	t011
4	+	+	+	7-14 days	+	+	+	t011
5	-	-	-	7-14 days	-	-	-	-
6	-	-	-	7-14 days	-	-	-	-
7	+	+	+	7-14 days	+	+	+	t011
8	+	-	-	7-14 days	-	-	-	t034
9	+	+	+	7-14 days	+	+	+	t034
10	-	-	-	7-14 days	-	-	-	-
11	+	+	+	7-14 days	+	+	+	t011
12	+	+	+	7-14 days	+	+	+	t011
13	+	+	+	7-14 days	+	+	+	t011
14	-	-	+	7-14 days	-	-	-	t011
15	-	-	-	7-14 days	-	-	-	-
16	+	+	+	7-14 days	-	-	+	t108
17	+	+	-	7-14 days	-	+	+	t108
18	+	+	+	7-14 days	-	+	-	t034
19	+	+	+	7-14 days	+	+	+	t011
20	+	+	+	>14 days	+	+	+	t034
21	+	+	+	>14 days	+	+	+	t1451
22	-	-	-	7-14 days	-	-	-	-
23	+	-	+	7-14 days	-	-	-	t011
24	-	-	+	7-14 days	-	-	+	t011
25	+	+	+	7-14 days	+	+	+	t011
26	+	+	+	7-14 days	+	+	+	t011
27	+	+	+	<7 days	+	+	+	t011
28	+	-	+	7-14 days	-	+	-	t011
29	-	-	-	7-14 days	-	-	-	-
30	-	-	-	7-14 days	-	-	-	-
31	+	+	+	7-14 days	-	+	+	t034
32	+	+	+	7-14 days	+	+	+	t011
33	+	+	+	7-14 days	+	+	+	t011
34	+	+	+	7-14 days	+	+	+	t1197
35	+	+	+	7-14 days	-	-	+	t034

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Investigation of MRSA transmission between pigs and the environment following intra-nasal inoculation

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Abstract

Meticillin-resistant Staphylococcus aureus (MRSA) ST398 has not been detected in pigs in Ireland. However, other strains of MRSA, including MRSA t002, have been isolated from animals and humans in Ireland. The aim of this study was to determine if nasal colonization of pigs with a non-ST398 strain of MRSA could be reproduced using intra-nasal inoculation and to investigate subsequent transmission of this strain. Six pigs were inoculated intra-nasally with 2×10^9 cfu MRSA t002. Six days post-inoculation these pigs were washed and moved to a clean house with 15 unexposed pigs (In-contact group). Another 15 unexposed pigs were added to the vacated house (Environment group). The inoculated pigs and In-contact group were moved to a clean house every 2 days to minimise exposure to MRSA in the environment. Nasal swabs were taken for MRSA culture from all pigs and counts were undertaken from samples from inoculated pigs. Inoculated pigs were euthanized 5 weeks post-inoculation and exposed pigs were euthanized 4 weeks post-exposure to MRSA. Upper respiratory tract samples were taken at postmortem for MRSA culture. All inoculated pigs were MRSA-positive for 5 days post-inoculation. Thereafter the number of positive pigs decreased with no inoculated pig positive after 17 days post inoculation. However, MRSA was isolated from three inoculated pigs following postmortem culture. Ten of the 15 pigs from the Environment group were MRSA-positive during the first 3 days post-exposure but all 15 were negative on subsequent samplings. Only 3 of 15 pigs in the In-contact group were MRSA positive during the first 3 days post-exposure; 4 other pigs in this group were MRSA-positive on subsequent samplings. This study demonstrates that, experimentally, pigs can become colonised with a non-ST398 strain of MRSA which can be transmitted by both direct contact with colonised pigs and by environmental exposure alone. However, transmission by both routes appeared to be inefficient and risk of persistence was low under these conditions.

Introduction

While meticillin-resistant Staphylococcus aureus (MRSA) has been isolated from pigs and their environment in several European countries, MRSA has not been detected in pigs in Ireland thus far (1, 2). MRSA has been isolated from companion animals and horses in Ireland and a high proportion of staphylococcal bloodstream infections in humans in Ireland are caused by MRSA (3, 4). In humans the primary site of carriage of S. aureus and MRSA is the anterior nares (5). In pigs, MRSA carriage is usually determined by nasal swab culture. However, the importance of staphylococcal carriage in other sites has not been assessed. Pharyngeal carriage of S. aureus has been described in humans and the tonsillar tissue of the pharyngeal area is an important site of entry and replication for several porcine pathogens (6, 7). Transmission of MRSA between humans usually occurs as a result of direct contact. While those carrying or infected with MRSA can shed the bacterium into their environment, the importance of the environment as a source of MRSA for the colonisation of humans is debated (8). In pig production settings, where large numbers of animals are kept in relatively unclean environments, the environment may play an important role in the transmission of MRSA. The aim of this study was to determine if a strain of MRSA, present in animals and humans in Ireland, could be used to experimentally reproduce nasal colonisation in pigs. An associated aim of this study was to determine the relative importance of animal- and environmental-sources in the transmission of MRSA. We also sought to determine if the pharyngeal tissues of the pigs were a significant site of colonisation for S. aureus and MRSA.

Materials and Methods

Forty pigs from a commercial pig-herd were selected for inclusion in this study based on the results of nasal swab culture for S. aureus. All 40 pigs were S. aureus-positive on at least 2 sampling occasions prior to the start of the trial.

The inoculating strain, MRSA t002, was grown on blood agar overnight then suspended in sterile phosphate-buffered saline.

Six pigs were inoculated intra-nasally with 2×10^9 cfu MRSA by syringe application to the nasal cavity. These 6 pigs were allowed to contaminate their environment for a period of 6 days. On day 6 post-inoculation (PI), the 6 pigs were removed from their house, washed in a disinfectant solution to remove MRSA from the body surface and moved to a new house.

Fifteen pigs (In-contact group) were added to the house now containing the 6 inoculated and washed pigs. All 21 pigs in this house were moved to a clean house every 2 days and the vacated house was disinfected. This group of pigs was primarily exposed to MRSA via nasal shedding from the inoculated pigs. Fifteen pigs were also added to the contaminated house vacated by the 6 inoculated pigs on day 6 PI (Environment group). These pigs were exposed to MRSA from the environment only. Four negative-control pigs were inoculated intra-nasally with sterile saline and housed separately from the other groups.

Nasal swabs were taken from inoculated pigs for MRSA enrichment culture and MRSA direct counts on Brilliance MRSA agar. Nasal swabs were taken from both groups of exposed pigs and negative-control pigs for MRSA enrichment culture only. Nasal swabs were taken daily for the first 3 days PI and post-exposure (PE) and then every 2-3 days. Environmental samples were taken from a 0.372 m2 portion of a shelf in each pig-house for MRSA enrichment culture and direct counts. This shelf was not in direct-contact with the animals in the house.

At postmortem examination, samples of the nasal skin, nasal mucosa, palatine tissue and para-epiglottic tissue were taken from each pig. MRSA direct counts and enrichment culture were carried out on all samples. In addition, S. aureus enrichment culture and direct counts were carried out on all samples.

Results (Table 1)

All 6 inoculated pigs were MRSA-positive on enrichment culture for 5 days PI with detectable counts of MRSA in each pig on at least one occasion. No further counts of MRSA were detected in the inoculated pigs after day 6 PI and the number of pigs MRSA-positive on enrichment culture decreased thereafter. All 6 inoculated pig were MRSA-negative on enrichment culture after day 17 PI. MRSA was detected in the palatine tissue of 2 pigs at postmortem examination. MRSA was detected in both the nasal mucosa and para-epiglottic tissue sample in one inoculated pig.

Seven of the 15 pigs (In-contact group) exposed to MRSA by contact with the inoculated pigs were MRSA-positive on enrichment culture between days 1 and 30 post-exposure (PE). Six of these pigs were MRSA-positive on only one occasion while the seventh pig was MRSA-positive on 2 sampling occasions. One pig was MRSA-positive on culture of the para-epiglottic tissue sample at postmortem.

Ten of the 15 pigs (Environment group) exposed to MRSA in the environment were MRSA-positive on enrichment culture between days 1 and 3 PE. Four of these pigs were MRSA-positive on more than one occasion during that period. None of the 15 pigs were MRSA-positive after day 3 PE. One of the 15 pigs was MRSA-positive on culture of the nasal skin sample at postmortem examination.

The environment of the inoculated pigs was MRSA-positive on days 1-3 PI with detectable counts on each occasion. MRSA were not detected in the environment of the inoculated pigs immediately prior to their removal from this house and their replacement with un-inoculated pigs. MRSA were not detected in the environment of any of the houses for the remainder of the study.

Direct counts of S. aureus were recorded on all 40 palatine tissue samples at post mortem with a median count of 4×10^5 cfu/gram of tissue. S. aureus was less frequently detected on enrichment culture of the other 3 tissue samples taken at postmortem and the median S. aureus count for each of these samples was zero.

Table 1: Number of inoculated and exposed pigs MRSA-positive on enrichment culture of nasal swabs and post-mortem tissue samples

Days post-inoculation or post-exposure	Inoculated pigs	Pigs exposed to MRSA-inoculated pigs	Pigs exposed to MRSA-contaminated environment
Day 1	6/6	2/15	6/15
Day 2	6/6	0/15	3/15
Day 3	6/6	1/15	6/15
Day 5-10	6/6	2/15	0/15
Day 11-28	2/6	2/15	0/15
Total no. pigs positive	6/6	7/15	10/15
Post-mortem samples			
Nasal skin	0/6	0/15	1/15
Nasal mucosa	1/6	0/15	0/15
Palatine tissue	2/6	0/15	0/15
Para-epiglottic tissue	1/6	1/15	0/15
Total no. of pigs positive at postmortem examination	3/6	1/15	1/15

Discussion

The inoculated pigs in this study appeared to be successfully colonised with MRSA for a period of at least 5 days. Since these pigs were kept in a clean environment and MRSA was not detectable in the environment after day 3 PI, these results are unlikely to reflect contamination from the environment. One pig was MRSA-positive on 9 separate sampling occasions and was also MRSA-positive on culture of 2 tissue samples at postmortem examination; it is likely that this animal was persistently colonised with MRSA. A recent study suggested that intra-nasal inoculation may not be an efficient method of reproducing nasal colonisation in pigs (9); therefore, the results of this study may not reflect the true colonising capacity of this strain of MRSA in pigs. Successful colonisation with S. aureus is dependent on several host and microbial factors. In this study, individual variation between pigs in their susceptibility to colonisation may have influenced the results observed. In the pigs exposed to MRSA by exposure to inoculated pigs, transmission of MRSA occurred but only to a limited extent. The number of inoculated pigs shedding MRSA and the numbers of bacteria shed may not have been sufficient to establish colonisation in the in-contact animals.

The number of pigs (10 of 15) testing positive for MRSA from the group exposed to MRSA in the environment alone suggests that transmission of MRSA from the environment to pigs occurs. It has been suggested that environmental survival of MRSA may be strain-specific (10). It is possible that the inoculating strain of MRSA t002 was not adapted to survival in the environment of the pig-house and so transmission of MRSA did not occur after day 3 PE.

A previous study found greater numbers of S. aureus in the tonsillar tissue of the pharynx of pigs compared with the nasal cavity (11) and is in agreement with our findings. It is possible that the pharyngeal tissues represent a significant site for the colonisation of pigs with S. aureus and MRSA. Since most studies rely on demonstrating MRSA on nasal swab culture, this finding has implications for sampling strategies and interpretation of the results of such studies.

Conclusions

Intra-nasal inoculation can be used to reproduce colonisation of pigs with MRSA t002; however, the efficiency of this method is questionable and is likely to be influenced by individual variation between animals. Transmission of MRSA t002 can occur as a result of direct-contact between pigs and contact with a contaminated environment. In this study, transmission by either route did not appear to result in persistent colonisation. The tissues of the pharynx may be a significant site of carriage of S. aureus and MRSA in pigs.

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Infection kinetics and host specificity of Methicillin-resistant Staphylococcus aureus (MRSA) in pigs

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Abstract

In this study, we investigated the colonisation kinetics and host specificity of three different clonal lines of MRSA (ST8, ST9 and ST398).

MRSA prevalence on skin, nasal mucosa, conjunctiva, feacal shedding and distribution patterns of MRSA in internal organs in weaning piglets are studied.

Introduction

The transmission of MRSA and its different clonal lines in practice, as well as the influencing factors of a transmission are largely unclear in the field of animal husbandry (2). It is known that MRSA can pass from animals to humans. Moreover people with direct daily contact to animals such as farmers, holders of livestock and pets, veterinarians and the staff in slaughterhouses show a higher colonization with MRSA than those without frequented contact to animals (1).

With regard to MRSA the number of studies in larger domestic animals is increasing. However these are mainly field studies dealing with the prevalence of MRSA (3) carried out in practice, with naturally contaminated animals in farms, barns and slaughterhouses. There are no previous reports about the rate of recovery of MRSA in internal organs with regard to animal experiments with large domestic animals.

Material and Methods

A pool of 58 piglets were randomly divided into four test groups and one control group. Three test groups were infected with MRSA ST8, MRSA ST9 and MRSA ST398, respectively. The fourth group was a fusion of MRSA ST398 infected and non infected "sentinel" animals.

Clinical symptoms, the nasal, conjunctival and skin colonisation of MRSA, faecal excretion and organ distribution of MRSA, as well as different environmental samples were examined.

Results

After nasal application with MRSA piglets of all four test groups showed no clinical signs of an MRSA infection. MRSA was present on the nasal mucosa, skin and conjunctiva in all four test groups, including all sentinel animals. Likewise, faecal excretion and internal colonization of MRSA ST8, ST9 and ST398 could be shown in each group. Colonization was less efficient with the MRSA ST9 strain (originated from the poultry food chain) as indicated by a lower proportion of positive nasal swabs and a numerically reduced colonization of internal organs, feces and skin, in comparison to the ST8 and ST398 groups.

Discussion

MRSA strains of the clones ST8, ST9 and ST398 were able to colonize the nasal mucosa and to real infect different inner organs of all pigs and, furthermore, to contaminate the environment throughout the whole study period. However, results of our study suggest existing strain specific colonization mechanisms of the different MRSA types that might be associated with a certain degree of host specificity.

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Methicillin Resistant Staphylococcus aureus (MRSA) in market age pigs on-farm, at slaughter and retail pork

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Abstract

This study was conducted to determine the occurrence and prevalence of methicillin resistant Staphylococcus aureus (MRSA) in finishing pigs on-farm, at lairage and assess the likelihood of carriage at slaughter and retail levels. A cross-sectional study targeting ten cohorts of commercial swine farms was conducted for carriage of MRSA. Paired nasal and peri-anal swab samples (n=24/farm) were collected from market age pigs on-farm and the same batch of pigs were followed and sampled at the lairage before slaughter and carcass swabs at post evisceration stage before chilling. Pork samples from the same batch of pigs were collected at retail market. We assessed phenotypic and genotypic relatedness from the various sources. Conventional cultural methods using oxacillin resistance screening agar was used. Antimicrobial resistance was tested to a panel of 21 antimicrobials. PCR was used to detect the presence of species-specific gene (nuc) and methicillin resistance marker gene (mecA). The genotypic relatedness of isolates was determined using the Pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). One or more MRSA positive pigs were detected in five of the ten herds (50%). The prevalence of MRSA in pigs was higher at lairage and ranged from 0% to 54.2% per farm compared to that same batch of pigs on-farm (0% to 12.5%). The proportion of MRSA positive isolates recovered from nasal swab samples was relatively higher (4.8%) compared to peri-anal samples (2.7%). We detected MRSA in 1.6% (4/240) of the carcass swab and 3.7% (5/135%) of the retail pork samples. Genotypically similar isolates were detected from farm to the retail chain based on PFGE. Using MLST, ST398 was detected from farm, lairage and retail pork. In addition ST5, ST9, ST39 and ST72 were detected at different points of sampling.

Introduction

In recent years, occurrence of livestock-associated methicillin-resistant Staphylococcus aureus (MRSA) referred to as multi-locus sequence type (ST) 398 has been reported among pigs and pig farmers in the Netherlands (Voss et al., 2005; Huijsdens et al., 2006), Canada (Khanna et al, 2008) and in the United States (Smith et al., 2009). When MRSA-positive food animals such as pigs are slaughtered, carcasses could easily be contaminated with MRSA and consequently meat from these animals might get contaminated. A study conducted by de Boer and colleagues (2009) on the prevalence of MRSA in raw retail meat products including pork, beef, veal, lamb and poultry indicated that 11.9% (264/2217) of the samples were contaminated with MRSA. Another study conducted in Louisiana on 120 retail meat samples also reported that while more than 45% of pork and 20% of beef were positive for Staphylococcus aureus, MRSA was identified from five pork and one beef meat samples (Pu et al., 2009). In general data on MRSA prevalence and occurrence in pig production units, slaughtered pigs and retail meat in the U.S. is very limited.

Materials and methods

Study design and sample collections: A serial cross-section sampling design was used on batches of market-age pigs in Ohio. A total of ten farms were identified and one main factor for recruitment was farms that slaughter pigs in plants and products that are sold within a known retail outlet where tracking products to retail would be reasonably conducive. We collected paired nasal and peri-anal swabs from randomly selected 24 pigs per farm from 10 farms (n=480). Nasal swabs from anterior nares and peri-anal swabs were collected from each identified pig on-farm and at lairage from animals prior to stunning. A matching 24 carcass swab samples were then collected from the same batch of slaughtered pigs at post-evisceration stage (n=240). In addition, a total of 131 retail pork samples [n=12-15 per batch] were collected from grocery stores in the same locality.

Isolation and identification: For the isolation and identification of Staphylococcus aureus, we followed selective culture methods using oxacillin resistance screening agar. Identification of the Staphylococcus species was performed at the USDA-ARS, Bacterial Epidemiology and Antimicrobial Resistance Research (BEAR) Laboratory, Athens, Georgia using the Vitek 2 system (bioMerieux, Durham, NC) and the Vitek 2 Gram-positive identification cards according to manufacturer's directions.

Antimicrobial susceptibility testing: The antimicrobial susceptibility of all Staphylococcus aureus isolates including MRSA was tested at the USDA-ARS (BEAR) Laboratory. Minimum inhibitory concentrations (MIC) for staphylococci were determined by broth microdilution panels using the SensititreTM semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH) and the SensititreTM Gram-Positive Plate GPN3F according to the manufacturer's directions. Results were interpreted according to CLSI (Clinical and Laboratory Standards Institute) guidelines when defined. The antimicrobials tested include: ampicillin, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, gatifloxacin, gentamicin, lavofloxacin, linezolid, oxacillin, penicillin G, streptomycin, synercid, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin.

Molecular characterization and genotyping: PCR was used to detect the presence of species-specific gene(nuc) and methicillin resistance marker gene (mecA). Multiplex PCR (Kondo et al., 2007) was used to determine and characterize staphylococcal cassette chromosome mec (SCCmec) types and mecA gene carriage on Staphylococcus aureus isolates recovered from the various samples during the study period.

Pulsed-field gel electrophoresis (PFGE) was conducted in selected MRSA isolates (n=40) as described (Mulvin et al., 2001) using the cfr91 macrorestriction enzyme. The isolates were selected randomly based on origin, sample type, stage of sampling and antimicrobial resistance profiles. Out of the 40 PFGE typed MRSA isolates we randomly selected 21 isolates based on origin, sample type, stage of sampling and antimicrobial resistance profiles for multi-locus sequence typing (MLST).

Results and Discussion

Occurrence and prevalence of MRSA in pigs on-farm, at lairage, carcass and retail pork: Of the total ten herds included in this study, one or more MRSA positive pigs were detected in five of herds, Table 1. We detected that the prevalence of MRSA was relatively higher in pigs after transportation (11.3%) compared to on-farm prevalence (2.9%), before transportation. The proportion of MRSA positive samples was relatively higher in nasal swabs (4.8%) compared to peri-anal swabs (2.7%) collected from batch of pigs on-farm and at lairage.

The same batch of pigs sampled on farm and before stunning were followed and carcass swabs taken before chilling and of the 240 carcass swabs we examined, 4 (1.6%) tested MRSA positive, Table 1. The same batch of carcass were followed to retail level and a total of 131 retail pork samples were included in this study of which 5 (3.8%) were MRSA positive and the pork samples originated from herd # I, III and IX (Table 1).

Table 1: MRSA in pigs on-farm, at lairage, carcass swabs and retail pork

Batch #	Number of pigs		Carcass swabs (n=240)	Retail pork (n=131)
	on-farm (n=240)	lairage (n=240)		
I	3/24 (12.5%)	13/24 (54.2%)	0/24	1/15 (6.7%)
II	0/24	1/24 (4.2%)	0/24	0/15
III	3/24 (12.5%)	11/24 (45.8%)	3/24 (12.5%)	2/15 (13.3%)
IV	0/24	0/24	0/24	0/12
V	0/24	0/24	1/24 (4.2%)	0/14
VI	1/24 (4.2%)	1/24 (4.2%)	0/24	0/14
VII	0/24	0/24	0/24	0/12
VIII	0/24	0/24	0/24	0/12
IX	0/24	1/24 (4.2%)	0/24	2/12 (13.3%)
X	0/24	0/24	0/24	0/12
Total	7/240 (2.9%)	27/240 (11.3%)	4/240 (1.7%)	5/131 (3.8%)

Antimicrobial resistance profiles: MRSA isolates recovered from various stages of sampling were highly multidrug resistant (MDR), resistance ranging from three to up to 11 antimicrobials, Table 2. The isolates were resistant to penicillin (96.3%), ampicillin (93.6 %), oxacillin (90%), tetracycline (76.4%), clindamycin (72.7%), erythromycin (62%), gentamicin (52%) and <3% resistance was detected to gatifloxacin, lavofloxacin, synercid, streptomycin and trimethoprim/sulfamethoxazole. All MRSA isolates tested were 100% susceptible to the antimicrobial effects of ciprofloxacin, daptomycin, linezolid, rifampin and vancomycin.

Table 2: Antimicrobial resistance patters of MRSA isolates recovered from various sampling stages

Batch #	Antimicrobial resistance pattern (# isolates	Origin (# of isolates)
III	AmCeCaErGmOxPnStSyTeSXT* (1)	NS-L[1]
III	AmCaErGfGmLfOxPnSyTeSXT (1)	PAS-L[2]
III	AmCaErGmOxPnStSyTeSXT (1)	NS-L
III	AmCeCaErGmOxPnTe (1)	NS-L
I, II, III	AmCaErGmOxPnTe (47)	NS-F[3] (5), NS-L (25), PAS-L (11), CS[4] (2), RP[5] (4)
IX	AmCeCaErGmOxPn (2)	RP (2)
III,IX	AmCaErGmOxPn (4)	CS (1), RP (3)
III	AmCaErGmPnTe (2)	NS-L (2)
III, IX	AmCaErOxPnTe (2)	NS-F (1), NS-L (1)
VI	AmCaErOxPn (2)	NS-F (2)
I, V	AmCaOxPnTe (17)	NS-F (1), NS-L (8), PAS-L (7), CS (1)
III	AmGmOxPnTe (1)	NS-L (1)
III, V	AmErOxPn (6)	CS (6)
V	AmOxPnTe (2)	CS (2)
I	AmCaPnTe (1)	PAS-L (1)
III	CaErPnTe (1)	RP (1)
VI	AmOxPn (4)	NS-F (1), NS-L (3)
I	AmPnTe (6)	NS-F (1), PAS-F[6] (1), NS-L (2), RP (2)
I	AmPn (1)	PAS-F (1)
III	Pansusceptible (1)	PAS-L (1)

*Am=ampicillin; Ce: ceftriaxone, Ca: clindamycin; Er: erythromycin; Gf: gatifloxacin; Gm: gentamicin, Lf: lavofloxacin; Ox: oxacillin; Pn: penicillin G; St: streptomycin; Sy: synercid: Te: tetracycline; SXT: trimethoprim/sulfamethoxazole
[1]NS-L: Nasal swabs at lairage; 2PAS-L: peri-anal swabs at lairage; 3NS-F: nasal swabs on-farm; 4CS: carcass swabs; 5RP: retail pork; 6PAS-F: peri-anal swabs on-farm

Molecular characterization and genotyping: Of the total MRSA isolates tested for SCCmec types, type II (7%), IV (5.5%), V (16.2%), and non typable (4%) were detected. However, the majority of the MRSA isolates (66.7%) did not belong to the known types. In these groups of isolates their mec gene complex and/or ccr gene complex have been amplified but did not match to the known groups and suggest the need for further study. The pulsed-field gel electrophoresis (PFGE) genotyping on selected MRSA isolates indicated the presence of genotypic relatedness among few isolates recovered on-farm and lairage as well carcass and retail pork. Among selected isolated genotyped using MLST, ST398 was detected on those isolates recovered from farm, lairage and retail pork. In addition ST5, ST9, ST39 and ST72 were detected at different points of sampling. Finding MRSA in carcass and retail pork may have some food safety implication. Co-occurrence of enterotoxin genes is underway to assess the likelihood of foodborne intoxication.

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Quantitative exposure to livestock-associated MRSA ST398 of pig slaughterhouse workers

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Abstract

Objectives: To quantify livestock-associated MRSA (LA-MRSA) exposure to workers in pig slaughterhouses and assess associated risk factors for carriage in slaughterhouse workers.

Methods: A cross-sectional study in three Dutch pig slaughterhouses was undertaken. Nasal swabs of 341 participants, surface wipes, air, and glove samples were screened for presence of MRSA. MRSA was quantitatively determined on gloves and in air samples by culturing and real-time PCR.

Results: 3.2% of the participants were defined as nasal MRSA carrier. MRSA positive workers were predominantly found at the start of the slaughter process. Major risk factors for carriage were working in the lairage and working in the scalding and dehairing area. Most nasal isolates (73%) belonged to the LA-MRSA clone ST398. MRSA ST398 positive environmental samples were found throughout the slaughter process. A clear decrease was seen along the slaughter line in the number of MRSA positive samples and MRSA colony count per sample.

Conclusions: This study showed that working in the lairage area and scalding and dehairing area were the major risk factors for MRSA carriage in pig slaughterhouse workers, while the overall prevalence is low. Occupational exposure to MRSA decreased along the slaughter line and paralleled the risk of carriage. These results can be used to model occupational risk of MRSA carriage in related occupations with meat contact, such as butchers and cooks, which likely will also be very low.

Introduction

Livestock-associated Methicillin-resistant Staphylococcus aureus (LA-MRSA) has been increasingly prevalent in veal calves, pigs, and veal and pig farmers over the last decade. MRSA prevalence estimates in persons working or living on livestock farms in the Netherlands go up to 33% (1-3). However, MRSA prevalence was much lower in family members living on the farm (8%) and in veterinarians (12.5%)(2, 4). Human-to-human LA-MRSA transmission is still considered low compared to non-livestock-associated strains (5).

Most LA-MRSA studies aimed at people working or living on farms, but the question was raised whether slaughterhouse personnel also have increased LA-MRSA carriage risk, as they too come into contact with living animals, dead animals and meat products. Furthermore, it is unclear what the MRSA carriage risk is for other occupations with meat contact, such as butchers and cooks. Recent studies showed LA-MRSA prevalence to be around 5% in poultry and pig slaughterhouse personnel, and contact with live animals was established as the major risk factor for MRSA carriage (6, 7). Quantitative data on MRSA exposure from animals and their derived meat products to slaughterhouse workers however, are still lacking.

The study presented here quantifies MRSA load in air and on hands as an exposure estimate of workers in pig slaughterhouses, and assesses MRSA carriage risk factors. Results will be discussed in the light of implications for occupational health and consumer risks. The results can be used to model the occupational risk for other occupations with meat contact, such as butchers and cooks.

Material and Methods

A cross-sectional study was undertaken in three Dutch pig slaughterhouses in 2009 and 2010. Nasal swabs were collected from 341 workers and analysed to determine MRSA carriage by culturing of MRSA (standard procedure as described in (8)). MRSA was confirmed with a mecA specific PCR (9, 10). The genetic diversity of non-ST398 MRSA colonies cultured from nasal swabs was determined with MLST and spa-typing (11, 12).

Furthermore, MRSA exposure in the air was measured by means of pumps taking active air samples, and by analysing the filters according to the procedures described above. Gloves of the workers were collected and presence of MRSA was quantitatively determined by culturing of MRSA, using a standard dilution curve, and by qPCR targeting ST398, SCCmec and mecA (9, 10, 13).

Questionnaires were used to collect data on potential risk factors and confounders: gender, age, smoking, country of birth, working area within slaughterhouse, contact with livestock outside of the slaughterhouse and recent antibiotics use. The data were analysed by means of logistic regression (SAS version 9.2).

Results

Eleven participants (3.2%) tested positive for nasal MRSA carriage. Most nasal isolates (73%) belonged to the LA-MRSA clone ST398. MRSA positive workers were predominantly found at the start of the slaughter process, i.e. in the lairage area, scalding and dehairing area, or evisceration area. Not surprisingly, major risk factors for carriage were working in the lairage area and scalding and dehairing area (OR: 5.6 and 4.1, respectively).

LA-MRSA positive glove samples were found throughout the slaughter process, with the highest levels at the front end. A clear decrease was seen along the slaughter line in the number of MRSA positive samples and MRSA colony count per air or glove sample, either by culturing or by qPCR calculated to estimated equivalent CFUs. Relatively twice as many culture positive gloves were found in the lairage and scalding and dehairing areas, when compared to the evisceration and cutting area. No culture positive gloves were found in the processing, packing and dispatch area. Quantitative PCR resulted in positive glove samples in the lairage, scalding and dehairing, evisceration, cutting, and processing area, with a clear decrease in average CFU's per sample along the processing line. Culture and qPCR positive MRSA air samples were found, but at low concentrations and only in the lairage area, and scalding and dehairing area.

Discussion

The study described here, confirmed that slaughterhouse workers were at low occupational risk of becoming MRSA carriers. The main risk factor was working in the lairage or scalding and dehairing area, in other areas exposure was found to be lower, both in air and glove samples. The overall carriage risk is considerably lower in slaughterhouse workers than in pig and veal farmers or veterinarians.

Research has shown that LA-MRSA prevalence in veal farmers decreased significantly after considerably reduced contact with veal calves, e.g. during the holiday or the empty-barn period between production rounds (14). Slaughterhouse workers may also be intermittent carrier. Therefore, they may, for example, loose the bacteria over the weekend as, so far, the bacteria seem to prefer the animal host over human hosts. Future studies should aim at establishing the duration of MRSA carriage in slaughterhouse workers.

General hygiene measures may help reducing risk of colonization or infection, such as regularly changing of gloves, and washing and disinfection of hands (all these facilities are provided in slaughterhouses). In addition, face masks could be properly worn to prevent inhaling LA-MRSA, especially in the lairage and scalding and dehairing areas. These hygiene measures could also be applied in other occupations with frequent contact with raw meat, such as butchers and cooks.

Previous studies have shown that MRSA could be found in meat samples, albeit in low concentrations and prevalence (15-17). It should be noted here, that the study by Pu et al. (2009) did not find zoonotic clones of MRSA, but the common hospital-acquired clone USA100 and the common community-acquired clone USA300. The study by De Boer et al. (2009) also found a significant part of the isolated MRSA strains from meat samples were non-ST398 strains. Of the strains isolated from gloves and air samples in the current study, 95% and 100% belonged to ST398, respectively. It remains unclear what the source is of the non-ST398 strains, but other sources than the animals or from outside the slaughterhouse are likely.

Although in the study described here the meat itself was not sampled, and therefore no conclusions can be drawn concerning meat products, qPCR positive glove samples were found in the cutting and processing areas. However, no positive samples were found in the packing or dispatching areas. Again, the number of positive samples was very low, as was the concentration of MRSA found in these samples. The supposed risk for occupations with contact with raw meat and even

consumers, is likely very low if they adhere to the standard (kitchen) hygiene protocols concerning raw meat products, and if they cook their meat properly (18). The data provided with this study will be used to model this risk.

Conclusion

This study showed that working in the lairage, scalding and dehairing area were the major risk factors for MRSA carriage in pig slaughterhouse workers, with a low overall prevalence. Occupational exposure to MRSA in air and on hands decreases along the slaughter line and parallels the risk of carriage. The results can be used to model occupational risk of MRSA carriage for other occupations with contact with raw meat.

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Observations on the distribution of monophasic Salmonella Typhimurium on pig farms in Great Britain

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Abstract

Ten pig herds were visited and intensively sampled to determine the within group prevalence, distribution of contamination and numbers of Salmonella organisms excreted by infected pigs. The distribution of infection was highly variable but on all farms with breeding pigs the breeding herd was involved, even though the occurrence of the organism was greater in growing and fattening pigs. Infection was less common in farrowing areas. Involvement of wild birds and contamination of soil on outdoor units was frequently found. Numbers of organisms excreted were typically low but levels of up to 106 cfu/g were found in a small number of samples. The role of breeding pigs was clearly illustrated by the finding of five different serovars, including monophasic S.Typhimurium, in a single batch of replacement gilts delivered to one outdoor breeding farm.

Introduction

Monophasic variants of Salmonella Typhimurium (mST) have emerged in pigs and other species in many countries during the last twenty years, but the emergence of phage type DT193 variants with resistance to ampicillin, streptomycin, sulphonamide and tetracycline in most European countries has been particularly dramatic, and has resulted in a substantial number of human cases (EFSA, 2010). The reason for the emergence and rapid spread of mST in UK pigs since 1996 is unknown but one hypothesis could be increased involvement of breeding pigs or higher numbers of organisms shed in faeces, leading to more rapid spread of infection. This study was therefore begun in order to investigate qualitatively and quantitatively the distribution, and subsequent persistence, of infection on a series of different types of pig farms.

Materials and Methods

Ten commercial pig herds (3 outdoor breeding, 3 indoor breeder finisher, 1 outdoor grower, 4 outdoor finisher, 2 indoor finishers) in which monophasic S.Typhimurium S.4(5)12:i:- or S.4,12:i:- had been isolated or suspected were visited by the authors. A combination of naturally pooled faeces (taken with a large gauze swab), individual faeces (60 per epidemiological group or if less than 60 pigs in the group a number of faeces equivalent to the number of pigs was taken) and wildlife and environmental samples were taken and returned to the laboratory on the day of collection. Culture of the swab samples was begun on the day of collection but individual samples were held at 4oC until the next day when 2 g was aliquoted and tested. The remainder of the individual samples was retained until a Salmonella result was obtained then a maximum of 40 samples from each farm as semiquantified by a dilution-enrichment technique as described by Wales et al (Wales et al., 2006). Salmonella culture was carried out using a modification of ISO6579 : Annex D, using Rambach agar as the single isolation medium. Salmonella isolates were serotyped and a selection were also phage typed.

Results

A mixture of Salmonella serovars was found in pig areas on all farms except E and F, where only monophasic S.Typhimurium (mST) was present. Only the qualitative results from breeding farms are shown in table one because of space limitations but other data will be presented at the conference. In larger farms both regular mST and 'Copenhagen' variants were sometimes found and phage types DT193 and DT120 could be present concurrently. Monophasic and regular S.Typhimurium was more likely to be found in weaned and fattening pigs rather than the breeding herd but in most cases a low to moderate prevalence was also found in breeding pigs, particularly gilts. Involvement of wild birds, particularly on outdoor units, and rodents was identified. Pooled water, transport vehicles and various environmental samples were

often positive. On Farm C, which held a newly established breeding herd set up on new ground, five different serovars of Salmonella (S.4,5,12:i:- DT193, ST DT untypable, S.Anatum, S.Infantis, S.Derby) were previously found in batches of gilts delivered from a breeding company. Some, but not all, of these serovars were later found amongst maiden gilts and boars sampled on the farm. Prevalences of mST excretion in batches of animals varied from less than 2% to 100% (in growing pigs) and estimated numbers of organisms per gram of faeces ranged from 1-106, with highest numbers more likely to be found in gilts and pigs in service areas. A wide range of serovars other than mST was found on most farms and these often dominated, especially in adult pigs.

Similar serovars were found in wild birds, rodents and environmental samples as were found in individual pig faeces samples. On outdoor units soil and wallows were particularly likely to be contaminated if there was significant infection in the pigs. Salmonella was also found in empty pens that had been cleaned and disinfected, illustrating the need for improved procedures.

Discussion

The observations from this series of visits confirm that mST is likely to behave in a similar way to regular ST (Davies and Wray, 1997), although the high level of involvement in human cases in the absence of a regular poultry host is unusual (VLA, 2010). Some of these farms have been visited on a second occasion, 3 - 4 months after the first visit, and mST has persisted on all of these except Farm J (data not shown) which was a batch farm in which the replacement batch of pigs carried serovars other than mST, although this was still found in the environment around the pig houses as well as in the range area of two free-range broiler flocks on the same premises. Spread from pigs into other species is a major concern, especially for chicken breeding and laying flocks which are likely to be slaughtered if such strains are found. To date, a small number of conventionally-housed chicken breeding flocks, free-range laying flocks and free-range broiler flocks in GB have been found with mST and the organism has also been reported from numerous cattle herds (VLA, 2010). All of the poultry and cattle herds with mST that have been visited by the project team have had pigs on the same or adjacent holdings. Pig-related serovars of Salmonella have also sometimes been found in wild bird faeces on such farms. Unlike the situation in pigs, mST in cattle herds appears to reduce rapidly, especially in closed herds when ST vaccine has been used. There are however indications that procedures such as moving outdoor herds to new land, acidification of feed and use of liquid feed may be associated with a reduction in the prevalence of infection in positive herds. Further studies will aim to follow the course of infection over a longer time period and will attempt to link the characteristics of the farm infections with management practices and molecular genetic profiles of the strains.

Conclusions

Monophasic Salmonella Typhimurium in pig populations appears to be a significant potential new public health threat and a source of infection for other species. Information on effective means of control is urgently required.

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Table 1: Breeding Herds – pooled faeces / environmental samples – no.mST(no. other serovars)/no. samples taken [%mST](% other serovars.)

	A°	B°	C°	D¹	E¹	F¹	Total
maiden gilts	2(4)^V/8[25](50)	0(0)/4[0](0)	33(10)^D(4)^V(2)^V(2)^R(2)/46[71.7](21.7)	1(4)^D/12[8.3](33.3)	0(0)/1[0](0)	2(0)/2[100](0)	38(14)/65[58.5](21.5)
in pig gilts	0(2)^V/2[0](100)	1(1)^V/14[7.1](7.1)	-	5(4)^D/14[35.7](28.6)	0(0)/1[0](0)	4(0)/4[100](0)	10(5)/33[30.3](15.2)
service area	0(6)^V/12[0](50)	0(1)^/5[0](20)	-	0(12)^D/14[0](85.7)	2(0)/8[25](0)	-	2(13)/27[7.4](48.1)
dry sows	0(22)^V(20)^V(2)/22[0](100)	1(3)^d,V/17[5.9](17.6)	-	3(30)^D/44[6.8](68.2)	0(0)/4[0](0)	8(0)/10[80](0)	12(33)/75[16](44)
farrowing sows	5(13)^V(2)^V(11)/21[23.8](61.9)	0(2)^/64[0](3.1)	-	0(5)^D/18[0](27.8)	0(0)/21[0](0)	5(0)/10[50](0)	5(7)^/113[4.4](6.2)
weaners	-	3(0)/28[10.7](0)	-	0(7)^D/37[0](18.9)	0(0)/8[0](0)	3(0)/3[100](0)	6(7)/76[7.9](9.2)
growers	-	-	-	19(0)/49[38.8](0)	18(1)^T/43[41.9](2.3)	4(0)/4[100](0)	41(1)/96[42.7](1)
finishers	-	-	-	7(5)^D/24[29.2](20.8)	-	9(0)/9[100](0)	16(5)/33[48.5](15.2)
wild birds	7(20)^V/43[16.3](46.5)	0(0)/17[0](0)	17(0)/22[77.3](0)	1(3)^D/9[11.1](33.3)	0(0)/4[0](0)	1(0)/2[50](0)	26(23)/97[26.8](23.7)
rodents	-	-	-	3(1)^D/6[50](16.7)	1(0)/6[16.7](0)	1(0)/1[100](0)	5(1)/13[38.5](7.7)
transport	-	0(0)/3[0](0)	2(0)/4[50](0)	-	-	10(0)/10[100](0)	12(2)/19[63.2](10.5)
pooled water	-	0(0)/4[0](0)	-	3(5)^D/8[37.5](62.5)	0(0)/8[0](0)	1(0)/1[100](0)	4(5)/21[19](23.8)
misc env.	-	1(1)^/41[2.4](2.4)	5(3)^D(2)^R/15[33.3](20)	-	0(2)^/8[0]0(25)	4(0)/9[44.4](0)	10(6)/73[13.7](8.2)
						Overall total:	187(122)/699[26.8](17.5)

General key: mST = monophasic Salmonella Typhimurium; Salm. = Salmonella; O outdoor herd; I indoor herd; env. = environmental sample

Serovar key: T S.Typhimurium; d S.Derby; L S.London; V S.Virchow; r S.Reading; m S.Bovismorbificans

Table 2: Breeding Herds – individual faeces samples – no.mST(no. other serovars)/no. samples taken [%mST](% other serovars)

	A°*	B°	C°	D¹	E¹	F¹	Total/Mean
maiden gilts prev.	4(0)/19[21.1]	4(1)^N/60[6.7](1.7)	167(0)/364[45.9](0)	ND	ND	0(0)/4[0](0)	171(1)/424[40.3](0.2)
mean cfu score (range)	1.75(1-2)	1.2(1-4)	1.42(1-4)	ND	ND	0	1.09(1-4)
in pig gilts prev.	22/22(100)	3(0)/60[5](0)	-	ND	ND	3(0)/11[27](0)	6(0)/71[8.5](0)
mean cfu score (range)	18(3-6)	1.3(1-2)	-	ND	ND	1.0(1)	6.77(1-6)
service area prev.	51/59[-](86.4)	1(1)^J/60[1.7](1.7)	-	0(7)^P/50[0](14)	0(0)/9[0](0)	-	1(8)/119[0.8](6.7)
mean cfu score (range)	3(1-6)	1.0(2)	-	1.4(1-2)	0	-	1.35(1-6)
dry sows prev.	59)/59(100)	0(1)^P/60[0](1.7)	-	0(15)^P/60[0](25)	ND	2(0)/38[5.3](0)	2(16)/158[1.3](10.1)
mean cfu score (range)	4.5(3-4)	1.0(1)	-	1.8(1-4)	ND	1.0(1)	2.08(1-4)
farrowing sows prev.	51/80(63.8)	0(0)/60[0]0(0)	-	0(1)^P/60[0](1.7)	0(0)/19[0](0)	1(0)/6[16.7](0)	1(1)/145[0.7](0.7)
mean cfu score (range)	1.4(1-3)	0	-	NA	0	1.0(1)	0.6(1-3)
weaners prev.	-	4(0)/60[6.7](0)	-	0(5)^P/60[0](8.3)	1(0)/50[2](0)	30(0)/30[100](0)	35(5)/200[17.5](2.5)
mean cfu score (range)	-	1.25(1-3)	-	1.8(1-4)	1.0(1)	2.3(1-4)	1.59(1-4)
growers prev.	-	-	-	14(0)/60(23.3)(0)	7(0)/101[7](0)	9(0)/9[100](0)	30(0)/170[17.6](0)
mean cfu score (range)	-	-	-	1.2(1-3)	2.0(1-5)	1.1(1-2)	1.43(1-5)
finishers prev.	-	-	-	5(0)/60[8.3](0)	see growers	41(0)/60[68.3](0)	46(0)/120[38.3](0)
mean cfu score (range)	-	-	-	1.0(1)	-	1.8(1-3)	1.4(1-3)

* not included in prevalence totals as serotyping not done on all isolates

292(31)/1407[20.8](2.2)

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Differences in risk factors for Salmonella serotypes in breeding pigs in Portugal

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Abstract

The EU Regulation No 2160/2003 imposes a reduction of the prevalence of Salmonella in food producing animals, like pigs. The Member States Salmonella serotypes prevalence varies. So could also vary the risk factors association with different Salmonella serotypes. The aim of this study is to assess if these differences are present in the Portuguese pig production system. The data used in the study refers to the baseline survey for the prevalence of Salmonella in breeding pigs in Portugal. A total of 1670 pen fecal samples, from 167 herds, were tested. Of these 170 samples were positive to Salmonella. The serotypes found were grouped under two groups for the purpose of this study, as follows: 27% S. Typhimurium or serotype 4,5,12:i:-, 73% other serotypes. Along the samples collection a questionnaire about the herd management and potential risk factors was applied. As data follows a hierarchical structure (pen samples – first level - nested in herds – second level) a multinomial multilevel analysis of the dataset was carried out using generalized linear mixed models (GLMM) with Markov chain Monte Carlo methods. Three categories for the outcome variable were specified: i) no Salmonella, ii) serotype Typhimurium or serotype 4,5,12:i:-, iii) other serotypes. Comparing to "no Salmonella" as reference the significant associations (p<0.05) found for "serotypes Typhimurium or serotype 4,5,12:i:-" were: mixed age in the pen, herds with 203 or more breeding pigs, pen samples with more than 10 animals/pen. For the "other serotypes": control of rodents, region of the country, semen from other sources than insemination centers, maternity pens versus mating pens, and feed from external or mixed source. A control plan design to reduce the prevalence of Salmonella should take these results in consideration to improve effectiveness.

Introduction

Salmonella is one of the major causes of food-borne disease in the European Union (EU) in the past years (EFSA, 2010). With the aim to control this agent the EU approved legislation (EU Regulation No 2160/2003) that imposed a reduction on the prevalence of this agent in food production animals, like pigs. To set the target of this reduction for each country it was decided to carry on baseline surveys in the EU to estimate the prevalence of Salmonella sp. in some food production animals. In pigs the baseline study was done at abattoir level (collection of lymph nodes of pigs slaughtered) and at herd level (collection of pen fecal samples of breeding pigs). These studies show that the prevalence of Salmonella positive holdings with breeding pigs in the European Union was 31.8% (28.7% for breeding holding and 33.3% for production holdings). Also the results showed that the prevalence of different serotypes varies between countries. For instance in Portugal 9.1% of the breeding holdings were positive to Salmonella Typhimurium and 33.3% were positive to other serotypes than Typhimurium and Derby, while in Ireland these numbers were 17.5% of prevalence for the both cases (EFSA, 2009). So we have different countries with different profiles in terms of serotype prevalence. This should be taken in consideration in control programs at herd level as they could improve the effectiveness of these programs because we might have differences in risk factors for different serotypes. Along the sample collection it was also collected information regarding management practices and potential risk factors. Some of the known risk factors in the literature are linked to: 1) biosecurity measures, 2) herd management practices, 3) feeding practices, 4) health disorders among others (Fosse et al., 2009). But all these known risk factors did not take in consideration possible differences between serotypes. We wonder if there are differences between risk factors for different serotypes or groups of serotypes. The aim of the study was to search for potential risk factors for the shedding among two different groups of serotypes of Salmonella sp. using pen fecal samples from breeding pig farms representative of the Portuguese reality.

Material and Methods

The sampling frame, the diagnostic testing methods, the sample collection procedures, and the timelines of this cross sectional study were specified in the Commission Decision 2008/55/EC. The target population was holdings constituting at least 80% of the breeding pig population in the Member State. The target population was 4522 herds with a total of 204 584 breeding pigs and 1 827 533 pigs in total (known population in 2007). These herds were divided by Regions. In each region herds with 50 or more breeding pigs, breeding holdings and production holdings were identified. The sample was calculated using expected prevalence of 50%, desired confidence level of 95%, accuracy of 7.5% and then applied a finite population correction factor, with an increase in 10% for each group. The sample size was formed by 174 swine herds. The choice of herds to sample was random and proportional to the distribution of herds along the regions of the country. The samples were collected between November 2008 and January 2009 by the herd veterinary assistant. The samples were sent to laboratory for detection of Salmonella (using method described by Annex D of ISO 6579). The Salmonella strains isolated from positive samples were serotyped by the national Reference laboratory for Salmonella according to Kaulfmann-White scheme Along the collection of the sample a questionnaire was applied to collect information about the herd management and potential risk factors. The variables collected concern pen and herd data, like for example: type of housing, number of animals that contributed to the sample, if it was detected diarrhea in the last three months, production phase, foor type, region of the country, number of breeding pigs, biosecurity measures among others.

Before statistical treatment some variables were recoded into new variables with lesser categories to have a reasonable sample size in each category and other variables were merged when biological arguments allowed this procedure. The outcome variable is the result to the presence of Salmonella in each sample, and was classified in three categories: i) no Salmonella, ii) serotype Typhimurium or serotype 4,5,12:i:-, and iii) other serotype. Given the similar characteristics between serotype Typhimurium and serotype 4,5,12:i- one group was created; the remaining serotypes were merged together because we could not analyze each serotype individually given the low number of cases per serotype. After this treatment we found that, among the 170 positive pen fecal samples, 27% were positive to S. Typhimurium or serotype 4,5,12:i:-, and 73% were positive to the remaining serotypes. As the data follows a multilevel structure, pen fecal samples (first level) nested in swine herds (second level) and the data were analyzed using a generalized linear mixed model (GLMM). We used a Monte Carlo Markov Chain (MCMC) method applied to GLMM described in the package MCMCglmm (Hadfield, 2010) of R free software (R Development Core Team, 2010). The outcome variable follows a categorical distribution with a logit link function. The regression slopes of fixed effects and the random effects were assessed for each separated category in the outcome variable. The final adjusted multivariable models were manually built using a backward and forward elimination process. The results then were converted to odds ratio (OR) and the 95% OR credible interval (OR CI) were calculated. The first 5000 samples were discarded as the burn-in period and the following 500 000 samples were used for posterior inference, with a thin interval of 10. The convergence was assessed by visual inspection of time series plots. Priors for Bayesian multinomial multilevel regression were expressed for fixed effects as a multivariate normal distribution with zero mean vector and a diagonal variance matrix with large variances (1e+10), for residuals the priors matrix were constraint to one for variance and 0.5 to the covariance as is recommended in bibliography for a categorical outcome (Hadfield, 2010). For random effects the prior was 0.5 to all the (co)variance matrix. The fit of the model was assessed by calculating the deviance information criterion (DIC) (Hadfield, 2010).

Results

A total number of 1670 samples were tested, belonging to 167 herds. Out of these samples 170 were positive to Salmonella detection, which belongs to 76 herds. Among the positive samples 23% were Salmonella Typhimurium positive followed by Salmonella Rissen (19%). The final results of the multilevel multivariable model are shown in Table 1 for the different outcomes. The coefficients and the corresponding OR are adjusted for all variables in the model. The DIC of the final model was 950.

Table 1: Final multinomial multilevel model, with coefficient, standard deviation (SD), OR and 95% credible interval (CI) for outcome serotypes Typhimurium or 4,5,12:i- and other serotypes.

Variable	Typhimurium or 4,5,12:i-				Other serotypes			
	Coefficient	SD	OR	95% OR CI	Coefficient	SD	OR	95% OR CI
Region of the herd								
Alentejo	Reference	-		.	Reference	-		.
Center	-1.2	1.6	0.3	0.01-6.5	1.6	0.7	4.9	1.2-22.6
Lisbon and Tagus Valley	-0.4	1.2	0.7	0.06-7.8	1.0	0.6	2.8	0.8-10.7
North	0.4	1.9	1.5	0.03-66.7	2.8	0.9	16.4	3.1-104.6
Number of breeding pigs								
<203	Reference	-		.	Reference	-		.
≥203	2.3	1.0	10.0	1.6-109.9	0.5	0.4	1.7	0.8-3.9
Source of semen								
Center for artificial insemination - CAI	Reference	-		-	Reference	-		-
Own boar+CAI	0.4	0.9	1.4	0.2-8.4	1.2	0.5	3.3	1.3-8.4
Boar from another herd	4.2	1.8	69.4	3.0-3904	1.7	0.9	5.5	1.0-33.1
Control of rodents								
No	Reference	-		.	Reference	-		.
Yes	-2.7	1.9	0.06	0.001-1.7	-2.2	0.7	0.1	0.02-0.4
Number of animals/pen								
=10	Reference	-		-	Reference	-		-
>10	1.7	0.8	5.4	1.1-30.6	0.7	0.4	2.0	0.9-4.6
Age of the breeding sows								
Only gilts or gilts and others	Reference	-		.	Reference	-		.
Without gilts	-2.0	0.8	0.1	0.03-0.57	0.2	0.3	1.3	0.7-2.5
Breeding sector room								
Mating	Reference	-		-	Reference	-		-
Gestating	0.1	0.6	1.1	0.4-3.5	-0.2	0.4	0.8	0.4-1.6
Mixture of animals of different sector	-0.08	1.8	0.9	0.02-31.8	1.0	0.8	2.6	0.5-11.7
Maternity	-1.2	0.7	0.3	0.1-1.2	-1.1	0.4	0.3	0.2-0.8
Replacement breeders	-1.2	1.2	0.3	0.02-2.8	0.2	0.7	1.2	0.3-4.8
Source of feed								
Exclusively own	Reference	-		-	Reference	-		-
Not exclusively own	0.8	1.3	2.2	0.2-33.1	2.2	0.7	9.3	2.5-44.7

Discussion

It can be seen from the analysis of Table 1 that there are different risk profiles for the two groups of Salmonella created. Concerning the category "other serotype" the risk associations were the following: region of the herd (samples from herds in the North and Center Region have higher odds of being positive than samples from herds in the Alentejo Region, probably because herds in the Center and North regions are more closed together); purchase semen from another herd or to use semen from own boar are risk factors when compared to the purchase of semen from insemination centers (where the quality and safety of semen is higher), this association has not been reported in literature, probably because that in the majority of the countries the semen comes from insemination centers; and pens where the pigs feed are not exclusively home produced (linked to exotic serotypes like the ones that are isolated in commercial feed), similar association was also found in other study ((Benschop et al., 2008). The protective associations found for "other serotype" were: control of rodents (the role of rodents in the transmission of this agent was also highlighted in others studies) (Skov et al., 2008) and maternity pens compared to mating pens (this could be justified by the hormonal changes in the sow at mating) (Nollet et al., 2005). For category "Typhimurium or 4,5,12:i:-" the risk associations found were linked to the size of the herd: 203 or more breeding pigs in the herd (similar association was found for Salmonella sp. in finishers) (Poljak et al., 2008) and the number of animals/pen (the greater the number of animals in the pen the easier is the transmission of infection between pigs). A protective association, pens without gilts, was found (older pigs with more resistance to infection). In this

category "Typhimurium or 4,5,12:i:-" the purchase of semen from another herd was also considered a risk factor but the wide credible interval (probably because of the high odds ratio and also the relatively small number of positive pen fecal samples in this variable category) indicates that this association should be a matter of further studies. Furthermore when this variable was removed from the analysis the other risk associations found remained statistically significant.

Conclusion

The majority of risk associations found were different between the groups of serotypes and as we had 13.8% herds with at least one sample positive to serotype Typhimurium or serotype 4,5,12:i:-, and the rest of the 31.7% herds positive to other serotypes, these should be taken in consideration when implementing a control program to Salmonella sp. as we have different herd risk profiles. To achieve a reduction on the prevalence, the measures of future control program should be cost-effective and adapted to country characteristics and serotypes. In this context this study gave valuable information that should be incorporated in future control plans for this agent in breeding pigs in Portugal.

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Occurrence and epidemiology of Salmonella enterica in two slaughterhouses and cutting plants in Spain

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Abstract

This study aimed at investigating the Salmonella occurrence in two Spanish pig slaughterhouses analysing the lairage, slaughter line, carcasses and the cutting plants thereof. The results obtained showed a decrease of Salmonella prevalence from the lairage to the cutting plant. The high levels of contamination in holding pens and several points of the slaughter line point them out as the major risk sources of Salmonella spreading within the slaughterhouse. The main Salmonella serotypes previously reported in Spanish finishing pig farms were found at both slaughterhouses. S. Typhimurium isolates recovered from different points of the slaughter chain were typed by MLVA.

Introduction

Salmonella is one of the major concern food-borne pathogens worldwide. Although eggs or poultry products are the main sources of contamination, Salmonella is also often associated with the consumption of pork and products thereof [1]. The high Salmonella prevalence in Spanish swine production [2] together with the forthcoming Spanish swine Salmonella Control Programme entail the establishment of suitable control measures. Finishing farms as well as slaughterhouses should be the main targets where these measures should be implemented since several studies have demonstrated the importance of the slaughter process in the spreading of Salmonella [3, 4]. There is no current data available regarding Salmonella prevalence and serovars in carcasses and slaughterhouses in Spain. The objective of this study, carried out in two Spanish slaughterhouses, was to improve the knowledge of the prevalence and serotypes in holding pens, different points of the slaughter line, cutting plants and also in carcasses before and after chilling process in order to find critical control points at slaughter level.

Materials and Methods

Two slaughterhouses, identified as slaughterhouse A and B which processed 400 and 375 pigs per hour respectively were investigated at the beginning, middle and end of the work week. Holding pens, the slaughter line and randomly selected carcasses at the end of the slaughter line (before chilling), were sampled on Monday, Wednesday and Friday while the cutting plant of each slaughterhouse and the same carcasses after chilling and cooling procedures were sampled the following working day (Tuesday, Thursday and the following Monday respectively). Holding pens were sampled in four rounds: (I) before the entry of pigs, (II) at half of the working day, (III) at the end of the working day and (IV) after cleaning procedures. Several points of the slaughter line and cutting plant (Table 1) were sampled throughout the working day.

Sample collection and processing were carried out as previously described [5]. Samples were processed by following the ISO standard methodology 6579:2002/Amd1:2007. A single isolate from each positive sample was serotyped by slide agglutination according to Kauffmann-White scheme using commercial antisera. S. Typhimurium and S. 4,[5],12:i:- isolates were further typed by MLVA.

Results

Holding pens. Each sampling day, eleven holding pens were evaluated in both slaughterhouses. The overall results show that 26 out of 66 (39.4%) of the holding pen floor surfaces were already contaminated by Salmonella before the staying of the pigs. Contamination was higher during the working day, with 98.5% and 80.3% of Salmonella positive samples among those collected in the holding pens at half and end of the working day respectively. After the cleaning procedures, Salmonella was recovered from the floor surface in 42 out of 66 holding pens (63.6%). It is noteworthy that no

Salmonella contamination was recorded in the sampling carried out on Monday in the slaughterhouse A while the eleven holding pens evaluated in the same visit to slaughterhouse B were contaminated by Salmonella. Moreover, in those sampling days in which the number of positive pens was high at the start of the working day, a higher number of positive pens were found during the other three sampling rounds.

Slaughter line. Ten different points within the slaughter line were evaluated (Table 1), most of them at both slaughterhouses. In total, 66 samples were collected in each of the three visits to slaughterhouse A. In slaughterhouse B, 46 samples were collected from the slaughter line in two of the sampling days while only 38 were recovered during the first visit to this abattoir. Mean prevalence of Salmonella positive samples was 33% (66 positive samples out of 198) in slaughterhouse A and 47.7% (62 positive samples out of 130) in slaughterhouse B. No statistically significant differences were found among different visits to slaughterhouse A (χ^2= 2.9, p= 0.23). However, the prevalence of Salmonella contamination varied among different visits in slaughterhouse B (χ^2= 6.3, p= 0.04) where it is remarkable that 60.9% of the samples collected had a positive result in the sampling performed at the middle of the work week.

Carcasses. Salmonella was recovered from 179 out of 446 carcasses evaluated before chilling (40.4%). No statistical differences were found in the prevalence of contaminated carcasses between both slaughterhouses although there were significant differences among different visits to the same slaughterhouse. In a similar way, 445 carcasses were evaluated after chilling and 48 gave a positive result (10.8%). The prevalence of Salmonella contamination was compared before and after chilling in 323 carcasses that were sampled at both stages. In those carcasses evaluated twice, Salmonella contamination was reduced from 45.8% to 10.8%, which means a significant decrease in Salmonella contamination after chilling (χ^2= 95.64, p< 0.001).

Cutting plant: Seventeen points, most of them in both slaughterhouses, were analysed. They were classified in three groups including machinery (surface and organic matter from saws and derinding machines), operators (hands and implements) and surfaces (conveyor belt at different processing points) (Table 2). Salmonella was identified in 17 out of 174 (9.8%) samples collected in slaughterhouse A and 30 out of 172 (17.4%) collected in slaughterhouse B. Contamination was more frequent in machinery (7 positive samples out of 54) (13%) followed by surfaces (4 positive samples out of 60) (6.7%) and operators (3 positive samples out of 90) (3.3%) in slaughterhouse A. On the other hand, Salmonella was detected in 20 out of 96 surface samples (20.8%) as well as in 3 out of 23 machinery samples (13%) and 5 out of 36 operator samples (13.9%) in slaughterhouse B.

Nine different serotypes were found; S. Rissen was the predominant serotype detected in 36.3% of the positive samples followed by S. 4,[5],12:i:- (17.6%), S. Typhimurium (14.8%) and S. Derby (14.5%). Main Salmonella serotypes identified in holding pens, slaughter line, carcasses and cutting plants are shown in Table 2.

A total of 8 and 6 different MLVA profiles were identified in the second and third visits to slaughterhouse A. Isolates with the same MLVA profile were found in several points of the slaughter chain and also one of the patterns was shared by isolates from both visits.

Discussion

Several studies have indicated the role that the slaughter process can play in the spreading and dissemination of Salmonella [3, 4, 5]. The proposal of this study was to identify critical points surrounding the slaughter process by analysing Salmonella prevalence from the lairage to the cutting plant at the beginning, middle and end of the work week. The evaluation of the lairage showed high levels of Salmonella contamination in holding pens. This contamination is consequence of the faecal shedding of Salmonella by the pigs housed there and it increased during the working day. What is more, we were able to demonstrate that the routinely cleaning procedures used in both abattoirs were unsatisfactory. These results are in agreement with previous studies [6] and confirm that the lairage can constitute an important source of new infections particularly when cleaning and disinfection procedures are not carried out properly. An improved cleaning protocol including the use of disinfectants and a proper instruction of the personnel are the main tools to decrease Salmonella contamination at this stage.

Several points of the slaughter line were included in the study to find out which ones were the most relevant in the dissemination of Salmonella. The prevalence of Salmonella contamination was high in those points where actions were performed by hands and implements of operators: the evisceration and the kidney, lard, fat and tonsil removal points. At some of these points, a tendency towards an increase in contamination throughout the working day was observed, probably related to an accumulation of Salmonella in implements. In slaughterhouse B, some samples collected after the sterilization gave a positive result showing the inefficiency of the cleaning and disinfection protocols carried out.

Taking into account the high prevalence of Salmonella infection in Spanish slaughter pigs [2], a relatively high prevalence

of Salmonella contamination in carcasses was expected. Mean prevalence was similar between both slaughterhouses. Nevertheless, the fact that it varied significantly among different visits to the same slaughterhouse demonstrates that carcass prevalence can be affected by the pig status and also by several internal factors of the slaughtering process. Moreover, a reduction in the percentage of Salmonella contaminated carcasses was observed in both slaughterhouses after their chilling and cooling. According to this, it seems that these chilling and cooling procedures should be done before carcass processing in order to limit Salmonella contamination in the latest steps of the pork production chain. What else, this result reveals the importance of taking into consideration the time of the sampling when comparing contamination results among different slaughterhouses or countries.

Also, different points of the cutting plant were analysed including machinery, operators (hands and implements) and surfaces, particularly conveyor belts. Salmonella was also isolated from several of these samples although, in general, a lower frequency of contamination in comparison with previous facilities of the same slaughterhouse was observed.

Main serotypes found during the present study were similar to those previously reported as the most frequent in Spanish fattening pigs [2] S. Rissen, a serotype particularly related to pigs from the Iberian peninsula, was the most prevalent one in all investigated stages followed by S. 4,[5],12:i:-, S. Typhimurium and S. Derby. It is noteworthy that S. Bredeney was the predominant serotypes found in one of the sampling days in the slaughterhouse B showing that Salmonella population within the slaughterhouse varies. MLVA profiles showed the spreading of particular types from the lairage to the cutting plant. The variability of serotypes and MLVA profiles within a sampling day and among different visits indicates that Salmonella population at the slaughterhouse is in constant change.

Conclusion

The present study aimed at investigating Salmonella contamination during the slaughter process in two Spanish abattoirs. The high contamination detected in lairages and slaughter lines implies that they constitute risk points and actions to reduce the contamination at both stages should be taken. Carcass contamination was frequent at the end of the slaughter line but it decreases after chilling and cooling procedures. Finally the main serovars found in Spanish farms are also found in the abattoir indicating a Salmonella flow from farm to the slaughterhouse and its spreading in the slaughter process.

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Table 1. Salmonella contamination at different points of the slaughter line and cutting plant of two commercial Spanish pig slaughterhouses.

	Slaughterhouse A		Slaughterhouse B	
	No positive samples	% positive samples	No positive samples	% positive samples
Slaughter line				
De-hairing	-	-	1	8.3
Bung Dropper	4[a]-0[b]	10[a]-0[b]	4[a]-3[b]	33.3[a]-25[b]
Evisceration	15	33.3	7	58.3
Kidney extraction	14	46.7	7	58.3
Lard removal	17	37.8	14	82.7
Trimming	16	53.3	6	50
Splitting saw				
Saw	1	11.1	-	-
Water	-	-	0	0
Organic matter	2	22.2	7	87.5
Mammals removal	-	-	4	50
Tonsils removal	-	-	5	62.5
TOTAL	66	33.3	58	49.1
Cutting plant				
Machinery				
Organic Matter	7	38.9	2	11.1
Surface	0	0	1	1.7
Operators	4	4.4	5	13.9
Working surface	4	6.7	96	20.8
TOTAL	17	8.3	30	17.4

a Sample collected before sterilization
b Sample collected after sterilization

Table 2. Main Salmonella serotypes recovered in different facilities of two commercial Spanish pig slaughterhouses. Number and percentage of isolates in each evaluated level are reported.

Serotype[1]	Holding pens	Slaughter line	Carcass non-chilled	Carcass chilled	Cutting plant	TOTAL
Bredeney	24 (14.6%)	17 (10.7%)	23 (15.0%)	6 (15.4%)	3 (10%)	73 (13.3%)
Rissen	55 (33.5%)	58 (36.5%)	61 (39.7%)	15 (38.5%)	9 (30%)	198 (36.3%)
Derby	43 (26.2%)	13 (8.2%)	19 (12.3%)	2 (5.1%)	2 (6.7%)	79 (14.5%)
4,[5],12:i:-	16 (9.8%)	48 (30.2%)	19 (12.3%)	5 (12.8%)	8 (26.7%)	96 (17.6%)
Typhimurium	22 (13.4%)	13 (8.2%)	28 (18.2%)	11 (28.2%)	7 (23.3%)	81 (14.8%)
TOTAL	164	159	154	39	30	546

[1]S. Mkamba, S. Clackamas, S. Anatum and S. Agama were the other serotypes found in less than 2% of the positive samples.

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EU-wide baseline survey on the prevalence of Salmonella in holdings with breeding pigs, 2008 - prevalence and factors associated with Salmonella positivity

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Abstract

In order to reduce the incidence of human salmonellosis, European Union (EU) legislation foresees the setting of Salmonella reduction targets for food producing-animals including breeding pigs. To set such a target, an EU-wide baseline survey was conducted in 2008 to determine the prevalence and diversity of Salmonella in holdings with breeding pigs across Member States (MSs). A total of 1,609 breeding holdings and 3,508 production holdings from 24 EU MSs, plus Norway and Switzerland, were included in the survey. In each randomly selected holding, one fresh voided pooled faecal sample was collected from every 10 randomly chosen pens of breeding pigs. All samples were tested for presence of Salmonella and the isolates were serotyped. The EU prevalence of Salmonella-positive holdings with breeding pigs was 31.8%, all but one of the 24 participating MSs detected Salmonella in at least one holding. The EU prevalence of Salmonella-positive breeding holdings was 28.7%, varying from 0% to 64.0% among MSs. The EU prevalence of Salmonella-positive production holdings was 33.3%, while the MSs' prevalence varied from 0% to 55.7%. Salmonella Derby and Salmonella Typhimurium were the most frequently isolated serovars. Salmonella Typhimurium monophasic isolates 1,4,[5],12:i:- were also found in several MSs. Breeding pigs may be an important source of dissemination of Salmonella throughout the pig-production chain. In addition to supporting the setting of the EU Salmonella reduction targets and assessing the impact of Salmonella transmission originating from holdings with breeding pigs, these results may also be used in the future to evaluate the impact of control programmes.

Introduction

In 2008 an EU-wide baseline survey was carried out to estimate the prevalence of Salmonella spp. (Salmonella) in holdings with breeding pigs. The aim of the survey was to obtain comparable data for all MSs through harmonised sampling schemes. The specific objectives of this survey were: 1) to estimate the prevalence of Salmonella-positive holdings with breeding pigs at EU level and for each MS individually; 2) to investigate the effects of factors potentially associated with the occurrence of Salmonella in holdings with breeding pigs; and 3) to investigate the Salmonella serovar distribution and determine the most frequently occurring serovars in breeding pigs across the EU. According to Regulation (EC) No 2160/2003 on the control of Salmonella and other zoonotic agents (EC, 2003), which aims to reduce the incidence of food-borne diseases in the EU, results of such a survey will inform the setting of the Community target for the reduction of the prevalence of the infection in breeding herds of pigs. A EFSA's scientific report part A on the analysis of this baseline survey published in December 2009 (EFSA, 2009) included the prevalence estimates at EU level and for each MS as well as the analyses of the most frequently identified Salmonella serovars in holdings with breeding pigs across the EU MSs, Norway and Switzerland. A Part B report on the analyses of factors associated with Salmonella–positive pens in holdings with breeding pigs is expected to be published by EFSA in July 2011. Main results from Report A are presented and discussed in this paper.

Material and Methods

The survey took place in the EU between January and December 2008 and targeted a population of holdings (preferentially housing at least 50 breeding pigs) constituting at least 80% of the breeding pig population in a MS. In each MS, holdings were randomly selected from the breeding holdings and production holdings group. A total of 1,609 breeding holdings and 3,508 production holdings from 24 EU MSs, plus Norway and Switzerland, were included in the survey. In each selected holding, samples were collected from 10 selected pens of breeding pigs over six months of age. One

pooled faecal sample was collected from each of the 10 selected pens. Samples were tested by the National Reference Laboratory (or an authorised laboratory) using the latest ISO 6579 Annex D method (ISO, 2007). At least one isolate from each positive sample was to be typed according to the Kaufmann-White Scheme. Further details on survey design are described in the Commission decision 2008/55/EC (EC, 2008).

Data on breeding holdings and production holdings were analysed separately, and the following four outcomes were considered: a)   positivity for Salmonella; b) positivity for S. Typhimurium; c) positivity for S. Derby; and d) positivity for serovars other than S. Typhimurium and/or S. Derby. A holding was considered positive if at least one of the 10 pooled faeces samples tested positive, and negative otherwise. Prevalence was estimated for each MS as the proportion of test positive breeding/production holdings out of the total number of holdings tested. At EU level, the prevalence was estimated using only the data from pig holdings with at least 50 breeding pigs. In the estimation of the EU prevalence, MSs were considered as strata and the proportion of sampled breeding/production holdings, i.e. the sampling fraction, was not constant across MSs. In order to account for disproportionate sampling among MSs, the EU level prevalence was estimated as a weighted mean of MSs' prevalences. To this end, each MS' prevalence was weighted by the reciprocal of the sampling fraction for breeding/production holdings. The EU prevalence was estimated using SAS 9.2, PROC SURVEYREG. The estimated prevalences at MS level and EU level do not account for imperfect sensitivity or specificity of the test. A finite population correction was used to calculate a 95% Confidence Interval (CI) for prevalence estimates at MS and EU level.

Data on holding- and at pen-level factors potentially associated with Salmonella-positivity of pens in breeding and production holdings were collected during the survey using a mandatory questionnaire at the time of sampling in the holdings. The following factors were considered: date of sampling, type of breeding/production holdings, holding size, gilt/boar replacement policy, and delay between the sampling date and testing date at the laboratory, number of pigs per pen, age category of the pigs, sex of the pigs, production stage, indoor/outdoor production, individual housing, floor type, all in/all out and cleaned production, origin of the feed, type of diet, and feed/water supplement. Two factors potentially associated with the sensitivity of the sampling and testing method were also considered (type of sample and delay between sampling and testing). Results of the analysis of the factors potentially associated with pen positivity will be presented in a Report part B to be published by EFSA in July 2011.

Results

In this survey, the EU prevalence of Salmonella-positive holdings with breeding pigs (all holdings, including both breeding and production holdings) with at least 50 breeding pigs was 31.8% (95% CI: 30.0; 33.7). The EU prevalence of holdings with breeding pigs positive to the other Salmonella serovars or groups of serovars was: 7.0% (95% CI: 5.9; 8.0) for Salmonella Typhimurium; 9.0% (95% CI: 7.9; 10.1) for Salmonella Derby; and 19.8% (95% CI: 18.3; 21.3) for serovars other than S. Typhimurium and/or Derby. One MS (Finland) and the Norway did not detect any Salmonella in their surveyed holdings. Figure 1 illustrates the prevalences of Salmonella-positive breeding holdings (left) and production holdings (right) for each participating country and at EU level (dashed lines).

The association between Salmonella prevalence in breeding and in production holdings is illustrated graphically in Figure 2. The scatter diagram shows that the prevalence of Salmonella-positive production holdings increases as the prevalence of Salmonella-positive breeding holdings increases, indicating that there is a positive correlation. This observation is notably clearer for countries with a prevalence above 5 % for either breeding or production holdings.

In total 1,303 Salmonella isolates originated from 452 Salmonella-positive breeding holdings, while 2,699 Salmonella-positive isolates originated from 950 Salmonella-positive production holdings. The frequency distribution of top five isolated Salmonella serovars in the survey, ranked by the number of positive holdings, is presented in Table 1.
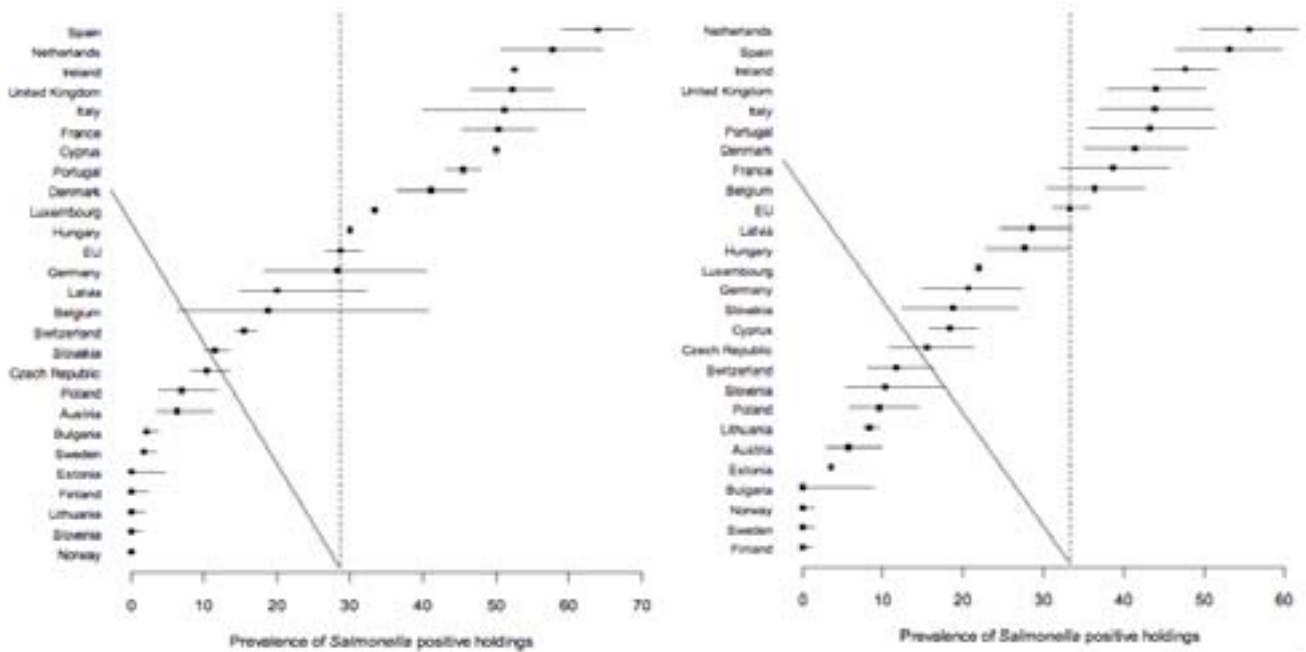
Figure 1   Prevalence of Salmonella-positive breeding holdings (left) and production holdings (right), with 95% CIs (horizontal lines), Salmonella EU baseline survey, 2008
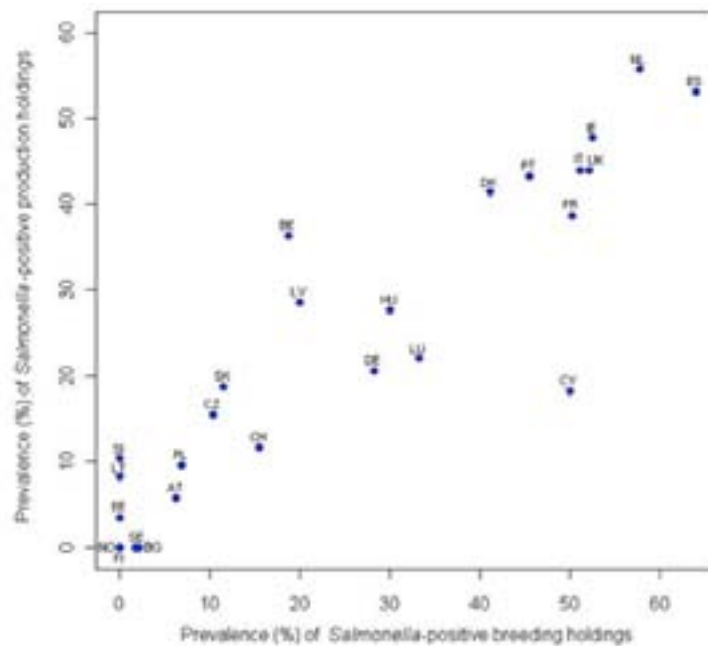
Figure 2   Scatter diagram of the prevalence of Salmonella-positive breeding holdings versus the prevalence of Salmonella-positive production holdings, Salmonella EU baseline survey, 2008

Table 1 Frequency distribution of the top five isolated Salmonella serovars in breeding and production holdings, ranked by positive holdings, Salmonella EU baseline survey, 2008

Salmonella serovars	Breeding holdings with serovars		Countries with serovars	*Salmonella* serovars	Production holdings with serovars		Countries with serovars
	N	%	N		N	%	N
S. Derby	134	29.6%	18	*S.* Derby	271	28.5%	20
S. Typhimurium	115	25.4%	17	*S.* Typhimurium	191	20.1%	16
S. Infantis	35	7.7%	7	*S.* London	90	9.5%	15
S. Rissen	33	7.3%	5	*S.* Infantis	58	6.1%	13
S. London	29	6.4%	8	*S.* Rissen	56	5.9%	6

Discussion

Approximately one third of holdings with breeding pigs were estimated to be infected with Salmonella in the EU and they represent a source of infection for other pigs – either breeding herds lower down the pyramid or directly to slaughter pigs. The variation in Salmonella prevalence among MSs was large and, overall, the findings of the present survey demonstrate the heterogeneity of the situation between the EU MSs. Explanatory factors for this variability should be investigated further as this may be of value to inform decisions on future control measures. Breeding holdings are at a crucial position at the top of the production pyramid and they may be an important source of dissemination of Salmonella infection throughout the whole production chain. Overall, the EU level prevalence of Salmonella-positive holdings seemed not to differ between breeding and production holdings.

The higher serovar diversity in production holdings may be due to the fact that more samples were collected in production holdings, but could also be attributed to the fact that breeding holdings typically breed their own replacement pigs whereas production holdings may buy in replacement breeding stocks from a wide range of breeding holdings, each of which may be infected with different Salmonella serovars. S. Derby was the most frequently isolated serovar at EU level in both breeding and production holdings, followed by S. Typhimurium. These two serovars, which were clearly predominating in holdings with breeding pigs, have been closely associated with pig breeding and production for many years (EFSA, 2006, 2007, 2008). S. Typhimurium-like strains, such as S. 1,4,[5],12:i:-, were reported by several MSs in breeding and production holdings. These strains have been isolated, with increased frequency, over the last 20 years and they have been recently indicated as variants deriving from S. Typhimurium (EFSA, 2010). Monophasic S. Typhimurium strains have been reported from pigs, cattle, poultry and humans. There have been major food-borne outbreaks involving this strain in humans in MSs and many non-European countries (Agasan et al 2002; Mossong et al 2007).

Conclusion

This baseline survey provided comparable estimates of the prevalence of Salmonella-positive holdings with breeding pigs and a description of the distribution of Salmonella serovars, across the EU. Breeding pigs may be an important source of dissemination of Salmonella throughout the pig-production chain. In addition to supporting the setting of the EU Salmonella reduction targets and assessing the impact of Salmonella transmission originating from holdings with breeding pigs, these results may also be used in the future to evaluate the impact of control programmes.

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Occurrence of human enteropathogenic Yersinia spp. in Belgian pigs and contamination of pork carcasses during slaughter

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Abstract

Human pathogenic Yersinia enterocolitica and Y. pseudotuberculosis typically cause enteric infections in humans, mainly young children. Pigs are the main animal reservoir for pathogenic Y. enterocolitica and infection in humans is often acquired by the consumption of contaminated pork. The aim of this work was to determine the contamination of pig carcasses with enteropathogenic Yersinia spp. in Belgium. Therefore, 180 pig carcasses were sampled in 9 different slaughterhouses. From each animal, tonsils, rectal content and carcass swabs were analysed for enteropathogenic Yersinia spp. using direct plating, selective enrichment and cold enrichment. All samples were taken after evisceration, but before chilling. Pathogenic Y. enterocolitica were isolated from the tonsils of 103 pigs (57.2%) and rectal contents of 36 pigs (20.0%). Twenty-eight pigs were positive for pathogenic Y. enterocolitica in both tonsils and rectal content, while 75 and 8 pigs were only Y. enterocolitica positive in tonsils and rectal content, respectively. All isolated Y. enterocolitica strains belonged to bioserotype 4/O:3. Tonsils and rectal content from 4 and 1 pig(s) were positive for Y. pseudotuberculosis, respectively. Regarding carcass samples, 76 (42.2%) pig carcasses were contaminated with enteropathogenic Yersinia spp. Pathogenic Y. enterocolitica were mostly recovered from the mandibular region (59/180), followed by the sternal region (31/180), medial site just before the sacrum (17/180), and pelvic duct (15/180). In conclusion, a high proportion of pigs carry pathogenic Yersinia spp. in their tonsils or intestines during slaughter. Moreover, a considerable number of pig carcasses is positive on one or more of the sampled carcass sites.

Introduction

Y. enterocolitica and Y. pseudotuberculosis are foodborne pathogens which generally cause enteric infections in humans (yersiniosis). Infections occur mainly in young children and usually manifest as acute gastroenteritis (Bottone 1997). Most yersiniosis cases are caused by Y. enterocolitica bioserotype 4/O:3 and 2/O:9. Pigs are regarded as the principal source of pathogenic Y. enterocolitica in the human food chain as they are the only food producing animals that regularly harbor these pathogenic types (Bucher et al. 2008; Fredriksson-Ahomaa et al. 2001). Y. pseudotuberculosis is also recovered from tonsils and intestines of healthy pigs, though to a lesser extent than pathogenic Y. enterocolitica (Laukkanen et al. 2008). The aim of this study was to determine the prevalence of enteropathogenic Yersinia spp. in tonsils and rectal content of Belgian pigs at slaughter. Moreover, carcass samples are taken to obtain qualitative and quantitative data on the contamination of pig carcasses with enteropathogenic Yersinia spp. during normal slaughter activities.

Material and Methods

Tonsils, rectal content and carcass swabs from 180 pigs were collected during 18 sampling visits in 9 different pig slaughterhouses (each slaughterhouse was visited twice). The annual number of slaughtered fattening pigs in these slaughterhouses varied from about 135,000 to 1,250,000. Each sampling visit, 10 animals were sampled (one every 15 minutes), starting from the beginning of slaughter activities. All samples were taken after evisceration, but before chilling. Carcasses were swabbed using cellulose sponges after splitting of the carcass. From each carcass, the following areas were swabbed: (1) pelvic duct, (2) split surface just before the sacrum, (3) sternal region (breast cut and surrounding skin), and (4) mandibular region (jowl). For the latter region, only the medial side of the mandibula was swabbed when the head was split, including submaxillary lymph nodes, but avoiding the area of the tonsils. When the head was intact and the skin (partly) removed, masseter muscles were also swabbed.

All samples were homogenized in peptone-mannitol-bile salts broth (PMB) and analyzed using (i) direct plating, (ii) selective enrichment and (iii) cold enrichment. (i) For direct plating, 500 μl of PMB homogenate was spread plated onto

a CIN plate, in duplicate. For tonsils and intestinal content, an additional CIN agar plate was inoculated with approximately 100 µl PMB homogenate using a spiral plater. (ii) For selective enrichment, 10 and 5 ml of PMB homogenate was transferred into 90 and 45 ml of irgasan-ticarcillin-potassium chlorate (ITC) broth for tonsils and rectal contents, and carcass samples, respectively. After 2 days enrichment at 25°C, a loopful was streaked onto CIN plates. Additionally, 100 µl was streaked onto another CIN agar plate after KOH treatment. (iii) For cold enrichment, the remaining PMB homogenate was incubated at 4°C for 7 and 14 days. After 7 days, the enriched culture was streaked onto a CIN agar plate. After 14 days enrichment, 100 µl was streaked onto a CIN agar plate after KOH treatment. All agar plates were incubated at 30°C for 24h and examined for Yersinia colonies using a stereo microscope with Henry illumination. Suspected colonies were streaked on a general nutrient agar and after 24h at 30°C transferred into urea broth, Kligler Iron Agar (KIA) and Tryptone Soy Broth (TSB).

The pathogenicity of Y. enterocolitica isolates was confirmed using a multiplex PCR with primers targeting the chromosomal virulence genes ail and yst and the plasmid virulence gene virF according to Harnett et al. (1996). Moreover, Y. enterocolitica serotype O:3 and O:9 were identified using primers targeting the rfbC and per gene, respectively (Jacobsen et al. 2005; Weynants et al. 1996). Y. pseudotuberculosis isolates were identified using a single PCR assay targeting the inv-gene according to Nakajima et al. (1992).

Results

Pathogenic Y. enterocolitica were isolated from the tonsils of 103 pigs (57.2%) and rectal content samples of 36 pigs (20.0%) (Table 1). Twenty-eight pigs were positive for pathogenic Y. enterocolitica in both tonsils and rectal content, while 75 and 8 pigs were only Y. enterocolitica positive in tonsils and rectal content, respectively. All isolated Y. enterocolitica strains belonged to bioserotype 4/O:3. Tonsil samples with countable numbers (n=67), were contaminated with a mean of 3.82 ± 1.21 log10 CFU/g and a maximum of 5.73 log10 CFU/g tonsillar tissue. Rectal content samples that were positive by direct plating (n=13) were contaminated with a mean of 2.91 ± 1.44 log10 CFU/g and a maximum of 6.11 log10 CFU/g.

Y. pseudotuberculosis was isolated from the tonsils of four pigs (2.2%). Tonsils from one pig were simultaneously infected with Y. enterocolitica and Y. pseudotuberculosis at concentrations of 4.20 and 4.43 log10 CFU/g tonsillar tissue, respectively. From one pig, Y. pseudotuberculosis was isolated from the rectal content, while solely pathogenic Y. enterocolitica were isolated from its tonsils.

Regarding carcass samples, 76 pig carcasses (42.2%) were contaminated with enteropathogenic Yersinia spp. Thirteen out of 76 positive pig carcasses (17.1%) did not carry pathogenic Yersinia spp. in the tonsils or rectal content. From two animals, the carcass was positive for Y. enterocolitica, while only Y. pseudotuberculosis was isolated from the tonsils. Moreover, in 51 animals, enteropathogenic Yersinia spp. were recovered from tonsils and/or rectal content while no pathogenic yersinias were recovered from the carcass surface. In total, 53 pigs were negative for enteropathogenic Yersinia spp. in the tonsils, rectal content or any of the sampled carcass sites.

Pathogenic Y. enterocolitica were mostly recovered from the mandibular region (59/180), followed by the sternal region (31/180), split surface (17/180), and pelvic duct (15/180). The mandibular region from one pig was simultaneously contaminated with pathogenic Y. enterocolitica and Y. pseudotuberculosis.

Table 1. Isolations of pathogenic Y. enterocolitica using different isolation methods from tonsils, rectal content and carcass swabs (n=180).

Isolation method Sample type	Direct CIN	Selective enrichment		Cold enrichment		Total (%)
		ITC + CIN	ITC + KOH + CIN	PMB + CIN (7d)	PMB + KOH + CIN (14d)	
Tonsils	78	70	79	79	99	103 (57.2%)
Rectal content	13	15	17	19	31	36 (20.0%)
Carcass swabs						
Pelvic duct	6	4	4	6	11	15 (8.3%)
Split surface	3	5	6	2	14	17 (9.4%)
Sternal region	1	3	10	2	26	31 (17.2%)
Mandibular region	17	10	17	9	56	59 (32.8%)

Most Y. enterocolitica and Y. pseudotuberculosis isolates were recovered after 14 days cold enrichment. For tonsil samples, direct plating detected 76% of positive tonsil samples. The sensitivities of direct plating for all other sample types (rectal content and different carcass swabs) varied from only 3 to 40%.

Discussion

A high proportion of pigs at slaughter carry enteropathogenic Yersinia spp. in tonsils and/or intestines, resulting in a high number of contaminated carcasses. Enteropathogenic Yersinia spp. are also isolated from several carcasses that are negative in either tonsils or rectal content, suggesting cross-contamination of carcasses during slaughter.

Contamination of the pelvic duct is most likely to occur during removal of the intestinal tract either directly from intestinal content or cross-contamination from a contaminated knife or bung cutter. Sealing of the rectum with a bag reduces carcass contamination (Laukkanen et al. 2010; Nesbakken et al. 1994); however, it is not a common practice in Belgium as none of the slaughterhouses applied this procedure. Moreover, in 8 pigs, the pelvic duct was positive while there were no pathogenic Y. enterocolitica isolated from the rectal content, which indicates cross-contamination.

The split surface of the carcass probably gets contaminated during splitting of the carcass. In this study, the head was split in 49% of the sampled carcasses. When splitting the head, the splitting machine inevitably also cuts the tonsils and can become contaminated, leading to the transfer of the pathogen to the split surface of the next carcass. However, during sampling it was observed that the splitting machine also makes contact with the tonsils if the head is not split. Eleven carcasses were positive at the split surface when the head was split (n=89). Considering carcasses from which the head was not split (n=91), 6 carcasses were positive at this site. In one slaughterhouse, tonsils were systematically cut out with the plug set and an incision was made in the neck to avoid contact of the splitting machine with the head. In this slaughterhouse, none of the 20 sampled pigs was positive at the split surface.

In 17% of the carcasses, pathogenic Y. enterocolitica were isolated from the sternal region. Four carcasses that were positive at this location were negative in both tonsils and rectum. The sternal region may get contaminated during opening of the thoracic cavity, evisceration, and removal of the plug set or following manipulation of the carcass.

The highest contamination was found in the mandibular region. This contamination might originate from the pig itself (such as tonsils, oral cavity or tongue) or may also be attributed to cross-contamination. Namely, in 12 animals, the mandibular region was contaminated with pathogenic Y. enterocolitica while the tonsils were negative. For instance, Nesbakken et al. (2003) indicated that incision of the submaxillary lymph nodes during veterinary inspection represents a risk for cross-contamination with enteropathogenic Yersinia.

Conclusion

A high proportion of pig carcasses are contaminated during slaughter. Taking into account that these pathogens are able to multiply at refrigerated temperatures, a considerable part of pork carcasses represent a potential risk for public health.

Therefore, further research is needed to elucidate the predominant factors related to the contamination of carcasses with enteropathogenic Yersinia spp. during slaughter.

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Microbial and serological effects of vaccination of sows and suckling piglets with an attenuated live Salmonella vaccine

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Abstract

The aim of the study was to investigate the use of the orally applicated attenuated Salmonella vaccine Salmoporc® (which is already licensed for runners and fattening pigs) in three days old suckling piglets. In particular, the tolerance, colonisation kinetics, humoral immune response, protection against challenge infection with Salmonella Typhimurium DT104 and a possible interference of maternal antibodies on the success of vaccination have been investigated. The results of the study show that oral application of Salmoporc® to three-day-old suckling piglets in combination with the oral vaccination at the time of weaning is very well tolerated and irrespective of the immune status of the sows also suitable to induce a protective immune response. This immune response is able to prevent clinical symptoms of salmonellosis and significantly reduces the colonization of internal organs and the excretion of the S. Typhimurium DT104 challenge strain in faeces. The results further show, that the vaccination of three days old piglets with Salmoporc® has no effect on the results of serological tests within the frame of Salmonella-monitoring.

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The use of antimicrobial substances in food animals
The big picture

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Abstract

The paper gives rather a broad overview on the development of the use of antibiotics and chemotherapeutics from there very beginning until today. The paper deals with scientific facts known in the medical and veterinary community, but also with speculations and "gut-feeling" demands of activist groups that criticise e.g. intensive animal production procedures as "factory farming". The discussions about the need to curb the problem of bacterial resistance and the various attempts to regulate the use of antibiotics in food animals are summarised, and the paper underlines that veterinarians carry the major responsibility for the most desirable use of antimicrobial substances, the "prudent use of antibiotics", where, when antimicrobials have to be applied, the application leads to the highest possible health effect and the lowest possible resistance in the bacteria accompany and/or threaten humans and animals. The paper, however, also argues that meeting demand for reducing the overall amount of antibiotics used in food animals needs a different approach: instead of only asking veterinarians to refrain from an overuse (especially the routine use) of antibiotics mainly in pigs and poultry, a concerted action of the farming community together with their consulting veterinarians is necessary with the clear target to significantly increase the health of the food animal populations by optimising the herd and flock health management, which automatically will result in a measurable reduction of reliance of the food animal production on antibiotics. Concluding, the paper speculates that there will be no significant reduction in the amount of antimicrobials used in food animals, unless farmers and veterinarians find new approaches to investing money in the health of herds and flocks, i.e. paying veterinary services for maintaining the animals' health rather than for curing their diseases.

THE breakthrough in battling bacterial disease

The development of sulphonamides by Gerhard Domagk and the discovery of penicillin by Alexander Fleming have been celebrated as milestones in mankind's attempts to reduce premature death and pain and suffering due to disease. There are no hard data about the percentage of the causes of death prior to the establishment of any state health system, but it can be argued that infectious diseases have been the major death causes for thousands of years. This is definitively true for the centuries when, due to an increase of human populations moving and commingling throughout Europa and to the start of urbanisation without any coordinated sanitation, the main epidemics that killed millions of people were Plague, Cholera and Typhoid. These bacterial epidemics had an exponentially higher death toll than any viral epidemic. There are no hard data to prove of disprove, if not many a million of the many millions of deaths due to the 1918 Hongkong Flu epidemic were due to secondary bacterial infections that maybe dramatically increased the number of fatalities. There are speculations that the number of people that lost their lives in wars due to the direct effect of a weapon is manifold smaller than the number of wounded people that later died due to the bacterial infections caused by their being wounded. In other words the fact that mankind suddenly was able to cure bacterial infections and to fight life-threatening bacteria (chemotherapeutics/ antibiotics and disinfectants) was celebrated as man's victory over infectious disease.

The triumphal march, disillusionment and finally a realistic view

Whereas in the early years of the availability of sulphonamides and antibiotics, only life-threatening infections of humans were treated, but soon more and more application areas were added: less harmful infections in humans, bacterial diseases in animals, more and more non-fatal diseases in humans up to the treatment of just "annoying" infections such as common colds, growth promotion ("non-therapeutic use") in food animals, and the routine prophylactic and "metaphylactic" use in large scale food animal production units. This development of expanding the use of antimicrobial substances would have remained being welcomed and undisputed, if there were not the phenomenon of acquired bacterial resistance in bacteria species that naturally are highly sensible to certain antimicrobial substances. When the first incidences of bacterial resistance were empirically recognised, little was known about the mode of effect the antimicro-

bial substances and nothing about the molecular mechanisms that determine the inefficiency of the so far fully potent drugs. It took some decades of finger-pointing (especially between the medical and veterinary profession) and learning that there has to be a hierarchy in the use of antimicrobial substances before the development of a realistic view on how to maintain the "sharpness of this valuable tool against bacterial disease" especially for those antimicrobial groups that are of critical importance in human medicine.

The initial hope that the threat of bacterial pathogens to human health had been banned the by the use of antimicrobial substances was shattered. However, exactly this experience led, to increased research activities into the mechanisms of the development of bacterial resistance through either the acquisition of a plasmid or a single point mutation. The major step in developing a general understanding that maintaining the efficacy of whichever antimicrobial is the WAY we use them in human and veterinary medicine was that it was comprehended that ANY use of ANY antimicrobial compound leads to a selection pressure in the targeted (and the simultaneously occurring) bacterial populations towards bacteria that are less vulnerable (sensitive) and withstand the bactericidal or bacteriostatic mechanisms, and that it is necessary to develop intelligent strategies to minimise the MAGNITUDE of the selection pressure. This understanding was also supported by the relatively late realisation that bacterial populations are by far faster to adapt to antimicrobial compounds than humans are able to find or develop and produce new compounds.

Strategies to curb bacterial resistance

The most important step in guiding medical and veterinary users of antimicrobial substances as treatment of bacterial infections was the development of the concept of the "prudent use of antibiotics", which is defined as applying antimicrobials in a way that leads to the highest possible health effect in humans or animals and to the lowest possible resistance in the bacteria that are exposed to the compound. WHO and FAO, but also many national medical and veterinary associations, chambers and international organisations such as FVE for veterinary medicine have issued guidelines on the prudent use of antibiotics. They all may differ in some details, but the following major basic principles are common for all prudent-use-guidelines:

- use only licensed antimicrobials only as much and as long as necessary and as little and as short as possible;
- select targeted antimicrobials according to the natural sensitivity of the identified bacterial species;
- use the chosen antimicrobial compound in the highest possible dosage and over the shortest possible period of time, which is yet long enough to minimise the selection of resistant bacterial strains;
- base this decision as often as possible on laboratory results on the actual sensitivity (clinical breakpoint) of the disease-causing bacterial strains;
- refrain from using broad-band antibiotics wherever possible;
- refrain from using antimicrobial groups of critical importance for human medicine in non-life-threatening bacterial infections of humans and in veterinary medicine.

These guidelines for the prudent use of antibiotics are nowadays broadly accepted in the medical and veterinary professions and they have definitively led to a higher degree of compliance with practices that are known to reduce the development of bacterial resistance (including a better supervision of the application of antibiotics in feed and water by the farmers!).

However, to which degree the magnitude of bacterial resistance in veterinary medicine has been influenced by the principles of the prudent use of antibiotics is more or less unknown, since the existing data on bacterial resistance are hardly comparable not only from country to country, but also over time. The decision of the EU to command the EFSA (based on Article 33 of the Reg. [EC] 178/2002) to carry out a standardised collection and analysis of data on zoonoses, antimicrobial resistance and food-borne outbreaks, is a major precondition for a meaningful measurement of the development of bacterial resistance specially in bacteria that cause zoonoses and food-borne diseases in humans. Unfortunately, the period of time, which EFSA needs to harmonise this data collection and to demonstrate make sound decisions on which measures which country needs to take, is longer than we need quiet down the still growing criticism with the use of antibiotics in food animals.

Measures in animal production beyond the "prudent use"

The still growing societal concerns expressed by public health authorities and organisations such as WHO, CDC and EFSA, is nowadays increasingly taken up by NGO's targeting modern animal production systems. And their "cause" is, unfortunately, supported by an increasingly public discussion about multi-resistant Salmonella strains, MRSA in food

producing animals (laMRSA), and ESBL. Only one quote from the Global Edition of the New York Times (March 23, 2011) illustrates the general perception of the deficiencies in the use of antibiotics in general, but also in food producing animals: "...But antibiotics are frequently misused – overprescribed or incorrectly taken by patients, and recklessly fed to farm animals".

As responsible researchers used to asking for sound data-based analyses we tend to ignore statements that lack reasonable evidence (show me the data that prove that the use of … has led to…). However, in the light of our consensus on the fact that ANY use of antimicrobial substances leads to bacterial resistance as well in the targeted bacterial strains as in all other bacteria that are exposed to the antimicrobial in question, we need to agree on the demand for reducing today's amount of antibiotics and chemotherapeutics used in general in food animals as much as possible as long as we achieve the necessary health effect in case of acute infections.

But here we need to accept that the rules for the prudent use of antibiotics are NOT able address the reliance of animal production on the routine use of antimicrobials. If bacterial disease is occurring in any animal population, it is the ethical duty of veterinarians to apply antimicrobial substances, of course following the rules of their prudent use. It has been ignored for too long that field veterinarians, although society expects them to consult farmers to optimise their husbandry systems and their management skills regarding animal health, have not the legal "power" to enforce their recommendations.

Especially in countries, where veterinarians are the only source of drugs for the farmers, it is widely believed that this fact is the major reason for a comparatively high consumption of antimicrobial substances in food animals. At first glance this seems to be supported by the relatively low amount of antibiotics (measured in mg/kg meat produced) in Norway, Sweden and Finland. However, Austria has the same low consumption, and it should not be forgotten, that the Scandinavian countries have a long tradition of animal health schemes including long-standing and successful Salmonella monitoring and reduction programmes. And, the wide range from about 50 mg/kg meat produced in Ireland and Denmark up to more than 250 mg/kg in Greece and the USA shows that there must be other factors that much more influence the quantities of antimicrobial substances that are used in food animal populations than just the way how farmers are supplied with drugs.

Not only well-proven experiences of every single veterinary practitioner, but also a growing number of scientific papers on the huge variability of the amount of antimicrobials used in food animals at the herd and flock level, tell us that the animal-health awareness of farmers and their management skills determine the health status of the herd or flock in question, which in turn, determines the necessary amount of antibiotics applied or prescribed by the veterinarian.

The results of our research projects on testing the so-called "Animal Treatment Index" and the Danish comparison of the use of antimicrobials per pig herd by measuring the Animal Daily Doses (ADD) prove that there is a need to revise the main stream attempts to force veterinarians to use less antimicrobials (which is twisting the arm of the wrong people) into first measuring the amount of antibiotics at farm level. Using a benchmarking system such the "Yellow Card Initiative", introduced last year in Denmark by the Danish Veterinary and Food Administration, will lead to concerted actions to measurably increase the health of the food animal populations, which "automatically" will lead to a reduction of routinely administered antimicrobials in food animals.

A new advisory tool to help practitioners reduce antibiotic consumption in pig herds

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Abstract

The present paper describes an advisory tool, Farm Facts, developed to assist veterinarians to reduce the consumption of antibiotics in pig herds. Farm Facts is a spreadsheet in three parts: The first part identifying the source of the main consumption in a herd, the second part giving a template for an action plan setting up a threshold for success, and the third part providing tables and graphs for follow-up according to the threshold after implementation of the action plan. Until now, Farm Facts has been used in herds for surveillance of results before and after implementation of vaccination programmes. As a typical example of successfull reduction of antibiotic consumption, results from a Danish finishing herd implementing vaccination against ileitis are presented. The follow-up with Farm Facts in this herd showed that the use of antibiotics was reduced by 64%, while keeping the level of daily weight gain and reducing the mortality. Farm Facts is a useful tool for implementation of alternatives to antibiotics in pig herds and visualisation of results to veterinarians and pig producers.

Introduction

Antibiotic consumption in Danish pig production has close attention of the public. Though the antibiotic consumption is low compared to many other pig producing countries (1), political pressure is laid on the farmers to reduce the consumption. Recently, a threshold level for an acceptable number of daily doses (ADD) per pig was set by the authorities with the yellow card system. Herds with ADD/pig above the threshold level are forced to imply action plans to reduce their antibiotic consumption to avoid penalties.

The present paper describes Farm Facts; an advisory tool for veterinarians for creation and follow-up on action plans for reducing antibiotic consumption in pig herds. Farm Facts is provided as a spreadsheet and consists of three parts: Part 1 identifies the focus area (which treatment contributes the most to the total antibiotic consumption), part 2 creates an action plan to reduce the identified treatment, and part 3 allows to follow-up and to compare selected parameters before and after initiating the action plan. These three parts will be described thoroughly in the method section below. Furthermore an example with data from a specific pig herd will be given in the results section.

Methods

Part 1: Focus area

Part 1 is based on data from the central, password protected database on prescriptions to Danish production animals, Vetstat. Every single prescription made by a veterinarian in Denmark is registered in this database, basically telling who prescribed what, when, and to whom. The prescriptions are categorized according to the age group and the diagnosis (table 1). Farm Facts helps the veterinarian to extract the data from the herds they serve and to prepare visuals to be presented to the farmers. Based on a data extract from Vetstat, the spread sheet in part 1 of Farm Facts calculates the number of ADDs per produced pig for all pig herds in a veterinary practice. The histograms are based on the categorisation of the prescriptions according to age group and diagnosis. Each pig herd gets two histograms: One comparing their ADD/pig to other herds (fig 1), and another showing diagnoses and treatments of their particular herd. The histograms can be used in the veterinary advisory work to compare the use of antibiotics between herds. In herds with a relatively high consumption of antibiotics, the veterinarian and the farmer can then identify diagnoses and treatments responsible for the main part of the consumption.
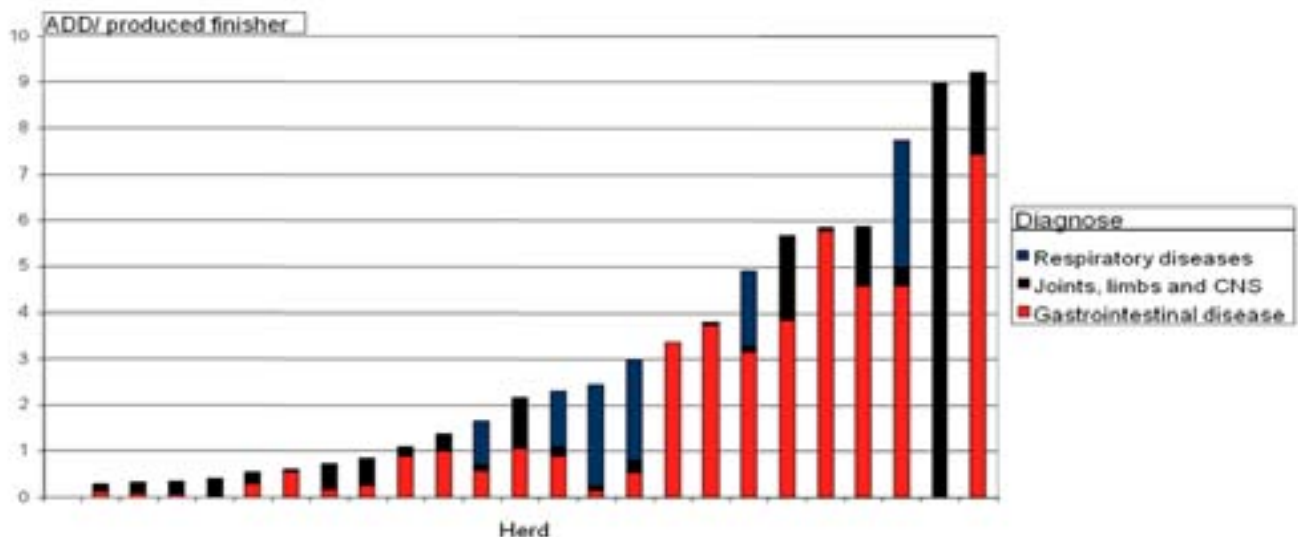
Fig. 1: Survey of use of antibiotics in herds served by one veterinarian.
Each pillar shows the total number of daily doses (ADD) prescribed per produced finishing pig in one particular herd. Used for identification of herds with a high consumption of antibiotics.

Part 2: Action plan

After the herds with a relatively high consumption of antibiotics are identified, the veterinarian and the farmer can devel-ope an action plan for reduction. The spreadsheet in part 2 of Farm Facts contains a template for the action plan, formed as an agreement between veterinarian and farmer to reduce a particular part of the antibiotic consumption in the herd. The challenge for veterinarian and farmer is to select the preventive measures that will reduce the disease problems, which nessecates the use of antibiotics. Therefore, Farm Facts has a built-in drop-down menu with suggestions for alternative ways to reduce diseases, including management changes, feeding strategies and vaccination programmes. These suggestions are grouped according to age group of pigs and disease categories as defined in Vetstat. Using the template, each action plan is prepared specificly for one age group in the herd. The action plan has a pre-defined running period, to which the farmer should stick to in order to give the preventive measures time to work. However, because no farmer wants to take out the antibiotics and get diseased pigs or bad production figures as a result, the action plan also includes success criteria based on selected parameters. If these criteria for disease occurrence or production level are not met, the action plan should be re-evaluated. Again, Farm Facts has a drop-down menu with suggestions for disease and produc-tion parameters within the different age groups and diagnoses. The action plan is designed to fit one page, and it should be printed out and placed in the stable; easily accessible for everyone taking care of the pigs.

Table 1: Categorization of prescriptions for Danish veterinarians for registration in the Vetstat database on antibiotic consumption in pig herds.

Category	Code	Definition
Animal species	15	Pigs
Age group	55	Sows and piglets (mean 250 kg)
	56	Weaners (mean 15 kg)
	57	Finishers (mean 50 kg)
Disease	10	Reproduction/ urinary disorders
	11	Udder/MMA
	12	Gastro-intestinal diseases
	13	Respiratory diseases
	14	Joints, limbs and CNS
	15	Metabolism/circulation

Part 3: Follow up

As the farmer implements new preventive measures to reduce antibiotic consumption, part 3 in Farm Facts allows to analyse the development of imporant parameters of success. These parameters are recorded in regular intervals, chosen by the veterinarian and the farmer. Besides a drop-down menu with a choice of parameters within each age- and disease category, the spreadsheet contains a record table that will be sized according to the running period chosen for the action plan. Recordings can be made directly on a computer, or the sheet can be printed out. The key element in the follow-up is that the selected parameters need to be recorded both before and after the implementation of the the action plan. The length of the interval should be constant over the whole recording period. In that way, there will be a sound basis for comparison. Some of the records might cause additional work, e.g. for counting coughs or greasy piglet tails, but this will benefit the pigs by creating more focus on the problems. The records can be shown in a graph, showing the mean for the parameter before and after implementation of the action plan in relation to the threshold level of success (example in fig. 3).

Results

Until now, Farm Facts has only been used for introduction of vaccination programmes, because vaccination is a straight-forward and simple way to prevent diseases and thus reduce the need for treatment with antibiotics. The results from a finishing herd are presented as example below.

The herd is a conventional Danish pig herd producing finishers in weekly batches of 384 pigs, with a one-time-capacity of approximately 2300 pigs. Prophylactic medication with antibiotics is not used, but before the action plan, the herd had a steady consumption of oral tiamulin for treatment of diarrhoea. Besides these group medications, injectables were frequently used, and the mean total consumption for the 2300 pigs was 112 ADD per day. To reduce the use of antibiotics, a vaccination programme with oral vaccination against ileitis (Enterisol® Ileitis) was initiated. For 2 months after the initiation of the action plan, batch data on the mortality and the ADD (fig. 2) were recorded in the follow-up tables of Farm Facts. The threshold of success was an at least constant mortality and a reduction of antibiotics. The number of pigs delivered more than 13 weeks after entrance was recorded as a batch wise measure of slow growers.
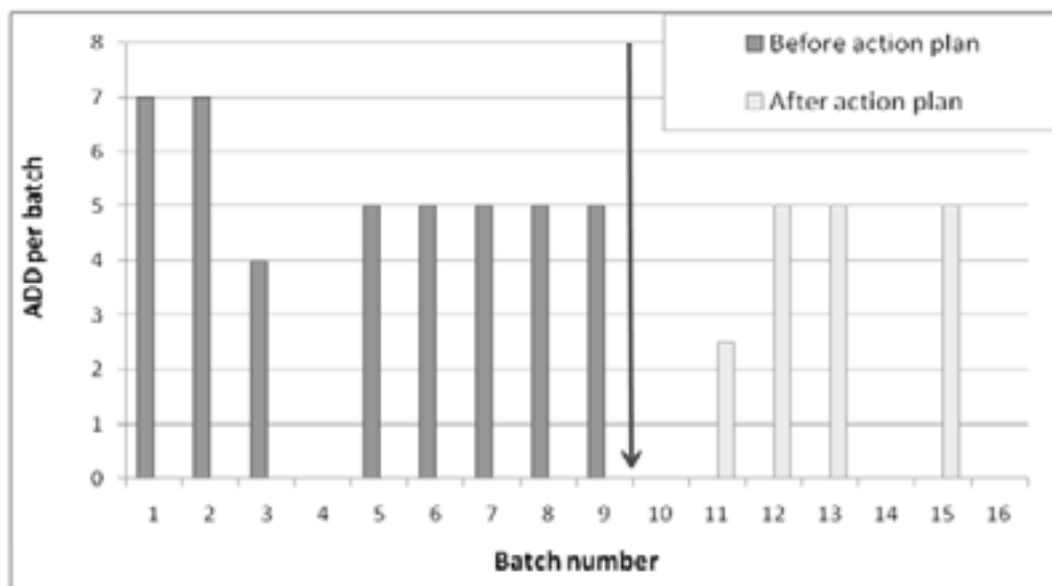
Fig. 2: Total consumption of antibiotics in a finishing herd before and after implementation of action plan (vaccination against ileitis). Arrow indicates the start of the action plan.

With vaccination, the mean total consumption of antibiotics was reduced by 64% to 40 ADD per day. Mortality decreased slightly (fig. 3), and the number of slow growers stayed constant. Despite the reduced use of antibiotics, the occurence of clinical diarrhoea decreased as well. It is of special importance for the farmer, that ADDs used as injectables dropped by almost 50%, from 5.8 to 3.1 ADD/day. This contributed only a little to the total ADD, but allowed the farmer to save working time.
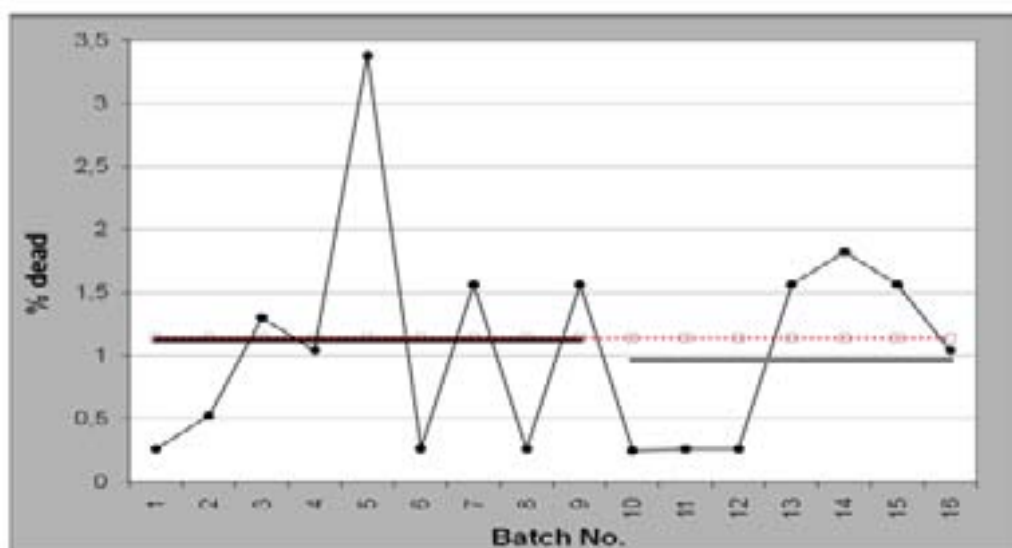
Fig. 3: Follow-up graph showing mortality before and after initiation of an action plan to reduce the use of antibiotics The black line shows mean mortality before the action plan, where pigs were frequently medicated, and the grey line shows mean mortality after implementation of the action plan (vaccination with Enterisol® Ileitis) and a 64% reduction in antibiotic use.

Discussion

With the current focus on reduced use of antibiotics in the pig production, pig producers and veterinarians has to look more into preventive measures instead of treatment. Farm Facts provides a platform for initiation and follow-up on action plans for implementation of additional preventive measures, ranging from management changes to vaccinations. It is developed to be used on the farm, involving everyone taking care of the pigs. As such, it does not deliver complicated statistical calculations, but illustrates in a simple manner, if things are moving in the right direction. This was the case after the introduction of vaccination against ileitis in the example herd, where the vaccination reduced the antibiotic consumption significantly, as shown in many other herds (2, 3, 4).

Conclusion

The advisory tool Farm Facts deliver a helpful tool for initiation of vaccination programmes and other action plans in pig herds seeking to reduce the consumption of antibiotics.

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The ResPig program as a tool for identifying risk factors affecting technical performance and post-mortem results at the slaughterhouse on Dutch pig farms and for restrictive antibiotic use.

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Introduction

The pig industry nowadays faces more complex diseases than ever before, such as Porcine Multi Systemic Wasting Syndrome and Porcine Respiratory Disease Complex. These disease entities are often caused by multiple infections combined with suboptimal conditions in the field of environment, management, biosecurity climatic conditions and more. Successful preventive plans include the identification of infectious and non infectious factors and preventive interventions based on this. Regarding PMWS, Madec formulated a list of 20 management measures and this was already quite successful in reducing the impact of that syndrome.

A poor understanding of the risk factors for infectious diseases often results in the unnecessarily prolonged use of preventive and curative antibiotic treatments, which is not good for human food safety and also allows the development and selection of more antibiotic-resistant bacteria (1).

ResPig is a digital diagnostic and monitoring program for veterinarians including regular cross-sectional serological investigations for the presence of PRRSV, PCV2, Actinobacillus pleuropneumoniae (App), Mycoplasma hyopneumoniae (M hyo), Influenza and Haemophilus parasuis. It includes also an objective scoring system for possible risk factors (environment, management, housing, biosecurity) for respiratory diseases and an economic module to estimate the financial loses due to infectious diseases and the financial effects of advised interventions. The program helps the veterinarian to take the necessary steps towards a structured approach to respiratory problems with restrictive use of antibiotics.

Because of the high number of participating farms and the uniform sampling protocols, it is possible to analyze the disease situation on a cluster of farms. Analysis of the ResPig databank provides information on possible relationships between the serological test results and the technical- and slaughterhouse performance parameters of finishers. When antibiotic use will be registered in ResPig it must also be possible tot determine relations between infectious diseases, management, biosecurity, housing and antibiotic use.

Material and methods

1. Data were used from tree hundred farms involving 936 cross-sectional serology results (sows, gilts, 5-, 10-, 16-and 22 weeks old pigs, 5 animals per group) in 2008 and 2009. Farms provided performance and slaughterhouse data and a vaccination history. Odds ratios were calculated between the serological results of the oldest fatteners and the technical and slaughterhouse data provided in the farm anamneses.

    The definitions for the technical performance parameters were taken from the farm comparison 2008-2009 of Agrovision's management system (calculates the average technical performance of Dutch pig farms) (2). The average VION farmingnet slaughterhouse scores for 2008 were used to determine the slaughterhouse definitions for pleurisy and pneumonia. These can be found in Table.1 together with the serological definitions.

2. Two multiplying farms measured their antibiotic used in daily doses per animal (DDD) (4) and their technical performance in delivered piglets per sow per year after implementation of the structural health approach with intervention plans aiming on optimization of biosecurity and preventive vaccination programs when necessary.

Table 1: serology definitions

definitions	samples	result
App +	average titer	≥ 14 omp elisa*
PRRS +	average titer	>0.4 idexx elisa
M hyo +	% samples positive	> 20% idexx elisa
Infl +	average titer	≥ 9 log2 HI
PCV2 +	average titer	≥ 10 log2 int.elisa

performance definitions

tech.-/slaughterhouse	result
high mortality	>2.5%
sub optimal ADG	<786 gr
high pleuritis	≥16.5%
high pneumonia	≥11.7%

*antibody Elisa on the 42 kD outer membrane protein of A. pleuropneumoniae (3)

Results

1.Table 2: Relationships: odds ratio between serology and peformance results

serology	techn. / slaughter results	O.R.*	P
App +	high pleurisies	4.7	(< 0.0001)
	high pleurisies + pneumonia	4.3	(< 0.0001)
PRRS +	high pleurisies	8.0	(= 0.0029)
	high pleurisies + pneumonia	6.2	(= 0.0028)
PCV2 +	sub optimal ADG	1.9	(= 0.0425)
M hyo +	high mortality	2.9	(= 0.0014)

* Significant associations (P<0.05) P-value based on Fisher's exact test

2.Table 3: structural health approach, antibiotic use and technical results

Farm		2008	2009	Δ
Farm 1: 545 sows	DDD (4)	32	8	25% ↓
	pigs/sow/year	26.8	28.5	6% ↑
Farm 2: 1200 sows	DDD (4)	62.2	56.4	9% ↓
	pigs/sow/year	26.5	28.9	9% ↑

Conclusions and discussion

Significant relationships were demonstrated between ResPig serology results for Actinobacillus pleuropneumoniae, PRRS, Mycoplasma hyopneumoniae and PCV2 and the technical performance and slaughterhouse data (table 2).

As expected, positive App serology in finishers was a risk factor for high levels of pleurisy with or without a high pneumonia score. PRRS infections were also a risk factor for both these slaughterhouse parameters. Secondary infections after PRRS-infections may be the explanation for this. Positive M hyo serology was a risk factor for high mortality in finishers. Early infections and disturbances in the clearance mechanism and integrity of the upper respiratory tract are possible reasons. A high PCV2 titer in finishers, demonstrating infection (without any indication of the actual viral load) was a risk factor for suboptimal Average Daily Gain (ADG).

The serology used in the ResPig protocols makes it possible to identify the risk factors which lead to poor results in technical performance during finishing and in the slaughterhouse so that veterinary advisers are better able to formulate more successful preventive programs.

Routine serological testing, identification of risk factors for respiratory diseases and subsequent adaptation of biosecurity and strategic vaccination interventions seem to be helpful in reducing antibiotic usage (table 3). This may contribute to better technical performance and might be beneficial for human food safety. An antibiotic use score will also be implemented in the ResPig program so that possible relations between infectious diseases, environment, housing, management and antibiotic use can be determined.

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The link between biosecurity and production and treatment characteristics in pig herds

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Abstract

It is believed that biosecurity influences production figures and health status of pig herds, nevertheless, few studies succeed in demonstrating and quantifying this relation.

In the present study, 95 randomly selected Belgian closed or semi-closed pig herds were visited to quantify the biosecurity status of the herd by means of a biosecurity scoring system (Biocheck) with a range from 0 (= total absence of biosecurity) to 100 (= perfect biosecurity). At the same visit additional data concerning herd, farmer, production characteristics and the use of antimicrobials was collected. The Antimicrobial use was quantified by calculating treatment incidences (TI).

The external biosecurity (preventing pathogens from entering the herd) ranged between 45 and 89/100 with an average of 65/100, whereas the internal biosecurity (reducing within herd spread of infection) score was on average 52/100 (min 18; max 87).

The number of sows and fattening pigs on a herd ware positively associated with the external biosecurity score. This indicates that on larger herds, more attention is paid to biosecurity. A negative association was seen between the internal biosecurity score and the age of the buildings as well as with the years of experience of the farmer. This indicates that biosecurity is higher on the agenda with younger farmers and in more modern herds.

A higher overall, external and internal biosecurity score had a significant positive influence on the daily weight gain of fattening pigs, indicating that a higher level of biosecurity positively influence production figures. No significant association was seen between the mortality rate of fattening pigs and biosecurity scores.

The internal biosecurity score, due to the subfactors 'Disease management' and 'Farrowing and suckling period', had a significant influence on the treatment incidence based on the used daily dose, indicating that an improved biosecurity is associated with a reduction of antimicrobial drug use.

Introduction

The term biosecurity comprises all measures to prevent pathogens from entering the herd and to reduce the spread of pathogens within the herd (Amass and Clark, 1999). Biosecurity can be divided into 2 different categories. External biosecurity deals with measures that prevent pathogens from entering the herd, while internal biosecurity is about preventing the within-herd spread of pathogens. To quantify the biosecurity situation on pig farms, a biosecurity scoring system was developed by the Veterinary Epidemiology Unit of the faculty of Veterinary Medicine, Ghent University, and incorporated in a free online application (www.biocheck.ugent.be) (Laanen et al., 2010).
It is believed that biosecurity influences production figures and the health status of pig herds. In the present study, this relationship was further explored.

Material and Methods

Ninety-five closed or semi-closed Belgian pig herds holding at least 80 sows and 400 fattening pigs were randomly selected from the national Identification and Registration (I&R) database of pig producing facilities (SANITEL-pigs, 2005). During a herd visit, the biosecurity status of the herd was quantified by means of a biosecurity scoring system (Biocheck)

with a range from 0 (= total absence of biosecurity) to 100 (= perfect biosecurity) for both external and internal biosecurity. Each biosecurity score is further subdivided into 6 subcategories, which are listed in Table 1. Additional information concerning herd (number of sows, number of fattening pigs, age of the buildings), farmer (years of experience) and production characteristics (daily weight gain and mortality of fattening pigs) and the use of antimicrobial drugs was collected. The use of antimicrobials was quantified by calculating the treatment incidence (TIUDDpig), according to Timmerman et al. (2006). The TIUDDpig expresses the number of pigs treated with one UDDpig (used daily dose in pigs) per1000 pigs. Only group treatments were taken into account.

Data were analyzed using a multivariable linear regression model. Pearson's correlation coefficients were calculated for detecting highly correlated variables. All analyses were performed using SPSS software (SPSS 19.0, SPSS Inc., Chicago, IL).

Results

The average external biosecurity score was 65 (min 45; max 89), whereas the average internal biosecurity was 52 (min 18; max 87) (Table 1).

Table 1. The subcategories of the Biocheck scoring system for external and internal biosecurity and the overall results for the 95 pig herds.

Subcategory	Average	SD	Min	Max
External biosecurity	**65**	**8**	**45**	**89**
Purchase of animals and semen	89	11	58	100
Transport of animals, removal of manure and dead animals	66	12	30	96
Feed, water and equipment supply	41	17	17	100
Personnel and visitors	64	12	24	100
Vermin and bird control	57	21	0	100
Environment and region	48	22	10	100
Internal biosecurity	**52**	**15**	**18**	**87**
Disease management	60	31	20	100
Farrowing and suckling period	61	20	14	93
Nursery unit management	56	24	0	100
Fattening unit management	64	26	0	100
Measures between compartments and the use of equipment	46	18	11	100
Cleaning and disinfection	38	27	0	95
Overall biosecurity	**59**	**10**	**36**	**88**

SD=Standard Deviation

The average data for the herd details (number of sows and fattening pigs, age of the buildings), farmer information (years of experience), production characteristics (daily weight gain and mortality of fattening pigs) and the use of antimicrobial drugs (TIUDDpig) are listed in Table 2.

Table 2. Results for the 95 pig herds concerning herd, farmer and production characteristics and the use of antimicrobial drugs.

Factor	Average	Median	SD	Min	Max
Number of sows	289	220	162	80	1000
Number of fattening pigs	1420	1250	877	400	7000
Years of experience of the farmer	21	20	9.9	1	42
Age of the buildings	32	30	17.01	2	129
Daily weight gain (gram/day)	680.6	676.0	71.1	486	870
Mortality (%)	3.55	3.00	2.19	1.00	11.50
TI_{UDDpig}	174.22	150.49	130.47	0.00	650.49

SD=Standard Deviation

No significant association was seen between the mortality rate of fattening pigs and any of the calculated biosecurity scores.

The overall biosecurity score was significantly associated with the TIUDDpig, which could be allocated to the internal biosecurity score. When further subdivided the two categories 'Disease management' and 'Farrowing and suckling period' were significantly associated with the TIUDDpig (Table 4).

Table 4. Influence of different parts of the biosecurity, herd and farmer characteristics on the TI_{UDDpig}.

	R²	Coefficient (β)	p-value
Overall biosecurity	0.037	-2.45	0.06
External biosecurity	0.015	-1.97	0.236
Internal biosecurity	0.040	-1.77	0.05
Detailed model			
Disease management (INT)	} 0.108	-0.90	0.04
Farrowing and suckling period (INT)	}	-1.43	0.03

INT=Internal Biosecurity

Discussion

The average biosecurity scores show that there is much room for improvement. On average, the scores for external biosecurity, which are mainly measures imposed on others (suppliers, etc) are higher than the scores on internal biosecurity, which are more related to work and management strategies of the farmers themselves.

The results from this study indicate the existence of a positive relation between the number of sows and fattening pigs on a herd and the level of biosecurity, indicating that on larger herds more attention is paid to biosecurity than on smaller herds. The larger a herd becomes the more professional and well managed it must become, yet it should be acknowledged that these relationships remain low indicating that other factors also play a role in the level of biosecurity. It is also noticeable that the biosecurity level (mainly internal biosecurity) increases with a decreasing age of the buildings. This illustrates that in more modern facilities, more attention is paid to biosecurity. The same holds for the years of experience of the farmer, suggesting that younger farmers are more aware of biosecurity.

When evaluating the link between biosecurity and the daily weight gain of fattening pigs, it appears that there is a positive association between the biosecurity status of the herd and the daily weight gain of fattening pigs. Although no causal relationship was proven, this suggests that a higher biosecurity could lead to a higher daily weight gain, and therefore to better production results. Parts of both the external and internal biosecurity play a role in the influence on daily weight gain. Also from an economical point of view is this interesting since investments in biosecurity might lead to a higher income through improved production results. The association between the daily weight gain and the number of fattening pigs shows that the bigger the herd, the better the production results are.

The negative association between the biosecurity score and the TIUDDpig shows that on herds with a higher biosecurity less antimicrobial drugs are used compared to herds with a lower biosecurity. This suggests that the use of antimicrobials on a herd could be decreased by increasing the biosecurity level. Noticeable is that only the internal biosecurity had a significant influence on the TIUDDpig suggesting that the amount of antimicrobials used on a herd is influenced by the spread of pathogens within the herd and not so much on the pathogens entering the herd. The internal biosecurity deals with work and management strategies on the herd itself meaning that the farmers have the power to interfere themselves in contrast to the external biosecurity where the situation is to some extent more dependent on others (suppliers, etc.) or on the location of the herd. Yet, although significant relations were found, the low R^2-values obtained indicate that a lot of unexplained variation remains present. Probably other factors, like the vaccination status of the herd, play a part in the amount of antimicrobial drugs used.

Conclusion

Improving the biosecurity level on a farm is a very useful tool in reducing the use of antimicrobial drugs and improving production figures. This is of interest to the individual farmer as well as to the whole pig-producing sector, since it will lower the costs, improve the profit and benefit the sustainability of pig production.

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Garlic reduces effect of Actinobacillus pleuropneumoniae infection in pigs

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Abstract

Lung diseases in pigs are among the most important health problem in pig husbandry, and generally treated with antimicrobials. To reduce the amount of antimicrobials used in pigs, we tested the preventive effects of freeze dried garlic added to feed on an infection with Actinobacilluspleuropneumoniae (APP).

Thirty male pigs of about seven weeks of age were aerosol challenged with APP serotype 2. Fifteen pigs received 5% (w/w) garlic added to their diet (Garlic group), from two days prior to infection until four days after infection. The others received the a standard diet (Control group).

There was no difference in the number of pigs with symptoms of lung diseases. 48 hours after infection, the number of white blood cells had significantly increased in the Control group but not in the Garlic group. In the Control group a positive correlation (p<0.001) was found between the occurrence of clinical respiratory disease signs and pathological findings. This correlation was not found in the Garlic group (p=0.15). We conclude that feeding high concentrations of freeze dried garlic does not prevent an APP infection, however, it affects the course and reduces the severity of an APP infection

Introduction

The development of antibiotic resistance possibly due to the use of antibiotics in animal production has resulted in a growing public concern (Rijsman et al., 2010). Organic farms aim to produce animal products without the help of antibiotics, mainly through preventive and stress reducing measures, but also by using alternative curative treatments. Lung diseases, endo- and ectoparasites and diarrhoea are the main health problems in Dutch organic pig herds. Actinobacilluspleuropneumoniae (APP) is a lung disease which is prevented by vaccination or treated with antibiotics (Van der Meulenet al., 2006). Dutch organic pig farmers are looking for alternatives to reduce the use of antibiotics and prevent APP. Garlic is a possible immunostimulant and has an anti-microbial activity (Sohnet al., 2009; Harjaiet al., 2010; Liu et al., 2010). Volatile sulphurous compounds are essentially responsible for the garlic smell of the exhaled air, and allyl methyl sulphide (AMS) is one of the most stable decomposition products of garlic in the body. AMS can exert antibacterial effects in the lungs (Hell and Kruse, 2007; Kyung and Lee, 2001). In vitro tests with AMS showed an antibacterial effect against APP serotype 9: it impaired the in vitro growth rate by 8% (unpublished data). The present study aims to test the preventive effect in vivo of freeze dried garlic added to feed on an infection with APP.

Material and Methods

Thirty male pigs of about seven weeks of age were aerosol challenged with APP serotype 2 (reference strain 1536) in a stainless steel incubator. Aerosols were introduced with an Aeroneb™ Pro micropump nebulizer (Aerogen Ltd., Galway, Ireland). 5 ml of the inoculum containing 1×10^9cfu/ml was completely dispersed within 30 minutes. Fifteen pigs received a 5% (w/w) garlic constituent of Enteroguard™ (Orffa Additives, The Netherlands) added to their diet (Garlic group), from two days prior to infection until four days after infection. The others received a standard diet (Control group). Body temperature, appetite, and behaviour of all pigs were monitored daily. White blood cells (WBC) were counted three days before the exposure and at day one and three after exposure. At four days after exposure the pigs were euthanized. Gross pathology findings were recorded, tissue samples were taken and lymph nodes were histologically and bacteriologically examined. AMS was measured in blood sampled from the pigs at the day just before the exposure and at day four after exposure.

The analysis of variance was applied to test the effects of the garlic diet on WBC count at days 1 and 2 after inoculation. Differences of lung weights between the two groups were analysed by using a two tailed Mann-Whitney U test.

Occurrence of pneumonia was compared between both groups by Fischer's exact test. For each treatment Spearman's rank correlation coefficient was used as a measure of association between the rankings of clinical sign severity and post mortem severity scores on the animals.

Results

Feed intake was temporarily reduced in the afternoon following the challenge with APP (Table 1). The AMS content in the garlic-fed group before challenge (i.e. 4 days after the 5% garlic administration), was lower (324 ± 128 nM) than at day 4 after challenge (i.e. after 9 days of administration (422 ±194 nM)). The differences between the average AMS content before and after challenge were not statistically significant. In the blood of the animals from the control group no AMS was detected above the minimum detection limit of 4 nM (Table 1).

Table 1: Average feed intake and standard deviation and average AMS content in blood of the pigs and standard deviation in the garlic fed group and in the control group.

Study	Feed intake (g/meal ± st. dev.)		AMS (nM± st. dev.)	
	before exposure	after exposure	before exposure	after exposure
Garlic	292 ± 30	263 ± 79	324 ± 128	422 ± 194
Control	291 ± 19	279 ± 58	< 4	< 4

In 35% of clinical recordings respiratory symptoms were observed in both groups, but no difference was seen between the two groups (Table 2). Increases in body temperature of >39.5°C were seen in 3 out of 15 pigs of the garlic-fed group and in 8 out of 15 pigs from the control group. Also, a lower percentage of recordings with an elevated body temperature were observed in the garlic-fed group (8.3% vs. 17.5%). In both groups, a significant increase of WBC count was observed at one day post infection (p < 0.05). However, at three days post infection, WBC count was still significantly elevated in the control group (p < 0.05), but not in the garlic-fed group.

One pig of the control group died 24 hours after inoculation due to severe pleuropneumoniae. During gross pathology examination, seven pigs of the control group had pleuropneumonia, pneumonia and/or pleuritis. In the garlic-fed group, five pigs had a pleuropneumonia, pneumonia and/or pleuritis. Inflammatory changes such as oedema or cell infiltrations can influence the relative lung weight. The mean relative lung weight was increased in the control group compared to the garlic-fed group (control group: 1.21 ± 0.15 %; garlic-fed group: 1.13 ± 0.10 %), indicating a protective effect in the garlic-fed group, reducing inflammatory changes after infection (p = 0.06).

A positive association between the occurrence of clinical respiratory disease signs and pathological findings was seen in the control group (p < 0.001), but not in the garlic-fed group (p = 0.15). It appeared that pigs from the garlic-fed group, which had shown clinical symptoms, had not necessarily developed pathological lesions.

Table 2: Summary of the results after challenge with Actinobacilluspleuropneumoniae serotype2

study	Respiratory symptoms	% animals with elevated body temperature > 39,5 °C[a]	Leucocyte index (%)[b]	Mean relative lung weight at day 4 post challenge (%)
Garlic	35%	8.3	63	1.13 ± 0.10
Control	35%	17.5	71	1.21 ± 0.15

[a] *Number of recordings with respiratory disease signs/total number of clinical recordings after challenge*
[b] *Percentage of recording of a WBC count > 20 G/l one and three days post infection*

Discussion

The clinical and pathological changes induced were mild and comparable with most APP infections in pig farms. The effect of garlic on the clinical symptoms could not be shown in this study. However the lower lung weight in the garlic group compared to the control group and the lower WBC counts for two days in the garlic group compared to the control group suggest a reduced inflammatory response. The effects of garlic in this study were achieved with 5% of freeze dried

garlic added to feed. In practice 1% is advised to be cost effective. Future studies are necessary to determine the dose effects relationship of garlic and prevention of the clinical signs of an APP infection.

Conclusion

It seems that feeding high concentrations of freeze dried garlic does not prevent an APP infection, however, it affects the course and reduces the severity of an APP infection.

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Risk management with regard to dioxin residues in pork meat

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Abstract

Maximum levels for dioxins have been established in 2001 in feed (feed materials and compound feed) and food of animal origin (fish, meat, eggs, milk and derived products). They are in force since 1 January 2002.
These maximum levels were complemented in 2006 with maximum levels for the sum of dioxins and dioxin-like PCBs in feed and food.
By the Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 feed business operators have to put in place, implement and maintain procedures based on the Hazard Analysis Critical Control Points (HACCP) principles. This means the identification of critical control points and the identification of, inter alia, possible chemical contamination.

In December 2010, German feed and food safety authorities were informed that several batches of fatty acids, which were meant to be used for technical purposes, contained higher levels of dioxins than allowed by EU feed law. These batches were mixed with other fats and subsequently used for the production of compound feed.
After the contamination occurred, contaminated feed was distributed to some 5000 farms in several areas of Germany, including more than 700 pig holders in Lower Saxony.
In light of the precautionary principle, the immediate blockage of these affected farms was an essential measure, whereby it was assumed that animals that had received contaminated feed would automatically yield contaminated food. Extensive sampling and analysis of feed und food followed, resulting in a number of non-compliant results. Unblocking of farms was done in a step-by- step, scientific based approach and took several weeks.
The presentation will give a short overview over the course of events and a description of the principles for the risk management by authorities and producers.

Risk of T. solium Transmission from Pork Slaughtered in Western Kenya

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Abstract

The tapeworm Taenia solium has been identified as an important public health issue in Latin America, Asia and across much of Africa, although the nature of global travel and migration puts all countries at risk of infection. Ingestion by people of infective eggs or proglottids from a T. solium carrier, can result in the aberrant larval infection; cysticercosis, with a particularly high burden of disease being associated with infection of the central nervous system; neurocysticercosis.

Understanding the risks associated pork production, preparation and consumption as is currently undertaken in many parts of the developing world is the first step to mitigation of such risks, ensuring a safe and viable pig industry in these countries and reducing the risk of parasite introduction to currently unaffected countries.

A study in Western Kenya focused on determining the prevalence of T. solium in pigs entering the food chain and was complemented by an ongoing community cross-sectional study which determined the pork eating and preparation behaviours within the same study area. Data from these studies, supplemented by the literature was used to inform a food chain risk built as a stochastic decision model.

This risk assessment model indicates that a significant number of potentially infective pork meals are taken in any one year in Western Kenya, in turn placing the wider community at risk of acquiring a T. solium cysticercosis infection through environmental contamination with eggs and proglottids. The effect of three potential mitigation strategies were modelled; with the initiation of a pen-side diagnostic test for T. solium infections used in abattoirs being the most effective to reduce the number of infective meals taken in a year.

HEV inactivation assessment using viable virus

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Abstract

HepatitsE is an acute icteric hepatitis caused by Hepatits E virus (HEV). HEV is transmitted by water supplies in developing countries.Recently, HEV contamination inconsumption water was also observed in a developed country(France). HEV is detected in pigs and several other animal species(e.g. wild boars and deer) and it is strongly suspected to be zoonotic,HEV has also been detected in the pork production chain:In a study conducted in a grocery inUSA 11% of livers testedwere HEV positive and similar data have beenobserved in Europe also.

People have beeninfectedwith HEV by eating raw or undercooked pork/deer meat. In France 5 people died after consuming raw pig liver sausages. HEV average mortality rate varies between 1 and 4%, but in pregnant women may increase up to 25%.

The risk of HEV infection via consumption of HEV-contaminated pig livers raises further public health concern.It is not clear whether cooking conditions are effective in inactivating the virus in the contaminated pig livers.

Only one HEV inactivation study was performed. The objective ofFeagins et al.(2007)was to determine if traditional cooking methods are effective in inactivating infectious HEV in contaminated commercial pig livers. Four of five pigs inoculated with HEV-positive liver incubated at 56 °C for 1 hour, developed an active HEV infection.

Our group reproducingFeagins experiment but replacing the use of live pigs with 3D cell culture. The results confirm Feagins' findings, showing that HEV can maintain its infectivity when heated at 56°C for 1hour. This research underlines the potential of the 3D cell culture system of replacing the traditional in vivo infectivity studies and emphasises the necessity for cooking pig liver before consumption.

Alternative method for knife disinfection with INSPEXX 200 is more efficient than 82°C water

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Abstract

EU regulations require disinfection of slaughter-equipment with water of at least 82°C. However, according EU regulation 853/2004, an alternative system having an equivalent effect may be used when equivalence can be shown. Ecolab's Inspexx© 200 (Inspexx) is a solution containing a stabilised blend of peracetic acid, peroctanoic acid and other organic acids – is shown to be an equivalent alternative. It is applied in water at ambient temperatures and does not need to be rinsed after application.

A practical trial in a Dutch pork abattoir was performed to study whether Inspexx is adequately effective in disinfecting knives used at slaughter. The standard method of disinfection with water of 82°C is used as reference whereas Enterobacteriaceae and mesophilic aerobic counts are outcome variables. Knives are immersed in hot water or Inspexx for 0, 1, 10, 30 and 60 seconds respectively.

Results clearly show that Inspexx reduces bacterial contamination of the knives significantly faster (within 1 second) than hot water, which needs at least 10 seconds. It is therefore concluded that Inspexx 200 can be used more effectively for the disinfection of knives used at slaughter.

The outcome of this study confirms a previous efficacy study performed on slaughter equipment. The latter study, in combination with safety and toxicity studies are the basis for official approval of the national authorities of several European countries to apply Inspexx as an alternative method for disinfection of cutting tools during slaughter. In this paper, we show that Inspexx is therewith applicable under practical conditions and a more robust alternative for disinfection of cutting tools used in dressing. Moreover, it can also save water and energy as water needs not to be heated to 82°C. Henceforth, Inspexx does not only improve meat safety, but it will also save environmental resources.

Introduction

According regulation 853/2004 (EU, 2004) slaughterhouses ' […] must have facilities for disinfecting tools with hot water supplied at not less than 82°C, or an alternative system having an equivalent effect'.

The use of water of 82°C can result in the coagulation of protein and melts fats, so that it can disperse over the surface of cutting tools, this can support the forming of biofilms and thus provide salmonella favourable conditions to survive in the slaughter environment. And subsequently, the abattoir has an optimal temperature for salmonella to multiply. Research (Swanenburg et al., 2001) has shown that slaughterhouses can have a house f ora of specific salmonella strains.

Ecolab developed a product based on the mixture of organic acids with the active substances; peracetic acid (POAA) and peroctanoic acid (POOA). This solution is marketed under the name Inspexx© 200 and is currently used as such in Europe and North America (USA and Canada). In previous studies it is shown that Inspexx can serve as an alternative method for disinfecting slaughter-robots during production. By improving the hygiene of slaughter-robots the microbiological results of carcasses improved as well (Oorburg, Holtslag et al., in prep).

Here, we studied whether Inspexx can serve as an alternative disinfection system for knives and whether it provides better microbiological results relative to conventional hot water disinfection.

Materials and Methods

Before commencing, several application points are identified (e.g. Achilles tendon incision, evisceration). The first part of the study required testing of knives, 2 trials per 3 days. During the first three days the effect of 82°C water is tested, disinfection is performed in the conventional knife sterilisers placed at the respective application points. During the other 3 days disinfection with Inspexx is performed in a knife steriliser located centrally. Knives from 5 different positions in the

slaughter line, i.e tendon incision, , intestine removal (evisceration), kidney, rework station, and loosening of the head are sampled. Samples are taken from either side of the blade; without disinfection, after 1 second at 82°C and after 1 min at 82°C.

In the second part of the study; the intervals 10 seconds and 30 seconds are tested on knives from the tendon and evisceration position, and the intervals 0, 1 second and 60 seconds are partly repeated. In this test, contrastingly to the first part of the study, one side is sampled before disinfection whereas the other side is sampled after disinfection.

Knife blades are sampled with Rodac plates (VRBG- en PCA). Each plate (3cm2 surface) is pressed on either sides of the knife blade. The maximum countable number per plate is 90 cfu.

Plate Count Agar (PCA) plates are incubated for 72 ± 3 hours at 30°C ± 1°C to subsequently enumerate Total Viable Count (TVC). Violet Red Bile Glucose Agar (VRBG) plates for 24 ± 1 hours at 37°C ± 1°C to subsequently enumerate enterobacteriacea.

Inspexx 200 solutions are produced by means of a special dosing equipment. Verification of the concentration is performed by means of conventional iodometric (Redox) titration. 10 ml of the solution is acidified with 0,5N H_2SO_4. 1,0N KI after which 2,0% starch solution is added. This mixture is subsequently titrated with 1,0N $Na_2S_2O_3$.

Results

The results of study 1 are shown in table 1. At 82°C after 1 second there is only a small reduction of TVC and enterobactericeae. Inspexx is already highly effective after 1 second.

Table 1: Total viable counts and enterobacteriaceae on knives after disinfection with hot water and Inspexx at different time intervals

Duration	82°C water n= 90	Inspexx n= 94
	Total Viable counts	
None	1,51±0,55	1,21±0,64
1 sec	1,34±0,69	0,19±0,39
1 min	0,01±0,06	0,003±0,03
	Enterobacteriacea	
None	0,57 ±1,07	0,56±0,74
1 sec	0,28 ±0,76	GA
1 min	GA	GA

counts expressed as log cfu/3cm² ± SD
GA = growth absent on all plates

The results of study 2 are shown in table 2. At 82°C, after 1 second, there is only a small reduction of TVC and enterobactericeae, and also at ten seconds disinfection is not fully effective. Inspexx is already highly effective after 1 second.

Table 2: disinfection with hot water at different time intervals

Time	82° C Before	After	Inspexx Before	After	n
		Total Viable Counts			
		Evisceration			
1 sec	1,87±0,18	1,71±0,53	1,59±0,68	0,61 ±0,65	10
10 sec	1,45±0,53	0,99±0,71	1,94± 0,04	0,11± 0,18	20
30 sec	1,48±0,42	0,10±0,21	1,68± 0,34	0,08 ±0,25	20
60 sec	1,64± 0,31	0,08±0,27	1,71 ±0,24	0,08 ±0,17	10
		Achilles tendon			
1 sec	1,95±0	1,96 ±0,0	1,78±0,0	0,63±0,61	10
10 sec	1,9±0,01	0,05±0,14	1,74±0,60	0,03±0,17	20
30 sec	1,86±0	0,12±0,43	1,96±0,0	0,0±0,20	20
60 sec	1,96±0	0,55±0,72	1,96±0,0	GA	10
		Enterobactericaeae			
		Evisceration			
10 sec	0,99±0,54	GA	0,75±0,62	0,04±0,16	15
30 sec	1,50±0,45	GA	0,71±0,41	GA	15
		Achilles tendon			
10 sec	1,55±0,41	0,07±0,19	1,68±0,44	GA	15
30 sec	1,67±0,27	0,07±0,21	1,83±0,17	GA	15

counts expressed as log cfu/3cm² ± SD
GA = growth absent on all plates

Discussion

The present results show that sufficient time, at least 10 seconds is crucial to achieve the required sterilising effect when using water at 82°C. This shows one of the problems slaughterhouses have at present, as it is difficult for employees during the daily routine to take sufficient time for disinfection, due to the high slaughter speed. Secondly, many slaughter-houses have problems in assuring the mandatory temperature of the sterilizers.

The present results prove that Inspexx is a better alternative for knife disinfection.

For Inspexx only 1 second immersion proved sufficient whereas dipping in hot water for 1 second did not have a significant effect. No significant difference between Inspexx and hot water is noticeable if immersion lasted 10 seconds or longer. As a consequence, Inspexx is highly effective in disinfecting knives, it is therefore more than equivalent to hot water.

Beside hygienic advantages Inspexx may also have sustainability advantages. Disinfection with hot water is expending high amounts of energy in order to bring and maintain water at the mandatory temperature. Secondly, hot water disinfection expends high amounts of water, as the sterilizers' temperature need to be maintained at a mandatory minimum.

High throughput of water at high temperatures often results in scaling, hence requiring higher throughput pressure and more energy consumption. Furthermore, high levels of humidity caused by hot water use results in condensation on walls, ceilings and other inaccessible areas which subsequently poses additional bacteriological risks.

Finally, it is common knowledge that use of hot water will result in coagulation of proteins on the cutting edges of knives causing bluntness of any cutting tool. Undoubtedly, this will require more effort from the employee to use his tools. Use of Inspexx does not result in coagulation of proteins on cutting edges hence, use of Inspexx prolongs use of knives.

Based on previous reports on efficacy (Oorburg, Holtslag et al, in prep), toxicological evaluations and safety assessments; the Dutch authorities have given permission for application Inspexx in slaughterhouses. This was followed by other EU member state authorities (for example Germany, Switzerland etc.).

Conclusion

Inspexx is highly effective in disinfecting knives; it is therewith more than equivalent to 82°C water. Besides improving hygienic quality, application of Inspexx can save water and energy.

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Public Health burden of exposure to microbes and parasites originating from pigs and pork

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Abstract

The production and consumption of pork still is an important source of human illness. Quantifying the burden of illness requires the integration of data from a wide variety of sources. First, relevant hazards need to be identified, based on sources such as outbreaks of human illness, and the occurrence of pathogens in pigs, pork and pork products. Then, the incidence of disease due to these pathogens in the population must be assessed. Reported cases only reflect a minor part of all illness and there are different approaches to estimating underreporting factors. The health impact of different pathogens varies widely in severity, duration and associated fatalities. Summary measures of population health, in particular the Disability Adjusted Life Years, are increasingly used to integrate all health effects into one metric. Most pathogens of interest do not only occur in pigs, but also in other food animals or other sources. Hence, the proportion of cases that is attributable to the pig reservoir or to pork consumption needs to be established as a next step. This presentation will summarize results from different national and EU-wide studies on the burden of illness due to pigs and pork, with a focus on non-typhoidal Salmonella spp. and Toxoplasma gondi. The WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) aims to assemble, appraise and report on the current, the projected as well as the averted burden of foodborne disease estimates at a global level. Several reviews have already been published, including a review on the global public health significance of Taenia solium.

It is increasingly recognized that humans are not only exposed to pathogens originating from the pig reservoir by handling or consumption of meat, but also by direct contact with live animals or by indirect environmental transmission. In some cases, such as MRSA, direct contact appears to be the dominant source of exposure, whereas for other pathogens (e.g. Campylobacter), the pathways are much more complex

Evaluation of the potential use of risk-based sampling to surveillance of antibacterial residues in Danish pork

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Abstract

Consumers consider presence of chemical substances in food products as undesirable. In Denmark, more than 20,000 samples are analysed each year for presence of antibacterial residues in Danish slaughter pigs, and these surveillance data indicate that the true antibacterial residue prevalence in Danish slaughter pigs is negligible. The question has been raised whether it would be possible to improve the cost-effectiveness of the surveillance programme. This was addressed in this study.

A Bayesian model was developed and used to investigate the impact of a potential risk-based sampling approach to the residue surveillance programme in Danish slaughter pigs. Residue surveillance data covering the period from 2005 to 2009 were used. The probability of detecting at least one confirmed sample presenting residues above Maximum Residue Limits (MRLs) was modelled, for different sample sizes and prevalence scenarios.

For the current prevalence scenario and a sample size of 20,000 samples, the probability of detecting at least one pig presenting residues above MRLs was high (>70%) but below 95%, due to the very low antibacterial residue prevalence in Danish slaughter pigs. However, potential risk-based scenarios suggest that, if sampling is targeted to high-risk slaughter pigs, where the prevalence is at least 10 times higher than the average prevalence in slaughter pigs in 2009, a higher probability of detection can be achieved, even when the sample size is reduced to 10,000 samples (>95%).

Use of a risk-based approach is likely to increase the cost-effectiveness of the overall antibacterial residue surveillance programme in Danish slaughter pigs. Further research should focus on the identification of high-risk pigs/herds potentially presenting a higher likelihood of non-compliance with antibacterial use requirements. High-risk pigs might be identified based on clinical appearance, and high-risk herds might be identified based on data describing antibacterial use and/or post-mortem meat inspection data. A similar approach might be considered for surveillance for antibacterial residues in Danish sows.

In conclusion, the model results suggest that if high-risk slaughter pigs can be identified, the number of samples from slaughter pigs can be largely reduced while achieving the same or increased probability of detection.

Introduction

Residues of pharmacological active substances or their metabolites might be found in food products from food-producing animals, as a result of veterinary use. Accordingly, Maximum Residue Limits (MRLs) for pharmacological active substances in foodstuffs of animal origin are established to assure high food safety standards (Regulation (EC) No 470/2009; Commission Regulation (EU) No 37/2010). EU Member States are required to implement residue surveillance in live animals and animal products (Council Directive 96/23/EC). The EU requires that 0.03% of the livestock population is tested for residues. Additionally, according to EU requirements, national residue surveillance plans should be targeted to high-risk animals.

In Denmark, a residue surveillance programme has been in place since 1972. Each year, 0.1% of the number of slaughter pigs and more than 1% of the sows slaughtered in the previous year are tested for antibacterial residues. The majority of

the samples are taken as a part of the slaughterhouses' own check programmes. Annually, more than 20,000 samples are collected from finisher pigs. Data collected over the last 10 years indicate that antibacterial residues in Danish finisher pigs are found at a very low prevalence corresponding to around 0.01% (Table 1).

The question arose whether it would be possible to improve the cost-effectiveness of the surveillance programme. This was addressed in a project conducted at the Danish Agriculture & Food Council in 2010.

Material and Methods

A Bayesian model was used to investigate the impact of a potential risk-based sampling approach to the residue surveillance programme in Danish slaughter pigs. Bayesian modeling is a statistical method where information obtained a priori (i.e. through experiments or test evaluations), expressed as probability distributions, is combined with the observed data to form the posterior distribution. The posterior distribution thus reflects both the uncertainty of the prior knowledge as well as the information in the data, i.e., the higher the sample size, the higher the precision of the prior distribution. There are several examples of the use of Bayesian analyses in modern veterinary epidemiology. Among the commonly used examples are the estimation of true prevalence (Branscum et al., 2004) and evaluation of diagnostic tests in the absence of gold standard (Branscum et al., 2005).

Residue surveillance comprises the use of screening and confirmatory tests. Danish surveillance data from 2005-2009 (Table 1) and prior knowledge about true prevalence (uniform distribution: zero to 0.01); screening test sensitivity (beta distribution: mode 0.90 and 5th percentile 0.80) and specificity (beta distribution: mode 0.95 and 5th percentile 0.90) were included in the model. For the confirmation test, sensitivity and specificity were assumed to be perfect. The priors and the data were combined in a model to estimate the posterior true prevalence of samples with residues above the MRL value. Using the posterior true prevalence for 2009 as a basis, different hypothetical risk-based scenarios were used to estimate the probability of finding at least one sample presenting antibacterial residues above MRLs for selected sample sizes. To illustrate targeted sampling to high-risk slaughter pigs, the prevalence of antibacterial residues was increased five and ten times, respectively, compared to the antibacterial residue prevalence found in slaughter pigs in 2009.

Table 1. Antibacterial residue surveillance data in Danish slaughter pigs, 2005-2009.

Year	Total number of samples	Number of positive screening/confirmed* samples	Substances found either during screening or confirmation
2005	19,912	4/0	
2006	18,965	2/1	Amoxixillin, benzylpenicillin
2007	18,416	2/1	Tilmicosin, benzylpenicillin
2008	23,615	3/2	Benzylpenicillin, doxycycline
2009	22,498	3/1	Doxycycline

* the residues found were above the MRL value for confirmed samples

The model and the simulations based on the model were carried out using OpenBUGS (Lunn et al., 2009). The first 10,000 iterations were discarded as burn in and the following 10,000 iterations were used for inference. Convergence was assessed by checking plots of the sampled values as time series, as well as auto-correlation plots (Toft et al., 2007). The probability of detecting at least one truly positive sample was estimated as the proportion of iterations where at least one positive MRL was detected by the screening test, i.e., was present in the sampled pigs and subsequently identified by the screening test.

Results

Table 1 presents the total number of samples collected in the Danish residue surveillance programme and the number of positive samples (residues above MRLs) in slaughter pigs from 2005-2009. Table 2 presents the posterior estimates for the probability of detecting at least one sample presenting residues above MRLs, for different sample sizes and for different prevalence scenarios.

For the current prevalence scenario (median 0.008, 95% CPI[0.001-0.027]) and a sample size of 20,000 samples the probability of detecting a pig presenting residues above MRLs was high (>70%) but below 95%, due to the very low

antibacterial residue prevalence in slaughter pigs. However, when prevalence is increased, resembling a targeted sampling approach to high-risk slaughter pigs, a higher probability of detection can be achieved, even when the sample size is reduced to 10,000 samples (>95%).

Table 2. Posterior estimates of the probability of detecting at least one confirmed sample presenting residues above MRLs for the current situation as well as two different prevalence scenarios and different sample sizes in Danish slaughter pigs

Surveillance scenario	MedianTrue Prevalence	Sample size	Probability of detecting at least one positive[b] sample (%)
Current	0.008[a]	20,000	72
		10,000	52
		5,000	33
Risk-based (x5)	0.04	20,000	96
		10,000	90
		5,000	77
Risk-based (x10)	0.08	20,000	99
		10,000	97
		5,000	90

[a] *median true prevalence found in Danish slaughter pigs in 2009 (95% CPI [0.001-0.027]*
[b] *residues confirmed above the MRL value*

Discussion

In this study, we used a Bayesian model to combine the prior knowledge about the individual components of the Danish antimicrobial residue surveillance programme and the surveillance data, into an assessment of the true prevalence of residues above MRL. This enabled us to give an estimate of the probability of detecting at least one positive sample, while taking into account the uncertainty known about the individual components of the system.

The efficiency of the surveillance programme was modelled as the ability of detecting at least one sample presenting residues above MRLs. This was chosen because it reflects the current number of positive findings which is between one and two (Table 1). Hence, it will be feasible to explain to consumers and trade partners that the safety of the programme remains unchanged, because approximately the same number of positive samples will be identified in a hypothetical risk-based programme as in the current programme.

According to the current surveillance programme, the prevalence of residues of antibacterials in Danish finisher pigs is very low (~0.01%). A risk assessment by Baptista et al. (2011) - presented elsewhere in this proceeding - shows that the human health risk associated with antibacterial residues in Danish pork is low to negligible in sows and negligible in slaughter pigs. Hence, food safety is secured; however, the current surveillance does not seem to be very cost-effective. Our results strongly indicate that a risk-based approach to antibacterial residue surveillance in slaughter pigs should be further investigated, targeting sampling to high-risk pigs or high-risk herds. Hereby, the cost-effectiveness of the overall antibacterial residue surveillance programme would be increased. This is in agreement with previous studies evaluating a risk-based sampling strategy for residue surveillance in Swiss calves (Presi et al., 2008).

Further research should focus on the identification of high-risk pigs or herds potentially presenting a higher likelihood of non-compliance with antibacterial use requirements. High-risk pigs might be identified based on clinical appearance, and high-risk herds might be identified based on antibacterial use and post-mortem meat inspection data. In the Netherlands, a risk-based sampling for residues in pigs is in place. Here, data from meat inspection are used (A. Jelsma, personal communication 2010). A similar approach is expected to be investigated in Denmark. Moreover, a new policy in Denmark called the "Yellow Card Scheme" has recently been implemented. Pig producers using more than twice the average of the antimicrobial consumption will receive a yellow card which among others implies restrictions on future use of antimicrobials (Anon., 2011). It has been suggested to include animals from yellow card farms in a future risk-based surveillance for residues.

Conclusion

A potential risk-based sampling approach to antibacterial residue surveillance in slaughter pigs might allow reducing the sample size largely, while increasing or maintaining the probability of detection. Risk-based antibacterial residue surveillance might be a more cost-effective approach compared to the current surveillance in Danish slaughter pigs.

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Modelling of Salmonella dynamics in the pig slaughterhouse

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Abstract

The burden of Salmonella entering pig slaughterhouses across the European Union (EU) is considered to be of public health significance. Therefore, targets will be set for each EU Member State (MS) to reduce the prevalence of Salmonella infection in pigs at slaughter. In order to meet the set target, each MS will need to develop a National Control Plan (NCP). As part of the evidence base for the development of NCPs, a Quantitative Microbiological Risk Assessment (QMRA) was funded under an Article 36 grant to support the scientific opinion required by the EC from the European Food Safety Authority (EFSA) and andopted by the BIOHAZ panel.

This presenation will detail our approach to a quantitative risk assessment for Salmonella in the pig slaughter chain. Attention will be devoted to the microbial processes involved in each of the phases during slaughter (e.g. inactivation, cross-contamination). For each of the microbial processess we describe how to incorporate variability (both over individual carcassess and over slaughterhouses), using the mathematics of recursive relations and Monte Carlo simulations.

We will demonstrate the suitability of such a quantitative model for implementations of interventions in the slaughterhouse environment. Furthermore we present some results, in terms of prevalences and concentrations throughout the slaughter chain, and compare these results to data available from the literature.

A Quantitative Microbiological Risk Assessment for Salmonella transmission in pigs in individual EU Member States

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Abstract

A farm-to-consumption quantitative microbiological risk assessment (QMRA) for Salmonella in pigs has been developed for the European Food Safety Authority. The primary aim of the QMRA was to assess the impact of reductions of slaughter-pig prevalence and the impact of important control measures applied at the farm and during transport, lairage and slaughter on the number of human cases of salmonellosis. The QMRA estimates the risk of salmonellosis and number of human cases for three product types: pork cuts, minced meat and fermented ready-to-eat sausages.

For four case study European Union Member States (MSs) the average probability of illness was estimated to be between 1 in 100,000 and 1 in 10 million servings given consumption of one of the three product types. The total numbers of cases attributable to the three product types was also estimated. The results from the intervention analysis suggest that specific slaughterhouse interventions are currently best placed to produce consistently large reductions in the number of human cases and that for high breeding prevalence MSs reducing infection on breeder farms would seem to be an important on-farm control measure.

Introduction

Under Article 36 of the European Parliament and Council Regulation (EC) No 178/2002 (EC, 2002), the European Food Safety Authority (EFSA) published a call and funded a "Quantitative Microbiological Risk Assessment (QMRA) on Salmonella in slaughter and breeder pigs". This QMRA was developed to provide evidence to a Scientific Opinion from the EFSA Panel on Biological Hazards (EFSA, 2010a) which would assist the EC on setting targets for Salmonella in Pigs and individual European Union (EU) Member States (MSs) with the development of a MS-specific National Control Plan (NCP). The full report of this QMRA is available on the EFSA website (EFSA, 2010b).

The aims of the QMRA were to assess the impact of (hypothetical) reductions of slaughter-pig prevalence and the impact of important control measures applied at the farm and during transport, lairage and slaughter on the number of Salmonella cases in humans; the sources of infection for fattening pigs at the farm level and the impact of transport, lairage and slaughter processes on the contamination of carcasses.

Material and Methods

In order to facilitate the investigation of interventions at different points of the food chain, a farm-to-consumption framework was adopted, so that we could model the prevalence of infection / contamination and the microbial load from the (breeding) farm to the point of consumption (exposure). The probability of human illness was then estimated by applying a dose-response model using the estimated amount of Salmonella bacteria ingested as an input.

EFSA requested that the QMRA should characterise the variability between EU MSs and, in particular, the inclusion of variability between MSs in their pig farms, slaughterhouses and consumption patterns; this presented numerous challenges. These challenges were overcome by the development of a generic model with a clearly defined set of parameters that may vary between MSs, the values of which can be easily input for any specific EU MS. To demonstrate the parameterisation and use of the model, four MSs were selected as case studies (MS1, MS2, MS3 and MS4).

The exposure assessment was split into 4 modules: Farm; Transport & Lairage; Slaughter & Processing and Preparation & Consumption. The output from one module is the input to the next and so collectively they model the entire farm-to-consumption chain. Efforts were made to take into account the natural variation of Salmonella infection and/or contamination in the modelling. This was done by, wherever possible, allowing for stochastic variation of parameter values. Consequently, as much as possible, variability within and between batches of pigs, farms, transport vehicles, slaughterhouses, cutting plants, retail outlets and consumer practices, both within and between MSs, was described. Three product types are included in the QMRA: pork cuts, minced meat and fermented ready-to-eat sausage. These products were chosen to represent a range of different production/preparation practices and consumption patterns, which will affect the Salmonella levels within these products at consumption and hence the probability of human illness. Within the mandate, EFSA were asked "to consider all serovars in pigs that are of human health significance", and hence the QMRA considered only Salmonella spp. as a group, rather than distinguishing between serotypes. The risk assessment was parameterised using data from the published and unpublished literature and, where necessary, expert opinion.

Results

The results of the QMRA are summarised in Tables 1 & 2. For all four MSs the average probability of illness is between 1 in 100,000 and 1 in 10 million servings given consumption of one of the three product types. MS2 is predicted to have a higher probability of illness. For all of the MSs, the product with the highest probability of illness per serving is fermented sausage. The lowest risk per serving is associated with pork cuts (MS1, MS2) and minced meat (MS3, MS4). The total number of cases attributable to each of the three product types was also estimated. However the QMRA appears to overestimate the number of cases, which can be attributed to a variety of factors including a lack of data regarding immunity and the dose response relationship, and the assumption that all Salmonella spp. were to be regarded as a potential public health threat.

Table 1: Baseline results from the QMRA: mean probabilities of illness by eating one serving of pork cuts, minced meat or fermented sausage in the 4 case study MSs.

Member State	$P_{illness}$		
	Pork Cuts	Minced Meat	Fermented Sausage
MS1	7.65×10^{-07}	8.84×10^{-07}	1.87×10^{-06}
MS2	1.86×10^{-05}	2.24×10^{-05}	4.25×10^{-05}
MS3	3.88×10^{-07}	2.32×10^{-07}	5.78×10^{-07}
MS4	2.55×10^{-06}	2.58×10^{-07}	4.29×10^{-06}

Table 2: Number of cases, per year, attributed to pork cuts (PC), minced meat (MM) and fermented sausage (FS), for the four case study MSs.

	MS1	MS2	MS3	MS4
No. of predicted cases by PC/year	520	9,802	1,162	1,384
No. of predicted cases by MM/year	125	11,148	182	56
No. of predicted cases by FS/year	375	4,298	165	1,246
Total no. of predicted cases (PC + MM + FS)/year	949	25,248	1,509	2,686
Predicted number of cases per 100,000 habitants	12	42	4	26

A key part of the QMRA was the investigation of interventions (for further information see Hill et al. 2011b). From the intervention analysis it was concluded that certain farm intervention mechanisms (such as reducing the susceptibility of the pig to infection, possibly by vaccination or organic acids) may produce significant changes in the slaughter pig prevalence, although evidence that specific farm interventions consistently work was sparse. In addition, considering the results from the farm model (see Hill et al 2011a), the model results lead us to suggest those MSs with a high breeding herd prevalence should focus on these herds in order to reduce the burden of infected new stock entering the weaning/growing/finishing stages (as these new stock are the main source of infection for slaughter pigs). Likewise it was concluded that MSs with low breeding herd prevalence should focus their attentions on reducing contamination of feed. From

the current evidence, it would appear that specific slaughterhouse interventions are currently best placed to produce consistently large reductions in the number of human cases. However, the multiple farm and abattoir intervention scenarios investigated here suggest that MSs can achieve larger reductions by targeting farm and slaughterhouse together. Reducing the prevalence at farm level is also considered important for preventing the transmission of Salmonella from pigs to other livestock species such as laying hens and broilers, where the prevention and control efforts are focused on the farm.

The intervention analysis described above highlighted a fairly proportional relationship between slaughter pig prevalence and risk of human illness. This was unexpected given the non-linearities included in the model, especially cross-contamination at the abattoir. However, further analysis (not shown here) has shown that arguably the most important non-linearity captured within the model, cross-contamination at the abattoir, is dominant only at lower levels of carcass contamination. These lowly-contaminated carcasses contribute only a small proportion of the overall risk (where most of the human risk comes from highly-contaminated carcasses), and hence cross-contamination is not important in terms of affecting human risk.

Discussion

Similar to other farm-to-consumption QMRAs (Havelaar et al 2008) the model probably overestimates the number of human cases. However it should be noted that there are uncertainties associated with the reported number of cases due to, for example, potentially significant under-reporting the level of which will vary between MSs. The validity of the model at earlier stages within the farm-to-consumption chain was assessed by comparing the QMRA estimated prevalence to the observed prevalence at the point of lairage (from EFSA slaughter pig baseline survey) and retail (MS surveys); the QMRA outputs were deemed to be plausible at these two points. Consequently, it is likely that factors such as the lack of data regarding immunity, the dose-response relationship and the assumption of all Salmonella spp. being equal are contributing to this potential overestimation. However the QMRA still allows for the prediction of the relative impacts of different interventions during the Farm, Transport, Lairage and Slaughterhouse stages, which was the main purpose of the QMRA.

During the development of the baseline model a number of data gaps/deficiencies were identified; some of which were assessed in an uncertainty analysis to have an important impact on the probability of illness. It is recommended that further data generation is undertaken in order to provide improved estimates for the parameters identified as uncertain and influential. The identification of such data gaps is a positive feature of any risk assessment model and many risk managers utilise such information to direct future research.

In relation to the intervention analysis, it is important to note that there was very inconsistent evidence to suggest whether any of the farm interventions can be consistently applied to produce either the required reduction in environmental contamination or the required increase in a pig's resistance to infection. Probably of extreme importance, but not investigated here, is the rate of uptake and correct application of interventions by farmers – if this is not universal across a MS the effect in reducing human illness will be reduced.

Conclusion

In conclusion, a QMRA has been developed that will assist the EC on setting targets for Salmonella in pigs and individual EU MSs with the development of a MS-specific NCP. The QMRA characterises the variability between EU MSs and in particular, the variability between pig farms, slaughterhouses and consumption patterns. This was achieved by developing a generic EU model with a clearly defined set of parameters that may vary between MSs, the values of which can be easily input for any specific MS model. Using the QMRA to perform an intervention analysis we have shown, theoretically, that large reductions in the number of pig-meat attributable cases of Salmonella within a MS can be achieved via intervention at either the farm and/or slaughterhouse level.

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Ranking of food safety risks in pork from organic and free-range production systems

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Abstract

The objectives of this semi-quantitative risk assessment were to identify, assess and rank food safety risks in outdoor pig production (organic and free-range) compared to indoor pig production (conventional) in Denmark. In addition, high-risk pork products would be identified. Finally, risk-reducing strategies for handling the identified agents would be suggested. Data were obtained from the literature as well as in-house statistics. Data describing tetracycline-resistant E. coli in outdoor pigs were available from the Qualysafe project. The OIE framework for risk assessment was applied.

No differences were identified between indoor and outdoor pig production in regard to Salmonella, Campylobacter, Y. enterocolitica and T. spiralis (for the latter, all carcasses are tested). Humans might acquire T. gondii from consumption of sub-optimal heat-treated pork (core temperature <61°C) or pork products not previously frozen, lightly cured (<3.7% salt), smoked, or fermented where the meat originate from finishers or sows in outdoor-productions. Most of the pork intended for such productions is frozen prior to processing, limiting the risk to humans. The largest uncertainty was related to the likelihood of survival of T. gondii in the above mention products. Further research is therefore required.

Introduction

Healthy food, environmentally-friendly production and animal welfare are regarded by the public as being taken care of in a better way in organic and free-range production systems compared to conventional systems. The environmental-exposure for production animals in these systems could, however, lead to transmission of pathogens from wild to domestic animals and subsequently to humans. Food safety might then be compromised.

Only about 1.3 % of the around 20 million pigs slaughtered annually in Denmark originates from a free-range or organic production system. It is, however, a political goal to double the agricultural area used for organic agriculture production in Denmark before year 2020. In free-range and organic productions, sows and piglets are kept outdoor all year on fields in huts that act as shelters. Most of the finishers are kept indoor where they get water and feed, but have access to the outdoor environment. Today, many of the zoonotic enteric agents that might be present in the environment of domestic pigs are, at least to some extent, controlled by blockage of the transmission ways i.e.; treatment of sewage and manure, heat treatment of feed, ground water supply. Denmark has obtained an official EU status as area with negligible prevalence of Trichinella spp in domestic pigs. Testing for Trichinella is still conducted for all pigs irrespective of production system, because of trade requirements.

In Denmark, most of the organic and free-range pigs are slaughtered at one specific abattoir and no specific hygienic measures are taken when slaughtering these pigs. The withdrawal time for animals treated with antimicrobials in organic is the double of what is required in the conventional production, limiting the incentive to use antimicrobials in this production system. About 67% of the Danish organic pork production is exported to Germany, France or the United Kingdom. Moreover, organic pork is imported from the Netherlands to Denmark for production of products such as cured and fermented sausages.

Many outdoor pig herds are small; and are therefore not included in Salmonella sero-surveillance programme (requires > 200 finishers per year). However, the pork from these pig-herds is covered in the microbiologic surveillance conducted at all Danish pig abattoirs.

The objectives of this semi-quantitative risk assessment were to identify, assess and rank food safety risks in Danish pig production (conventional versus organic and free-range). In addition, high-risk pork products would be identified. Finally, risk-reducing strategies for handling the identified agents were suggested.

Materials and Methods

Data were obtained from the literature as well as in-house statistics. Data describing tetracycline-resistant E. coli in outdoor pigs were available from the Qualysafe project.

Risk assessment framework

A risk assessment (RA) framework given by the OIE was adopted with some modifications, and data were identified by conducting a literature search. The hazards included were zoonotic pathogens in pigs described in the veterinary scientific literature. Use of antimicrobials, antibiotic residues in pork and development of antimicrobial resistance were also evaluated to a certain extent.

The outcomes from each of the three steps of the risk assessment; release, exposure and consequence, were categorised into four groups: 0-3 (Negligible, Low, Medium or High). A similar scale was used for uncertainty in the outcomes. The final relative risk estimates were obtained by multiplying outcomes from the three steps. These risks are only relevant in the context of comparing one to another.

Table 1. Matrix showing possible outcomes of the assessment of Release and Exposure

		Exposure			
		Negligible	Low	Medium	High
Release	Negligible	0	0	0	0
	Low	0	1	2	3
	Medium	0	2	4	6
	High	0	3	6	9

Table 2. Matrix showing possible outcomes of the assessment of Release, Exposure and Consequences

		Consequences			
		0	1	2	3
Release x	0	0	0	0	0
Exposure	1	0	1	2	3
	2	0	2	4	6
	3	0	3	6	9
	4	0	4	8	12
	6	0	6	12	18
	9	0	9	18	27

Results

Hazard identification

Hazards included were: Salmonella, Campylobacter, Y. enterocolitica, Verotoxin producing E. coli (VTEC) and Brucella suis, T. gondii, T. spiralis, Taenia solium, Ascaris suum, Cryptosporidium, Giardia and zoonotic virses: Hepatitis E virus (HEV), Infuenza virus and Noro virus. Use of antimicrobials, antibiotic residues and development of antimicrobial resistance were also evaluated to a certain extent.

Release/exposure assessment

At current, the most important risk of introduction of Salmonella into a pig herd in Denmark is regarded as being purchase of infected pigs. The environmental infection pressure of Y. enterocolitica seems also to be of less importance, also, pointing to trade of animals as the main factor for introduction of this zoonotic agent.

A high prevalence of Campylobacter in the live pig does not seem to play a role for food safety because of the major decimation of Campylobacter that happens during chilling (blast, tunnel or batch) of carcasses and further during storage at low temperatures. Campylobacter isolated from pork is mainly C. coli, and only 5-10% of the human cases of campylobacteriosis are caused by this serotype.

Studies of E. coli shows that the prevalence of tetracycline-resistance in E. coli is lower in the organic than in the free-range and conventional production – ref ecting the lower amount of tetracycline used in outdoor pig production compared to conventional.

Toxoplasma gondii, Brucella suis and Trichinella might be present in wildlife. Hares might be infected with B. suis, whereas rodents, foxes and wild boar might be infected with Trichinella. However, at current there are no free-ranged wild boars in Denmark, and the prevalence of Trichinella in foxes is around 0.1%. For T. gondii, the cat is the final host whereas pig, sheep, goat and poultry are intermediate hosts.

The prevalence of T. gondii in conventional pigs is low. In outdoor-reared pigs the prevalence is assessed as being higher – especially in the sows because they are exposed to infected cats and rodents for a longer time. The results of a project conducted in 2009 in Denmark indicated that about 40% of outdoor-reared pigs have antibodies against T. gondii.

Pigs that are kept outdoor could be exposed to hares that harbour Brucella suis. However, the prevalence of this infection is currently regarded as negligible in organic and free-range pigs in Denmark. The last reported case of brucellosis in an outdoor pig farm in Denmark was in 1999 – and the infection was regarded as originating from hares. Moreover, Trichinella has not been found neither in outdoor nor in indoor-reared pigs for more than 75 years.

The prevalence of VETC is very low and T. solium is not found in Denmark. Ascaris and inf uenza were assessed as not being food-borne. Contact with pig faeces could, however, be a risk. For Listeria and Noro-virus, other sources than raw pork was regarded as more important.

Table 3. Outcome of a semi-quantitative risk assessment and relative ranking of food safety hazards in pig production (probability: 0= negligible, 1= low, 2= medium and 3= high). The relative risks might be compared between indoor and outdoor production. The uncertainty is given in brackets

Hazards	Release	Exposure	Consequence	Relative Risk (uncertainty)	
				Free-range	Indoor
Salmonella	1	1	1	1 (1)	1 (1)
Campylobacter	3	0	1	0 (2)	0 (2)
Y. enterocolitica	3	1	1	3 (2)	3 (2)
T. gondii	2	2	3	12 (3)	3 (1)

Consequences of a human Toxoplasma gondii infection
The most common symptom of toxoplasmosis in humans resembles inf uenza. However, toxoplasmosis in pregnant women might lead to abortion or foetal abnormalities. The prevalence of congenital toxoplasmosis in newborn in Denmark were screened from 1997-2007, revealing that 1.6 per 10,000 liveborn infants with congenital toxoplasmosis. Immune-compromised, e.g. HIV patients could also be affected seriously by toxoplasmosis.

Risk ranking
No differences were identified between indoor and outdoor pig production in regard to Salmonella, Campylobacter, Y. enterocolitica and T. spiralis (all carcasses are tested). However, the risk associated with T. gondii is larger in pork from outdoor compared to indoor production (Table 3).

Specific risk products and risk mitigation
Humans might acquire T. gondii from consumption of sub-optimally heat-treated pork (core temperature <61°C) or not previously frozen, lightly cured (<3.7% salt), smoked, or fermented products ready-to-eat, originating from finishers or sows

in outdoor-productions. Freezing or heat treatment will kill T. gondii cysts present in the meat destined for such productions. The largest Danish producer of free-range and organic pork production reports that there is no production of risk products based on free-ranged or organic produced pork in Denmark. Additionally, eighty percent of the pork used for sausage production in Denmark is pre-freezed (-12°C) and hence, the risk for T. gondii cysts is reduced to a large extent. The largest uncertainty was related to the likelihood of survival of T. gondii in the above mention products. Further research is therefore required in particular with respect to effect of salt on the survival of the parasite.

Discussion

The zoonotic risk associated with pork from outdoor pig production seems to be very equal to pork from conventional production. The low environmental pressure in Denmark might be explained by blockade of important transmissions routes for Salmonella, i.e. heat treatment of feeding, treatment of sewage and use of ground water. Salmonella and Y. enterocolitica as well as other of the included pathogens (e.g. Cryptosporidium) seems to survive well in water and manure. There are, however, many data gaps in regard to recirculation of the included pathogens in the ecosystem. More data from Danish wildlife, sewage and water are needed before more definitive conclusions can be drawn about the level of pathogens in the ecosystem and the infection pressure from the environment.

The most important route of transmission of T. gondii to humans is considered contact with oocyst in cat faeces (contaminated soil, vegetables and handling of indoor cat-toilets). Eating under-cooked mutton is also recognized as a transmission route for T. gondii tissue cysts for humans. Contrary, pork is usually heat-treated to a core temperature >61°C. Therefore, pork has been ascribed a very low risk from the 1970's and onwards when the indoor system for pig production was almost fully adopted.

Only surface decontamination methods are implemented at abattoirs. Hence, Toxoplasma cysts (which are harboured on the inside of the muscle) are not eliminated during slaughter, if present. Cysts are killed during heat-treatment. There are, however, some products like lightly cured and smoked filets and fermented sausages mildly cured (~ low salt content) where T. gondii could survive if present. To control this, pork used for processing of such products might be subjected to freezing for 24 hours at -12°C. This is widely practiced in Denmark. Knowledge about survival of T. gondii in smoked, lightly-cured and/or fermented or dried products is, however, limited. This is unfortunate because consumption of rose-cooked pork is on the increase as well as consumption of pork products with a low content of salt.

Conclusion

No differences were identified between indoor and outdoor pig production in regard to Salmonella, Campylobacter, Y. enterocolitica and Trichinella. The risk of humans acquiring infection with T. gondii is slightly increased when consuming sub-optimally heat-treated pork (core temperature <61°C) or not previously frozen, lightly cured (<3.7% salt), smoked, or fermented products originating from finishers or sows in outdoor-productions compared to similar pork for indoor production. This risk is mitigated when the pork is frozen prior to processing, and this is widely

National baseline surveys to characterise processing hygiene and microbial hazards of Australian culled sow meat, retail pork sausages and retail pork mince

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Abstract

Pork products were sampled at retail to determine the impact of further processing on hazard levels to which consumers may be exposed, compared to carcases . Surveys of 116 fresh pork sausages and 148 fresh pork mince samples were purchased from supermarkets (n=87, n=105) and butcher shops (n=29, n=43), respectively. For sausages, concentrations of TVC averaged 4.6 log10 cfu/g. The E. coli prevalence was 16.4% (95% CI: 10.2-24.4%) with average count of 0.65 log10 cfu/g. The prevalence of coagulase positive Staphylococci was 3.4% (95% CI: 0.9-8.6%) and that for Listeria monocytogenes was 16.4% (95% CI: 10.2-24.4%), although none of the samples exceeded levels of 100 cfu/g. The prevalence of Salmonella was 8.6% (95% CI: 4.2-15.3%). For pork mince mean TVC were high (6.2 log10 cfu/g) and a 4 log variation in TVC was observed from product with the same number of days until the use-by-date. In addition commercial retail criteria, which specify a maximum TVC of 6 log10 cfu/g up to the end of shelf life, were not met by 54.1% of samples (including 50.5% of supermarket samples), an indication of possible cool chain issues. E. coli (6%), coagulase positive Staphylococcus (1.3%), Campylobacter spp. (2.7%) and Salmonella spp. (1.5%), were detected. No E. coli O157:H7, MRSA or Yersinia enterocolitica were detected (i.e. prevalence <2.5%).

Introduction

Based on consumer surveys, it is estimated that in Australia there are around 730 million annual servings of sausages alone, with a beef:pork ratio of 53:47. In addition, in recent years there has been a marketing push by the pork industry to encourage the use of minced meat to increase per capita consumption. Food borne outbreaks associated with pork in Australia are mostly due to Salmonellosis from the food service sector (Pointon and Horchner 2010).

Surprisingly, given the level of consumption, there have been few studies of the microbiology of comminuted meats in the retail chain, which could help indicate if improvements achieved in abattoir processing hygiene have translated to similar low levels in pork retail products or whether further improvements are required. Changes in marketing strategy to increase per capita consumption should take into consideration hazard levels and whether further processing steps will act as contributing factors to consumer risk. Consequently, between 2008 and 2010 national microbiological benchmarking studies were conducted on pork sausages and pork mince at retail.

Materials and Methods

Sampling Methods

A total of 116 pork sausages and 148 samples of pork mince were purchased nationally (x 0.5 Kg) from butcher shops (n=29 and n=43, respectively) and supermarkets (n=87 and n=105, respectively). Sample numbers were allocated approximately proportionally on a population basis to the five largest capital cities in Australia as determined by the Australian Bureau of Statistics 2006 Census (Anon., 2006). Sampling of butcher shops and supermarkets, in each city, was in the approximate ratio 1:3 to reflect estimated retail volumes and potential consumer exposure.

Sausage samples were tested for TVC, E. coli, Salmonella spp., Staphylococcus aureus, Listeria spp.
Mince samples were tested for TVC, E. coli, E coli O157:H7, Salmonella spp., Staphylococcus aureus, Methicillin Resistant Staph. aureus (MRSA), Campylobacter spp. and Yersinia enterocolitica.

Laboratory Methods

Samples were placed in insulated containers with ice bricks and transported overnight to the laboratory for testing within 24 hours of collection.

Total Viable Count (TVC) and E. coli Count: 25g of sow meat/mince, pork sausage or pork mince was diluted 1:10 (w/v) with buffered peptone water, stomached for 60 seconds and 10-fold serial dilutions prepared in Peptone Saline Solution (Media Production Unit, The University of Melbourne). Counts were performed using either 3MTM PetrifilmTM Aerobic Plate Count Plates (25°C/96hr) or 3MTM PetrifilmTM E. coli/Coliform Count Plates (37°C/24 hr) (3M Corporation, St Paul, Minnesota). Colonies were identified and counted as per manufacturer's instructions.

E. coli O157:H7: Samples were tested for the presence of E coli O157:H7 using the BIOCONTROL VIP® Gold for EHEC single step immunoassay (BIOCONTROL, Bellevue, Washington). Mince (25g) was added to 225 mL of pre-warmed modified Tryptone Soya Broth (BIOCONTROL, Bellevue, Washington) homogenised for 120 seconds using a stomacher. This broth was incubated for 18 to 28 h at 35 - 37°C. A 100 µL aliquot was then examined as per the manufacturer's instruction.

Salmonella spp. The remaining 1:10 (w/v) dilution, as described under the TVC/ E. coli section above, was examined as per Australian Standard (AS) 5013.10-2004. All colonies identified as Salmonella spp. were forwarded to The Salmonella Reference Centre at The Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia for serotyping. Staphylococcus aureus: Aliquots (1 mL) from each serial dilution were inoculated onto either 3M™ Petrifilm™ Staph Express Count Plates (3M Corporation, St Paul, Minnesota) and incubated at 37°C for 24 h Colonies were identified and counted as per manufacturer's instructions.

MRSA: A loopful of the overnight buffered peptone enrichment was streaked onto a Brilliance MRSA agar plate (Oxoid, Thebarton, South Australia) and incubated for 18-20 hours at 37°C. The plate was then observed for typical colonies as per the manufacturer's instructions. Suspect colonies were streaked for purity onto Nutrient Agar and screened by polymerase chain reaction (PCR) as per Jonas et al. (2002).

Listeria spp.: A second 25 g sample of sausage meat was examined for the presence or absence of Listeria species following FSIS MLG 8.05 – Isolation and identification of Listeria monocytogenes from red meat, poultry, egg and environmental samples. Levels of Listeria organisms were determined by spread inoculating 0.1 mL aliquots of the 1:10 dilution prepare above onto plates of Modified Oxford (MOX) Agar (Oxoid Ltd., Basingstoke Hampshire England) with the limit of detection 100 cfu/g.

Campylobacter spp.: Mince (25g) was weighed aseptically into a stomacher bag and examined as per AS 5013.6-2004: Examination for specific organisms – Campylobacter.

Yersinia enterocolitica: Mince (25 g) was diluted 1:10 (w/v) in tris-buffered peptone water and homogenized in a stomacher for 1 min with the examination carried out following Roberts D and Greenwood M. 2003. The identity of suspect colonies was confirmed using Microbact 24E incubated at 25°C for 48 h. (J Holds, SA Pathology, Adelaide, South Australia pers. comm.). Those cultures presumptively identified as Yersinia enterocolitica were sent for confirmation, biotyping and serotyping by the Salmonella Reference Centre at the IMVS, Adelaide, South Australia.

Results

A summary of the results of the surveys are presented in Table 1. Mean log10 TVC(cfu/g) in sausages and retail mince was 4.6 and 6.2, respectively. Mean log10 E. coli concentration was 1.63 and 1.33, being detected in 16.4 and 10.1% of samples. Salmonella spp. were isolated from 8.6% and 1.5% of sausages and mince samples, respectively. Serovars included S. typhimurium (3: pt 8, 108, 197), S. rissen (1), S. infantis (2), S. sofia (2), S. agona (1), S. derby (1), S. ohio (1). L. monocytogenes was isolated from 16.4 % of retail pork sausages. E. coli O157:H7, MRSA and Yersinia enterocolitica were not detected in the products tested (Table 1).

Table 1. Summary of overall microbiological results for pork sausages and retail pork mince.

	Pork Sausages # (%)	Pork Mince # (%)
N	116	148
Log_{10} TVC(cfu/g)	4.6	6.2
E. coli prevalence	19 (16.4)	15 (10.1)
Log_{10} *E. coli* (cfu/g)	1.63	1.33
E coli O157:H7	Nt	0 (<2.5)
Salmonella[1]	10 (8.6)	2 (1.5)
L. monocytogenes [2]	19 (16.4)	Nt
Listeria (other)	42 (36.2)	Nt
Staph (Coag. Pos)	4 (3.4)	2 (1.3)
MRSA	Nt	0 (<2.5)
Campylobacter spp.	Nt	4 (2.7)
Yersinia entercolitica	Nt	0 (<2.5)

[1] *Includes S. typhimurium (3: pt 8, 108, 197), S. infantis (2), S. rissen (1), S. sofia (2), S. agona (1), S. derby (1), S. ohio (1).*
[2] *All positive L. monocytogenes < 100cfu/gm. Nt= not tested*
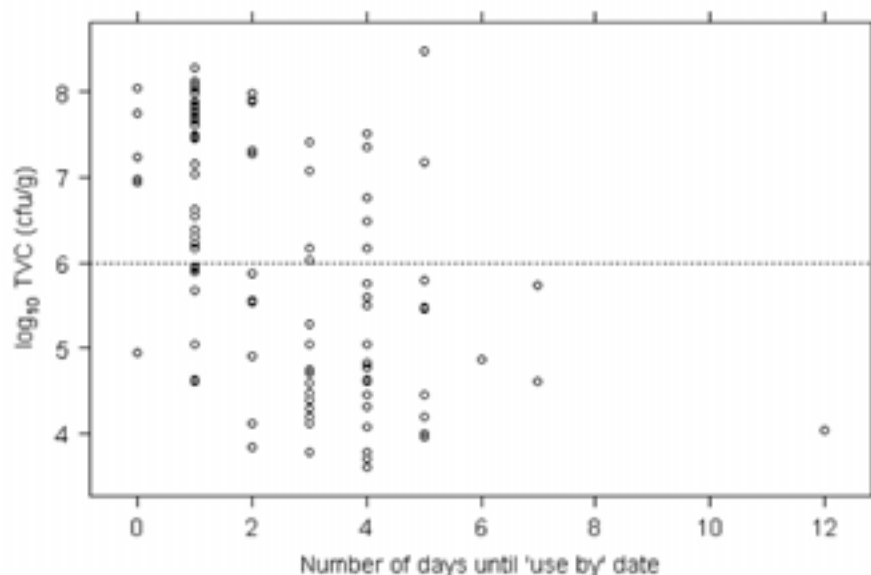
Figure 1. Scatter plot of pork mince TVC versus number of days until 'use by' date

Discussion

A recent National Finisher Baseline Survey (Hamilton et al 2010) indicated Australian pig carcases hygiene was good, with a mean E. coli prevalence of 20.8% and a -0.79 mean log10 cfu/cm2. Mean Salmonella prevalence was 0.4% (Hamilton et al 2011). However, comminuted meats such as sausages and mince by their very nature are problematic from a food safety point of view. Their production entails considerable mixing of lots, potentially leading to wider distribution of contamination and hence increased potential for consumer exposure.

The survey results show that for sausages there is considerable contamination, probably associated with the use of carcase trims, and in the case of retail butcher shops the potential for cross contamination. This point is demonstrated by the isolation of S. sofia, a chicken associated serovar (Pointon et al 2008), from pork sausages and mince in this study. The sausage Salmonella prevalence of 8.6% is considerably lower than the 24% reported in a similar Brazilian study (Mürmann et al 2011) and higher than 2.9% reported from Ireland (Broughton et al 2004). It remains a potential food safety issue if sausages are improperly cooked, when process control is absent at major community events.

For mince the Salmonella levels are relatively low (1.5%) despite the processing hygiene indicators (TVC and E. coli prevalence and counts) being similar to sausages (Table 1). An explanation may be the source of the raw material.

Supermarket mince constitutes the predominant source consumed, and is generally processed centrally from shoulder primal cuts. This may explain why mince Salmonella detection rates more closely re**f**ect carcase levels (Hamilton et al 2011). Similar studies have reported prevalence in pork mince ranging from 0.3% (Delhalle at al 2009) to 12.5% (Duffy et al 2001)

Mince, which unlike sausages is not allowed to contain preservatives, had a mean log10 cfu/g of 6.2. Of particular concern was the fact that for pre-packaged mince with the same number of days to the use-by-date there was a 4 log variation in TVC across samples. In addition commercial retail criteria, which specify a maximum TVC of 6 log10 cfu/g up to the end of shelf life, were not met by 54.1% of samples, including 50.5% of supermarket samples (Fig 1). This is a very significant result that indicates an underlying problem in either mincing hygiene, cool chain abuse and/or an overly optimistic shelf life claim. While mince is widely promoted for use in Bolognese style dishes marketing promotions should be aware of the potential risk associated with end-uses that have less certain hazard reducing steps such as fresh pork burgers.

Conclusion

This study is a prudent reminder that even when carcase hygiene is high by international standards, final hazard levels in retail products and potential consumer risk are subject to a number of contributing factors including carcase levels, use of trim or primal cuts, cross contamination, cool chain temperature abuse and added ingredients.

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Modelling the use of different enforcement strategies to improve food safety

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Abstract

According to the General Food Law, food producers are responsible for the production of safe products. Safe in this regard is often interpreted as compliance to EU food safety legislation. The level of compliance between companies differs and can be improved by measures such as education or sanctions. In order to determine the effectiveness of various enforcement strategies on the level of compliance we developed a simulation tool using Agent Based Modelling (ABM) as a method. This ABM tool allows to simulate with actions and reactions between autonomous agents, yielding an emerging overall effect. This emerging effect will give valuable insight in how the overall behaviour of the system and the individual behaviour of agents mutually depend on each other. As a case study, we focused on the use of antibiotics within primary pig production. The agents in this case were defined as individual farmers and food safety inspectors. Two groups of farmers were indicated: a cooperative, law-abiding versus an egoistic, more fraudulent group of farmers. We looked at the effect of a bonus-malus system and the use of education on these two groups. The ABM approach visually demonstrated that social and financial stimuli are important factors influencing the level of compliance. Furthermore, a certain amount of law-abiding behaviour is needed in combination with a minimum number of food safety inspectors to achieve a pre-set level of compliance and therefore a certain level of food safety.

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Association between serological salmonella monitoring in breeding herds and meat-juice prevalence in sow herds with production of finishers

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Introduction

Several epidemiological studies have studied risk factors for salmonella infections in finisher pigs. Most of these studies have focused on the risk for having a high prevalence, measured either bacteriologically or serologically.

Surveillance programs have been implemented in several European countries, measuring the salmonella prevalence in meat-juice samples. The rationale is that herds should decrease the salmonella prevalence by instituting salmonella reducing production and management strategies, including hygiene, all in-all out production and manipulating the gut flora by the use of organic acids, fermented liquid feed and coarse ground, non-pelleted feed.

Little emphasis has so far been put on the importance of spreading salmonella from herd to herd by the transport of pigs, be it gilts and boars for breeding or weaners and growers for finisher production.

This study investigates the effect of introducing gilts or boars from nucleus and multiplier herds with differing salmonella prevalences into sow herds with a production of finisher pigs.

Material and Methods

Data were collected in 3 different but overlapping time periods from two groups of herds, herds selling gilts and boars and sow herds with own production of finisher pigs for slaughter (integrated sow herds).

Integrated sow herds were divided into two groups based on microbiological results in 2007, herds with an isolation of Salmonelle Typhimurium from pen fecal samples, and herds without isolation of S. Typhimurium.

Serological results from herds selling gilts were obtained from the Danish Zoonosis Register for 2008. 10 blood samples are taken each month from these herds, and analysed in the Danish MIX-ELISA.

For each gilt producing herd, a seroprevalence was calculated as the number of samples with an OD% above 20, dividided by the total number of samples.

From the Danish Movement Database all herds receiving gilts or boars from the gilt producing herds in the period from July 1. 2008 to July 1. 2009, and the number of gilts and boars transported to the integrated sow herd from the gilt producing herd was calculated.

From the Danish Zoonosis register, results from meat-juice samples from the integrated sow herds were obtained for the period October 1. 2008 to September 30. 2009. To avoid the bias introduced by the Danish risk based surveillance, only the first sample taken each month was used in this study.

All integrated sow herds receiving gilts or boars from more than one gilt producer was excluded from the study.

The final data set included 158 gilt producers selling gilts or boars to 646 integrated sow herds.

Initial logistic regression analyses were done using Proc Glimmix (SAS Inst.), including the effect of gilt producer as a random effect, and handling overdispersion on the sow herd level by introducing a residual variation on the sow herd level as a random effect. The dependant variable was the proportion of positive meat juice samples per sow herd.

Two predictors and their possible interaction were investigated statistically. The effect of previous isolation of Typhimurium in the sow herd, and the effect of introducing pigs from gilt producers with different levels of Salmonella, measured as seroprevalence.

Several combinations and transformations of prevalence and number of gilts introduced were investigated, until a satisfactory and interpretable model was found.

To further explore the results, proc nlmixed (SAS Institute) was used to model a zero-inflated model. The code used for the model was modified from Stevenson (2005).

In the zero-inflated model, risk factors can be modelled for two different risks at the same time, the risk of being a positive herd and the risk of having a higher prevalence if positive.

This provides valuable information on the mechanisms affecting the salmonella level in pig herds.

Results

55 (8.5 %) of the sow herds had a positive pen fecal sample in 2007. 5634 meat juice samples were found from the sow herds. 302 meat juice samples were positive.

The best and most interpretable model for the effects of prevalence in the gilt producing herd and number of gilts or boars transported to the sow herd was obtained by calculating the number of seropositive gilts or boars being introduced by multiplying the number of gilts or boars transported by the prevalence from the salmonella surveillance from the gilt producer: Number of salmonella seropositive gilts or boars introduced (referred to as salmonella positive gilts)=Prevalence in gilt producing herd*number of gilts moved to the sow herd.

The final logistic regression model had the following form:

Logit $(y/n)=\alpha+\beta_1$*logN(salmonellapositive gilts+1) + β_2*(positive pen fecal sample in 2007) + β_3*interaction between the two predictors+γ*sow herd+residual variation (1 is added to number of salmonella positive gilts to avoid problems taking the logarithm).

The interaction between the two predictors was significant (p=0.02). For ease of reading, the table is broken up into sow herds with and without salmonella positive pen fecal samples in 2007. The OR for gilts moved into the herd is presented as the OR for doubling the number of positive gilts.

Table 1 summarizes the results from the logistic regression model, and figure 1 displays the results graphically.

The effect of salmonella positive gilts was significant (p=0.01) in sow herds without isolation of Typhimurium in 2007, but insignificant in sow herds with isolation of Typhimurium (p=0.79).

Figure 1. Results from logistic regression model (Glimmix).
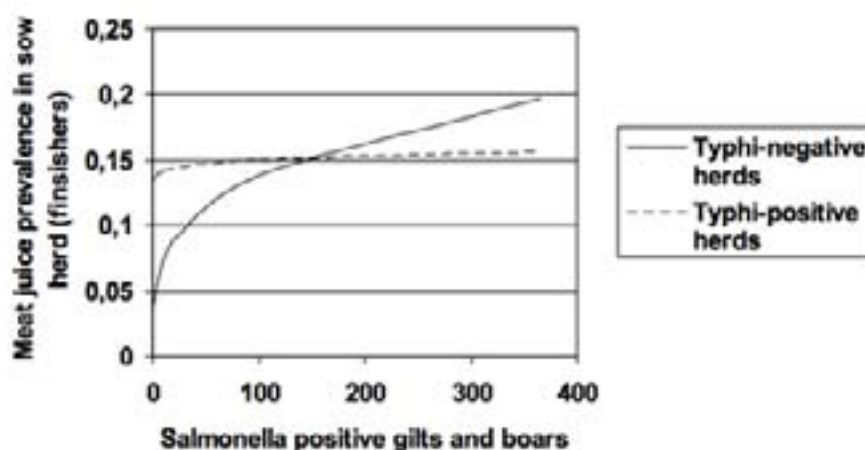
There is little or no effect of introducing negative gilts into positive sow herds, whereas there is a clear efffect of introducing positive gilts into herds without isolation of S. Typhimurium in 2007.

Introducing more salmonella positive gilts and boars into the herd increases the prevalence of positive meat-juice samples in finisher herds from the integrated sow herd.

The results from the zero-inflated models are also presented in table 1, following the same procedure as for the logistic regression model. Figure 2 and 3 presents the results graphically for the zero-inflated model.

The risk of being a positive herd is not influenced by introducing positive gilts into an integrated sow herd (p=0.86), where S. Typhimurium already has been diagnosed, whereas the risk of becoming a positive sow herd increases considerably with the number of positive gilts introduced into herds where S. Typhimurium had not been diagnosed the preceeding year (p=0.02).

The association between prevalence in positive sow herds and introduction of positive gilts is much weaker. For sow herds with previous isolation of Typhimurium the effect was far from being significant (p=0.55), but for herds with no previous isolation, the effect was significant (p=0.03), although relatively small.

Figure 2. Risk for being a positive sow herd depending on number of salmonella positive gilts and boars introduced to an integrated sow herd.
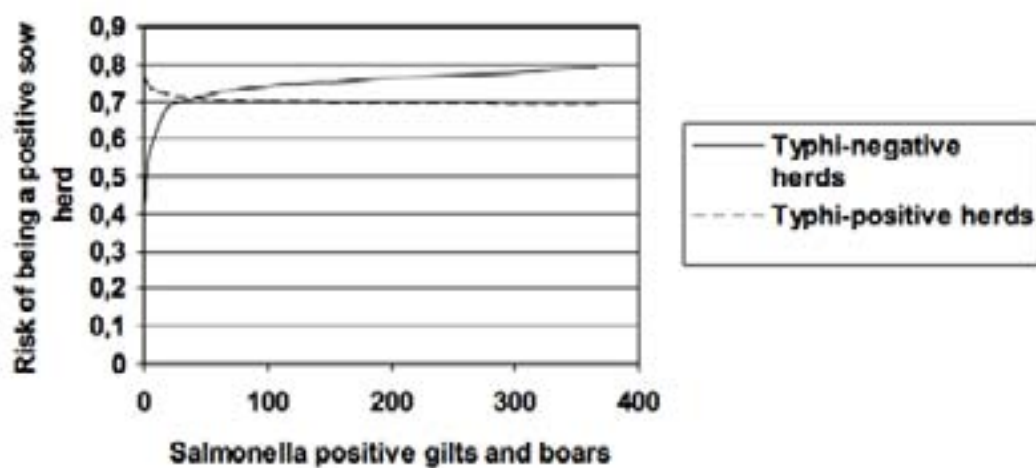
Figure 3. Association between prevalence in positive sow herds and number of salmonella seropositive gilts and boars introduced to an integrated sow herd
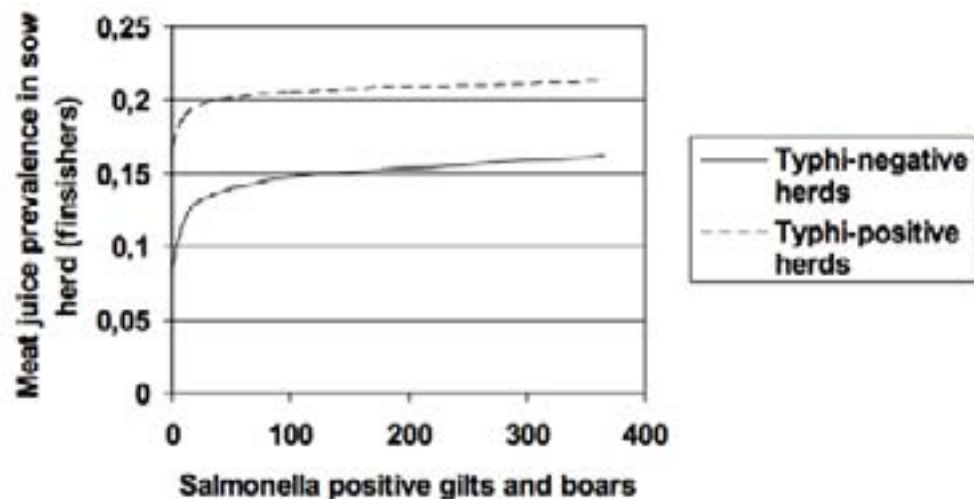
Table 1. Results from logistic regression models

Glimmix model	Sow herds without isolation of Typhimurium in 2007		Herds with isolation of Typhimurium in 2007	
	Estimate (CL)	OR (CL)	Estimate (CL)	OR (CL)
Intercept	-3.33		-1.89	
Log(salpos. gilts+1)*	0.33(0.20-0.45)	1.3(1.2-1.4)	0.03(-0.20-0.27)	1.02(0.9-1.2)
Zero-inflated model +/- part				
Intercept	-0.41		1.1621	
Log(salpos.gilts+1)*	0.37(0.07-0.68)	1.3(1.1-1.6)	-0.05(-0.61-0.51)	-1.0(0.7-1.4)
Zero-inflated model prevalence part				
Intercept	-2.44		-1.64	
Log(salpos.gilts+1)*	0.17(0.02-0.32)	1.1(1.0-1.3)	0.07(-0.17-0.31)	1.0(0.9-1.2)

*OR calculated as $2^{estimate}$, so it expresses the OR for doubling number of positive gilts introduced.

Discussion

A relatively high number of sow herds were positive even when they did not receive gilts or boars from salmonella positive gilt producers. This probably reflects historic introductions into the sow herds before the observation period in this study, or introduction from other sources of infection.

The majority of sow herds were not sampled microbiologically in 2007, so the sow herds without an isolation of S. Typhimurium constitute a mixture of salmonella positive herds and salmonella negative herds, whereas sow herds with a positive S. Typhimurium isolation are positive.

This bias will most probably lead to an underestimation of the effect of bringing in positive gilts to a negative sow herd.

The fact that there was no effect of introducing salmonella positive gilts into sow herds with previous isolation of Typhimurium stresses the importance of protecting negative herds, if a reduction strategy for Salmonella has to be successful.

The risk of becoming a positive sow herd increases relatively fast with an increasing number of salmonella positive gilts. Herds that buy in replacement gilts will often introduce a much higher number of gilts than the relatively low number of gilts needed to pose a risk for the sow herd.

In this study 65 % of sow herds introduced more than 50 gilts pr year. If these gilts come from a positive gilt producer, then even at a relatively low prevalence, a high number of seropositive gilts will be introduced to the sow herd, almost inevitably infecting the sow herd.

Effectively this means that gilt producers should be declared as positive or negative, and negative sow herds should only get their replacement gilts from negative herds.

For positive sow herds little seems to be gained by restricting the introduction of positive gilts. And if a high number of sow herds are already positive, then the over all effect of restrictions on the gilt producer's trade will be limited, unless it is possible to eradicate salmonella from the positive sow herds. A strategy that so far has been shown to be very difficult without depopulation of the sow herd.

Conclusion

Introducing gilts from salmonella positive gilt producers poses a risk for becoming a positive sow herd, whereas the quantitative relation between seroprevalence in positive sow herds and introducing a high number of seropositive gilts is relatively weak.

Introducing seropositive gilts into a sow herd that already is positive, measured as a S. Typhimurium-positive pen fecal sample the previous year had little or no effect on the prevalence in the sow herd.

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Relation between antimicrobial use and resistance in Belgian pig herds

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Abstract

The aim of this study was to determine the link between the characteristics of antimicrobial therapy and occurrence of antimicrobial resistance in Escherichia coli of clinically healthy pigs exposed to antimicrobial treatments.

A total of 918 Escherichia coli isolates were obtained from faecal samples, collected from 50 pig herds at the end of the fattening period and susceptibility was tested towards 15 different antimicrobial agents, using the disk diffusion method. The Antimicrobial Resistance Index (ARI) of each isolate was calculated, as the number of antimicrobials to which resistance was found divided by the number of drugs tested. The antimicrobial resistance percentage per class (ARclass) was defined as the percentage of E. coli strains clinically resistant to that specific class. Data on group level antimicrobial use in the sampled herds was collected and quantified as treatment incidences (TI) based on the used daily dose pig (UDDpig) and the animal daily dose pig (ADDpig) (number of pigs treated with one ADDpig or UDDpig/1000 pigs at risk/day). The UDDpig/ADDpig ratio gives an indication of the correctness of dosing.

The TIADDpig for group level use was 235.7 per 1000 pigs at risk per day, whereas the TIUDDpig equaled 200.7. This means that in reality, fewer pigs were treated with the same amount of antimicrobials than theoretically expected and thus antimicrobials were generally overdosed. Generalized linear regression analysis showed a significant relation between the TIADDpig and the ARI (p< 0.01), whereas there were no significant links for the TIUDDpig (p> 0.05). Analysis of the antimicrobial resistance for -lactam antimicrobials and tetracyclines suggests that the effect of correct or incorrect dosing on resistance development was different for the different antimicrobial classes tested. Besides the amount of administered antimicrobial agents, the frequency of drug administration may play a role in the selection of antimicrobial resistance in commensal E. coli.

Introduction

The emergence of resistant bacteria is a well documented threat to the effectiveness of therapy in both human and veterinary medicine (Schwarz et al., 2001; Catry et al., 2003). Besides several bacterial-associated factors, the characteristics of an antimicrobial therapy are believed to contribute to the rate and the extent of antimicrobial resistance development (Schwarz et al., 2001; Catry et al., 2003). The selection pressure exerted by the use of antimicrobial agents might be infuenced by several features such as dose, duration of treatment and frequency of therapy, but few data are available to support these hypotheses. The goal of antimicrobial therapy is to help an animal in its cure from disease by a reduction or elimination of pathogenic bacteria. Yet, selection pressure will also be exerted on the commensal bacterial fora during metaphylactic and prophylactic antimicrobial use. So a high selection pressure will be exerted mainly on the commensal fora. The aim of this study is to investigate the link between the characteristics of antimicrobial therapy and occurrence of antimicrobial resistance in Escherichia coli exposed to antimicrobial treatments.

Material and Methods

Study design, data and sample collection

Fifty closed or semi-closed pig fattening herds were randomly selected from the Belgian farm-animal identification and registration database (SANITEL, 2010) and visited between January and October 2010. The herds held at least 150 sows and 600 fattening pigs. On each herd 20 randomly selected fattening pigs were sampled within 3 weeks before slaughter. Faecal samples were taken after rectal stimulation and were put in a sterile recipient. The samples were inoculated the same day of collection.

All data on antimicrobial group level treatments applied between birth and time of sampling of the selected animals were

collected retrospectively. A group treatment was defined as each prophylactic or metaphylactic administration of antimicrobials to all the animals of the same production group. For each group treatment, following data were gathered: product name, duration of therapy, dose applied, administration route, age of the treated animals and body weight at time of treatment (estimated by means of a standard growth table).

Data on the external and internal biosecurity level on the herds were obtained by means of the biocheck biosecurity quantification system as described in detail in Laanen et al. (2011).

Bacteriological analysis and susceptibility testing
The isolation, identification and susceptibility testing of Escherichia coli was performed as previously described in Persoons et al. (2010).

Following antimicrobial groups were tested (agents): -lactam antimicrobials (ampicillin, amoxicillin-clavulanic acid and ceftiofur), phenicols (chloramphenicol and florfenicol), tetracycline, aminoglycosides (gentamicin, streptomycin, apramycin, neomycin and kanamycin), quinolones (enrofloxacin, nalidixic acid), sulfonamides (sulfadiazine), pyrimidines (trimethoprim).

Data were dichotomized into resistant or susceptible by allocating the intermediately susceptible isolates to the susceptible group.

Quantification of drug consumption
Antimicrobial drug consumption was quantified as treatment incidences (TI) based on the animal daily dose pig (ADDpig) and the used daily dose pig (UDDpig). TIADDpig and TIUDDpig (number of pigs treated with one ADDpig or UDDpig/1000 pigs at risk per day or number of days during which a pig is administered antimicrobials in a theoretical live of 1000 days) were calculated based on the acquired data, according to the method described by Timmerman et al. (2006). The UDDpig/ADDpig ratio of each antimicrobial treatment gives an idea of the correctness of dosing. A variation of 0.2 under or above 1 (range 0.8-1.2) was considered as correct dosing. The 'frequency of therapy' is a count for every new start of an antimicrobial therapy administered during a pigs' lifetime.

Data analysis
The Antimicrobial Resistance Index (ARI) for each isolate was calculated as the number of antimicrobials to which resistance was found divided by the number of drugs tested. The average ARI per herd was used in further analysis.

Antimicrobial resistance percentage per antimicrobial class (ARclass) was defined as the percentage of E. coli strains resistant to that specific class. For those antimicrobial classes where more than one antimicrobial was tested, a strain was determined "resistant" if it was resistant against at least one antimicrobial of that class.

A generalized linear regression model was developed to study the possible relationship between the TI based on ADDpig or UDDpig, the UDDpig/ADDpig ratio, the frequency of therapy and the external and internal biosecurity scores on the one hand and ARI on the other hand. In this analysis a combination of all antimicrobial treatments was used as a measure of the total consumption on the herd.

For the seven different antimicrobial classes a logistic regression model with antimicrobial resistance per class (ARclass) as a binomial outcome variable and the potential risk factors listed above as covariates was performed. The antimicrobial treatments tested in this analysis were restricted to the ones corresponding to the class of the dependent variable.

All statistical analyses were performed in SPSS 19.0 (SPSS inc., Chicago Illinois, USA).

Results
An average isolation success of 18.4 isolates per 20 sampled pigs per herd was achieved, resulting in a total of 918 Escherichia coli isolates.

The average TIADDpig for group level use was 235.7 per 1000 pigs at risk per day (SD = 222.7, Min = 0, Max = 1322.1), whereas the average TIUDDpig equaled 200.7 (SD = 136, Min = 0, Max = 699). This means that on average, fewer pigs were treated with the same amount of antimicrobials than theoretically possible which suggests that the antimicrobials are on average overdosed. A significant positive relation between the TIADDpig and the ARI (p< 0.01) was seen, whereas a link could be found between the TIUDDpig and the ARI (p= 0.085). Likewise higher TI showed higher ARclass for both the -lactam antimicrobial agents as for the tetracyclines. Analysis of other antimicrobial classes included in the susceptibility testing, could not be performed since no sufficient data on antimicrobial use of other classes were available.

Lower values for ARI after correct dosing compared to under- or overdosing (ARI equals 0.16, 0.18 and 0.22 respectively) were seen. Yet these differences are borderline non-significant (p = 0.07).

Table 1. Results of the logistic regression model for correctness of dosing for the β-lactam antimicrobial class and the tetracyclines (OR = Odds Ratio; AR_{class} = Antimicrobial Resistance per class of antimicrobials)

Antimicrobial class	Correctness of dosing	AR_{class}	OR	p-value
β-lactams	Under	42,8%	Ref.	
	Correct	42,2%	0.98	>0.05
	Over	40,9%	0.93	>0.05
Tetracyclines	Under	62.1%	Ref.	
	Correct	31.6%	0.28	<0.05
	Over	79.2%	2.32	<0.01

Ref.: reference category

Table 2: results of the logistic regression model for frequency of treatment for the β-lactam antimicrobial class and the tetracyclines (OR = Odds Ratio; AR_{class} = Antimicrobial Resistance per class of antimicrobials)

Antimicrobial Class	Frequency of antimicrobial therapy	AR_{class}	OR	p-value
β-lactams	0	23.2%	Ref.	
	1	37.0%	1.94	<0.01
	2	47.9%	3.04	<0.01
	3	36.2%	1.88	<0.05
	4	39.7%	2.17	<0.05
	5	26.3	1.18	>0.05
	6	47.4	2.97	<0.05
Tetracyclines	0	54.7%	Ref.	
	1	68.4%	1.79	<0.01
	2	57.9%	1.14	>0.05

The correlation between the ARI and the external (r = -0.16, p = 0.12) and internal (r = -0.18, p = 0.07) biosecurity was slightly negative.

Discussion

Moderate to high resistance percentages were seen for respectively β-lactam antimicrobials and tetracyclines even when these antimicrobials had not been used (0 level of frequency of therapy). High resistance to tetracycline in commensal E. coli from pigs has been reported several times (Hendriksen et al., 2008). The use of tetracyclines in swine production has been considerable since their discovery (Chopra and Roberts, 2001; Schwarz et al., 2001). As such the high antimicrobial resistance prevalence, even without tetracycline treatment, is not surprising.

The observed relation between TI and ARI indicates that the amount and number of days during which a pig is administered antimicrobials seems to be of importance for the presence of antimicrobial resistance. A TI_{UDDpig} lower than the TIADDpig reflects an average overdosing of antimicrobial treatments. A misevaluation of the bodyweight at moment of administration (Timmerman et al., 2006), intentional overdosing to aim at less disease or lack of precision could also be possible causes. The stronger link between TI_{ADDpig}, (quantifying the duration of selection pressure assuming a correct dosing) in comparison to the TIUDDpig (quantifying the true duration of selection pressure) and ARI could be an indication that the total amount of antimicrobials give is more influential than the actual duration of the selection pressure. Moreover, also the frequency of treatment seems to play a role in the degree of antimicrobial resistance both for β-lactam antimicrobials as for tetracyclines.

Although under discussion, antimicrobial concentrations lower than prescribed concentrations are thought to create a favourable opportunity for less sensitive bacterial populations, resulting in a exponential increase of the number of resistant bacteria after treatment (Catry et al., 2003). This could explain the higher AR_{class} for the tetracyclines after underdosing tetracyclines compared to correct dosing. Yet, a higher AR_{class} for the tetracyclines was also found after overdosing tetracyclines. These findings require more detailed research to ascertain the biological relevance. In this study, no effect

of correct or incorrect dosing on the AR$_{class}$ was seen for the β-lactam antimicrobial class. Different ways of spread of resistance and mechanisms of acquiring antimicrobial resistance between tetracyclines and β-lactam antimicrobials of the tested E. coli isolates could possibly explain these observed differences.

Higher correlations were found for internal biosecurity than for external biosecurity and ARI. Most likely this is due to the lower antimicrobial use in herds with a higher internal biosecurity (Laanen et al., 2011), consequently leading to a lower antimicrobial resistance of E. coli.

Conclusion

The use of antimicrobials selects for the acquisition and spread of antimicrobial resistance. The hypothesis that incorrect dosing is a risk factor for antimicrobial resistance selection is probably drug dependant whereas it seems that the frequency of drug administration may play a role in the selection of resistance to β-lactam antimicrobials and tetracyclines. A higher biosecurity score is related to less antimicrobial resistance.

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Salmonella in pigs and pork and their antimicrobial resistance - 10 years of surveillance in Germany

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Abstract

Salmonella from pigs and pork have been submitted to the National Reference Laboratory for the Analysis and Testing of Zoonoses (Salmonella) (NRL Salm) for a number of years. This study retrospectively analyses the data of Salmonella and their antimicrobial resistance generated between 2000 and 2009. A total of 4163 isolates from pigs and 1839 isolates from pork submitted to the NRL Salm were serotyped and tested for antimicrobial resistance using the broth microdilution method. Minimum inhibitory concentrations (MIC) were evaluated using epidemiological cut-off values as provided by EUCAST (www.eucast.org) at the time of interpretation (2010).

The majority of isolates from pigs and pork belonged to three serovars: S. Typhimurium (66 and 52%), monophasic variant of S. Typhimurium (1,4,[5],12:i:-) (11 and 10%) and S. Derby (7 and 10%). In both origins the number of S. Typhimurium decreased by roughly 50% while monophasic variant of S. Typhimurium increased from zero to 32 and 26%, respectively. The proportion of S. Derby varied between 5 and 12% in both origins.

Antimicrobial resistance in S. Typhimurium was high. In pigs there was a slight decrease in resistant isolates from 97 to 88% over the ten year period. In pork, the proportion of resistant isolates was lower but remained constant at about 80%. In the monophasic variant of S. Typhimurium susceptible isolates were rare (3%) and the majority (>80%) of isolates was resistant to streptomycin, ampicillin, sulfamethoxazole and tetracycline. In S. Derby, resistance was substantially lower (55%) compared to S. Typhimurium and the monophasic variant of S. Typhimurium. Resistant isolates to 3rd generation cephalosporins were rare (<1%) in pigs and absent in pork. However, in 2008 four isolates (0.8%) and in 2009 seven isolates (2%) indicated a potential emergence of resistance to this group of antimicrobials. Resistance to fuoroquinolones was constantly on a low level in pigs and pork (3 to 4%). Similarity of trends in serovar and resistance patterns of animal and food derived isolates supports the assumption of vertical transmission along the food chain.

Introduction

Resistance in zoonotic pathogens potentially hampers treatment of severe human infections. Food is an important source for Salmonella causing human infections. Moreover, Salmonella in food often originates from animals that harbour Salmonella or from cross contamination during slaughter and processing. It was the purpose of this study to investigate the development of antimicrobial resistance in Salmonella from pigs and pork over a 10 year period.

Material and Methods

Isolates had been submitted for diagnostic purposes to the NRL Salm between 2000 and 2009. At the NRL isolates were confirmed as Salmonella and serotyped. Antimicrobial resistance testing was carried out using the broth microdilution method according to CLSI- standards by the National Reference Laboratory for Antimicrobial Resistance. All isolates were evaluated using the same epidemiological cut-off values as provided by the European Committee on Antimicrobial Susceptibility Testing (www.eucast.org) at the time of analysis (2010). More details as well as test ranges can be obtained from the reports published by BfR (www.bfr.bund.de).

Results

A total of 4163 isolates from pigs and 1839 isolates from pork submitted to the NRL Salm were serotyped and tested for antimicrobial resistance. Figures 1 and 2 display the proportion of the most frequent Salmonella serovars based on the frequency observed in 2009 for pigs and pork, respectively. S. Typhimurium was the most frequently detected serovar in most years. However, its proportion decreased from more than 80% to less than 50% in pigs and from more than 60% to

below 40% in pork. Its share was partly replaced by the monophasic variant of Salmonella Typhimurium named S. 1,4,[5],12:i:- that increased from 0% in 2000 to 30% (pigs) and 25% (pork) in 2009. In contrast, the proportion of S. Derby was more or less constant over time.
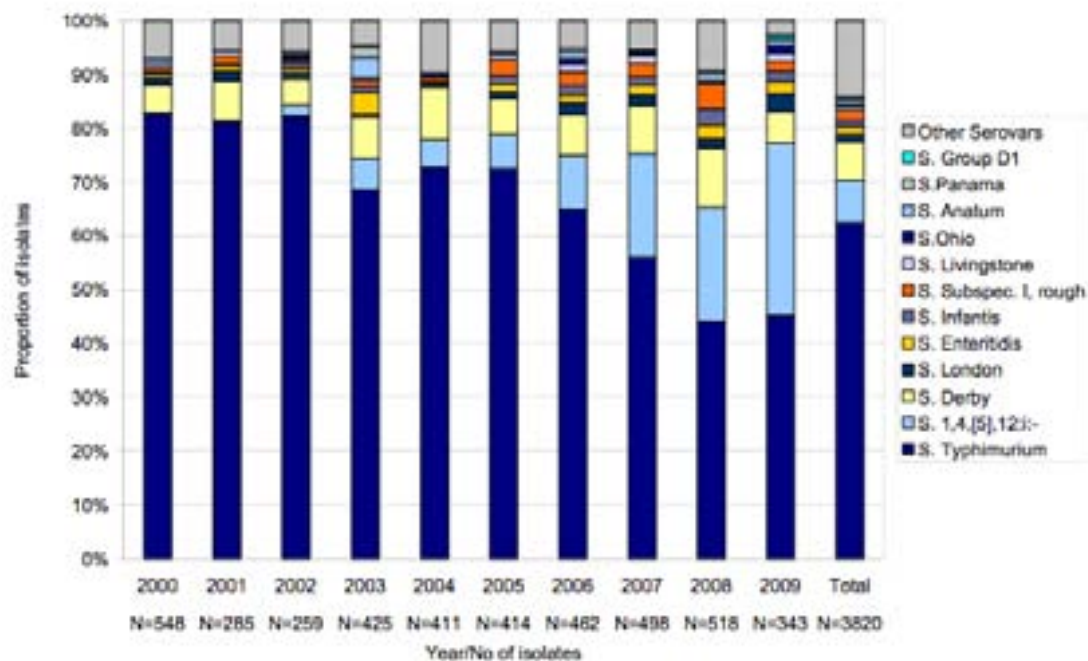
Fig. 1. Proportion of the most frequent Salmonella serovars among the submitted isolates originating from pigs in Germany (2000 – 2009)
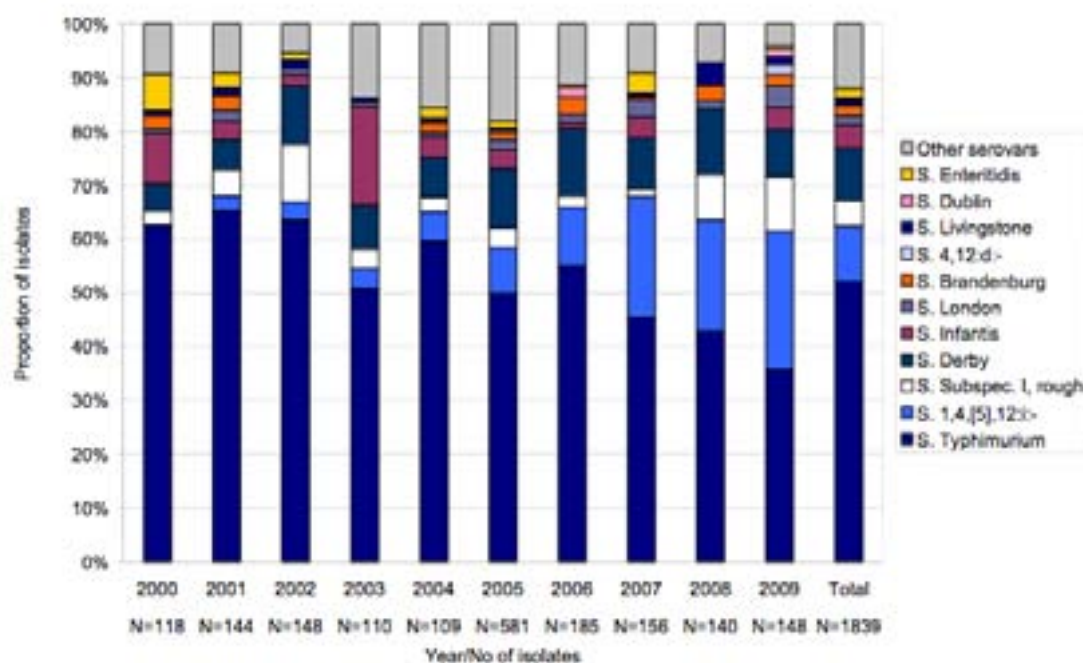
Fig. 2. Proportion of the most frequent Salmonella serovars among the submitted isolates originating from pork in Germany (2000 – 2009)

In 2009, 81% of the Salmonella isolates from pigs were resistant to at least one class of antimicrobials and 75% to more than one. The majority of resistant isolates is linked to the serovar S. Typhimurium and his monophasic variant S. 1,4,[5],12:i:- that were resistant in 87.1% and 96.4% of isolates and multiresistant in 81.9% and 94.6%, respectively. The respective resistance rates for S. Derby were 40% and 35%. In contrast, most isolates (7/8) of S. Enteritidis were fully susceptible.

In 2009, S. Typhimurium was resistant to aminopenicillins, streptomycin, tetracycline and sulfamethoxazole (73-83%). Resistance to amphenicols (34-40%), trimethoprim (32%) and kanamycin (13%) was less frequent. Only 2 isolates were resistant to third generation cephalosporines and (fuoro-)quinolones.

The resistance pattern of the monophasic serovar S. 1,4,[5],12:i:- was similar to S. Typhimurium with a predominance of a fourfold resistance to ampicillin, streptomycin, sulfamethoxazole and tetracycline. Few isolates were resistant to cephalosporines (2/110, 1.8%) und ciprofoxacin (1 isolate). Resistance to amphenicoles and trimethoprim was less frequent than in S. Typhimurium. In contrast, resistance to streptomycin, ampicillin, sulfamethoxazole and tetracycline was even higher than in S. Typhimurium.

S. Derby was less frequently resistant to all of the substances tested. None of the isolates was resistant to third generation cephalosporines or (fuoro-)quinolones.

Overall the proportion of resistant and multiresistant Salmonella isolates in pigs decreased slightly over the years (from 93 and 82% in 2000 to 81 and 75% in 2009). Resistance to amphenicoles and (fuoro-)quinolones tended to decrease in S. Typhimurium (from 56% in 2000 to 35% in 2009). On the other hand, resistance to gentamicin and kanamycin slightly increased. Resistance to third generation cephalosporines was on a low level in all years but tended to increase between 2007 and 2009.

Resistance of S. Derby fuctuated substantially over the years (between 11 and 60%). This may in part be attributed to the limited number of isolates and variations in the submission pattern.
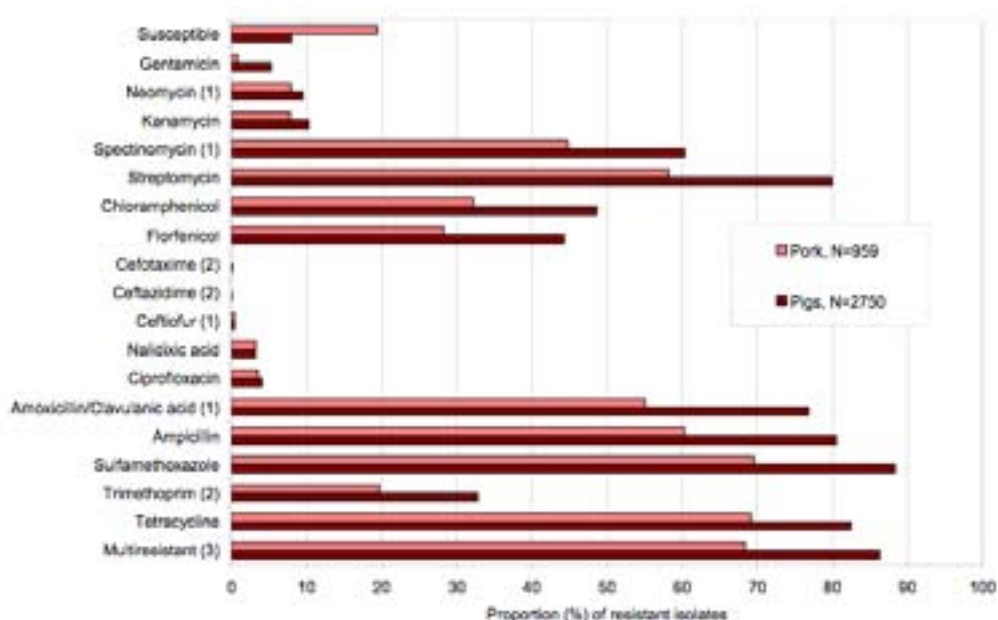
Fig. 3: Resistance of S. Typhimurium from pigs and pork in Germany to selected antimicrobials (2000 – 2009)

In pork, the proportion of resistant (67%) and multiresistant (52%) isolates was lower over the years compared to isolates from pigs (Figure 3). This also applied specifically for S. Typhimurium and S. 1,4,[5],12:i:-. However, the observed resistance pattern was similar, which is in line with the observed similarities in serovar patterns. There were some fluctuations, so that no clear trend could be observed for resistance and multiresistance. Interestingly, the changes in resistance observed in isolates from pigs were not observed in those from pork. The overall level of resistance to amphenicoles and especially to (fluoro-)quinolones had been lower from the beginning and there was only a slight additional decrease concerning amphenicoles.
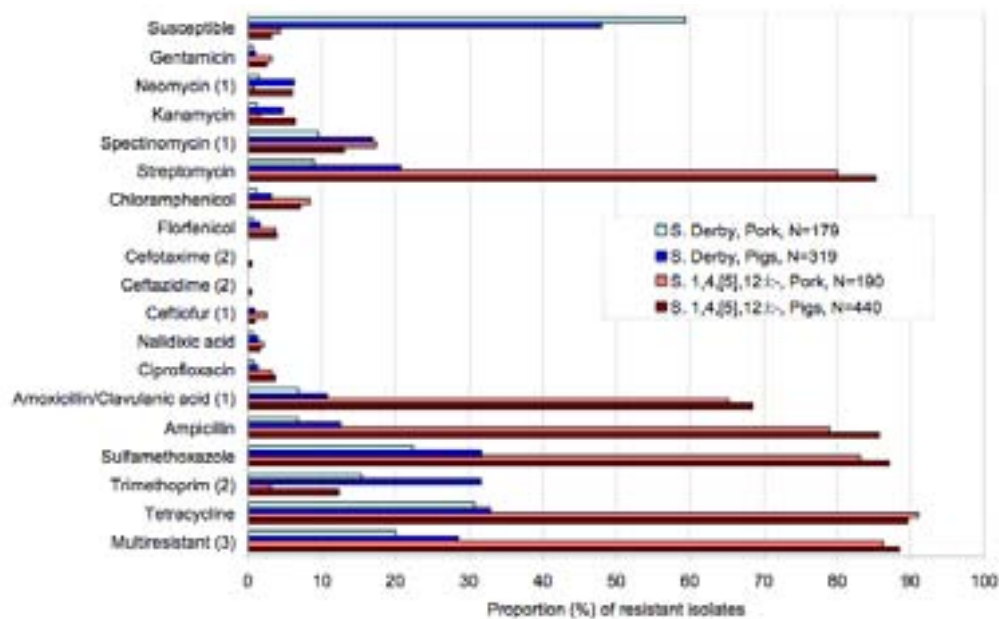
Fig. 4: Resistance of S. Derby and S. 1,4,[5],12:i:- from pigs and pork in Germany to selected antimicrobials (2000 – 2009)

Discussion and conclusions

There were great similarities between isolates from pigs and pork concerning the serovar and resistance patterns. This underlines that isolates in food often originate from primary production and cross contamination during slaughter and processing. Overall, isolates from food were less resistant than those from primary production. This difference was not fully attributable to a different serovar pattern because differences were also observed within the same serovars (S. Typhimurium and S. 1,4,[5],12:i:-). A potential factor could be that meat is more or less uniformly derived from one age group of pigs while isolates from pigs are derived from all age groups including piglets and weaners that might have a different degree of exposure to antimicrobials leading to the selection of more resistant strains. In addition, insufficient information was available on the origin of the meat (national production or imported products.

In isolates from pigs, the decrease in resistance is a positive development. The decrease in resistance to chloramphenicol can be attributed to a long term effect of the ban of this substance from animal production in Europe. The decrease in resistance to f uoroquinolones was less expected as f uoroquinolones are licensed for use in pigs, although not for oral medication. However, limited information is available on the amount of f uoroquinolones used in pig production in Germany, which underlines that consumption data are urgently needed.

The slight increase in isolates resistant to 3rd generation cephalosporines warrants attention, as 3rd and 4th generation cephalosporines are considered critically important for human medicine.

More data on the resistance of the isolates can be obtained from extensive reports published by BfR (www.bfr.bund.de).

Use of heavy metals in swine feed and its association with the co-selection of metal tolerant and multi-drug resistant Salmonella

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Abstract

This study was conducted to characterize the role of chemical interventions, biocides and heavy metal micronutrients, in particular, in swine production systems on the emergence of heavy metal and biocide tolerant Salmonella and its association with antimicrobial resistance. A total of 353 Salmonella isolates with different antimicrobial resistance profiles identified from 36 barns exposed to three different classes of biocides were analyzed. The sources of isolates included feed (n=30), fecal (n=226), and environment (n=97) samples that were systematically selected. The minimum inhibitory concentrations (MIC) of each isolate against heavy metals copper (Cu) and zinc (Zn) was determined on Mueller-Hinton-II (MH) agar plates containing serial dilutions of copper sulfate (1-32mM) and zinc chloride (0.25-16mM). A non-parametric Wilcoxon Rank Sum test for trends across ordered groups (Stata 10, College Station, TX) was used to determine association between concentration of metal in feed and MIC. The most common MDR patterns among the more heavy metal tolerant isolates were AmClStSuTe (n=81) and AmStTeKm (n=58), which are common multi-drug resistance patterns found in swine production systems. There was a significant association between the concentration of copper in feed and the MIC of isolates recovered from fecal samples for copper ($p<0.001$). Heavy metal tolerance was also significantly associated with distinct multi-drug resistance types. The odds of finding high Zinc MIC were 15 times higher for the AmClStSuTe R-type than AmStTeKm (Chi-square= 47.2; $p<0.05$). On the other hand, the odds ratio for association between copper tolerance and R-type AmStTeKm was 4.6 (Chi-square=17.9; $P<0.05$). No association between biocide use and heavy metal tolerance was detected in this study. Unique genes that encode for tolerance to copper and zinc and physical linkage to antibiotic resistance determinants are being investigated. The findings in this study suggest that the use of copper in swine feed results in higher tolerance of Salmonella strains to copper which in turn co-selects for antimicrobial resistance.

Introduction

Non-typhoidal Salmonella serovars are among the most important foodborne bacterial pathogens worldwide. Many strains of commonly occurring Salmonella serovars have been shown to exhibit multi-drug resistance (MDR), however, the role of non-antiotic chemical agents as a selective pressure for emergence and persistence of antimicrobial resistance is poorly understood (Aarestrup and Hasman, 2004). In commercial swine production systems, heavy metals such as copper , zinc, manganese, and others in feed are used to assist with normal growth of pigs and to provide cytotoxic effects on bacteria (NRC, 1998). The role of chemical interventions, biocides and heavy metal micronutrients, in particular, in swine production systems on the emergence of heavy metal and biocide tolerant Salmonella and its association with antimicrobial resistance is not well known. The present study investigates the occurrence and significance of tolerance to copper and zinc, its association with the concentration in swine feed and co-selection with antimicrobial resistance in Salmonella isolates recovered from swine production units.

Materials and Methods

Study design and sample collections: This was part of a study conducted to study the role of specific classes of biocides and heavy metals (copper and zinc) in the occurrence and persistence of multidrug resistant Salmonella in swine production units. Briefy, a longitudinal randomized control study was conducted on 36 swine barns that originated from three production systems and 12 farms of independent production pyramids. Feed (pooled one per barn) and fecal (48 per barn) samples collected from the 36 barns were tested for copper and zinc concentrations. Samples were collected in four replicates. The details of the study design and sample collections were previously described (Zewde et al., 2009).

Salmonella isolation and antimicrobial susceptibility testing: Salmonella was isolated using conventional culture and identification systems (Gebreyes et al., 2004). Briefy, buffered peptone water was added at a 1:9 W:V for each of the samples and incubated at 37C for 24h. A 100uL suspension was transferred to Rappaport Vassiliadis media and further incubated at 42C over night. A loopful of the suspension was streaked on to XLT-4 selective media and incubated at 37C for 24h. Presumptive isolated black colonies were tested for via triple sugar iron (TSI) and urease reactions. Further confirmation was done by serogrouping using uni- and polyvalent antisera. Antimicrobial susceptibility testing was done using kiby-Bauer disc diffusion testing. A total of 353 Salmonella isolates with different antimicrobial resistance profiles identified from 36 barns exposed to three different classes of biocides were analyzed. The sources of isolates included feed (n=30), fecal (n=226), and environment (n=97) samples that were systematically selected.

Minimum inhibitory concentrations (MIC): The minimum inhibitory concentrations (MIC) of each isolate against heavy metals copper (Cu) and zinc (Zn) was determined using a agar gel dilution system on Mueller-Hinton-II (MH) agar plates containing two fold serial dilutions of copper sulfate (1-32mM) and zinc chloride (0.25-16mM). The pH of the copper media was adjusted to 7.2 and the zinc to 5.5 to allow for solubility of the metal in the media. Zinc susceptibility was recorded at 4mM and 8mM and copper susceptibility at 2mM, 4mM, 16mM , 20mM, and 24mM. Two MH plates of each dilution were inoculated with the selected isolates at a uniform concentration using repeat inoculators and incubated overnight at 37°C. MIC was determined by the absence of growth at a given concentration. A non-parametric Wilcoxon Rank Sum test for trends across ordered groups (Stata 10, College Station, TX) was used to determine association between concentration of metal in feed and MIC.

Results and Discussion

The most common MDR patterns among the more heavy metal tolerant isolates were AmClStSuTe (n=81) and AmStTeKm (n=58), which are common multi-drug resistance patterns found in swine production systems. There was a significant association between the concentration of copper in feed and the MIC of isolates recovered from fecal samples for copper (p<0.001).

Heavy metal tolerance was also significantly associated with distinct multi-drug resistance types. The odds of finding high Zinc MIC were 15 times higher for the AmClStSuTe R-type than AmStTeKm (Chi-square= 47.2; p<0.05), Fig 1a. On the other hand, the odds ratio value for association between copper tolerance) and MDR AmStTeKm was 4.6 (Chi-square=17.9; P<0.05), Fig 1b.
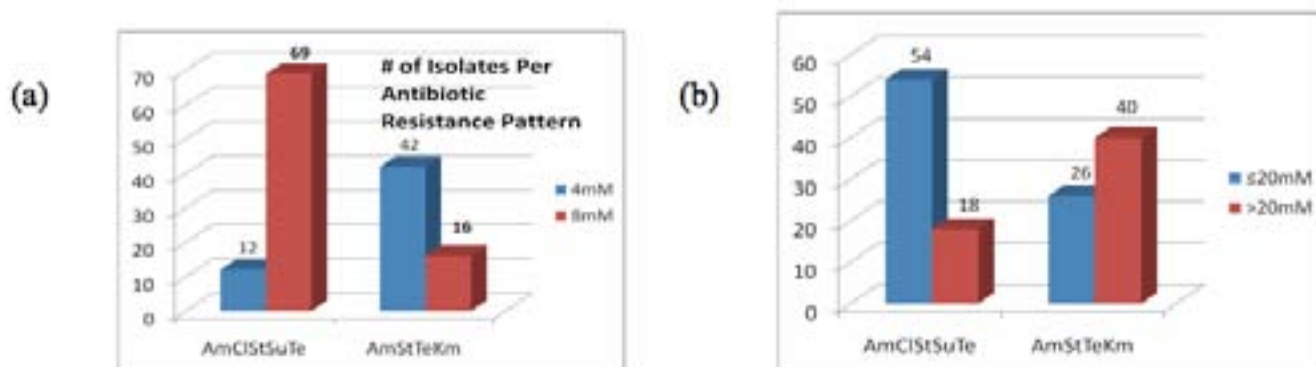
No association between biocide use and heavy metal tolerance was detected in this study. Unique genes that encode for tolerance to copper and zinc and physical linkage to antibiotic resistance determinants are being investigated. The findings in this study suggest that the use of copper in swine feed results in higher tolerance of Salmonella strains to copper which in turn co-selects antimicrobial resistance. The findings imply that the use of heavy metals in swine feed may contribute to the persistence of multi-drug resistant Salmonella of pork safety significance.

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POSTERS

Tracing back to Sources of MAIC Using Farm records and Lab Techniques

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Summary

For the implementation of tracing back systems (particular agents, unwanted observation and other), detection techniques should be available. For MAIC, a step-by-step approach was established, combining visual observation, lab based PCR-identification, tracing back to the farm of origin and finally the search for potential ports of entry:

- Recording of observations as part of internal or veterinary recording system
- Using a PCR based identification procedure, the agent could be identified
- Based on the ID of the farm, farms at risk could be traced back
- Investigations for ports of entry on farm level may follow.

Introduction

Complexity of food chains requires complex detection-, verification- and inspection systems, all of them must get organised in such a way, that "failures" or "unwanted observations" of any kind are detected wherever along the lines. Only then, corrective measures can be taken.

All such unwanted items require techniques adapted to detection and/or verification of their presence or absence. Prior to implementation, techniques should be assessed with respect to their efficacy (sensitivity and specificity), for their applicability at the position intended, the availability of personnel skills and (if needed) the lab capacity.

For food animals, ante and post mortem inspection still relies on pathological and clinical observation, which has been done since the beginning of the last century in Europe and elsewhere. Basic principle was the indication of pathological observations for a disease or the presence of an agent, which may cause this lesion. Still today, ante and post mortem inspection rely on such indicative values.

Amendment of Statutory Meat Inspection in the EU: From its origin, cutting of the mandibular lymph nodes (LN) was intended to detect tuberculosis in both, cattle and swine, and for both categories, it is still mandatory: For cattle to detect bovine Tuberculosis, for swine, to detect abscesses possibly caused by agents of the Mycobacterium avium intracellulare Complex (MAIC, Table 1). Evidence for human relevance of MAIC is available (immunocompromised persons and children). Also found in LN abscesses is Rhodococcus equi, which should be considered as of risk for humans, too (1).

Table 1: M. avium and M. intracellulare (2)

Family	Mycobacteriaceae
Genus	Mycobacterium
Species	Mycobacterium avium
	ssp. avium
	ssp. paratuberculosis
	ssp. silvaticum
	ssp. hominissuis
Species	Mycobacterium intracellulare

However, visual inspection is not reliable enough, with respect to sensitivity as well as specificity, and abscesses indicating MAIC are supposed to be notoriously underreported.

As abscesses are not indicative for MAIC, microbiological confirmation is desirable, but the slow microbiological detection procedure for members of this family asks for more rapid techniques.

In addition, cutting of LN carries the risk of contamination, in particular, if other tissues (e.g., tonsils) are going to be cut simultaneously. A high percentage of tonsils and gut lymph nodes of finisher pigs may harbour Salmonella, Campylobacter and to a smaller extend Yersinia or Listeria (3).

So, replacement or improvement of an inaccurate (from the information point of view) and risky (from the contamination point of view) inspection procedure with more reliable techniques is required. It was the aim of this study to implement a daily practice system of

- Detecting abscesses, simultaneously tracing back to the respective farm of origin
- Laboratory based confirmation of the agent from suspicious LN
- Feeding back from the suspicious amount of lesions to the farm
- Sampling at the farm, detecting and closing possible ports of entry

Material and Methods

Information from post mortem inspection: Post mortem data (2005 to 2009) of the veterinary services and recorded via inspection terminals were scrutinised for lesions possibly indicating MAIC infections. Data stem from two cooperating farmer associations (fattening pigs), all finishers were slaughtered at one single abattoir.

Data of interest were transferred into a separate table, calculation was done using PASW for Windows: Identification number of the farms, number of animals shipped from these sites and the observations from both, cutting mandibular LN and palpating the gut LN.

Lab data: Sampling and techniques used: In addition, in the year 2007 LN samples with visible and suspicious lesions from finishers were taken during meat inspection from both locations, mandibular and gut LN at the same abattoir.

44 LN with lesions (of them, 11 from the guts) were processed the day after collecting. From these, samples were taken from visually unaffected tissue and in parallel from an abscess directly. Tissue preparation was done as follows:

- Homogenisation with a Retsch- Mixer Mill (MM 2000, Manufacturer Retsch, Germany) and using a one-way stainless steel grinding ball
- DNA- extraction with the aid of a commercial kit (High Pure Template Preparation Kit, Roche) with a few adaptations (4)
- PCR on a Thermocycler Trio (Biometra, Göttingen, Germany) with the primer- pair AV 6/7 (5). The protocol was slightly re-arranged.
- Nested PCR with several variations in the basic protocol was done with the primers AVNF (5'-cga ccg ccg gga cct aac g) and AVNR (5'-gcg ccg acg acc acc aca t)
- Gel-Electrophoresis and documentation with INTAS digital Video- System.

Results

Results of post mortem inspection: Farms were arranged in a list according to the number of suspicious lesions in the mandibular- or gut LN. For every year and both inspection sites, 4 farms with the highest number of suspicious lesions were identified, i.e. in total 20 farms for the mandibular lesion and another 20 farms for the lesions located in the gut lymph nodes. 5 farms appeared 2 times (Table 2):

Table 2: Farm of Origin being Suspicious for MAIC

Farm 235	Guts 2005 / Guts 2009
Farm 187	Guts 2008 / Guts 2009
Farm 91	Mandib. 2005 / Mandib. 2007
Farm 104	Mandib. 2008 / Guts 2008
Farm 7	Mandib. 2006 / Guts 2008

Lab results (Identification and detection techniques): Most lymph nodes examined stem from the mandibular LN (after cutting). Irrespective of the matrix, 28 out of 44 LN were positive for MAIC, MAIC was not obtained from 16 LN.

Positive LN: 28 out of 44

Negative LN: 16 out of 44

Positive results were obtained from tissue without any visible lesion, too (Table 3). However, it should be noticed, that all LN were affected with suspicious lesions. Results indicate, that a PCR based procedure works despite the presence of tissue. However, sensitivity has not yet been assessed.

Table 3: MAIC from 27 Tissue Pairs (Identical LN): Tissue with and without Visible Abscesses

no lesion visible	from abscess lesion	Pairs
MAIC positive	MAIC negative	1
MAIC negative	MAIC positive	10
MAIC positive	MAIC positive	7
MAIC negative	MAIC negative	9

Discussion

The food chain covers the total production sequence, i.e., the whole life of animals, from incoming goods to the farm up to the animals being shipped for slaughter. This MAIC- inspection approach combines daily observation, lab based examination, tracing back and search for ports of entry. It is based on daily practice in a commercial food chain (fattening pigs) in Northern Germany. In detail:

Observation during post mortem inspection:
- Even under the assumption of underreporting and low detection sensitivity, our results indicate, that farms at risk may be detected with the veterinary service inspection routine. However, the risk of contamination remains.
- Visual observation depends on personal inspection capability. Microabscesses must be expected to be overseen, lab based techniques are needed.

Development of lab procedures:
- Here, a PCR was used, tissue background noise was overcome with some modifications in DNA extraction and PCR protocols. Yet, sensitivity has not been investigated.
- LN examined here, did not belong to animals from the farms: In this part of the project, the lab protocol as such was intended.

Tracing back to the farm of origin and possible re-arrangements (biosecurity measures):
- Using the total of observation records over several years from the farms with an identity number, shipments with a high burden of MAIC suspicion were identified, opening the option of tracing back. So, the farm was open for consideration, too.
- Based on this, incoming goods and biosecurity measures on a farm with a high number of pigs with suspicious lesions may be scrutinised in order to eliminate the initial port of entry (not yet done).

An assessment of MAIC with respect to human health remains still open, consequently, inclusion of the MAIC agent into surveillance systems is still undecided and up to risk assessment. Meanwhile, collection of data including fine-tuning of detection systems should be already the issue of the day.

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Case studies: Tuberculination in pig herds suspected of infection with Mycobacterium avium

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Abstract

Mycobacterium avium, both subspecies hominissuis (MAH) and subsp. avium (MAA), are considered a significant zoonotic hazard in pigs. Therefore special attention is given to detect the presence of this hazard in pigs during post mortem meat inspection. Herds delivered at slaughter were monitored on blood antibodies against MAH. Herds with an antibody respons against a MAH infection were visited. Initially a questionnaire assessing relevant risk factors for MAH was applied. Additionally to the questionnaire in several herds intracutaneous tuberculination was carried. Positive results in tuberculination in 3 different herds in the Netherlands, Belgium and Germany are presented; two farms where compost was used and one farm where the pig holding was adjacent to a big broiler farm. Twice the presence of MAH and once MAA was bacteriologically confirmed. When the supply of compost was stopped in two herds no positive tuberculination was present anymore. The other herd with the adjacent broiler fock ceased its activities as a pig producer.

When preventive measures are an active part of daily farm management MAH can be controlled at farm level effectively. Screening blood of slaughter pigs on the presence of MAH antibodies can be used to identify true positive herds. Serological surveillance is presently applied in the newly developed supply chain meat inspection in Germany and The Netherlands.

Salmonella monitoring: Faster and better information for fatteners

Christiane Butschen

Abstract

The Erzeugergemeinschaft Rheinland w.V., located in Moers, is a so-called "Systemberater" for the German quality assurance system, "Qualität und Sicherheit" (QS). One of its tasks is to support the organisation of Salmonella monitoring for its 233 fatteners. This includes the distribution of information about the actual salmonella status.

EG Rheinland participates in work package 2.1. within the Interreg IV A "SafeGuard" project. The overall aim of this work package is to analyse salmonella results proactively in a database called "EGR-Backbone" in order to inform farmers faster in case of changes in the salmonella antibody status. Prior to this project, EGR-farmers only got information about the average result of the last 60 samples of the past year on a quarterly basis.

Since the end of 2010, sample data from EGR-fatteners are imported from the "QS-Salmonella database" into the "EGR-Backbone". There the system creates different flexible reports for advisors as well as for farmers. For example, the "EGR-Backbone" reports give more attention to the last results (90-31 days and last 30 days back from last sample date) by weighting them and calculating a trend. If the trend shows a negative result, the farmer receives a report automatically. Another important point is that sampling takes place regularly. If, for some fatteners, no samples have been taken for more than 8 weeks, they will be informed by the system. Besides farmers, advisors also get an overview about all farms in order to monitor the fatteners more efficiently.

Farmers as well as advisors remain continually informed about the Salmonella antibody status from the actual batches of finished pigs and can therefore take corrective actions earlier than would otherwise be possible.

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A retrospective analysis of Salmonella isolation trends from pigs in Great Britain since 1994, with special reference to monophasic S. Typhimurium and antimicrobial resistance trends

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Abstract

The numbers of Salmonella reports from pigs in Great Britain have reduced considerably since the mid- 1990s, when up to 384 positive epidemiological group reports (incidents) per year were recorded, and numbers have been relatively stable since 2003 with less than 200 incidents reported per year. S. Typhimurium has been the most common serovar throughout the study period (between 58 and 75% of incidents). S. Derby, which was the second most common serovar for many years, has shown a downward trend since 2007, accounting only for 5% of incidents in 2009. At the same time, monophasic strains of S. Typhimurium have been on the rise since 2006. S. 4,5,12:i:- went from 0% in 2005 up to 6.2% of incidents in 2009, whereas S. 4,12:i:-, after showing a small peak in 1997, has also increased since 2007 and accounted for 1.2% of incidents in 2009. Throughout the 1990s, the most commonly seen phage type among S. Typhimurium isolates was DT104, but numbers declined sharply from 1998 onwards. Since 2002, U288 has been the most commonly seen phagetype in S. Typhimurium with up to 67.7% of all S. Typhimurium incidents being U288. DT193 has been increasing since 2003, with up to 41.5% of S. Typhimurium incidents in 2008. At the same time as the number of DT193 incidents in S. Typhimurium increased, an increase in the number of monophasic strains of S. Typhimurium occurred, with the majority of those isolates being DT193.
The percentage of S. Typhimurium isolates from pigs showing resistance to six or more antimicrobials has increased from 19.7% in 1995 to 47.6% in 2009, with a peak of 76.8% in 2007. Resistance of S. Typhimurium to compound sulphonamides, ampicillin, streptomycin and chloramphenicol increased considerably between 2002 and 2007, while resistance to tetracycline has been at a high level since the beginning of the study period. The most common resistance pattern observed in S. 4,5,12:i:- (ampicillin, streptomycin, compound sulphonamides, tetracycline) was seen in 72.7% of isolates in 2009. In species other than pigs, S. 4,5,12:i:- has, so far, shown a significant increase in cattle only, and the first isolates from poultry were only reported in 2010.

Introduction

Human salmonellosis is one of the most common foodborne diseases in the UK, and contaminated pork may be an important source of infection for humans. It has been estimated in various European countries that 10–20% of all cases of salmonellosis in humans are related to the consumption of pork (Borch et al., 1996; Berends et al., 1998; Steinbach & Hartung, 1999; EFSA, 2010).

Pigs can be infected by several Salmonella serovars but symptoms are rarely seen, making infection difficult to recognize, thus risking the spread of infection to the rest of the herd and causing Salmonella to enter the food chain.

The majority of Salmonella serovars are biphasic and express two distinctive f agellar phases but some isolates do not express either phase 1 or phase 2 f agella and are therefore classed as monophasic. In recent years, such monophasic strains of S. Typhimurium (namely S. 4,5,12:i:- and S. 4,12:i:-) have been observed in several animal species including pigs.

Material and Methods

The Salmonella Unit at The Animal Health and Veterinary Laboratories Agency is the Salmonella Reference Laboratory for Veterinary Salmonellosis in the UK. The data for this study period was sourced from Salmonella surveillance data from Great Britain from 1994 to 2009.

Characterisation of the Salmonella isolates in this study was done by serotyping according to the White-Kauffmann-LeMi-

nor scheme. Phagetyping of S. Typhimurium was carried out using the HPA, Colindale Bacteriophage updated Anderson typing designations for S. Typhimurium, and Antimicrobial Resistance was determined using a disc diffusion technique on Oxoid "Isosensitest" agar.

Results

Salmonella incidents:

The numbers of Salmonella reports from pigs in Great Britain have reduced considerably since the mid- 1990s, when up to 384 positive epidemiological group reports (incidents) per year were recorded, and numbers have been relatively stable since 2003 with less than 200 incidents reported per year. S. Typhimurium has been the most common serovar throughout the study period (between 58 and 75% of incidents). S. Derby, which used to be the second most common serovar for many years, has shown a downward trend since 2007, accounting only for 5% of incidents in 2009. At the same time, monophasic strains of S. Typhimurium have been on the rise since 2006. S. 4,5,12:i:- went from 0% in 2003 up to 6.2% of incidents in 2009, whereas S. 4,12:i:-, after showing a small peak in 1997, has also increased since 2007 and accounted for 1.2% of incidents in 2009. Fig. 1 shows trends of S. Typhimurium, S. Derby, S. 4,5,12:i:- and S. 4,12:i:- incidents in pigs in Great Britain between 1994 and 2009.

Fig. 1:
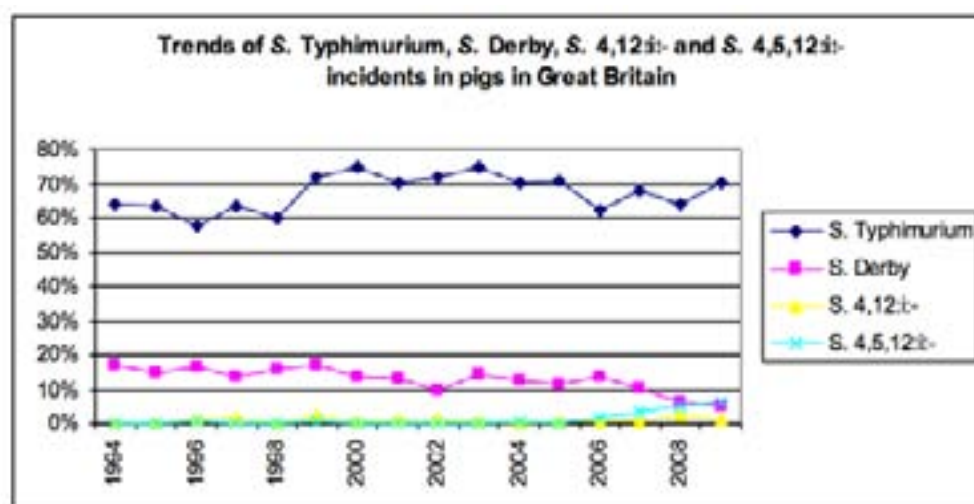
Phagetyping trends:

There has been a significant shift in the phage types associated with S. Typhimurium in pigs during the period 1994 to 2009. In the mid to late 1990's, the predominating phagetype was DT104, peaking at 79% of S. Typhimurium incidents in 1997 and then decreasing sharply to 28.5% in 1999 and has declined further to 5.6% of S. Typhimurium incidents in 2009. Phagetype U288 was first seen in 1997, and numbers rose sharply since 2002. U288 has been the predominant phagetype since 2002, peaking at 67.7% of S. Typhimurium incidents in 2007. Since 2008, numbers have declined, with 44.4% of S. Typhimurium incidents in 2009 being U288. Fig. 2 shows phagetyping trends (in terms of the proportion of S. Typhimurium incidents represented by each phage type) of S. Typhimurium in pigs between 1994 and 2009. At the same time as the number of DT193 incidents in S. Typhimurium increased, an increase in monophasic strains of S. Typhimurium could be observed, which predominantly belong to DT193, with 73% of S. 4,5,12:i:- incidents being DT193 in 2009 (data not shown).

Fig. 2:

Phagetyping trends of *S.* Typhimurium in pigs
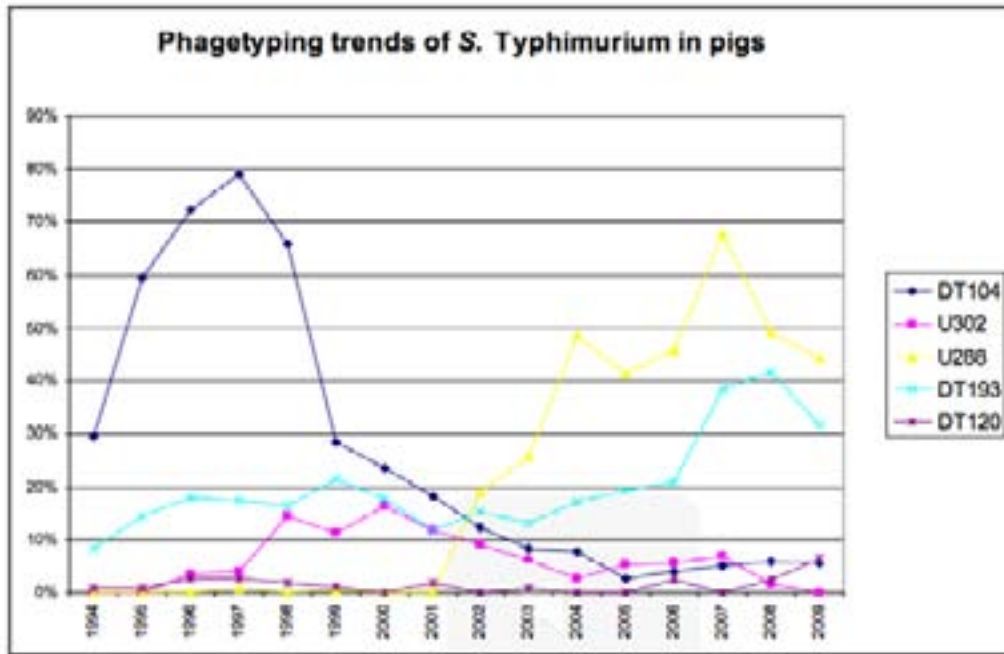
Resistance trends:

Resistance of S. Typhimurium to compound sulphonamides, ampicillin, streptomycin and chloramphenicol increased considerably between 2002 and 2007, whereas resistance to tetracycline has been at a high level since the beginning of the study period. Fig. 3 shows resistance trends in S. Typhimurium over the study period. The percentage of S. Typhimurium isolates from pigs showing resistance to six or more antimicrobials has increased from 19.7% in 1995 to 47.6% in 2009, with a peak of 76.8% in 2007 (fig. 4).

The most common resistance pattern observed in S. 4,5,12:i:- (ampicillin, streptomycin, compound sulphonamides, tetracycline) was seen in 72.7% of isolates in 2009.

In species other than pigs, S. 4,5,12:i:- has, so far, shown a significant increase in cattle only, and the first isolates from poultry were reported in 2010.

Fig. 3:

Resistance of *S.* Typhimurium isolates against a panel of antimicrobials

Fig. 4:

Proportion of *S.* Typhimurium isolates showing resistance against 6 or more antimicrobials
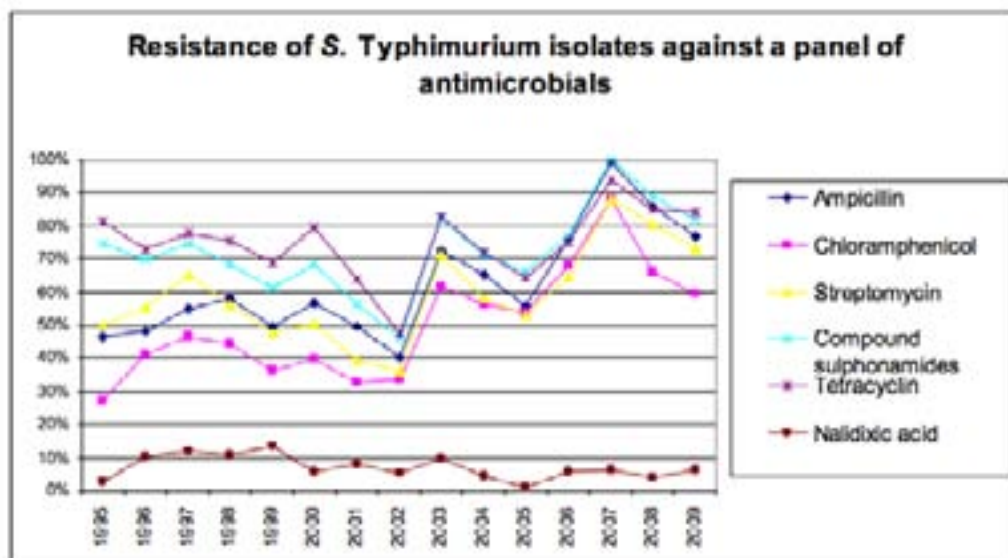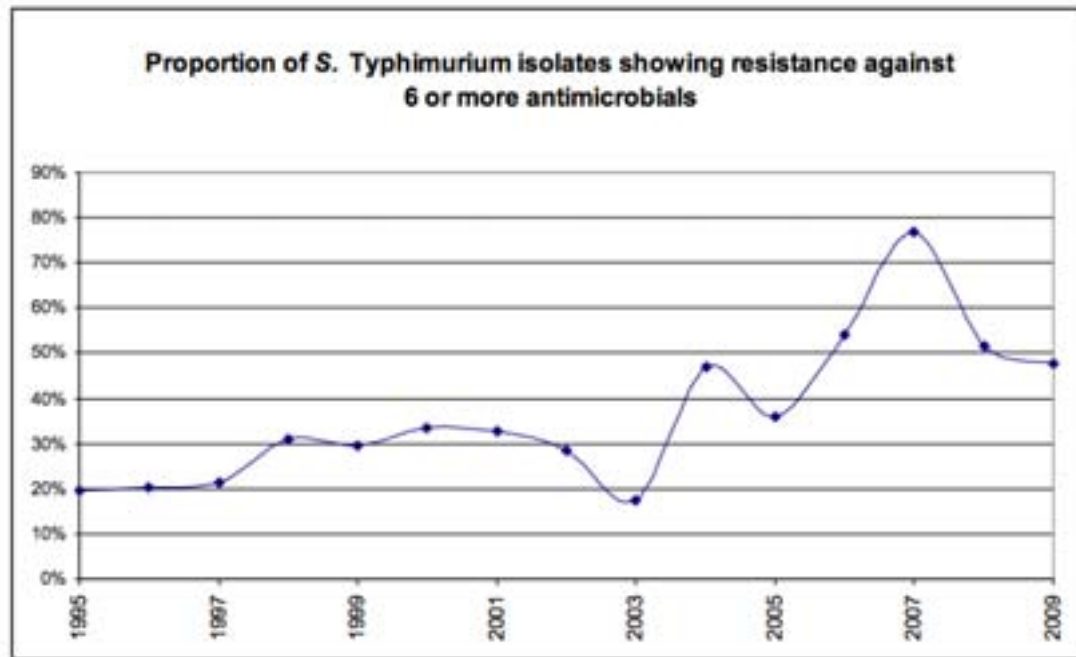
Discussion.

It is not unusual for certain Salmonella serovars or phage types to rise and fall over time and be replaced by other strains, which is shown here for pigs in Great Britain. In some cases, this is a slow process (as in the case of S. Derby in pigs), but it can become epidemic in nature, as shown by S. Typhimurium DT104. However, the emergence of multi-resistant S. Typhimurium DT104 was not confined to one species, and was seen across a wide range of animal species and humans. In England and Wales, infections in humans with S. Typhimurium DT104 went from ~200 in 1990 to >4000 in 1996 (Threlfall et al., 1998), followed by a significant decline to 2090 cases in 1998 (Threlfall et al., 1999). The situation in pigs shows a comparable trend, whereas the proportion of S. Typhimurium DT104 in cattle remained at a fairly high level since the early 1990s and only started to decline in 2007 (VLA, 2009). This is likely to be related to the higher rate of development and dissemination of new phage types in the pig industry. The reason for the fall in specific resistances and resistance to six or more antimicrobials is unclear, but the former is likely to be largely related to the emergence of S. Typhimurium and mST DT193 strains with tetracycline resistance only and the latter due to the predominance of the tetra-resistant phenotype (ASSuT) amongst such strains. The occurrence of mST S.4,12:i;- in the late 1990s is interesting as this corresponds with reports of the emergence of mST in Spain. Unfortunately, no further typing was carried out on these strains and they have not been retained.

Conclusion

Large scale pig production has traditionally been associated with the cyclical occurrence of high levels of S. Typhimurium and antimicrobial resistance. The direct relevance of this to public health is uncertain but the simultaneous occurrence of mST DT193 in pigs and humans in many countries suggests that the porcine source is important. The reason for the rapid emergence of mST DT193 across Europe since 2007 is unclear, but the organism must have some fitness advantage in competition with existing strains. The most likely explanation is limited 'herd immunity' in the early stages of national and international dissemination as the virulence of mST in pigs appears to be comparable with other S. Typhimurium strains. The role of the pig industry as a source of S. Typhimurium contamination of the environment, animal feed and poultry f ocks should also not be under-estimated.

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Clostridium difficile in pork and retail meat in Texas

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Abstract

The incidence and severity of disease associated with toxigenic Clostridium difficile (Cd) have increased in hospitals in North America from the emergence of newer, more virulent strains of Cd. Toxigenic Cd has been isolated from food animals and retail meat with potential implications of transfer to humans. The objective of the present study was to determine the prevalence of Cd in pork from sausage manufacturing plants and retail meat in Texas, and to compare two different enrichment techniques for isolation of Cd from meat. We detected 23 Cd isolates from 243 meat samples (9.5%) from three sausage manufacturing plants and five different retail meat outlets from 2004 to 2009. Twenty-two isolates were toxin A+, toxin B+, binary toxin+, and were characterized as toxinotype V, PFGE type-NAP7 or "NAP7-variant". Susceptibilities to 11 antimicrobial agents in this study were similar to those reported previously for toxinotype V isolates, although our results suggested somewhat less resistance than reported for other meat, animal, or human clinical toxinotype V isolates. Comparison of the enrichment techniques demonstrated that an extended enrichment of 15 days produced 23 isolates whereas a 7 day enrichment method produced 11 isolates (P = 0.03).

Salmonella sp. in edible offal (liver and tongue) from pigs slaughtered for consumption

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Abstract

During this study, 120 samples from slaughtered pigs (tongue swabs, n=40; liver swabs, n=40; liver parenchyma, n=40) were collected in a slaughterhouse. Salmonella sp. was isolated using conventional microbiological methods and strains were analyzed using serotyping, antimicrobial susceptibility testing and macrorestriction profiling (MRP) by Pulse Field Gel Electrophoresis (PFGE), to identify clonal relationships and potential contamination sources. The highest prevalence of Salmonella sp. was observed in tongue samples (10/40; 25%), followed by liver swab (5/40; 12.5%) and liver parenchyma samples (4/40; 10%). XbaI macrorestriction allowed defining 8 genotypes (MRP) among the 3 analyzed serotypes: S. Rissen (5), S. Typhimurium (2) and S. 4,[5],12:i:- (1). Strains with the same MRP were observed in tongue swab samples collected in different days, suggesting common contamination sources and the persistence of Salmonella sp. clones in the slaughterhouse. Also, the presence of the same MRP in liver parenchyma and in liver and tongue swabs samples seems to indicate that the pig is one of the possible vehicles of Salmonella sp. to those edible products. The results observed in this study underline the significance of Salmonella sp. contamination in pork tongue and liver that will become available for direct or indirect (through meat products) human consumption. They also suggest that measures should be taken in order to improve hygienic conditions to minimize Salmonella sp. contamination during the slaughter process and along liver and tongue processing and selling chain.

Introduction

Salmonella is an important cause of food-borne illness in humans and in recent years the increasing number of studies in this area of research has highlighted the importance of pork as a source of human salmonellosis. However, the information about Salmonella sp. distribution on the two most important pig edible offal (tongue and liver) is very scarce and, to our knowledge, this is the first study performed in Portugal on this subject. In the pig, infection within the intestinal tract may be followed by invasion of the gut cells. Infection is established in the intestinal lymph nodes and, afterwards spread to the liver is observed. Therefore, the presence of Salmonella sp. within the hepatic parenchyma is an incontrovertible evidence of pig infection. In opposite, the presence of Salmonella sp. in the tongue and liver surface mainly re**f**ects the contamination of these offal during slaughtering Contaminated offal, livers and tongues, will become available for human consumption. The major aim of the present survey was to estimate the prevalence of Salmonella–contaminated livers and tongues in pigs slaughtered in Portugal.

Material and Methods

During this study, samples from 40 slaughtered pigs were collected in one slaughterhouse along 8 sampling days during 5 months (5 pigs each visit). From each pig the following samples were collected after removal of the pluck (evisceration) and before chilling: liver swabs, tongue swabs and liver parenchyma. The liver and the tongue swabs were performed according to Swanenburg et al. (2001) and a piece of parênquima of approximately 30g was collected with aseptically material. A total of 120 samples were collected. All the samples were individually packed in a sterile labelled recipient and transported under refrigerated conditions to the laboratory where Salmonella sp. isolation was performed on the same day, according to ISO 6579:2002. Subsequently, presumptive Salmonella sp. isolates were serotyped according to the Kauffmann-White scheme in the "Laboratório Nacional de Investigação Veterinária" (Lisbon, Portugal), the Portuguese Reference Laboratory for Salmonella. Antimicrobial susceptibility testing was performed as recommended by

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the Clinical and Laboratory Standards Institute (CLSI, 2008). All Salmonella sp. isolates were tested against a total of 13 antimicrobial agents: ampicillin (10 μg), amoxicillin-clavulanic acid (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), chloramphenicol (30 μg) and trimethoprim-sulfamethoxazole (25 μg).

PFGE genotyping, was performed as described before by Vieira-Pinto et al. (2006). After PFGE, the gel was stained with ethidium bromide, photographed under UV transilumination with ImageMaster VDS DE 230 VAC (Pharmacia Biotech). The BioNumerics software (version 4.61, Applied Maths, Kortrijk, Belgium) was used to register the macrorestriction profiles, normalize densitometric traces, calculate Pearson product-moment correlation coefficient -r- and perform cluster analysis by the UPGMA algorithm.

Results

A total of 19 Salmonella-positive samples were identified. The highest Salmonella sp. prevalence was observed in tongue swab samples (10/40; 25%), followed by the liver swabs (5/40; 12.5%) and the liver parenchyma samples (4/40; 10%). A total of 7 (17.5%) livers were positive for Salmonella. In 54.6% of the sampled pigs, Salmonella sp. was observed at more than one sampling site.

Following isolation and presumptive identification, Salmonella sp. strains were analyzed by serotyping, antimicrobial testing and macrorestriction profiling, aiming to identify clonal relationships and assess for the correlation between the presence of Salmonella sp. in different pig samples, in order to unravel routes of dissemination and potential contamination sources.

Among the positive samples, 3 serotype were identified, namely S. Rissen (15/19; 79%), S. Typhimurium (3/19; 16%) and S.4,[5],12:i:- (1/19; 5%).

Table 1 presents the distribution of the Salmonella sp. serotype and antibiotic resistance profile (ARP) among the positive samples, and additional epidemiological data, regarding slaughter date and farm. XbaI macrorestriction allowed defining 8 MRP among the 3 analyzed serotypes. The results of Salmonella sp. isolation from the different samples, combined with the serological, antimicrobial resistance and MRP results, may allow us to disclose hypothetical relationships between Salmonella- positive samples:

- S. Typhimurium isolated from the liver parenchyma and tongue swab of the same pig harbour the same MRP/ ARP (Fig. 1–A), suggesting a common source of Salmonella sp. in life (before slaughter) and/or tongue contamination by the pig itself during slaughter process.
- A strain of S. Rissen isolated from the liver parenchyma of one infected pig presented the same MRP/ARP identified in the liver swabs from pigs slaughtered in the same day at the same farm (Fig. 1–B); this scenario points to the pig as the possible source of liver contamination with Salmonella sp.
- Strains of S. Rissen with the same MRP/ARP were also observed in tongue swabs from different days (Fig. 1–C), suggesting common contamination sources and the persistence of Salmonella sp. clones along the slaughter process or in the lairage.

Table 1 - Serotype and antibiotic resistance profile (ARP) of the Salmonella sp. isolates and additional epidemiological data related to the slaughter date and the farm of origin.

Date	Pig	Farm	Liver parenchyma Serotype	ARP	Liver Swab Serotype	ARP	Tongue swab Serotype	ARP
20 Jan	1-2	F	-	-	-	-	-	-
	3-5	A	-	-	-	-	-	-
27 Jan	6-10	C	-	-	-	-	-	-
03 Feb	11-15	E	-	-	-	-	-	-
10 Feb	16 -20	F	-	-	-	-	-	-
24 March	21	G	-	-	-	-	S. Typhimurium	AML, S, TE
	22-25	G	-	-	-	-	-	-
	26	G	S. Rissen	TE	-	-	S. 4,[5],12:i:-	S, C, TE
	27	G	S. Rissen	TE	S. Rissen	TE	S. Rissen	TE
28 March	28	G	-	-	-	-	S. Rissen	TE
	29	G	-	-	S. Rissen	TE	-	-
	30	G	-	-	S. Rissen	TE	S. Rissen	TE
	31	B	-	-	-	-	S. Rissen	TE
	32	B	-	-	-	-	S. Rissen	TE
05 May	33	B	S. Rissen	AML, SXT, TE	-	-	S. Rissen	TE
	34	B	S. Typhimurium	AML, NA, S, C, TE	S. Rissen	TE	S. Typhimurium	AML, NA, S, C, TE
	35	B	-	-	S. Rissen	AML,SXT,TE	S. Rissen	TE
19 May	36 - 40	D	-	-	-	-	-	-
TOTAL	40	7	4/40 (10%)		5/40 (12,5%)		10/40 (25%)	
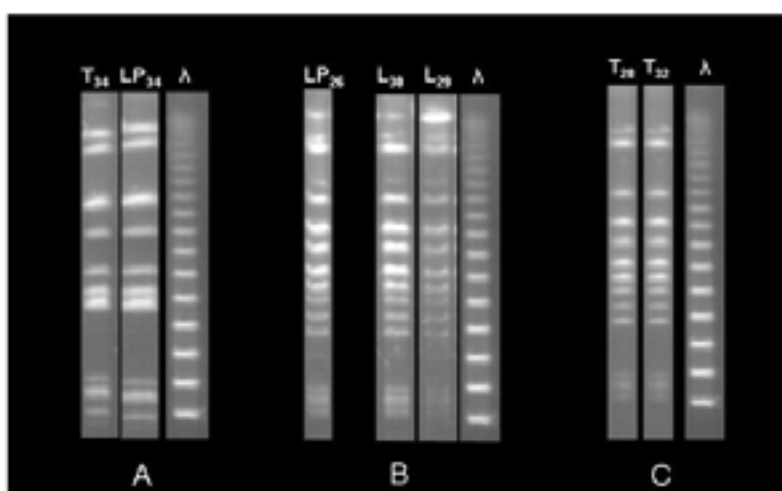
Figure 1 – Examples of MRP patterns observed in Salmonella sp. isolated from different samples (T – Tongue swab; LP – Liver parenchyma; L – Liver swab)

Discussion

In this study, the highest Salmonella sp. prevalence was observed in the tongue swab samples (10/40; 25%). This value was higher than previously observed by Swanenburg et al. (2001) who reported a prevalence of 9.3%. The presence of Salmonella sp. in the surface of the tongue may indicate not only a potential contamination by ingestion of contaminated material before slaughtering but may also suggest a contamination during the slaughtering process, for instance during evisceration, tonsils extraction or during meat inspection. Strains of Salmonella Rissen with the same MRP/ARP were obtained from tongue swabs of different days (Fig.1–C), suggesting common contamination sources and the persistence of Salmonella sp. clones along the slaughter process or in the lairage. Thus, pointing to a possible presence of a residential Salmonella f ora in the slaughterhouse (EFSA, 2008a). In order to avoid that, a regular accurate cleaning and disinfecting of all equipment and installation should be carried out (Swanenburg et al., 2001).

As previously reported by Peterson et al. (2002), the oral cavity of pig's head is frequently contaminated with pathogenic bacteria. According to our results, the tongue also seems to have an important level of Salmonella sp. contamination, underlining that additional hygienic measures should be adopted during the evisceration process that involves the removal of the tongue together with the pluck. According to Olsen et al. (2001), the presence of Salmonella sp. in the pig's oral cavity, including the tongue, may inf uence the carcass contamination. About this subject, Peterson et al. (2002) adviced that the head should be removed from the carcass at an early stage of the slaughter process and transported separately to a specific cutting room. Authors also suggested that the head meat and tongue should be heat treated before being approved for meat products.

With respect to the results of the Salmonella sp. prevalence in the liver swabs (12.5%) observed in this study, which gives information about hygiene during the slaughter process, were high than the results observed by Swanenburg et al. (2001), which reported an prevalence of 9.3%. Nowadays, according to the Regulation (EC) N.°854/2004, the post-mortem inspection procedure of the pigs liver and its lymph nodes includes visual inspection and palpation. This means that the routine liver palpation during sanitary meat inspection, may lead to cross-contamination through the inspector's hands.

Results from the liver parenchyma (10%) which gives information about infection of the pig before slaughter slaughtering, on the farm, during transport or in the lairage, were very similar to the observed for slaughter pigs infected with Salmonella sp. in ileo-caecal lymph nodes (10.3%) reported on the baseline survey on the prevalence of Salmonella in slaughter pigs, in the EU (EFSA, 2008a).

It is also important to point out that the presence of Salmonella infection in the intestinal lymph nodes, which are removed from the carcass and are not consumed, may only represent a limited public health threat, whilst a contaminated liver is likely to be a greater risk due to dissemination via the food chain.

The results observed in the liver samples highlight the importance of this offal as a vehicle of Salmonella sp. to the food chain, suggesting that hygienic and technical measures should be adopted in order to reduce the risk of cross-contamination and human infection. For instance, during meat inspection the liver should be incised only when strictly necessary and the knife used by the Official Veterinarian during its activity should be carefully and properly disinfected in order to avoid/reduce cross-contamination.

Among the positive samples Salmonella Rissen (15/19; 79%), was the most prevalent serotype, which is in accordance to the EFSA Report (2008a) that indicates S. Rissen as a serotype frequently isolated from slaughter pigs' lymph nodes in Spain and Portugal. Although S. Rissen doesn't seems to be a risk and a cause for Salmonella infections in humans within the European Union, the authors believe that the relevant frequency of occurrence along the pork production chain in Portugal, should incite to further studies on the epidemiology and virulence traits of this serotype.

The presence (3/19; 16%) of S. Typhimurium observed in this study should be emphasised, as it is the second most frequently serotype isolated from reported human salmonellosis in the EU, with increasing importance comparatively to the previous year (EFSA, 2010a). In the reported food-borne Salmonella outbreaks in 2008, pig meat and products thereof, were important food sources and may have contributed to the significant increase in S. Typhimurium outbreaks in humans. Salmonella 4,[5],12:i:- (1/19; 5%) was also detected in the present study. This monophasic S. Typhimurium has been increasing identified in the EU since 2006 as being involved in major food-borne outbreaks in humans in MSs and many non-European countries. The emergence of these new S. Typhimurium strains in pig populations and their subsequent spread to other animal species and humans is of public health significance (EFSA, 2010b). Therefore, it is recommended a special attention and concern to the presence of this serotype along the pork production chain.

Conclusions

Theis study underlines the significance of pig liver and the tongue as a potential vehicle of Salmonella sp. along processing and selling chain and also to the final consumer. The findings of this survey are expected to highlight the importance of the improvement of hygienic and practical measures in order to minimize Salmonella sp. cross contamination and multiplication along liver and tongue processing and selling chain in order to safeguard human health.

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Comparison of characteristics important for survival in pork processing environments of Salmonella Typhimurium, S. Derby, S. Infantis and S. Brandenburg

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Abstract

Salmonella is the causative agent of salmonellosis. In general, salmonellae infections in humans are foodborne. In particular food products of animal origin are an important cause of salmonellosis. Epidemiological studies have shown that in Europe up to 15-20% of all human cases of salmonellosis were associated with consumption of pork. A study by the EFSA revealed that 10.3% of the slaughter pigs are positive for Salmonella. Salmonella infection in slaughter pigs can result in contamination of pork. Contamination can occur either directly by the content of the intestines, or indirectly by cross-contamination during the processing by contact with contaminated surfaces. Serovars frequently found on carcasses at the end of the slaughter process are S. Typhimurium, S. Derby, S. Infantis and S. Brandenburg. Knowledge on the survival of these serovars in processing environments is needed to develop better strategies for control in order to minimize the risk of cross-contamination during processing. S. Typhimurium is one of the most widely studied Salmonella serovars and several characteristics that are important for survival in pork processing environments have been described. However, not much is known about the survival characteristics of S. Derby, S. Infantis and S. Brandenburg. Therefore, biofilm formation, survival on stainless steel and at different water activities, and resistance against disinfection treatment, which are considered important characteristics for survival in pork processing environments, were analysed for these serovars, and compared with S. Typhimurium. Biofilm formation was analysed under different conditions and on different surfaces, which revealed that these factors do influence biofilm formation. Although all strains used in this study were isolated from slaughter pigs or in the pork processing environment, differences between and within the serovars were observed. This study provides a broad analysis and comparison of survival characteristics of Salmonella serovars in the pork processing environment and the obtained insights may support development of strategies for control of Salmonella in pork processing environments.

Herd-level risk factors influencing serological Yersinia prevalence in fattening pig herds

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Abstract

Yersiniosis is the third most frequent zoonosis reported in the European Union with pork as an important source. Identifying risk factors in swine production which may decrease the risk of pork production contamination during pre-harvest is an important step prior to controlling Yersinia spp.. Therefore, management strategies and production processes which might be associated with fattening pigs testing seropositive for pathogenic Yersinia spp. were investigated on 80 fattening pig farms. Although more than 70 farm characteristics were included in the risk assessment, there were only a few which seemed to be connected with serological prevalence: housing on a fully slatted floor and the use of municipal water were observed in herds with low serological Yersinia prevalence, whereas recurring health problems and a low daily weight gain compared to the mean of the herds included in the study were identified more often in herds with a high prevalence.

Introduction

In humans, yersiniosis is a gastrointestinal infection caused by Yersinia (Y.) enterocolitica, and to a lesser degree by Y. pseudotuberculosis. Infection with Y. enterocolitica is recognised as a high-risk food-borne zoonotic hazard with public health relevance, having the highest risk for pork consumers (2, 16). Pigs are asymptomatic carriers of pathogenic Y. enterocolitica and tonsillar tissue is the most reliable sample to detect Y. enterocolitica in slaughter pigs (19). Pigs were also identified as regular carriers of Y. pseudotuberculosis. But the agent has seldom been isolated from fattening pigs in European countries (14).

Serological testing is preferable to bacteriological methods on the basis of practicability, time-saving aspects, and costs. There is a strong association between Y. enterocolitica positive tonsil culture and seropositivity (11, 12), whereas serological results did not correlate to bacteriological findings in faeces (22). Investigations into antibodies based on YOPs (Yersinia outer membrane protein) present a broad diagnostic tool to detect pathogenic Yersinia infected pigs on the farm (6), because all pathogenic Yersinia spp. (Y. pestis, Y. pseudotuberculosis, and pathogenic Y. enterocolitica) carry the 70-kb virulence plasmid (1), which encodes the secretion of YOPs.

Antibodies against Yersinia spp. cannot be found in all pig herds (22). The objective of this study was to find those herd factors associated with the detection of antibodies against pathogenic Yersinia antigens in fattening pig herds.

Material and Methods

The serological within-herd Yersinia prevalence of 80 fattening pig herds from Lower Saxony, Germany, was taken for risk analysis. Prevalences were estimated based on serological results of 30 slaughtering pigs per herd.

Detecting antibodies against Yersinia was performed using a commercial ELISA (enzyme-linked immunosorbent assay) test based on recombinant Yersinia outer membrane proteins. The Pigtype® Yopscreen ELISA was applied according to the manufacturer's instructions (Labordiagnostik Leipzig, Germany). A basic cut-off of optical density (OD %) 20 was used.

A standardised risk-factor questionnaire was adopted from another study (SALINPORK (10)) and modified to include 74 questions on herd size and type, housing conditions, management practice, feeding practice, and production parameters. Since serological results originate from two studies, interaction effects were accounted in a multivariate logistic regression model. The Wald chi-square test was performed to calculate the relation between farm factors and serological prevalence. As the serological prevalence within the herds represented a bimodal distribution, the herds were divided in two categories: category I with low within-herd prevalence ($\leq$ 20 % of the tested pigs were positive) and category II with high within-herd prevalence (> 20 % of the pigs were positive).

Results

The serological within-herd prevalence of Yersinia varied from 0 % to 100 %. 16.3 % (n=13) of the herds had no serological reactors. 25 % (n=20) of the investigated herds were merged into the category I and 75 % (n=60) of the herds belong to the category II. Most herds had a seroprevalence above 90 % (52.5 %, n =42) (Fig. 1).
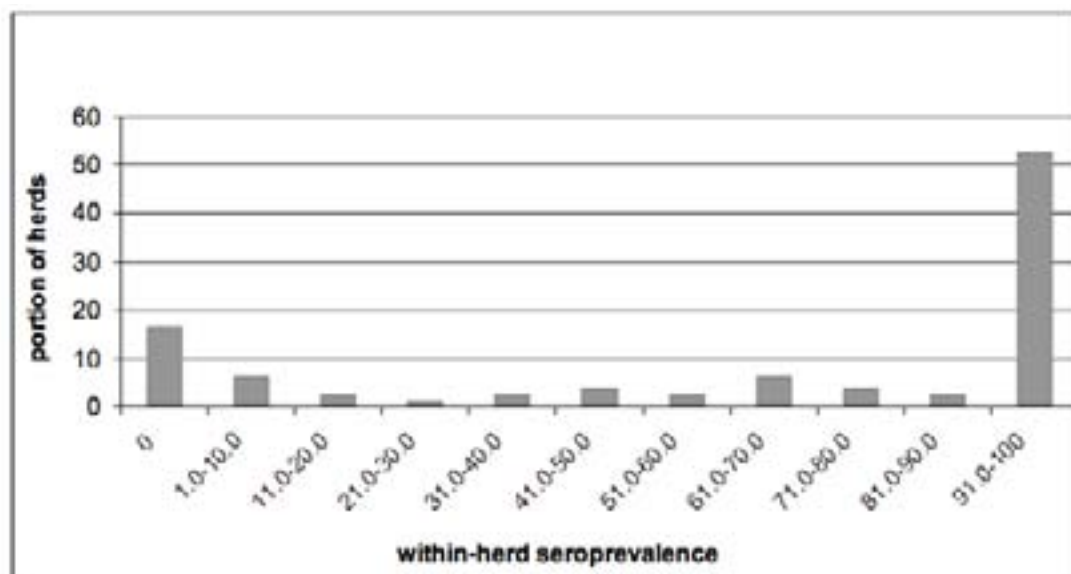
Figure 1: Frequency distribution of within-herd Yersinia seroprevalence of 80 fattening herds

Although over 70 parameters were gathered from each farm for risk analysis, only four farm factors were associated with the serological prevalence of Yersinia spp. (Tab. 1). Farms which housed the pigs on fully slatted **f**oor and which offered municipal water mainly indicated low within-herd Yersinia prevalence (category I). In contrast, farms with high within-herd prevalence (category II) often recorded recurring health problems in the fattening herd and the daily weight gain was inferior to those herds with low Yersinia prevalence.

Figure 1: Frequency distribution of within-herd Yersinia seroprevalence of 80 fattening herds

Variable	Category I (portion of herds in %)	Category II (portion of herds in %)	p-value	odds ratio	CI*
Exclusive housing on fully slatted floor	65.0 % (N=20)	36.7 % (N=60)	0.0336	0.316	0.11 – 0.92
Exclusive use of municipal water	75.0 % (N=20)	44.1 % (N=59)	0.0342	0.284	0.09 – 0.91
Recurring health problems in the herd	15.8 % (N=19)	49.1 % (N=55)	0.0219	4.902	1.27 - 20.00
Daily weight gain below the mean of 708 g	27.8 % (N=18)	71.7% (N=53)	0.0072	6.610	1.67 – 26.21

CI = confidence interval

Discussion

Bacteriological findings in faeces of finishing pigs showed that 80 % of 20 farms had at least one animal infected with Y. enterocolitica (15), which is in accordance with the serological results in the presented study. Relating to the number of infected pigs, there were 64.2 % serological positive animals. Thibodeau et al. (2001) found that 66 % of pigs at a slaughterhouse showed serological evidence of previous infection (20). Bacteriological findings of pathogenic Y. enterocolitica in tonsils of slaughtered pigs in Germany confirm these data (3).

The applied ELISA cannot distinguish between infections with different pathogenic Yersinia spp.. Y. pestis, the etiological agent of plague, is not found in Europe Y. pseudotuberculosis is especially found in production systems where pigs have contact with the outside environment, e. g. organic production (7). Against, pathogenic Y. enterocolitica was found more

frequently in pigs from conventional housing (13), like the herds included in this study. It is assumed that the presented serological results were mainly caused by infection with Y. enterocolitica, although an infection with Y. pseudotuberculosis cannot be completely ruled out.

Y. enterocolitica is transmitted from infected faeces or picked up from the floor of a contaminated pen (5). Fully slatted floor do not enable the oral intake to such an extent like partially slatted and solid floor. The risk factors "recurring health problems in the herd" and "low daily weight gain" might be associated. A low daily weight gain might be the consequence of recurring health problems, because illness causes inadequate feed intake and weight loss e.g. diarrhoea. Diarrhoea might lead to an increased shedding of the agent, causing a spread of the infection on the farm. Unfortunately, the information concerning the cause of health problems was invalid for analytical purposes. Virtanen et al. (2011) detected a relation between higher carriage and shedding prevalence of Y. enterocolitica to the use of tetracycline. They speculated that the need for tetracycline on farms reflected also the lower health status of pigs on these farms, which in consequence would be associated with the Y. enterocolitica prevalence (21).

A low serological within-herd prevalence was detected in farms exclusively using municipal water. Using this water was also discovered to be a protective factor for carriage and faecal shedding of Y. enterocolitca in pigs (21). Case-controlled studies in humans have identified drinking untreated water as a risk factor for Y. enterocolitica infection, but strains mainly belong to biotype A (17), which do not carry the virulence plasmid. A coherence between drinking untreated water from wells and streams and a infection with Y. pseudotuberculosis infections was described in Japan (4).

The findings of Laukkanen et al. (2009) might also be connected with drinking water. They found "drinking from a nipple" and "wet feeding" to be risk factors associated with a high bacteriological prevalence of Y. enterocolitica in slaughtered pigs (8). Although "wet feeding" was also included in the presented analysis, it could not be demonstrated as a risk factor. Fattening pig herds were found to have higher prevalence of antibodies to Yersinia than conventional farrow-to finish herds (18). The suggested beneficial effect of integrated or closed herds might have been included in the question relating to the number of suppliers, but there was no association with seropositivity. Skjerve et al. (1998) also demonstrated under-pressure ventilation and manual feeding of slaughter pigs to be protective factors, whereas using own vehicles for transporting animals to the abattoir, keeping clean and unclean sections in herds separate, using straw and daily observations of a cat with kittens increased the risk. Not all of these factors were requested in the presented study, but use of own transport for slaughter pigs and contact with other farm animals or pets on the farm were not apparent risk factors.

Further farm factors which were associated with high bacteriological prevalence of Y. enterocolitica were presented by Laukkanen et al. (2009). High prevalence was associated with the absence of coarse feed or bedding, which in turn seemed to represent typical factors for organic and small conventional farms (8). In the presented study, no association between herd size and seropositivity could be detected. Other described risk factors, such as production capacity (8), no access of pest animals to pigsty (8) and medicated feed (9) could not be supported by our findings.

Conclusion
The epidemiology of Yersinia spp. on swine farms is complex. Further investigations should necessarily include testing the presumed risk factors to evaluate their potency to affect the prevalence in fattening pig herds.

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Proceedings - Posters

Clostridium difficile in a farrowing pen

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Abstract

Clostridium difficile is an important cause of enteric disease in humans. In pigs Clostridium difficile can cause neonatal enteritis and can be isolated from faeces from diseased and healthy animals. According to recent research, isolates from humans and animals show genetic and phenotypic overlap. In The Netherlands, strains isolated from diseased piglets were indistinguishable from strains isolated from Dutch patients. These strains belonged to ribotype 078. Because pigs can either be clinical hosts and/or may be a possible reservoir more understanding of the epidemiology of Clostridium difficile among pigs is needed. The objectives of this study were to specify whether, how and when newborn piglets get infected by Clostridium difficile for the first time. With this intention, six sows, their farrowing crates and litters (71 piglets) at one farm were sampled around the day of birth of the piglets. Within 48 hours after birth, all sampled 71 piglets at the farm became positive for Clostridium difficile ribotype 078. Moreover, all sows became positive within 113 hours after birth of the piglets and the farrowing crates were intermittently positive during the sampling period. This research shows that the sow, the farrowing crate, the air and the teats of the sow are possible transmission routes of Clostridium difficile ribotype 078.This information might help to advise farmers on taking measures against Clostridium difficile infections in neonatal piglets.

Introduction

Clostridium difficile (CD) is an anaerobic Gram-positive, spore-forming bacterium. It is widely distributed and can be found in soil, water, intestinal tracts of animals, and even on meat CD has been found in a wide variety of animal species e.g. pigs, calves, dogs, horses, ostriches, elephants, ratites, cats and mice.

Isolation by culturing and toxin detection are the main methods used in the laboratory for diagnosis of CD associated disease (CDAD). For typing of CD various techniques are used, as PCR ribotyping, pulsed-field gel electrophoresis and toxinotyping (Weese, 2010). Using PCR-ribotyping, CD can be divided in more than 300 different ribotypes.

In piglets, an infection with CD can cause neonatal enteritis. Pigs, 1-7 days old, are affected and may show diarrhoea, although, some pigs are obstipated. (Songer et al., 2006) The CD ribotype mostly found in neonatal piglets in The Netherlands is ribotype 078 (Keessen et al., 2010).

Debast et al. (2009) isolated CD ribotype 078 strains from diseased piglets from two Dutch pig-breeding farms with problems of neonatal diarrhoea and from Dutch human patients. The ribotype 078 strains were indistinguishable. Therefore a common origin of human and animal strains could be considered.

Because pigs can be either clinical hosts of CD and/or possible reservoirs for humans, more understanding of the epidemiology of CD among pigs is needed. The objective of this study was to determine how soon after birth, CD ribotype 078 could be isolated from newborn piglets. In addition, the newborn's environment, e.g. sows and farrowing crates, were sampled for CD to determine by which routes piglets get infected.

Materials and methods

Sampling

The research was conducted at a Dutch pig breeding farm with 200 sows, where Clostridium difficile ribotype 078 was known to be present. Sows, the environment and piglets (normally born or after caesarean section) were sampled.

Sampling before and after normal parturition: at the moment the sows (6) entered their farrowing crate, approximately one week before parturition, a stool sample of the sow and a sample of the farrowing crate were collected. Within 15 hours after birth of the piglets, the sow, its environment and the neonatal piglets were sampled. A rectal stool sample was taken of the sow, the environment was sampled using electrostatic cloths and piglets were sampled using rectal swabs. All 72 piglets from these 6 litters got an ear tag and were monitored for illness, as diarrhoea, during the sampling period.

Subsequently, the newborn piglets, the sow and the environment were sampled once a day until Clostridium difficile was detected. The teats of the sow and the ambient air of the farrowing compartment were infrequently sampled.

Another six sows and their environment were sampled before delivery; their piglets were not monitored, because first samples of the newborn piglets could not be taken within 15 hours post partum. Sampling is described in Hopman et al, 2011.

Culturing

All rectal and environment samples were cultured for CD using an enrichment broth as described by Rodriguez-Palacios et al. (2007). After enrichment in Clostridium difficile moxalactam norfoxacin (CDMN) broth (broth produced by Mediaproducts, The Netherlands), the culture broth was homogenized and two ml was transferred into a sterile tube. The broth was mixed with two ml 96% ethanol and left at room temperature for >60 minutes (alcohol shock to select for bacterial spores). After centrifugation (4000 x g for 10 min), the supernatant was discarded and the sediment was plated onto commercially-prepared Clostridium difficile agar (Clostridium difficile agar (CLO agar), Biomérieux). These culture plates were incubated anaerobically, using gaspaks (GasPak EZ Anaerobe Container System Sachets, BD) and anaerobic jars, at 37 oC for at least 48 hours.

Colonies characteristic for CD were identified by morphological criteria, the characteristic horse-manure odour and Gram-staining. Confirmation of identification of CD was done at the University Medical Center in Leiden. Genetic identification of CD was done by an in-house PCR for the presence of the gene encoding glutamate dehydrogenase (gluD) specific for CD. All strains were further investigated by PCR-ribotyping

Results

Presence of Clostridium difficile 078 in sows ante en post partum, piglets and the farrowing crate ante partum are described in table 1.

Table 1
Presence of Clostridium difficile ribotype 078 in sows, her individual farrowing crate and her piglets

	Litter (# piglets sampled) (# piglets born dead):					
	One (10) (0)	Two (11) (0)	Three (10) (0)	Four (14) (1)	Five (12) (1)	Six (15) (3)
Sow:						
ante partum	-ᵇ	-	-	-	-	-
≤1 hour p.pᵇ.	ndᶜ	nd	-	-	-	nd
10 - 26 hour p.p.	-	-	-	-	+	-
32 – 48 hour p.p.	-	+	-	-	nd	+
	First + at 56 hrs p.p.		First + at 113 hrs p.p.	First + at 72 hrs p.p.		
Farrowing crate:						
ante partum	+ᵈ	lost sample	-	+	+	+
≤1 hour p.p.	nd	nd	-	-	-	nd
10 - 26 hour p.p.	-	+	-	+	+	+
32 – 48 hour p.p.	+	+	+	+	+	+
Piglets:						
≤1 hour p.p.	nd	nd	1/10	1/14	0/12	nd
10 - 26 hour p.p.	0/10	4/11	8/10	8/13ᵉ	11/12	13/15
32 – 48 hour p.p.	10/10	11/11	10/10	13/13	12/12	15/15

a (-) CD 078 not found after culturing, b (p.p.) post partum, c (nd) not done, d (+) CD 078 found after culturing, e one piglet died
On teats of two sows, Clostridium difficile 078 was found ante partum. All samples of the teats taken post partum, were positive for CD 078. Ante partum the air had been sampled in one farrowing pen and was found to be positive for CD 078. All air samples of the farrowing pens, taken post partum, were positive for CD 078 at the moment of sampling.

Discussion and conclusion

This study demonstrated that all sampled newborn piglets, irrespective of the presence of diarrhoea, got infected with CD ribotype 078 within two days after birth. Within this herd, just one ribotype, CD 078, was isolated, not only from neonatal piglets, but also from sows, ambient airsamples, and from the environment of the piglets. CD seems to be able to spread easily between sows, piglets and the environment. In the present study, only one ribotype was found. It is possible that pigs are infected with more ribotypes and that more ribotypes are present at the farm but that the isolation techniques are more sensitive for CD ribotype 078 and other ribotypes are overlooked. Our findings suggest that all piglets become infected with one ribotype. Little is known about the spread, contamination and infection of CD through aerosols in piglets. At the selected farm, the farrowing pens were always cleaned, after weaning of the piglets, using alkaline foam cleaner. Occasionally, Halamid® was used. CD 078 was found on the floor, in the air and under boots (data not shown), so it might be concluded that the cleaning and disinfection protocol used at this farm, is inadequate to kill CD or the protocol is not executed properly. To control CD 078 at farms, effective hygiene and disinfection procedures should be established. Environmental disinfection should be performed using sporocidal agents, which ideally contain chlorine.

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Variety of Clostridium difficile PCR ribotypes in pigs arriving at the slaughterhouse

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Abstract

Food products of animal origin might play a role in interspecies transmission of C. difficile. In pigs, Clostridium difficile can cause neonatal enteritis and can be isolated from faeces from both diseased and healthy animals. To determine the prevalence of C. difficile in Dutch pigs arriving at the slaughterhouse a pilot study was conducted at one slaughterhouse in the Netherlands. Rectal faecal samples were taken from fifty slaughtering pigs from ten farms just after the pigs were sedated. These samples were examined using a real time PCR (BD GeneOhmTM Cdiff Assay), in combination with culturing after enrichment. Using real time PCR, none of the faecal samples were found to be positive for C. difficile while after culturing 14 samples (coming from pigs from nine different farms) were found to be positive for C. difficile. The positive samples derived from 9 different farms and encompassed seven different ribotypes.

Introduction

Clostridium difficile has been found in meat samples destined for human consumption. This suggests that food products may play a role in interspecies transmission of C. difficile (11, 12, 13).

In Canada, 60 retail ground meat samples were analyzed for the presence of
C. difficile using an enrichment broth. C. difficile was isolated from 20% (12) of these samples purchased over a 10-month period in 2005. Eleven isolated strains were toxigenic (10). In Tucson, Arizona, 88 retail meat samples were collected and analyzed for the presence of C. difficile. Forty-two percent (37) of these samples yielded C. difficile i.e. 41.3 % of the analyzed porcine meat samples. (12) In Austria, three percent of ground meat samples tested positive for C. difficile, two of the isolates were identified as Austrian Isolate-57 and one isolate was identified as ribotype 053. These isolates only came from mixed beef and pork samples. (5) De Boer et al. (2011) could not isolate C. difficile from 63 pork samples in the Netherlands in 2011 (2).

Little is known about the route of contamination of pork or the risk of foodborne transmission. Metcalf et al. (2010) suggested different routes like contamination of pork originating from pigs arriving at the slaughterhouse or through humans working in food production (8).

The prevalence of C. difficile has been investigated among 165 pigs arriving at the slaughterhouse in Switzerland. None of these pigs were positive for C. difficile (3). There seems to be an age dependent decline in colonization rate of pigs with C. difficile as was reported by Weese et al. (2010). At day 2, 74% of the piglets were colonized while on day 62 only 3,7 % were positive for C. difficile (15). Contamination of pork originating from pigs arriving at the slaughterhouse is only possible if pigs at that age are colonized with C. difficile. Therefore, the colonization rates of pigs at the time of slaughter are more relevant to human exposure than those earlier in life (15). To determine the colonization rate of pigs with C. difficile arriving at the slaughterhouse a pilot study was carried out at one slaughterhouse in the Netherlands.

Materials and methods

Fifty rectal faecal samples taken of Dutch pigs, just after they had been electrically stunned and bled at a slaughterhouse situated in the southern part of the Netherlands, were tested for the presence of C. difficile. All animals arriving at the slaughterhouse were processed in their original peer group. Therefore it was possible to collect five rectal samples randomly within one herd. Five samples per pig farm were collected resulting in the acceptance of ten Dutch farms in this study. The number of 50 pigs from 10 farms was chosen because this project was a pilot study to determine an estimate of the colonization rate of pigs with C. difficile.

These rectal faecal samples were examined using a real-time PCR (BD GeneOhmTM Cdiff Assay) directly on faecal samples, in combination with culturing after enrichment.

BD GeneOhmTM Cdiff Assay
BD GeneOhmTM Cdiff Assay is an in vitro diagnostic test for direct and qualitative detection of C. difficile toxin B gene in human stool specimens from patients suspected of having C. difficile infection (CDI). This assay has not yet been validated for use in faeces from animals. The test is based on real-time PCR and performed directly on stool specimens, and in case of faeces from pigs, directly on faeces.
Faeces was collected and transported directly to the laboratory (under cooled conditions) where the manufacturer's instructions for human samples were followed.

Culturing, identification and confirmation of C. difficile
All faecal samples were cultured for C. difficile using an enrichment broth as described by Rodriguez-Palacios et al. (2007) (10). The culturing protocol was followed as described by Hopman et al. (2010) (4). Approximately 1 g of faeces was placed into 9 ml of Clostridium difficile moxalactam norfoxacin (CDMN) broth (broth produced by Mediaproducts, the Netherlands) and incubated anaerobically at 37oC for 24 hours. After broth incubation, the broth was homogenized and 2 ml was mixed with 2 ml 96% ethanol in a sterile tube and left at room temperature for a minimum of 60 minutes (alcohol shock to select for bacterial spores). After centrifugation (4000 x g for 10 min), the supernatant was discarded and the sediment was plated onto commercially prepared C. difficile agar (CLO-agar, Biomérieux). These CLO-plates were incubated anaerobically at 37 oC for 48 hours.
Colonies characteristic for C. difficile were identified by their morphological shape, their characteristic horse-manure odour and positive Gram-staining. Only when culturing revealed colonies characteristic for C. difficile, a colony was sent for confirmation of identification at the National Reference Laboratory for C. difficile at Leiden University Medical Center. Genetic identification of C. difficile was done by an in-house PCR for the presence of the gene encoding glutamate de-hydrogenase (gluD) specific for C. difficile (9). All strains were further investigated by PCR ribotyping based upon Bidet et al. (2000) (1). Detection of toxin genes was done as described by Van den Berg et al. (2007) (13)

Results
C. difficile was cultured from 14 (28%) of 50 faecal samples, derived from pigs originating from nine different farms (table 1). Seven different ribotypes were found. which all were positive for TcdA and TcdB. The real-time PCR (BD GeneOhmTM Cdiff Assay) performed directly on faecal samples gave no positive results.

Discussion and conclusion
Present results demonstrate that C. difficile can be found in Dutch pigs at the moment of slaughtering. In total, 14 out of 50 samples were positive for C. difficile (28%). Pigs, from nine of ten examined farms, proved to have C. difficile in their faeces. Seven different ribotypes were found: C. difficile ribotype 005, 013, 015, 035, 062, 078 and a C. difficile belonging to an unknown ribotype. This variety of C. difficile PCR ribotypes in pigs is a remarkable finding since rarely other PCR ribotypes than PCR ribotype 078 have been isolated from piglets in the Netherlands (6). PCR ribotype 045 has been isolated from a few piglets on farms that housed predominantly PCR ribotype 078 positive piglets (results not shown). Except for PCR ribotype 078, the other PCR ribotypes found in this study are not belonging to the seven most frequently found PCR ribotypes in human C. difficile infections (data from the National Reference Laboratory for C. difficile). Data on the prevalence of found PCR ribotypes among other animals in the Netherlands are limited, but a recently completed study indicated that ribotype 005 was present in horses and pigs, ribotype 015 in sheep and ribotype 035 in horses (7). It is possible that pigs acquire C. difficile from the environment during transport to the slaughterhouse. More research is needed to find out whether this is route of contamination plays a role in the acquisition of C. difficile by pigs.
Our results are in great contrast with the findings of Hoffer et al. (2010) who found no positive pigs among 165 pigs sampled at the slaughterhouse (3). Colonization percentages are of course infuenced by the methodology used to detect C. difficile. Hoffer et al. did not use the same enrichment broth as was used in present study (3). In present study a specific C. difficile enrichment broth containing moxalactam norfoxacin and 5% horse blood as described by Rodriguez-Palacios et al. (2007) was used (10). Hoffer et al. used brain heart infusion broth (Difco, Becton Dickinson, Sparks, MD) which is a general-purpose liquid medium used in the cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria.

The real-time PCR used direct on faecal samples was not sensitive enough to determine the presence of C. difficile in pigs because no positive samples were found, while 14 cultures after enrichment were positive for C. difficile (culturing without enrichment was not performed). The real-time PCR has not yet been validated for use in pigs and is predominantly used in stool specimens of human patients already suspected of having CDI. Possibly the number of C. difficile bacteria in porcine faecal samples is low explaining why the real-time PCR gave no positive signals but also inhibiting factors in pig faeces could inf uence the results of this test. Culturing after enrichment, although it is time-consuming, seems to be the best technique to check for C. difficile positive slaughtering pigs.

Finding C. difficile in faeces of pigs raises the question whether contamination pre-, during or post-processing can explain contaminated retail products. Explanations can be found by doing further research at slaughter and processing.

Present data indicate that pigs arriving at the slaughterhouse in the Netherlands are positive for C. difficile in their faeces. Found variety of ribotypes is remarkable because at farm level only a limited number of ribotypes has been found.

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Proceedings - Posters

Carriage of Campylobacter by sows and spread to fattening pigs in farrow-to-finish farms

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Abstract

We carried out a one-year study, in 2008, at 53 farrow-to-finish farms in Brittany, France, to determine the proportion of sows excreting Campylobacter and to determine whether Campylobacter excretion by fattening pigs on these farms was related to transmission from sows. We also determine the genotypes of the Campylobacter isolates.

Ten samples of feces from sows were collected from randomly selected sites (maternity, service and gestation areas) on the 53 farrow-to-finish farms. Sampling was also carried out during the fattening stage (four samples per farm) on 27 of the 53 farms. Feces were 10 fold diluted and direct streaking was done on Karmali plate. Plates were then placed at 37°C during 48h in microaerobic atmosphere. Campylobacter isolates were identified by PCR and typed by PFGE. Campylobacter was detected in 25.1% of the 530 samples from sows, and 69.8% of the 53 pig farms had at least one positive sample (of 10 taken). Campylobacter was detected in 15.4% of the 168 samples from fattening pigs and 62.9% of the 27 farms studied had at least one positive sample (of 4 taken). All the Campylobacter isolates belonged to the C. coli species. They displayed a very high level of genetic diversity, also inside farms and few genotypes were common to several farms. Only few genotypes were common to both fattening pigs and sows. However, samples from fattening pigs at which Campylobacter had been detected in feces from sows were more likely to have a positive feces sample than those from farms at which the bacterium had not been detected in feces from sows.

This study provided recent valuable information on the occurrence of Campylobacter in farrow-to-finish farms and on its spread between sows and fattening pigs.

Introduction

Campylobacter sp. is one of the most frequent causes of human enteritis in industrialized countries. The main source of human Campylobacter infections, as highlighted by many epidemiological studies, is the consumption of contaminated food — particularly raw or insufficiently cooked poultry products (Moore et al., 2005). Pork meat has also been implicated in human Campylobacter infection. Friedman et al. (2004) in the USA identified the consumption of non poultry meats, such as hamburgers, pork roasts and sausages, as a high risk factor for sporadic Campylobacter infections. Pigs are a natural reservoir of Campylobacter, with a prevalence of infection superior to 50%, (Minvielle et al., 2007), with Campylobacter coli the predominant species.

The goals of this study were to determine the proportion of sows excreting Campylobacter at farrow-to-finish pig farms, to determine whether Campylobacter excretion by fattening pigs on these farms was related to transmission from sows and to analyze the species and genotype diversity of the Campylobacter population found on these farms.

Material and Methods

Samples. 53 farrow-to-finish farms from Brittany, France, were sampled from January to December 2008. Ten samples of feces (representing each at least 10 sows in the room) were realized randomly at different sites (maternity, service area, gestation) in each farm; each site being represented at least one time in a farm. When sows were in individual housing in a room; at least 10 sows were considered in the sample. Sampling was also carried out during the fattening stage (four samples per farm) on 27 of the 53 farms.

Campylobacter detection. We carried out only direct streaking tests with our fecal samples. For each sample, 25 g of feces was diluted 1:10 in peptone-buffered water and 1 ml was streaked directly on three Karmali plates. Plates were incubated at 37°C in a microaerobic atmosphere for 48 h. Typical colonies on Karmali were then sub-cultured on blood agar plates for 24h at 37°C for Campylobacter confirmation as described in the ISO 10272 method and for species identification and genotyping.

Species identification. DNA extraction was done by blowing the cells at 95°c for 10 min. Multiplex-PCR (Wang et al. 2002) was used to confirm the genus of the bacterial isolates and to identify them to species level (Campylobacter jejuni, C. coli, C. lari, C. fetus, and C. upsaliensis). PCR products were visualized by the electrophoresis of 10 μl aliquots of each amplification product, for 3 hours at 100 V, in a 2% agarose gel stained with ethidium bromide.

Pulsed-field gel electrophoresis (PFGE) and analysis of electrophoretic profiles. DNA preparation, restriction endonuclease digestion and PFGE were carried out as described by the Campynet protocol (Rivoal et al., 2005). Two profiles, corresponding to the restriction profiles obtained with SmaI and KpnI, were obtained for each isolate. Electrophoretic patterns were compared using BioNumerics® (Applied Maths, Sint-Martens-Latem, Belgium). Similarities between profiles were determined by calculating the Dice correlation coefficient, with a maximum position tolerance of 1%. A dendrogram based on the combined results for Kpn1- and Sma1-digested DNA (KS) was constructed. Strains were clustered by the unweighted pair-group method using the arithmetic mean (UPGMA) (Struelens et al., 1996). The Simpson's index (D) was determined as described by Hunter (1990).

Results

Finally, 25.1% 95%CI [20.8-29.3] of the 530 samples from sows were tested positive for Campylobacter and at least one of the ten samples taken was positive in 37 farms among the 53 farms (69.8% 95%CI [56.3-83.2]). Low levels of contamination were found within the positive farms, with 71.7% of the farms for which a positive result was obtained having no more than three positive samples. The excretion of Campylobacter by sows was not associated with the stage of the sows at which samples taken; 18.8 %, 28.2%, and 22.2% of the fecal samples carried the bacteria at the service area stage, the gestation stage and the maternity stage, respectively. For fattening pigs, 15.4% 95%CI [7.8-23.0] of the 168 samples tested positive for Campylobacter and at least one of the four samples taken tested positive for Campylobacter on 62.9% 95%CI [44.0-81.8] of the 27 farms.

All the Campylobacter isolates belonged to the C. coli species. Simpson's index was high, D=0,998 95%CI [0,997-1,000], consistent with a high degree of genetic diversity in the Campylobacter population from pig. They displayed a very high level of genetic diversity, also inside farms and few genotypes were common to several farms. In 12 cases, isolates shared the same genotype. In 10 of these cases, the isolates with identical genotypes were obtained from the same farm. In only two cases isolates with identical genotypes came from different farms: isolates 08MD0081, 08MD0082 (farm no.75) and isolate 08MD0388 (farm no. 260), on the one hand, and isolate 08MD0139 (farm no. 120) and isolate 08MD0169 (farm no. 122), on the other. Diversity of genotypes from sows inside farm could be high. The number of genotypes varied from one to height. In 16 farms, only one or two genotypes were found. In 14 farms, 3 to 5 genotypes were identified, and in 7 farms more than 6 genotypes.

Only few genotypes were common to both fattening pigs and sows. However, excretion of Campylobacter by sows was also recorded at 14 of the 17 farms (of 27 tested) on which Campylobacter excretion by fattening pigs was detected (table 1). The risk of fattening pigs excreting Campylobacter in their feces was higher on farms at which Campylobacter excretion by sows was observed.

Discussion

In our study, 25.1% of feces samples from sows tested positive for Campylobacter. C. coli was the only Campylobacter species present. Our results are similar to those of previous French studies (Magras et al., 2004; Minvielle et al., 2007). Leblanc Maridor et al., (2008) showed that if pigs were orally inoculated simultaneously with several species of Campylobacter, C. coli was the species with the strongest colonizing capacity.

Sows in France are thus a reservoir of Campylobacter and could be a source of contamination of the piglets. Wehebrinck et al. (2008) reported, for a farm in Germany, that 33.8% of the sows and 64.7% of the fattening pigs excreted Campylobacter. Finally, Campylobacter was detected in 77% of the 1448 feces samples from sows taken at American farms (Wright et al., 2008). In our study, 69.8% of the farrow-to-finish farms exhibited at least one positive sample which is closed to 52.9% reported by Oporto et al. (2007) however, 71.7% of our positive farms had no more than three positive samples. This situation may result from effective control through the use of sanitary barriers within farms, limiting propagation of the bacterium between different areas of the farm.

The C. coli isolates from our pig farms displayed a high level of genetic diversity which is similar to other studies in which PFGE was used for typing (Laroche et al., 2008). Only in three cases isolates with the same genotype come from two different farms. This high level of diversity makes it difficult to identify a common origin of contamination for pig farms

affected by Campylobacter. Our work highlighted for some farms several genotypes indicating that numerous Campylobacter can circulate in the pig buildings of a farm and suggesting several sources of contamination. Soultos and Madden (2007) previously reported that piglets were initially contaminated with bacteria of the same genotype as those infecting their mothers. These authors considered the sows to be a source of piglet contamination. Magras et al., (2004) reached the same conclusion following the isolation of Campylobacter coli from 79% of fecal samples taken from sows on nine French farms.

Conclusion

This study provided recent valuable information on the occurrence of Campylobacter in farrow-to-finish farms and on its spread between sows and fattening pigs. Sows are a reservoir of Campylobacter and risk that fattening pigs excrete the bacteria increases if Campylobacter excretion by sows was observed in the same farms.

Acknowledgements

Table 1: Relationship between Campylobacter shedding by fattening pigs and sows from same farms (27 farrow-to-finish farms)

	No of farms with *Campylobacter*-negative fattening pigs	No of farms with *Campylobacter*-positive fattening pigs	total	% positive
No of farms with *Campylobacter*-negative sows	3	3	6	50.0
No of farms with *Campylobacter*-positive sows	7	14	21	66.6
Total	10	17	27	62.9

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Shedding of Listeria monocytogenes by sows in French farrow-to-finish pig farms: prevalence, serotype and risk factors of contamination

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Abstract

This work was undertaken in 2008 to estimate the prevalence of L. monocytogenes in French farrow-to-finish pig farms at the breeding pig level and to determine risk factors of contamination of sows by L. monocytogenes.

A total of 730 feces (10 per farm) were sampled from sows in 73 pig farms. 172 samples were also taken during the fattening stage, at 43 of the 73 farms (4 per farm). Detection of L. monocytogenes was carried out according to the ISO 11290-1/A1 method and isolates were serotyped. Generalized Estimating Equations were used in order to determine risk factors associated to contamination of sows by L. monocytogenes.

For sows, 46.6% of the farms and 11.3% of the samples were positive for L. monocytogenes. The 83 positive samples provided a total of 125 strains. Serotype 1/2a, 1/2b and 4b were the most prevalent serotypes with 41.6%, 36.0% and 20.8% of the strains, respectively. Of the remaining isolates, 1.6% were attributed to serotype 1/2c. Moreover, the serotype 1/2a, 1/2b and 4b were found in 21, 17 and 11 farms respectively. The serotype 1/2c was detected in only one farm. In 20 farms, only one serotype was found. In 11 farms, 2 serotypes were identified, and in 3 farms until 3 serotypes.

The prevalence in the fattening rooms was estimated at 25% and L. monocytogenes was confirmed in 14.5% of samples. The 33 strains collected belonged to four serotypes: 1/2a(30%), 1/2b(43%), 4b(24%) and1/2c(3%). The risk of fattening pigs excreting L. monocytogenes in their feces was higher on farms at which L. monocytogenes excretion by sows was observed (OR=33.51).

Different factors were associated to contamination of sows by L. monocytogenes: a food completely or partly made in farm, the production stages "service area" and "gestation area" and the period "autumn/winter". An antibiotic treatment during the 4 weeks before the sampling reduces the shedding of L. monocytogenes. This survey also showed that the sows were source of contamination by L. monocytogenes of finishing pigs.

Introduction

In 2009, there were 1,645 reported cases of human listeriosis in the European Union, making L.monocytogenes the fifth most important zoonotic agent implicated in human enteritis, in terms of the number of cases (EFSA and ECDC, 2011). The number of listeriosis cases in humans increased by 19.1 % compared to 2008. Serotypes 1/2a, 1/2b and 4b are to those usually involved in human listeriosis in Europe (Goulet et al. 2008).

Pig and pork products have been identified as the main source of human infection by L.monocytogenes. In various industrialized countries, pork products were specifically involved in listeriosis (Goulet et al., 1998; De Valk et al., 2001; Thevenot et al, 2006).

In 2000-2001, Beloeil et al. (2003) detected L. monocytogenes in 14% in fattening pigs of the 93 French farms investigated. In this study, no search for this germ was realized on sows of these same farms.

This work was undertaken in 2008 to estimate the prevalence of L. monocytogenes in French farrow-to-finish pig farms at the breeding pig level and to determine risk factors of contamination of sows by L. monocytogenes. Distribution of serotypes was also considered.

Materials and methods

Samples and questionnaire. Seventy-three farrow-to-finish Brittany farms were sampled in 2008. Ten samples of feces (representing each at least 10 sows in the room) were realized randomly at different sites (maternity, service area, gestation) in each farm. A total of 730 samples were collected for sows.

Samples were also taken during the fattening stage, in 43 of the 73 farms. Four feces samples (representing each at least 10 pigs in the room) were taken in 4 different fattening rooms chosen at random in the farm. A total of 172 samples were collected for fattening pigs.

General data relating to the farm and management of the pigs were recorded (total numbers of sows and of fattening pigs, etc.). Data concerning pigs related to a sample at the day of the sampling were collected (type of feed, origin of feed, antibiotic treatment, age of the sows, etc.).

Detection and serotyping. All the samples were analyzed for L. monocytogenes detection according to a modified protocol based on the standard NF EN ISO 11290-1 published in 2005. All the collected strains were serotyped with sera purchased from Eurobio (Les Ulis, France)

Statistical analysis from the questionnaire. The overall significance of the link between each explanatory variable and the outcome variable (presence/absence of L monocytogenes) was performed through Wald statistics for Type III GEE analysis (Horton and Lipsitz, 1999). Generalized Estimating Equations were computed with the GENMOD procedure of the SAS 9.1 software.

Results

For sows, 46.6% of the 73 farms and 11.3% of 730 the samples were positive for L monocytogenes. The 83 positive samples provided a total of 125 strains. Serotype 1/2a, 1/2b and 4b were the most prevalent serotypes with 41.6%, 36.0% and 20.8% of the strains, respectively. All the remaining isolates were of serotype 1/2c (1.6%). Moreover, the serotype 1/2a, 1/2b and 4b were found in 21, 17 and 11 farms respectively. The serotype 1/2c was detected in only one farm. The number of serotypes per farm varied from one to three (table1). In 20 farms, only one serotype was found. In 11 farms, 2 serotypes were identified, and in 3 farms until 3 serotypes

The prevalence in the fattening rooms was estimated at 25% (11 positive farms on 43). L. monocytogenes was confirmed in 14.5% of samples (25/172). The 33 strains collected belonged to four serotypes: 1/2a (30%), 1/2b (43%), 4b (24%) and1/2c (3%). Among the 10 farms in which sows and fattening pigs excreted L. monocytogenes, the different serotypes (1/2a, 1/2b, 1/2c and 4b) found in the sows were also recovered in the fattening pigs from the same farm except for 3 farms in which serotype 1/2b was found in sows but not in fattening pigs.

Excretion of L. monocytogenes by fattening pigs was also recorded at 10 of the 17 farms on which L. monocytogenes excretion by sows was detected (table 1). The risk of fattening pigs excreting L. monocytogenes in their feces was higher on farms at which L. monocytogenes excretion by sows was observed (OR=33.51). Moreover, we observed that more the number of positive samples is high among the sows higher is the probability that fattening pigs shed L. monocytogenes (Table 1).

Table 1: distribution of the 43 farms according their status in L. monocytogenes at the sows and fattening levels.

N° of farms		Status of farms at fattening pig level		Total
Status of farms at sows level		negative	positive	
Negative		25	1	26
positive		7	10	17
N° of samples + on the	1 to 3	7	6	13
10	More than3	0	4	4
		32	11	43

Different factors were associated to contamination of sows by L. monocytogenes: a food completely or partly made in farm, the production stages "service area" and "gestation area" and the period "autumn/winter". An antibiotic treatment during the 4 weeks before the sampling reduces the shedding of L. monocytogenes.

Table 2: the final GEE model for risk factor for Listeria shedding of sows

Variables	OR	95% CI
Origin of feed		
Commercial compounds	1.0	-
Home milled or mixed	3.18	1.34-7.56
Stage of sows at which samples taken		
Maternity	1.0	-
Service area	4.21	1.48-11.97
Gestation area	4.46	1.82-10.94
Use of antibiotic in the last 4 weeks		
Yes	1.0	-
No	2.19	1.05-4.57
Season		
Spring / Summer	1.0	-
Fall / Winter	3.76	1.48-9.51

Discussion

Our study showed that sows are a reservoir of L. monocytogenes. At the breeding pig level (sows), we found L. monocytogenes in 46.6% of the 73 farms and, 11.36% of the fecal samples collected were positive. Few works described excretion of L. monocytogenes by sows. A recent Canadian study (Farzan et al., 2010) explored the contamination of L. monocytogenes in different stages of production (finisher, sows and weanlings). They indicated that L. monocytogenes was not recovered from sow fecal samples and only infrequently found in the feces of weanling pigs and finisher pigs. They results are similar to those of Esteban et al., (2009); no L. monocytogenes was detected in the 17 swine herds studied.

At the fattening level, 25.6% of our 43 farms were positive of L. monocytogenes, with 14.5% of the collected samples positive. This prevalence is twice higher than that observed by Beloeil et al., (2003). In this previous French study realized in 2000-2001 on 93 farms, 14% of the farms had their finishing pigs' excreted L. monocytogenes. However, these results didn't reach the same conclusion than Fosse et al. (2011). No L. monocytogenes was detected in the 127 pooled fresh feces collected from 37 batches of fattening pigs originated from the 14 French farms considered in their work. Nevertheless, their study used only a direct streaking on ALOA which is less sensive.

Four serotypes were identified in these farms; the most dominant serotype was 1/2a, followed by 1/2b, 4b and 1/2c. Among these serotypes, three of them (1/2a, 1/2b and 4b) were also recovered from French pork-processing plants (Chasseignaux et al., 2001). However, the percentage of isolates with serotype 1/2c is higher on food products (Thevenot et al, 2006). Among the 10 farms in which sows and fattening pigs excreted L. monocytogenes, the different serotypes found in the sows were also recovered in the fattening pigs from the same farm. Moreover, in our study, we showed that the risk of fattening pigs excreting L. monocytogenes in their feces was higher on farms at which L. monocytogenes excretion by sows was observed (OR=33.51). This survey showed that the sows were source of contamination by L. monocytogenes of finishing pigs. This relation was not observed by Farzan et al., (2010).

Our work highlighted different factors associated to contamination of sows by L. monocytogenes: a food completely or partly made in farm, the production stages "service area" and "gestation area" and the period "autumn/winter". An antibiotic treatment during the 4 weeks before the sampling reduces the shedding of L. monocytogenes. In previous study (Beloeil et al., 2003), wet feeding during the fattening period was identified at a risk factor. This factor was not revealed in our study but we observed that from samples collected from pig provided in "wet feeding" the number of positive samples was about 11% when "wet feeding" was from commercial origin and about 35.3% when "wet feeding" was "food completely or partly made in farm". Among the different production stages, "maternity" seems to be more protector than the stages "service area" and "gestation area". Good hygiene practices could explain this result. However, we showed that an antibiotic treatment during the 4 weeks before the sampling reduces the shedding of L. monocytogenes. In our study, these two variables were linked; indeed 51% of the sows in "maternity" received an antibiotic treatment in the last 4 weeks before the sampling. Some earlier studies have reported that antibiotic treatment decreases carriage of Salmonella by pigs (Beloeil et al., 2007) and Campylobacter by poultries (Réfregier et al., 2001). The season fall/winter was identified as another risk factor in this study. L. monocytogenes may be subsisting in the environment because of its ability to grow and to survive at low temperature (Brisabois, 2008).

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Conclusion

This study provided recent valuable information on the occurrence of L. monocytogenes in French farrow-to-finish pig farms. This microorganism is prevalent in the sows (46.6%) and in fattening pigs (25%). Our study showed that sows are a reservoir of L. monocytogenes and could contribute to contamination of fattening pigs. Our work highlighted for some farms several serotypes suggesting several sources of contamination. Furthermore, the serotypes found in this study are identical to those usually involved in human listeriosis in Europe. The definition of risk factors of contamination of sows by L. monocytogenes can reduce the prevalence of L. monocytogenes in these farms and contribute to the overall reduction of human risk from consumption of pork products.

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Isolation of Salmonella spp. in pigs during transport, lairage, slaughterline and quartering

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Abstract

The aim of this study was to determine the prevalence of Salmonella in different points of the chain (transport, lairage, slaughterline and quartering). From eight consecutive assays a total of 134 Salmonella isolates out of 1180 different samples (11.3%) were recovered. The highest percentage of isolates were detected at the point of pre-scalding (30/80, 37.5%), caecal contents (18/80, 22.5%), trucks (13/60, 21.6%), tonsils (15/80, 18.7%), ileocaecal lymph nodes (13/80, 16.2%) and lairage (9/80, 11.2%). In the remaining points of sampling the number of isolates was minimal, being remarkable of 21 isolates obtained from different environmental samples (knives and surface of tables) (21/320, 6.5%) and 5 isolates in the quartering plant samples (5/80, 6.25%). Two more assays enhancing the pre-operational use of disinfectant on surfaces and irrigating ozone on carcasses were carried out. Serotypes more frequently isolated from four selected assays (64 isolates) were Bredeney, Rissen, Typhimurium, Montevideo, Israel, Derby, Emek, Choleraesuis, Durban, Kentucky, London and Sandiego. S. Typhimurium phage types U311, 193 and 104b were identified. Except for one assay, where Salmonella Bredeney was isolated 'from transport to quartering', different serotypes were isolate from the stages of sampling (trucks, lairage, slaughterline or quartering), which point to a different epidemiological source of those isolates. Although a lack of an evident cross-contamination was observed, our results have shown a high rate of isolates from transport, lairage and slaughterline environment which represent important critical points to acquire Salmonella infection both before and after slaughter of Iberian pigs.

Introduction

Salmonella is an important foodborne pathogen worldwide commonly recovered from pigs and pork in the European Union (EFSA, 2010). Spain occupies the second place in both pig and pork production and is considered as the highest pork consumer between the 27 member states of Europe (Marquer, 2010). Pigs may acquire the infection during transport and lairage and pig products may become contaminated during slaughterline and quartering process; stress factors during transport and lairage may induce Salmonella carrier pigs to shed the pathogen at a higher rate and increase the susceptibility of Salmonella-free pigs to infection (Mannion et al., 2010). Others studies have reported differences on Salmonella serotypes isolated and prevalence between farms and slaughterhouses (Hurd et al., 2001) suggesting that the transport and lairage are relevant risk factors in pigs at slaughter. Furthermore, cross-contamination in slaughterline has been reported as an important source of Salmonella contamination for pig carcasses (Swanenburg et al., 2001).

The aim of this research was to determine the presence of Salmonella spp. during Transport, Lairage, Slaughterline and Quartering (TLSQ) to improve Standard Sanitation Operates Procedures (SSOP) throughout the food production chain of Iberian pigs.

Material and Methods

During 2009 and 2010 eight systematic sampling (TLSQ1 to TLSQ8 assays) from ten Iberian pigs production units (5 Iberian commercial fed and 5 Iberian acorn-fed) were carried out. Ten finishing pigs from each pig herd were followed through one abattoir. Six different stages of the chain were tested: (i) trucks at its arrival to the slaughterhouse and after cleaning and disinfection (C+D), (ii) lairage prior entry of the pigs, and after departure to slaughter, (iii) ten pig

carcasses in different stages of slaughter-dressing, (iv) tonsils, ileocaecal lymph nodes and caecal contents, (v) environmental samples (slaughterline and quartering), and (vi) quartering samples (ham, shoulder and back). Moreover, two more assays (TLSQ9 and TLSQ10) were carried out as previously cited after a specific pre-operational disinfection program on quartering tables surfaces (dectocide H21R), and after an ozone treatment during the carcasses washing stage, respectively.

All samples were processed in peptone water (37 °C, 24 hours) and then transferred onto MSRV (Oxoid) (42 °C, 24-48 hours) according to ISO 6579: 2002. Finally, we conducted a double culturing on XLD and Chromogenic media (Oxoid) (37 °C, 24 hours). Moreover, sixty four isolates - from 4 out of 8 selected TLSQ assays - were serotyped and S. Typhimurium strains were phage typed by the National Reference Laboratory of Salmonella (Madrid, Spain).

Results

A total of 134 Salmonella isolates from 1180 different samples (11.3%) (Table 1) were recovered. The highest percentage of isolates were detected at the point of pre-scalding (30/80, 37.5%), caecal contents (18/80, 22.5%), trucks (13/60, 21.6%), tonsils (15/80, 18.7%), ileocaecal lymph nodes (13/80, 16.2%) and lairage (9/80, 11.2%). In the remaining points of sampling the number of isolates was minimal, being remarkable the isolation of 21 isolates from different environmental samples (knives and surface of tables) (21/320, 6.5%) and 5 isolates in the quartering plant from ham/shoulder/loin samples (5/80, 6.25%). Moreover, the higher number of isolates was obtained coinciding with the higher workload of the slaughterhouse, when more than 400 pigs were slaughtered. Two more specific assays, using disinfectant on quartering surfaces and ozone on carcasses, were performed at the end of our study (TLSQ9 and TLSQ10, respectively); only three and four isolates were detected, respectively, from caecal contents (5 isolates), ileocaecal lymph nodes (1 isolate), and carcass at post-scalding stage (1 isolate).

The distribution of serotypes isolated from environmental and pig samples (TLSQ3, 4, 6 and 7) was as follow: Bredeney (28 strains), Rissen (11), Typhimurium (8), Montevideo (4), Israel (4), Derby (2), Emek (2), Choleraesuis (1), Durban (1), Kentucky (1), London (1) and Sandiego (1). S. Typhimurium phage types U311 (5 strains), 193 (1) and 104b (2) were identified.

Table 1. Number of Salmonella isolates and prevalence levels (%) during Transport, Lairage, Slaughterline and Quartering process (TLSQ1 to TLSQ8) in the Iberian pig processing plant

TLSQ	T1	T2	L1	L2	S1	S2	S3	S4	S5	To	Ln	C	E	Q	Total
1°	2	1	2	0	11	0	0	0	0	2	6	7	2	0	33
2°	2	0	0	1	3	0	0	0	0	6	6	4	0	2	24
3°	0	0	3	0	0	0	2	0	0	0	0	2	8	1	16
4°	1	1	0	1	4	6	0	0	0	0	0	1	9	1	24
5°	0	0	0	0	0	0	0	0	1	1	0	1	1	0	4
6°	0	3	0	0	5	0	0	0	0	0	0	1	0	1	10
7°	1	1	0	0	6	1	0	0	0	6	1	1	1	0	18
8°	1	0	1	1	0	0	0	0	1	0	0	0	0	0	4
Total	7	6	6	3	30	7	2	0	2	15	13	18	21	5	134
N° Samples	26	34	40	40	80	80	80	80	80	80	80	80	320	80	1180
Prevalence	26.9	17.6	15	7.5	37.5	8.7	2.5	0	2.5	18.7	16.2	22.5	6.5	6.2	11.3

Key: T1, truck prior C+D; T2, truck after C+D; L1, lairage prior entry of the pigs; L2, lairage after exit of the pigs; Slaughterline (carcasses samples): S1 (pre-scalding), S2 (post-scalding), S3 (post-chilling), S4 (post-evisceration), S5 (post-washing); To, tonsils; Ln, ileocacecal lymph nodes; C, caecal contents; E, environmental samples; Q, quartering plant (ham, shoulder and loin)

Discussion

Our results show the potential role of transport, lairage and slaughterline for contamination of carcasses in the pre- and post-slaughter environment. Recently Mannion et al. (2010) reported the role of transport, lairage and slaughterline equipment in the dissemination of Salmonella in pigs in the pre- and post-slaughter environments in the Republic of Ireland. Three processing plants with a total of eight production units (sixteen finishing pigs each one) were investigated. From

911 samples 153 isolates were detected (16.8%) including environmental (71/177, 40%) and pig samples (62/734, 8.5%) (Mannion et al., 2010). Our results are in agreement with those detected in Ireland (11.3% vs 16.8%) although are different with respect to environmental samples (9.3% vs 40%), overcoat from lairage samples prior entry of the pigs (15% vs 80%).

The phenotypic characterization of the 64 selected isolates from TLSQ3, 4, 6 and 7 showed that except for one assay (TLSQ4) where Salmonella Bredeney was isolated 'from transport to quartering', pointing to a potential cross contamination between environmental and pig samples, different serotypes were obtained from each stage sampled (trucks, lairage, slaughterline or quartering), assuming that in our study the serotypes isolated at the different stages of the chain belonged to a different epidemiological source.

Several serotypes were 'uncommon' and different from those found in studies in free-range Iberian pigs and pigs in feedlots in Spain (Astorga et al., 2007; Gómez-Laguna et al., 2010): Montevideo, Israel, Emek, Durban, Kentucky and Sandiego. Previous studies have reported a disparity between the Salmonella serotypes isolated from slaughters and production units (Hurd et al., 2011). Mannion et al. (2010) reported different serotypes from two selected farms at abattoir, as follow: Derby, Infantis, Typhimurium, Bredeney, Panama and Give; being phage type 104b of S. Typhimurium the most frequently detected.

Conclusion

In conclusion this study showed the isolation of different serotypes of Salmonella spp. from samples of different source, which constitute a great risk for Iberian pigs both before and after slaughter. Thus, the HACCP must focus on intensive cleaning and disinfection programs in the pre-slaughter environment and the inclusion of new chemical agents or treatments which allow decreasing or eliminating the risk of Salmonella spp. infection or recontamination from the environment, which should be intensified when a higher workload is present.

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Serological Response of Swine to an Attenuated Salmonella enterica serovar Typhimurium Strain that Reduces Gastrointestinal Colonization, Fecal Shedding and Disease due to Virulent Salmonella Typhimurium

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Abstract

Swine are often asymptomatic carriers of Salmonella spp. Interventions are needed to limit Salmonella colonization of swine to enhance food safety. An attenuated Salmonella enterica serovar Typhimurium mutant strain (BBS 202) was tested in swine to determine whether vaccination could provide protection against wild-type S. Typhimurium challenge. Two groups of piglets (n=14/group) received an intranasal inoculation of BBS 202 or a PBS placebo at 6-weeks of age with a booster 2-weeks later. At 11-weeks of age, all pigs were challenged with the parental, wild-type S. Typhimurium by intranasal inoculation. Average swine rectal temperature (fever) was significantly decreased in BBS 202-vaccinated pigs at days 1 and 2 post-challenge with virulent S. Typhimurium compared to mock-vaccinated pigs. Fecal shedding of wild-type S. Typhimurium was significantly reduced at 2-days post-challenge in BBS 202-vaccinated pigs compared to mock-vaccinated pigs. Colonization of tissues within the gastrointestinal tract by wild-type S. Typhimurium was reduced in BBS 202-vaccinated pigs; a significant decrease in S. Typhimurium colonization of the ileal Peyer's patch region and ileocecal lymph nodes at 7-days post-challenge was observed. Serological analysis using the IDEXX HerdChek Swine Salmonella Test Kit indicated that all pigs were negative for antibodies to LPS derived from Salmonella serogroups B, C1, and D prior to challenge with wild-type S. Typhimurium. Thus, although vaccinated pigs had received two doses of BBS 202, antibodies from these pigs were not reactive to the LPS antigen in the ELISA test. However, sera from 85% of vaccinated and 78% of mock-vaccinated pigs were positive in the ELISA assay at day-7 post-challenge with wild-type S. Typhimurium. These preliminary results indicate that vaccination of swine with BBS 202 confers protection against challenge with virulent S. Typhimurium by reducing disease severity, pathogen fecal shedding, and gastrointestinal colonization but does not interfere with herd level monitoring for Salmonella spp., thereby allowing for differentiation between pigs naturally infected with Salmonella spp. and BBS 202-vaccinated swine.

Introduction

An estimated 1 million cases of foodborne illness are attributed to nontyphoidal Salmonella spp. each year in the U.S. at a predicted cost of $2.3 billion (Scallan, 2011; Frenzen, 1999). Nontyphoidal Salmonella spp. are a leading cause of hospitalization (35%) and death (28%), in the U.S., due to foodborne disease (Scallan, 2011). There are greater than 2,400 Salmonella serovars that are ubiquitous in the environment and many can colonize food producing animals and poultry as well as wild animals and birds without causing overt disease. In the U.S., ~100,000 cases of pork-associated, human salmonellosis occur each year with a social cost of $81 million annually (Miller, 2005). Swine 2006, the most recent study from the USDA's National Animal Health Monitoring System, reported that 52.6% of swine production sites (representing 94% of the U.S. swine inventory) were positive for Salmonella spp. Swine that are Salmonella carriers are a food safety risk for consumers of pork, an animal health risk to non-colonized/uninfected pigs, and an environmental risk due to fecal shedding of the pathogen into swine manure that is used as a soil amendment. Interventions are needed to limit the colonization of swine with Salmonella spp. and reduce the risks to public health, animal health and the environment. We investigated an attenuated Salmonella enterica serovar Typhimurium strain to determine whether administration of the potential vaccine strain would 1) protect swine against virulent S. Typhimurium challenge while 2) allowing the differentiation of infected and vaccinated animals (DIVA) using the IDEXX HerdChek Swine Salmonella Test Kit. Our results indicate that BBS 202-vaccinated pigs have reduced disease severity, pathogen fecal shedding, and gastrointestinal colonization due to virulent S. Typhimurium challenge, but swine vaccination did not interfere with the herd level monitoring system for Salmonella spp.

Material and Methods

The wild-type S. Typhimurium strain χ4232 and its attenuated derivative BBS 202 were used in this study. At 6-weeks of age, two groups of piglets (n=14/group), fecal-negative for Salmonella spp., received an intranasal inoculation of 1 ml PBS containing 1.0 X 109 CFU of BBS 202 or a PBS placebo. A booster of BBS 202 or PBS was given to the same groups of pigs 2-weeks later. At 11-weeks of age, all pigs were challenged with 1 ml PBS containing 1.0 X 108 CFU of χ4232 by intranasal inoculation. On 0, 1, 2, 3, and 7 days post-inoculation (dpi), the swine rectal temperature was monitored, fecal samples were obtained for quantitative and qualitative Salmonella culture analysis, and blood samples were obtained via the jugular vein. At 7 dpi, all swine were euthanized and necropsies performed to obtain tissue samples from the gastrointestinal tract (ileal Peyer's Patch, ileocecal lymph nodes, and cecum) for quantitative and qualitative Salmonella culture analysis. Serum antibody analysis to LPS antigen derived from Salmonella serogroups B, C1, and D was performed using the IDEXX HerdChek Swine Salmonella Test Kit. Statistical analysis was performed with SAS Analyst (Cary, NC) using the Two Sample t-test for the Means. Results were considered statistically significant when $P \quad 0.05$.

Results

A schematic for swine vaccination and challenge is shown below.
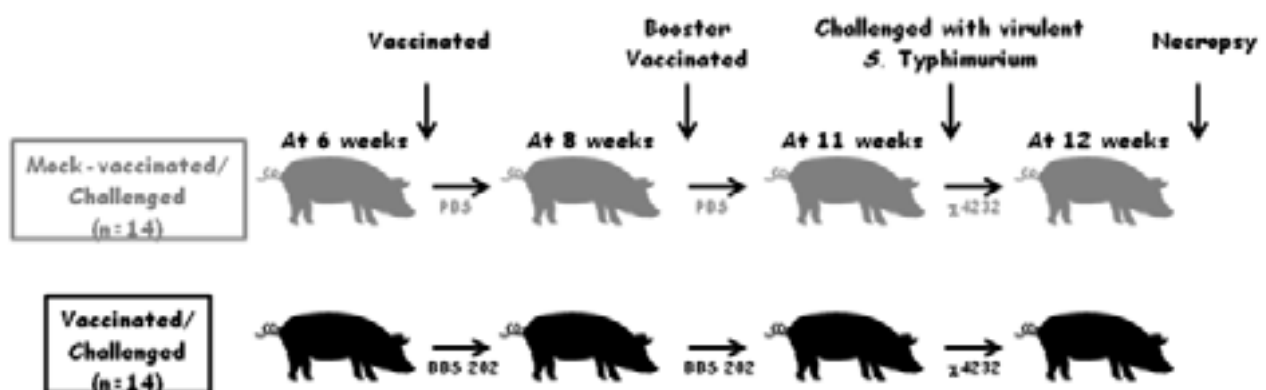
At 1 and 2 dpi, the swine rectal temperature was significantly increased in mock-vaccinated pigs compared to BBS 202-vaccinated pigs (Figure 1).

Figure 1. The average rectal temperature of BBS 202-vaccinated pigs is significantly lower at 1 and 2 days post-inoculation with wild-type S. Typhimurium compared to mock-vaccinated pigs. The swine rectal temperature was monitored on 0, 1, 2, 3, and 7 days post-inoculation. *Significant difference comparing BBS 202-vaccinated to mock-vaccinated pigs.
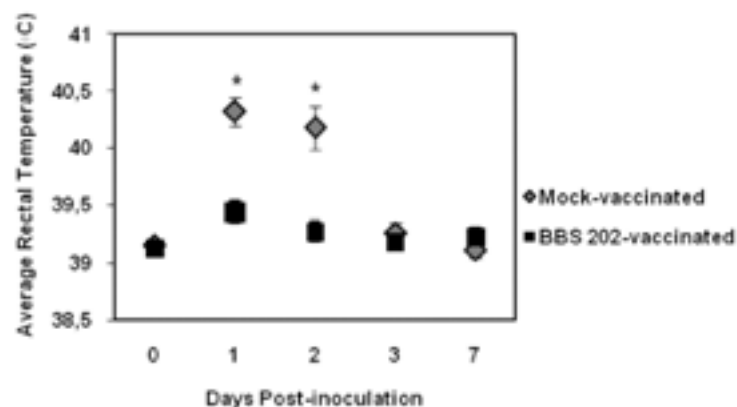
Swine fecal shedding of S. Typhimurium was significantly increased at day 2 post-challenge in mock-vaccinated pigs compared to BBS 202-vaccinated pigs (Figure 2).

Figure 2. Swine fecal shedding of wild-type S. Typhimurium is significantly lower at 2 days post-inoculation in BBS 202-vaccinated pigs compared to mock-vaccinated pigs. Swine fecal samples were obtained on 1, 2, 3, and 7 days post-inoculation for quantitative and qualitative Salmonella bacteriological analysis. *Significant difference comparing BBS 202-vaccinated to mock-vaccinated pigs at the indicated time point.
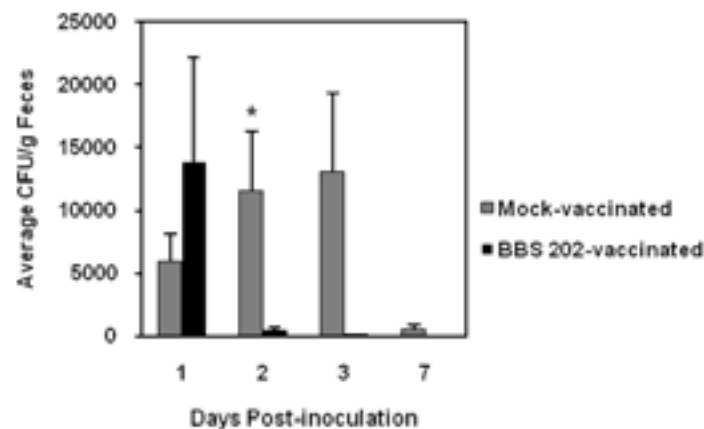
S. Typhimurium colonization of the ileocecal lymph nodes and ileal Peyer's Patch at 7 days post-challenge was significantly decreased in BBS 202-vaccinated pigs compared to mock-vaccinated pigs (Figure 3).

Figure 3. Colonization of the Ileocecal Lymph Nodes and Peyer's Patches by wild-type S. Typhimurium is significantly lower at 7 days post-inoculation in BBS 202-vaccinated pigs compared to mock-vaccinated pigs. At 7 days post-inoculation, all pigs were necropsied and tissue samples of the gastrointestinal tract [ileocecal lymph nodes (ICLN), Peyer's Patch (PP), cecum] were obtained for quantitative and qualitative Salmonella bacteriological analysis. *Significant difference comparing BBS 202-vaccinated to mock-vaccinated pigs for the indicated tissue.
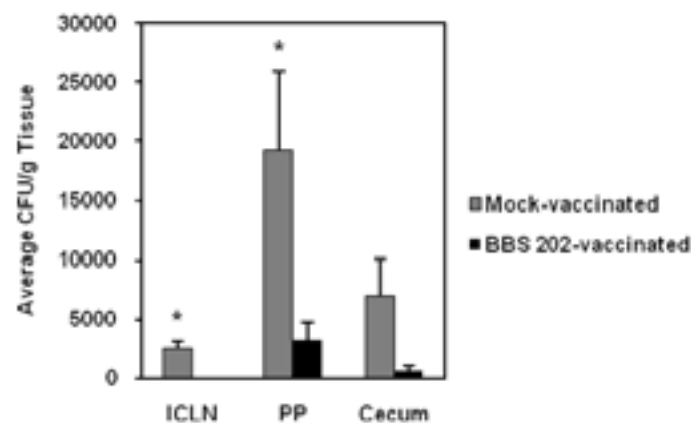
Swine serum was obtained from all 28 pigs at 11-weeks of age (5 weeks following initial vaccination with BBS 202 and the day of challenge) to determine the presence or absence of antibodies to Salmonella LPS antigen using the IDEXX HerdChek Swine Salmonella Test Kit; all swine were negative to LPS antigen by ELISA (Figure 4). At 12-weeks of age (7-days post challenge), swine serum was obtained to determine seroconversion to Salmonella LPS antigen following wild-type challenge. Sera from 85% of vaccinated and 78% of mock-vaccinated pigs were positive in the LPS ELISA assay at 12-weeks of age (7 dpi).

Figure 4. Serum from BBS 202-vaccinated swine is negative in the IDEXX HerdChek Swine Salmonella Test Kit 5-weeks after initial vaccination. Swine serum from all pigs at 11- and 12-weeks of age was assayed for Salmonella LPS antigen using the IDEXX test. The percent positive pigs at each time point are shown. *All pigs were challenged with wild-type S. Typhimurium at 11-weeks of age.
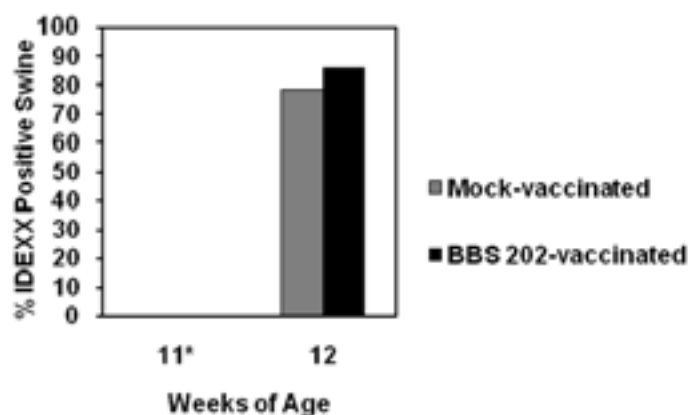
Discussion

Salmonella spp. can colonize the gastrointestinal tract of pigs without causing obvious disease. Swine that are Salmonella carriers pose a risk to public health, animal health and the environment. Vaccination of swine against Salmonella spp. may reduce pathogen colonization and carriage but may also interfere with swine herd Salmonella surveillance programs due to production of anti-Salmonella LPS antibodies. Our rationally attenuated S. Typhimurium vaccine strain was designed to overcome this potential vaccine limitation and allow the differentiation of infected and vaccinated swine. Indeed, vaccination with BBS 202 reduced disease severity, Salmonella fecal shedding and gastrointestinal colonization following challenge with virulent S. Typhimurium, without stimulating an immune response to S. Typhimurium LPS antigens. Furthermore, upon challenge with a virulent, wild-type S. Typhimurium strain, the majority of both BBS 202-vaccinated and mock-vaccinated swine had a seroconversion to a positive IDEXX ELISA reaction indicating a recent Salmonella infection.

Conclusion

Vaccination of swine with the S. Typhimurium BBS 202 vaccine strain reduces S. Typhimurium gastrointestinal colonization and fecal shedding but did not stimulate an anti-Salmonella LPS immune response that would compromise Salmonella surveillance programs for swine herds. Thus, vaccination with the S. Typhimurium BBS 202 vaccine strain permits the differentiation of Salmonella infected versus vaccinated swine.

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Risk associations for presence of Salmonella sp. in pen samples of breeding pigs in Portugal using binomial multilevel models

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Abstract

As Salmonella is one of the major causes of food-borne disease in the European Union (EU), EU approved legislation to achieve a reduction of the prevalence of this agent in the pig sector. To set the target for this reduction in each country it was decided to carry out baseline surveys in the EU to estimate the prevalence of the agent. The dataset analyzed in this work refers to the cross sectional baseline survey on the prevalence of Salmonella in breeding pigs in Portugal. A total of 1670 pen fecal samples from 167 herds were submitted to fecal culture. Of these samples 170 were positive to Salmonella. Along with the collection of samples a questionnaire was applied to collect information about the herd management and potential risk factors. As data follows a multilevel structure, pen fecal samples (first level) nested within swine herds (second level) a multilevel analysis was performed using generalized linear mixed models (GLMM). The outcome variable was presence/absence of Salmonella in the pen sample. The results show significant associations (p<0.05) at herd level: North Region versus Alentejo Region (OR=3.86), rodents control (OR=0.23), more than 90% of boars home-raised or no boars versus more than 90% of boars from an external source (OR=0.54), semen bought from other herd versus semen bought at insemination centers (OR=4.47) and herds with 170 or more sows (OR=1.82); at pen level: maternity pens versus mating pens (OR=0.39), feed from external or mixed source versus home source (OR=2.81) and more than 10 animals in the pen versus 10 animals per pen (OR=2.02). This study gave valuable information that should be incorporated in future control plans for this agent in breeding pigs in Portugal.

Introduction

Salmonella is one of the major causes of food-borne disease in the European Union (EU) in the past ten years (EFSA, 2010). Some cases are related to pork products. With the aim to control this agent the EU approved legislation (EU Regulation No 2160/2003) that imposed a reduction of the prevalence of this agent in food production animals, like pigs. To set the target of this reduction for each country it was decided to carry on baseline surveys in the EU to estimate the prevalence of Salmonella sp. in some food production animals. In pigs the baseline study was done at abattoir level (collection of lymph nodes of pigs slaughtered) and at herd level (collection of pen fecal samples of breeding pigs). These cross sectional studies also allowed the collection of information regarding management practices and potential risk factors.

Some of the known risk factors, summarized in systematic reviews (Fosse et al., 2009), are linked to: 1) biosecurity measures like potential biological vectors, as rodents, hygiene of hands, equipment and facilities, bought of animals from different suppliers, 2) herd management like herd size, batch production system, and housing - type of floor (partial slatted floor), type of pen partitions, 3) feeding practices like dry feed, purchasing feed, adding organic acids to feed, 4) health disorders like use of antibiotics, parasite infestations, health status of the herd among others.

The data were collected at the time of the baseline survey on the prevalence of Salmonella in breeding pigs in Portugal. The aim of this study was to search for risk factors of Salmonella in the pen fecal samples in this country.

Material and Methods

Sampling frame and collection of samples

The sampling frame, the diagnostic testing methods, sample collection procedures, and the timelines of this cross sectional study were specified in the Commission Decision 2008/55/EC. The target population was holdings constitut-

ing at least 80% of the breeding pig population in the Member State. The target population was 4522 herds with a total of 204 584 breeding pigs and 1 827 533 pigs in total (known population in 2007). These herds were divided by Regions. In each region herds with 50 or more breeding pigs, breeding holdings and production holdings were identified. The sample was obtained using expected prevalence of 50%, desired confidence level of 95%, accuracy of 7.5% and then applied a finite population correction factor, with an increase in 10% for each group. The sample size was formed by 174 swine herds. The choice of herds to sample was random and proportional to the distribution of herds along the regions of the country. The samples were collected between November 2008 and January 2009 by the herd veterinary assistant and sent to laboratory for detection of Salmonella (using method described by Annex D of ISO 6579). Positive samples were serotyped in the national Reference laboratory for Salmonella according to Kaulfmann-White scheme.

Data collection

During sample collection a questionnaire was applied to evaluate information about the herd management and potential risk factors. The variables collected concern pen data and herd data, such as: type of housing, number of animals that contributed to the sample, if it was detected diarrhea in the last three months, production phase, floor type, region of the country, number of breeding pigs, biosecurity measures among others.

Statistical analysis

Taking in consideration the questions about management of replacement breeding pigs and their source and also biosecurity measures it was created two new binary variables called "Good herd replacement policy" and "Good biosecurity measures". Some variables were recoded into new variables with fewer categories to have a reasonable sample size in each category. The outcome variable was presence/absence of Salmonella in the pen sample. As data follow a multilevel structure, pen fecal samples (first level) nested within swine herds (second level), were analyzed using a binomial generalized linear mixed model (GLMM) with a logit link function. The glmmPQL procedure of package MASS (Venables and Ripley, 2002) of R free software (www.R-project.org) was used. This type of model in this package is fit by the penalized quasi likelihood approximation. At first univariable models for each potential risk factor were considered. The variables with p 0.15 were selected to enter the multivariable model. The final multivariable model was found using backward and forward procedure to select the variables in the model and to control for confounding and interaction between variables. The regression coefficients were converted to odds ratio (OR) and the 95% OR confidence interval (CI) were calculated. The intra-class correlation factor (ICC) was calculated using the following formula (Twisk, 2006): ICC = $\sigma2between/ \sigma2between+(2/3)$, where $\sigma2between$ is the between group variance (herd variance).

Results

A total number of 1670 samples were tested (level 1), belonging to 167 herds (level 2) that filled the questionnaire. Out off samples collected 170 were positive to Salmonella detection, which belongs to 76 herds. Most of these positive samples were identified as Salmonella Typhimurium, followed by Salmonella Rissen. Results of the final multilevel multivariable model are shown in Table 1. The factors with higher risk of being positive to Salmonella with statistical significant differences (p<0.05) are: herds from North Region, herds with 170 or more sows, herds that use semen purchased in another herd, pens where feed is not exclusively from the producer, and pens with more than 10 animals/pen. On the other end factors with lower risk of being positive to Salmonella with statistical significant differences (p<0.05) are: herds which do rodent control, herds without boars or with >90% of boars homebred and maternity rooms pens. The ICC was estimated in 0.27, meaning that the herds account for 27% of the variation in pen samples.

Table 1: Binomial GLMM final multilevel multivariable OR and 95% CI for OR

Variable	Multivariable analysis		
	OR		
	Estimative	95% CI	p value
Region of the herd			
Alentejo	1.00		
Center	1.97	0.75-5.22	0.17
Lisbon and Tagus Valley	1.40	0.61-3.20	0.43
North	3.86	1.08-13.75	**0.04**
Number of sows			**0.04**
<170	1.00		
≥170	1.82	1.04-3.19	
Management of breeding boars			
more than 90% purchased	1.00		
Without boars or >90% homebred	0.54	0.30-0.97	**0.04**
10-90% purchased or homebred	0.93	0.38-2.30	0.88
Source of semen			
Center for artificial insemination – CAI	1.00		
Own boar+CAI	1.84	0.97-3.46	0.06
Boar from another herd	4.47	1.38-14.43	**0.01**
Control of rodents			**<0.01**
No	1.00		
Yes	0.23	0.09-0.59	
Number of animals per pen			**<0.01**
=10	1.00		
>10	2.02	1.19-3.43	
Breeding sector room			
Mating	1.00		
Gestating	0.78	0.53-1.15	0.21
Mixture of animals of different sector	1.55	0.62-3.89	0.35
Maternity	0.39	0.24-0.63	**<0.01**
Replacement breeders	0.81	0.26-1.81	0.61
Source of feed[a]			**0.02**
Exclusively own	1.00		
Not exclusively own	2.81	1.19-6.61	
Random effects[b]	Variance		
Herd	1.50		

[a] although variable with p>0.15 the variable was forced into the multivariable model
[b] variance at pen fecal level constrained to be 1 (binomial variance)

Discussion

In Portugal few studies about herd risk factors have so far been done (Baptista et al., 2010). Concerning the associations found with multivariable analysis in our study we will discuss the significant. Pens were the feed was purchased or mixture of purchased and own produced have higher risk (OR=2.81) of being Salmonella positive, this associations was also found in another study ((Benschop et al., 2008); feed is a source of potential transmission of Salmonella and in the HACCP plan of commercial feed this hazard should be taken in consideration, but feed is mainly associated with exotic serotypes and not with more prevalent serotypes (like Typhimurium). The role of rodents in the transmission of this agent was also highlighted in various studies (Skov et al., 2008) and this study also found a protective association for the herds that control rodents (OR=0.23), rodents are biological vectors of this agent and if not controlled could play an important role in their transmission within herds and between nearby herds. The number of sows is a measure the herd size and in this study herds with equal or more sows than the median have higher risk of being positive (OR=1.82); this type of association was found for herds concerning the finishers (Poljak et al., 2008) and is mainly associated with practices of mixture of pigs that happens in big herds. Mating pens showed higher odds of being positive than the maternity pens (OR=2.6) which is similar to the results found in a longitudinal study (Nollet et al., 2005) were it was detected more Salmonella shedding at mating than in the others sector of breeding sows, and it is justified by the hormonal changes in the sow at this time which contribute to a higher shedding of the bacteria. The results concerning the region (North with higher risk than the South) were surprising and need further investigation, ideal spatial analysis, to see if there is another factor that influences this result. Using expert opinion, one explanation could be that in the north the herds are more close together than in the south where the distance between herds is higher. To purchase semen from another herd is a risk factor when compared to the purchase of semen from insemination centers, where the quality and safety of semen is controlled

and tested. This association is not found in literature, to the best of our knowledge, maybe because in the majority of the countries the semen comes from insemination centers. This is a practice that is beginning to be more prevalent in Portugal. The management of breeding boars is also a risk factor and using homebred boars is safer than using purchased boars. This happens because the majority (80%) of the herds with more than 90% of boars from purchased use the semen from own herd or another herd, while in the herds without boars or more than 90% homebred that happens just in 52% of the herds.

Conclusion

This study allowed identifying significant risk associations as well as potential protective variables for the risk of Salmonella sp. presence in faeces in breeding pigs units in Portugal. So far, in Portugal, only another study about herd risk factors has been done (Baptista et al., 2010). The risk associations founded significant after this study should be evaluate in future longitudinal studies using these results as guidelines. To achieve a reduction on the prevalence of Salmonella, in the future, the control programs could take into consideration the information from both studies.

Acknowledgments

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Post harvest reduction of Salmonella by use of vaccination in growing pigs

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Abstract

This study was a randomized, blinded trial to evaluate effect of vaccine on post harvest Salmonella contamination rate of pig carcasses. Pig was the experimental unit.

Litters were assigned to treatment by farrowing date and parity. Piglets were double tagged, sex recorded and entire litters were either vaccinated (oral drench) or left as non-vaccinated controls. No movement of piglets between treatments was allowed. At weaning, control litters were placed on the top level of a truck, vaccinated pigs on the bottom level, transported to a wean-finish barn, and mixed within pen at the wean-finish barn.

At harvest, 100 animals per treatment were selected by random number and taken to a regional abbatoir. Pigs were loaded by treatment into separate compartments of a cleaned, disinfected trailer, transported three hours to the abbatoir, and held in adjacent cleaned, disinfected lairage pens overnight. Swabs for culture were taken from the transport vehicle and lairage pens.

After CO2 stunning, exsanguination, and dehairing, individual pig numbers were written on each carcass in edible ink and the tags removed. The peritoneal cavity of each carcass was swabbed with an individual, sterile sponge hydrated in buffered peptone water, and the ileocecal lymph node was collected. Both were immediately sent to the Iowa State University Veterinary Diagnostic Laboratory for culture. The following morning, the surface of the chilled carcass was swabbed per the plant's USDA process (jowl, midline, tailhead) by the same method. Positive culture samples were serotyped at the National Veterinary Service Laboratory. Salmonella Anatum and S. Muenchen were isolated from two environmental pen samples. Salmonella Mbandaka was detected in lymph nodes of non vaccinated pigs.

No Salmonellae were isolated from vaccinated pigs, a significant reduction from control pigs (Fisher's Exact P-value = 0.0332). Vaccination may be considered to improve the post harvest safety of pork.

Prevalence and characterization of Salmonella and Listeria monocytogenes in french raw pork meat at the distribution level

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Abstract

This study was undertaken in 2010 to estimate the occurrence of Listeria monocytogenes in raw pork meat at the distribution level in France.

A total of 320 samples (minced pork meat, pork chop, fillet and roast, and other various pieces) have been collected at the distribution level in various geographical areas throughout France. Sampling was done over the year. Detection and enumeration were conducted as described in ISO methods. All isolates were serogrouped by PCR and genotyped by a standardized PFGE method.

L. monocytogenes was detected in 12.8% of the samples (41 on 320). Eight samples could be numerated and number of ufc/g varied from 10 to 730. Minced pork meats were particularly contaminated (25%). The 159 isolates of L. monocytogenes were serogrouped by PCR; 57, 11, 75, 16 isolates were respectively from serogroup IIa, IIb, IIc and IV. PFGE, after ApaI and AscI restriction, generated 33 and 23 PFGE types respectively. Most often, isolates of L. monocytogenes from a same sample highlighted the same serogroup and the same PFGE pattern except one sample from which the 8 isolates were distributed in 3 serogroups and 5 PFGE patterns. Diversity of genotypes was higher among isolates from minced meat with 20 combined profiles. Few genotypes were common between minced meats, cut meats and others pieces.

This study provided recent valuable information on the occurrence of L. monocytogenes (13.3%) in raw pork meat at the distribution level. Minced pork meats were particularly contaminated and various genotypes of L. monocytogenes were found indicating that various source of meats and transformation of meat increase the risk to contaminate minced pork meat.

Introduction

The facultative intracellular bacterium Listeria monocytogenes is capable to cause a severe invasive illness in human (listeriosis). This ubiquitous bacterium is widely distributed in the environment and infection occurs through ingestion of contaminated food (Schlech, et al.,, 1983). In France, the joined efforts of the government and food producers have led to decrease significantly the incidence of listeriosis in the past 20 years and thereby the number of epidemics. However, the recent observation of increasing number of listeriosis cases (Goulet, et al.,, 2008) in most of the industrialized countries calls up to reinforce the epidemiological surveillance. Pork meat and processed pork products have been the sources of outbreaks of listeriosis in France and in other European countries during the past decade (Jacquet, et al.,, 1995; Jay, 1996; Loncarevic, et al.,, 1997; Goulet, et al.,, 1998). So, the aim of this study was to estimate for 2010 the occurrence of Listeria monocytogenes in raw pork meat and to characterize isolates. Several methods have been used to differentiate L. monocytogenes strains (Liu, 2006). We chose here to characterize the isolates by PCR (Kerouanton, et al., 2010) and by pulsed-field gel electrophoresis (PFGE), considered accurate for epidemiological investigations and of help for surveillance and control of listeriosis (Kerouanton, et al.,, 1998).

Material and Methods

Samples

A total of 320 samples (112 minced pork meats, 120 cut meats (pork chop, fillet and roast), and 88 other various pieces) have been collected at the distribution level in various geographical areas distributed on all France. Sampling was done over the year 2010.

Detection and enumeration of L. monocytogenes

Detection and enumeration were conducted with adaptation of the NF EN ISO 11290-1, NF EN ISO 11290-1/A1 and

NF EN ISO 11290-2 methods. Typical isolates on ALOA were subcultured on TSA-YE plates and stored at -80°C in glycerol peptone broth.

Molecular serotyping

DNA extraction was performed from fresh bacterial cultures on TSA-YE plates using Instagene kits (BioRad, France). The multiplex PCR assay method was performed following the recommandation of Kerouanton et al. (Kerouanton, et al., 2010). Listeria genus recognition was ensured by detection of the prs gene. The amplification mix of 25µl, with 0,5µl DNA, consisted of 1U of Taq DNA polymerase (Faststart, Roche); 1x fast-start Buffer without MgCl2, 2mM MgCl2, 0.2mM dNTPs, 0.4µM of each of the following primers: lmo0737 1 and lmo0737 2; lm31118 1 and lm31118 2; orf2110 1 and orf2110 2; and 0,2µM of the other primers prs1 and prs2, lip1 and lip2a. The cycling program consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C (40s), annealing at 53°C (45s), extension at 72°C (1min15s) and a final extension at 72°C (7 min).
Amplified PCR fragments were separated by 2% agarose gel electrophoresis in 1x Tris borate EDTA buffer and visualised by GelRed staining (Interchim, France))

PFGE Typing

DNA plugs were prepared from fresh bacterial cultures on TSA-YE plates. PFGE was performed according to the CDC PulseNet standardized procedure for typing L. monocytogenes (Graves, 2001). DNA was digested at 37°C for 4 h with two different macrorestriction enzymes, ApaI or AscI. Restriction fragments were separated in a 1% SeaKem Gold agarose gel, using the CHEF method in a CHEF-DRIII apparatus. The following electrophoresis conditions were used: voltage (6 V cm) 1; initial switch time, 4 s; final switch time, 40 s; run time, 21 h. XbaI-digested DNA from Salmonella Branderup H9812 was included, as a reference, in all PFGE gels (Hunter, et al.,, 2005).
Electrophoretic patterns were compared using BioNumerics® (Applied Maths, Sint-Martens-Latem, Belgium). Similarities between profiles were determined by calculating the Dice correlation coefficient, with a maximum position tolerance of 1%. A dendrogram based on the combined results for ApaI- and AscI -digested DNA (KS) was constructed. Strains were clustered by the unweighted pair-group method using the arithmetic mean (UPGMA) (Struelens, 1996). The Simpson's index (D) was determined as described by Hunter (Hunter, 1990).

Results

L. monocytogenes was detected in 12.8% of the samples (41 on 320). The prevalence was significantly more important in minced pork meat (25%) (Table 1). Eight samples could be numerated and number of ufc/g varied from 10 to 730.

Table 1. Prevalence of L. monocytogenes in pork meat

Pork meat	Number of sample			
	Positive	(%)	Negative	Total
minced pork meat	28	25	84	112
pork chop, fillet and roast (cut meat)	5	5	115	120
other various pieces	8	9	80	88
Total	41	12.8	279	320

By multiplex PCR, 159 isolates have been confirmed as L. monocytogenes. They were distributed in four serogroups (Table 2). The major serogroup identified was IIc (serotype 1/2c or 3c) with 75 isolates (47%). Others serogroups were IIa (57 isolates), IIb (11 isolates) and IVb (16 isolates). Serogroups IIa and IVb were found whatever the type of meat.

Table 2. Distribution of the isolates according to their serogroup and source.

	PCR serogroups				Total
	IIa	IIb	IIc	IVb	
minced pork meat	27	6	61	5	99
pork chop, fillet and roast (cut meat)	13	-	-	3	16
other various pieces	17	5	14	8	44
Total	57	11	75	16	159

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PFGE after ApaI and AscI restriction generated 33 and 23 PFGE types respectively (figure 1). The discriminatory index of the method for the combined results was of 0.93.

Figure 1. Distribution of the isolates according their PFGE patterns and source
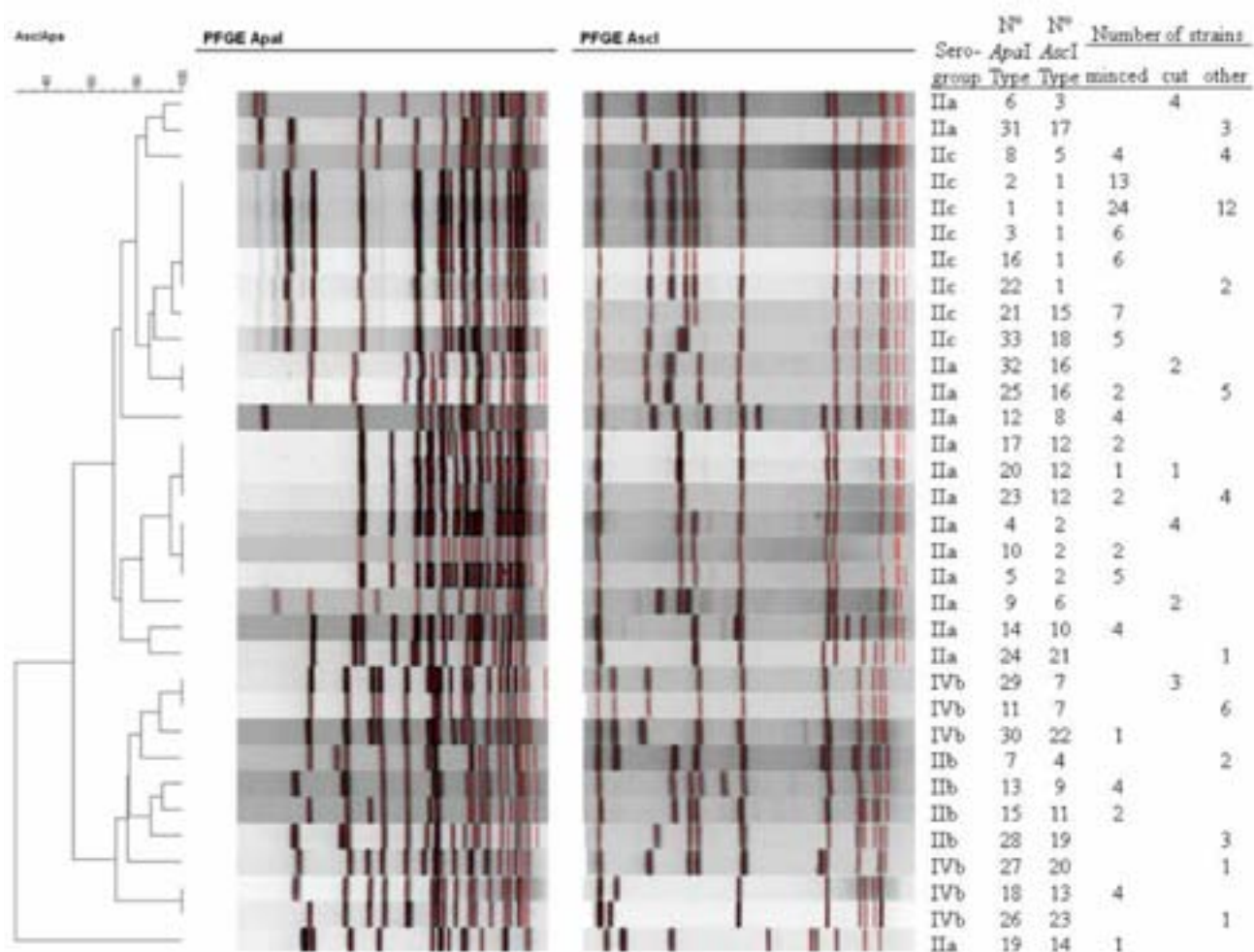
AscI/Apa	PFGE ApaI	PFGE AscI	Sero- group	Nº ApaI Type	Nº AscI Type	Number of strains		
						minced	cut	other
			IIa	6	3		4	
			IIa	31	17			3
			IIc	8	5	4		4
			IIc	2	1	13		
			IIc	1	1	24		12
			IIc	3	1	6		
			IIc	16	1	6		
			IIc	22	1			2
			IIc	21	15	7		
			IIc	33	18	5		
			IIa	32	16		2	
			IIa	25	16	2		5
			IIa	12	8	4		
			IIa	17	12	2		
			IIa	20	12	1	1	
			IIa	23	12	2		4
			IIa	4	2		4	
			IIa	10	2	2		
			IIa	5	2	5		
			IIa	9	6		2	
			IIa	14	10	4		
			IIa	24	21			1
			IVb	29	7		3	
			IVb	11	7			6
			IVb	30	22	1		
			IIb	7	4			2
			IIb	13	9	4		
			IIb	15	11	2		
			IIb	28	19			3
			IVb	27	20			1
			IVb	18	13	4		
			IVb	26	23			1
			IIa	19	14	1		

Diversity of genotypes was higher among isolates from minced meat with 20 combined profiles. Most often, combined PFGE was specific to one type of pork meat. Sixteen were only found for strains isolated from minced pork meat, 6 for strains isolated from cut meats (pork chop, fillet and roast), and 7 for strains isolated from other various pieces. Six of the seven combined profiles found among cut meat isolates were specific to this origin. Thus, only 3 profiles were common to minced pork meat and other various species of meat and 1 was common to minced and cut meats. None was common to the 3 origins. Most often strains from a same sample highlighted the same serogroup and the same PFGE pattern. Only 4 samples showed diversity of isolates and 1 was particularly poly-contaminated; the 9 isolates studied from this sample were distributed in 3 serogroups and 5 PFGE patterns.

Discussion
Occurrence of L.monocytogenes observed in this study is high (12.8%) with 41 positive samples on 320. But only 8 of the 320 samples could be enumerated for the presence of this pathogen which means that 33 samples were with less than 10 UFC/ g of meat.
Most of isolates (47.1%) in our study were from serogroup IIc (1/2c or 3c strains). These serotypes have been recently associated to pork meat in a Japanese study (Ochiai, et al.,, 2010).The percentage of isolates with serotype 1/2c was previously shown to be higher on pork products (Thevenot, et al.,, 2006). Furthermore, the serotypes found in this study are identical to those usually involved in human listeriosis in Europe (Goulet et al. 2008).
The isolates displayed a very high level of genetic diversity; 33 combined PFGE patterns were obtained, However only 8 PFGE combined ApaI/AscI patterns have been highlighted for the 75 IIc isolates; these serotypes 1/2c or 3c are known

to be genetically homogeneous (Ragon, et al.,, 2008).

Minced pork meats were particularly contaminated and various genotypes of L. monocytogenes were found; different sources of meat and transformation could explained this result.

Conclusion

This study provided recent valuable information on the occurrence of L. monocytogenes in raw pork meat at the distribution level (12.8%). Minced pork meats were particularly contaminated and various genotypes of L. monocytogenes were found indicating that various source of meats and transformation of meat increase the risk to contaminate minced pork meat. However, the predominance of serogroup IIc isolates, rarely associated with listeriosis, could suggested than pork meat is not the major food source of listeriosis.

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A study of the distribution of Salmonella serovars in an integrated pig company

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Abstract

A total of 3220 faecal samples from 161 pig farms (rearing and finishing units) belonging to an integrated pig enterprise were collected over a period of 18 months. Salmonella was found in 630 (19.5%) of the samples. At the farm level, 111 of 161 premises (69%) had at least one Salmonella- positive sample. 72.8% of rearing units and 66.6% of finishing units were positive for Salmonella; 61.4% of isolates were S. Typhimurium (387/630 isolates), and 25% of isolates were S. Derby (157/630). S. Panama, which was the third most common serovar (4.9% of isolates), is rarely found in pigs or other animals in the UK and appeared to be largely specific to this company, being found in the multiplier herd as well. A total of sixteen serovars were recorded within the study. Many of the serovars were found in breeding and multiplier herds from the company over several years, indicating that they were likely to be persisting or circulating within the integration. This study was carried out before the appearance of monophasic S. Typhimurium strains in pigs in the UK, and these were not found in the survey, but are now regularly reported from UK surveillance of pig herds, including from the integration involved in this study. Risk factor analysis suggested that increasing age of the pig farm was associated with increased likelihood of the presence of Salmonella and that finishing farms housing less than 500 pigs were associated with lower levels of S. Typhimurium than larger finishing farms.

Introduction

It is widely accepted that contaminated pork may be an important source of human salmonellosis, and it has been estimated in various European countries that 10–20% of all cases of salmonellosis in humans are related to the consumption of pork (Borch et al., 1996; Berends et al., 1998; Steinbach & Hartung, 1999; EFSA, 2010). Pork-related outbreaks of non-typhoidal salmonellosis in humans with fatal outcome have been described (Jansen et al., 2007).

Pigs can be infected by several Salmonella serovars, and the occurrence of these is also partly geographically determined (Loynachan et al., 2004; Fedorka-Cray et al., 2000). All serovars isolated from pigs are considered to be a potential hazard for public health by the European food safety authority (EFSA) (EFSA, 2006), although it is recognised that most individual serovars, apart from S. Typhimurium and possibly S. Infantis in some countries, currently play a minor part in human infections.

The occurrence of Salmonella serovars in pigs has changed significantly over the past decades. While Salmonella Choleraesuis was the predominant serovar in the 1950s and 1960s, it is rarely seen in Western Europe nowadays. Currently, the most commonly isolated non-typhoidal serovars in pigs and pork are Salmonella Typhimurium, including variant Copenhagen, and Salmonella Derby (Letellier et al., 1999; Davies et al., 2004; Gebreyes et al., 2004; Valdezate et al., 2005; EFSA, 2006; Rostagno et al., 2007). However, during the past five years, reports of monophasic strains of Salmonella Typhimurium (S. 4,5,12:i:- and S. 4,12:i:-) from pigs and humans have become more frequent in various European countries. The public health risk of Salmonella infection from consumption of contaminated pork depends on multiple factors including the level of infection in the pig herd (Nollet et al., 2005; Hill et al., 2003).

The aim of this study was to determine the extent of Salmonella infection within one big company and to monitor the change in serovars over a period of 14 years. During the initial study, a questionnaire was used aiming at identifying risk factors associated with the presence of Salmonella.

The main part of this study was undertaken between 1996 and 1998. Over the following years, several visits were done to multiplier, breeding, rearing and finishing farms belonging to the company, with the last visits to a breeding herd and two finishing herds done in 2010/2011. In addition, reports from voluntary surveillance within the company were assessed. The long period of time over which the study was performed offered a unique opportunity to investigate the persistence, disappearance and introduction of certain Salmonella serovars within the company.

Material and Methods

The first part of the study consisted of a survey including 161 farms belonging to the same integrated pig company. Of those, 59 were rearing farms and 102 were finishing farms. No nucleus, multipliers or breeder herds were included in the survey. Each farm was visited once by a veterinary surgeon who interviewed the farmer and completed a questionnaire containing sections on demography, farm structure and management details, herd details, antibiotic therapy and disease security/hygiene measures.

Samples of pooled faeces (10-15g) were collected from pig accommodation at up to twenty locations on the farm. Faeces samples were examined for Salmonella using a standard technique (Davies & Wray, 1997). Briefy; contents of the sample pot were transferred to a honey jar containing 225 ml buffered peptone water and incubated at 37°C for 18hrs +2hrs. 0.2ml of this culture was inoculated into the centre of a DIASALM agar plate and incubated at 41.5°C for 24 and 48 hours. The DIASALM cultures were subcultured to Rambach agar and incubated for 24 ± 4hrs. Suspect colonies of Salmonella were screened using polyvalent O and H antisera and later subjected to full serotyping.

After the initial study had finished, selected breeder and finisher farms belonging to the integration were visited periodically for 3 years, with some additional visits done in 2010/2011. During those visits, the following samples were taken: swabs of wallows and bird droppings and individual faecal samples (at least 60 samples per group). The samples were analysed as above, but during 2010/11 the DIASALM had been replaced by MSRV, to harmonise with EU requirements for primary production testing.

Results

Survey results:

A total of 161 farms were included in the survey and 3220 pooled pen faeces samples were collected and tested for the presence of Salmonella. Of the 3220 samples examined, 630 (19.5%) tested positive for Salmonella spp. S. Typhimurium (ST) was found in 387 (12%) of samples making it the most common serovar. A list of the serovars and the frequency with which they were found is shown in table 1. As this part of the study was conducted before the emergence of monophasic strains of S. Typhimurium in pigs in the UK, no such isolates were found, but 3 isolates of an aphasic group B strain were identified.

One hundred and eleven farms (111) (68.9%) had at least one Salmonella-positive sample; 43 of those farms were rearing farms and 68 were finishing farms.

The rearing farms in the survey ranged in size from 200 to over 2000 pig places, the mode being 500-800. Forty-three of the 59 (72.8%) rearing units had Salmonella. Of these, 35 (59.3%) were positive for S. Typhimurium.

The finishing farms in the survey also ranged in size from less than 200 to over 2,000 pig places. The mode in this case was 800-1,100. Sixty-eight of the 102 (66.6%) finisher farms had Salmonella. Of these 56 (54.9%) were positive for S. Typhimurium.

Although Salmonella was isolated from 68.9% of farms, all farms were not affected to the same degree. The proportion of samples positive for Salmonella at the farm level ranged from 5% to 95%.

The length of time for which a farm had been keeping pigs seemed to be a risk factor as such that farms which have had pigs for more than 30 years were more likely to be positive for Salmonella than farms which have had pigs for less than five years.

Over the next three years, a multipler herd, two breeding herds and five rearing/finshing herds were visited and the same serovars were found as in the survey. While S. Typhimurium and S. Derby are commonly seen in pigs in the UK, S. Panama appeared to be a company-specific serovar which is rarely found in other pig companies.

Visits in 2010/2011:

In 2010/2011, two finishing sites and one breeding site from the company were visited twice each.

The first visit to one finishing herd (outdoor) resulted in a high percentage of Salmonella-positive samples (338/429; 78.8%), with 57.8% of all isolates which were serotyped being S. London. The second most prevalent serovar was S. 4,5,12:i:- with 24.1% of all serotyped isolates. Other serovars found, but at low numbers, were S. 4,12:i:-, S. Bardo, S. Panama and S. Newport. Shortly after the visit, the herd was moved to a different field, and at the second visit, the number of Salmonella-positive samples had dropped significantly to 16.8% (87/518). Of the isolates which were serotyped, 38% were S. 4,5,12:i:-, 23.8% were S. 4,12:i:-, 21.4% were S. Typhimurium and 14.3% were S. London. In the other (indoor) finishing herd S. 4,5,12:i:- and ST that were identified at the first visit were replaced by S. Bovismorbificans and S. London at the second visit.

The first visit to the breeding site resulted in a low percentage of Salmonella-positive samples (30/573; 5.2%). Of the 27 serotyped isolates, 10 were S. London, 8 were S. 4,5,12:i:-, 4 were S. 4,12:i:-, 3 were S. Bardo (a variant of S. Newport) and 2 were S. Newport. Similar serovars with the addition of ST, S. Bovismorbificans and S. Panama were found at the second visit.

Discussion

Results from the survey show, that, in the case of finishing farms, the proportion of farms with S. Typhimurium was lower (32.0%) in farms with fewer than 500 pigs than in farms with more than 500 pigs (62.8%). This may be related to the greater diversity of sources of pigs for larger batch farms and suggests that restriction of unit size could be a possible control measure, albeit economically disadvantageous. The length of time that the holding had been in operation as a pig farm was identified as a risk factor, which suggests that long term persistence of Salmonella on farms is an important f actor in the epidemiology of infection. Especially in the case of S. Panama, which is hardly ever found in the UK apart from this one company, persistence within the company seems to have occurred for more than 14 years. The emergence of monophasic strains of S. Typhimurium could be seen within this company and seems to follow a national trend, with more than 7 % of all Salmonella isolates from pigs from the UK being either S. 4,5,12:i:- or S. 4,12:i:- in 2009, and preliminary data for 2010 supporting this trend. During the two visits in 2010/11 to a finisher herd, a significant shift in serovars was found between the first and the second visit. This might be due to several factors; first, the herd was moved to a different field between visits, which had not been used for pigs for a long time. Therefore, contamination of the soil with Salmonella was presumably very low or even negligible. Even though the pigs supplied to this finishing site came from the same breeding site, different batches of pigs might have different serovars and there was some shift in serovars within the breeding herd at the same time. There might also be a difference in susceptibility of different age groups to different serovars or sequential replacement of serovars according to exposure and partial immunity and clearance.

Conclusion

This study showed that some Salmonella serovars can persist in an integrated pig company over many years. In particular, S. Panama, which is a very company-specific serovar, was observed over a period of 14 years as a fairly low proportion of all serotyped isolates.

The emergency of monophasic strains of S. Typhimurium (namely S. 4,5,12:i:- and S. 4,12:i-) could also be observed and followed a national trend, whereas the prevalence of S. Typhimurium went down at the same time.

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Table 1. Salmonella serovars found during the survey and numbers of isolates

Serovar	Number of isolates:
S. Typhimurium	387
S. Derby	157
S. Panama	31
S. Goldcoast	19
S. Reading	14
S. Anatum	4
S. London	5
S. Agona	2
S. Manhattan	2
S. Enteritidis	1
S. Brandenburg	1
S. Bovismorbificans	1
S. Kentucky	1
S. Kimuenza	1
S. Schwarzengrund	1
S. 4,12:ː- (untypable)	3

The influence of good farming practice on the occurrence of Salmonella on pig farms

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Abstract

Compliance to good farming practice is a substantial issue to increase animal health and food quality in pork production. In this case-control study, as part of a general framework, farmers were asked six questions via a face-to-face question-naire, in order to determine their motivation for Salmonella control on their farms. The cases were in the so called Category III (n= 104) of the German Salmonella monitoring system; the controls were in Category I (n = 67). This system is based on the German law to the reduction of Salmonella on pig farms, where farms in Category III have sero-prevalence of > 40% and herds in Category I have a sero-prevalence < 20 %. After a first round of questions based on their motivation, the farms were divided into two groups: those with a "good" and those with a "poor" motivation. A significant difference in motivation between categories could not be determined. As a second step, 16 questions were asked to determine the routine of cleaning and disinfection (C+D-routine). Likewise, two groups were then established. No significant difference in C+D-routine could be established between the categories.

Introduction

Numerous studies concerning risk-factors for Salmonella infectionsin pigs and poultry have been conducted since the 1960s. Many of these studies have illustrated the importance of an efficient cleaning and disinfection routine (Davies and Wray 1995, Davies and Wray 1996, Madec et al. 1999). However, while these studies are meticulous in determin-ing the procedure in use and its efficiency, to the authors' knowledge, none of these studies allow for a judgment of the motivation of the farmer, i.e. it is simply assumed that motivation to reduce the Salmonella prevalence of a herd is high. The objective of this case-control studywas therefore twofold:

1) to verify that farmers are indeed highly motivated to reduce theirSalmonella problem
2) to verify an impact of motivation on the efficiency of a procedure

Although slight differences in motivation and C+D-routine were observed between the two groups, in an overall effect they were not found to be significant.

Material and Methods

Study farms were all located in the north-western part of Germany. Participation was voluntary and any farm taking part in the German Salmonella monitoring, regardless if farrow-to-finish or sole finisher, was applicable. The cases were in the so called Category III (n= 104, high sero-prevalence); the controls were in Category I (n = 67, low sero-prevalence). Each farmer was asked six questions in an interview based questionnaire. They were:

1. Do you consider Salmonella to be a problem on your farm?
2. Do you see a link between your Salmonella-status and your production performance?
3. Have you already implemented measures against Salmonella?
4. Have you planned (further) measures against Salmonella?
5. Do you use your Internet password to check on your Salmonella-status?
6. Were Salmonella detected in faeces samples in the last three months?

The farmers were scored for a "yes" to the individual questions, except for Question 6, where "no" scored (Table 1), and these points were added up to a sum-score. The cut-off for a "good" motivation was set at "7", farms with a lower score were ranged as "poor" motivation (Graph 1).

The farmers were also asked 16 questions concerning their routine of cleaning and disinfection (C+D) of a compartment.

1. Are all animals removed from the compartment?
2. Is the compartment allowed to damp prior to cleaning?
3. Is a high-pressure cleaner utilized?
4. Is warm water utilized?
5. Is a specific cleaner (soap etc.) utilized?
6. Is the compartment rinsed afterwards?
7. Is the compartment left to dry?
8. Is the compartment heated to room temperature before disinfection?
9. Is the solution for disinfection prepared freshly each time?
10. Is the concentration of disinfectant the same as recommended by the company?
11. Does the correct dosage reach the environment?
12. Is the disinfectant listed in the so called DVG-List (i.e. a disinfectant approved by the German Veterinary Medical Society (DVG))?
13. Is the disinfectant allowed to re-act the correct amount of time?
14. Is the amount of disinfectant used correct of the area to be disinfected?
15. Are leftovers from the disinfection removed?
16. Is it documented when and how the compartment was C+D?

The farmers were given scored for a "yes" to the individual questions (Table 2) and the points were added up to a sum-score. The cut-off for a "good" C+D routine was set at 25 points or above, farms with a lower score had a "poor" C+D routine (Graph 2).

The answers were compiled in a database (Microsoft Access 2003) and the statistical analysesdetermining percentages, odds ratios (OR), confidence intervals (CI, set at 95%)and the scores were performed via SAS 9.1 (SAS Institute Inc.).

Results

Table 1 shows the results of the "motivation"-questions.For each Category the amount (N) as well as the percentage (%) of "yes" and "no" answers are given, the cut-off was at "7".

Table 1: Results of the "motivation"-questions

Question	Maximum Score	Answer Category III		Answer Category I	
		N	Yes / No %	N	Yes / No %
1. Problem	2	60 / 44	57.7 / 42.3	6 / 61	9.0 / 91.0
2. Link	1	20 / 82	19.2 / 78.6	29 / 37	43.3 / 55.2
3. Measures 1	1	67 / 37	64.4 / 35.6	35 / 32	52.2 / 47.8
4. Measures 2	2	61 / 43	58.7 / 41.4	11 / 56	16.4 / 83.6
5. Internet	3	41 / 26	61.2 / 38.8	56 / 47	53.9 / 45.2
6. *Salmonella*	2	0 / 103	0.0 / 99.0	2 / 65	3.0 / 97.0

Note: Where the N does not add up to 104 for Category III or to 67 for Category I and the percentage does not add up to 100, information was missing.

Graph 1 shows the results of the "motivation" score, given in percentage. In Category III "good" N = 71, "poor" N = 33; in Category I "good" N = 49, "poor" N = 18.

Graph 1: Motivation-Score
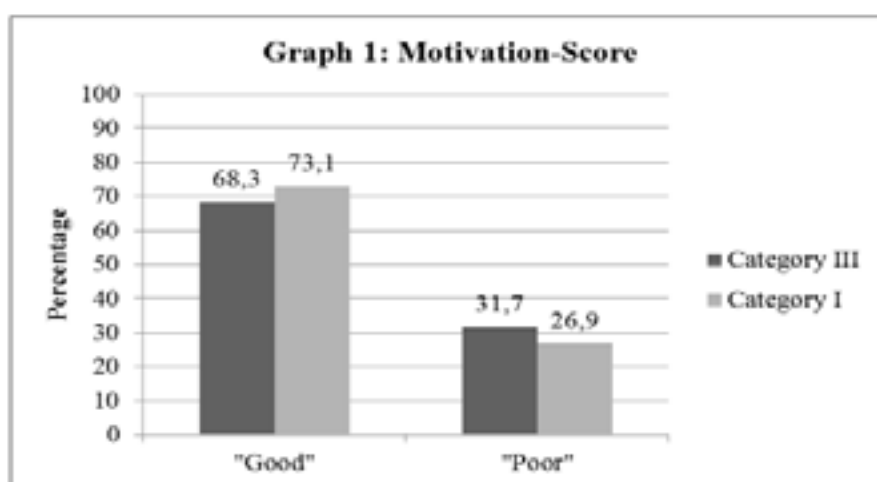
Table 2 shows the results of the "C+D-routine"-questions. Again, for each Category the amount (N) as well as the percentage (%) of "yes" and "no" answers are given.

Table 2 Results of the "C+D-routine"-questions

Question	Maximum Score	Answer Category III		Answer Category I	
		N	Yes / No %	N	Yes / No %
1. Animals	1	99 / 1	99.0 / 1.0	67 / 0	100.0 / 0.0
2. Damping	1	95 / 5	95.0 / 5.0	67 / 0	100.0 / 0.0
3. Warm Water	1	1 / 98	1.0 / 98.0	2 / 65	3.0 / 97.0
4. High Pressure	4	99 / 0	99.0 / 0.0	67 / 0	100.0 / 0.0
5. Soap	1	24 / 74	24.5/ 75.5	18 / 49	26.9 / 73.1
6. Rinse	1	15 / 83	15.3/ 84.7	15 / 52	22.4 / 77.6
7. Dry off	4	65 / 34	65.7/ 34.3	48 / 19	71.6 / 28.4
8. Heating	1	14 / 85	14.1 / 85.9	10 / 57	14.9 / 85.1
9. Solution	4	94 / 5	95.0 / 5.0	63 / 4	94.0 / 6.0
10. Concentration	4	92 / 7	92.9 / 7.1	64 / 3	95.5 / 4.5
11. Dosage 1	4	24 / 75	24.2 / 75.8	32 / 34	48.5 / 51.5
12. DVG-List	1	69 / 23	75.0 / 25.0	50 / 16	75.8 / 24.2
13. Reaction time	1	91 / 7	92.9 / 7.1	64 / 3	95.5 / 4.5
14. Dosage 2	4	87 / 9	90.6 / 9.4	63 / 3	95.4 / 4.6
15. Leftovers	1	29 / 70	29.3 / 70.7	16 / 51	23.9 / 76.1
16. Documentation	1	41 / 59	41.0 / 59.0	40 / 26	60.6 / 39.4

Note: Where the N does not add up to 104 for Category III or to 67 for Category I and the percentage does not add up to 100, information was missing.

Graph 2 shows the results of the "C+D-routine" score, given in percentage, the cut-off was at "25". In Category III "good" N = 73, "poor" N = 27; in Category I "good" N = 56, "poor" N = 11.

Graph 2: C+D-Score
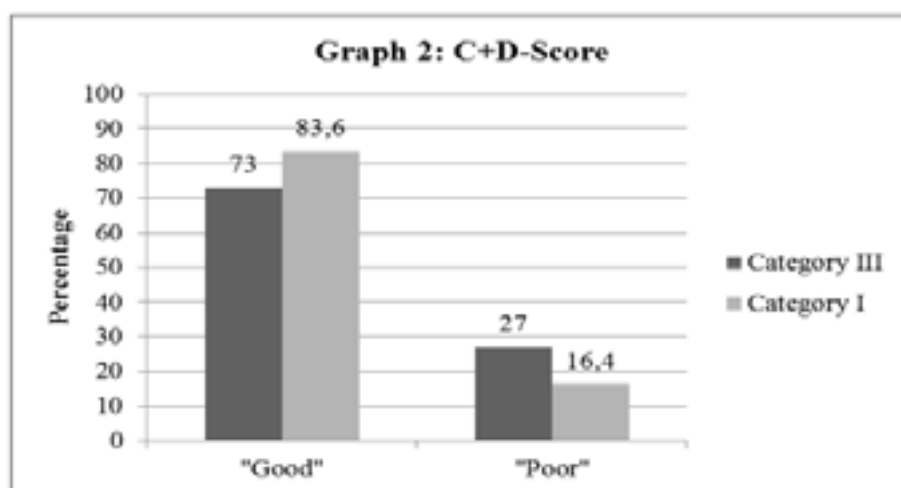
Neither the difference between the categories in motivation (OR 0.79, CI 95% 0.40 – 1.56)nor in the C+D-routine (OR 0.53, CI 95% 0.24 – 1.16)was found to be significant.

Discussion

As a general trend, the study did not reveal evidence, that motivation and case-control (i.e. Category) status are associated. However, this result is not free from different types of biases, namely selection, interview and (non-differential) misclassification.

First, it must be stated, that the farms in the control-group Category III are required by German law to implement measures against the Salmonella-problem of their herd. One may argue therefore that the controls are naturally highly motivated in order to change their Category-status, while the cases are highly motivated to maintain their present status. If this is true however, then there is a case of a non-differential misclassification and thus a dilution of the effect may be observed, i.e. the true value of the OR is decreased (Dohoo et al. 2003).

Secondly, participation in the study was voluntarily. This again may decrease the strength of effects.

It was however surprising to the authors that no significant difference between the C+D-routine of the categories could be determined, although a significant difference between residual Salmonella found in the environment had recently been observed (Gotter et al. 2011). Once again, the bias which is inherent in interviews concerning the social desirability of the answer must be pointed out (Meagher 2009). It is therefore possible that a different study approach, including perhaps the direct observation of the C+D-routine by the interviewer, may have led to different results.

Similarly, a more precise statement of some of the questions concerning the routine, for example how long a compartment was damped prior to cleaning, what kind of high pressure cleaner was used or what kind of disinfectant was utilized (acid or formaldehyde based) etc. might also have led to different results.

Conclusion

This study was not able to demonstrate a difference between cases and controls in regard to motivation and C+D-routine-procedures on the study farms. The hypothesis to explain this unexpected outcome is that the C+D procedures on the study farms are not "Salmonella-specific". There is still the need to raise the awareness in the farming community that the reduction of residual Salmonellacannot be achieved by only intensifying the C+D procedures, unless areas are included that so far have not been the target of "traditional" C+D-routines (biosecurity areas, hallways, loading ramps, transporters etc.). A different study approach, based not only on a questionnaire but also on direct observation, may be able to decrease the interview bias and therefore lead to different results. Further research in this direction is required.

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Proceedings - Posters

Growth and survival of exponential and stationary phase Salmonella during sausage fermentation

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Abstract

When raw meat is contaminated with enteropathogens, the growth state may appear in a mixture of phases. Survival for exponential and stationary phase cells differs, with stationary phase cells being generally more resistant. Our aim of this study was to investigate the survival of exponential and stationary phase Salmonella during freezing and to follow the survival/growth of these cells during subsequent sausage fermentation. Minced meat was inoculated with exponential and stationary phase Salmonella Thyphimurium, respectively, and frozen at -20°C for up to 35 days. The meat was thawed overnight at 5°C prior to sausage production. The sausages were fermented at 25°C and growth/survival was observed for 3 days. In the freezing period before sausage production, no reduction of stationary phase cells was observed after more than 35 days whereas exponential phase cells were reduced more than 1.5 log10 units. Despite this reduction, exponential phase cells was able to grow to the same level as the stationary phase cells during fermentation of sausages simulating failure of the starter culture. However, the pH drop caused by the starter culture prevented growth of both exponential as well as stationary phase Salmonella. These results show that failure of a starter culture to lower pH may lead to growth of Salmonella, independent of the phases.

Introduction

During slaughter raw meat will occasionally be contaminated with enteropathogens such as Salmonella and Yersinia. This can be a food safety problem in raw fermented sausages. Several Salmonella outbreaks have been linked to fermented sausages where epidemiological and molecular subtyping studies confirmed the ethiology (Bremer et al., 2004, Nygård et al., 2007, Bone et al., 2010).

The growth state of the bacteria during contamination of meat is unknown, but they may appear in a mixture of phases. The ability to respond to environmental changes is fundamental for bacterial survival. The stress sensitivity between exponential and stationary phase cells may be different. An acid challenge experiment in juice at different temperatures showed e.g. that the stationary phase cells were considerably more resistant than exponential growing cells and that the low temperature protection was only observed for the stationary cells (Gawande 2002).

Freezing of meat for sausage production arrest bacterial growth but it is questionable whether it can be used as a decontamination step as only 0.5 log unit reduction of stationary phase Salmonella Typhimurium was observed after freezing for 10 weeks at -20°C (Barrel, 1988).

In this study the aim was to investigate: 1) the survival of exponential and stationary phase Salmonella during freezing in minced meat and 2) survival/growth of exponential and stationary cells during sausage fermentation in sausages made of meat that had been frozen for different periods.

Material and Methods

Bacterial strains and preparation of inocula. Salmonella enterica serotype Thyphimurium DT12 and Salmonella enterica serotype Thyphimurium U292 isolated from an outbreak 2009 in Denmark were used. From stock culture, one loop full of culture was streaked onto blood agar plates and incubated over night at 37°C and then sub-cultivated by transferring one loop full of single colonies to 20 ml Luria Broth (LB). After 19 hours of incubation at 37 °C, the cells had reached stationary phase and was used directly for sausage production after dilution. To adjust the number of bacteria, the stationary culture was first diluted to OD600 = 0.1, which corresponds to a cell level of approximately 8.4 log10 cfu/ml. This culture was diluted to 7 log10 cfu/ml and was used as inoculum. For preparation of exponential cells, the 19 hours culture was diluted to 4 log10 cfu/ml. Subsequently the culture was grown for about 4.5 hours until the cells had reached 7 log10

cfu/ml (OD600 = 0.05) and immediately after used as inoculums for sausage production.

Sausage production. For sausage production, minced meat, with a fat content of 16-20%, was purchased from a local shop. The procedure for sausage production for exponential and stationary cells was identical. Initially, 1200 g minced meat was added an inoculum of 24 ml of exponential and stationary cells, respectively, resulting in a start level of approximately 4.5 log10 cfu. Then the inoculated meat was divided into bags with 300 g each. One bag was immediately used for sausage production; the other three bags were frozen at -18°C for 0, 7, and < 35 days, respectively, before sausage production.

For the sausage production the final batter consisted the Salmonella inoculated meat with the following ingredients added: NaCl (3 wt/wt %), dextrose (0.7%) dextrose and Na-nitrite (0 or 100 ppm) varied. Then the batter was divided into two portions: 150 g batter added starter culture (F-1 Bactoferm containing a mixed culture of Staphylococcus xylosus and Pediococcus pentosaceus, Chr. Hansen) and 150 g batter was without starter culture. The sausage batter was then stuffed into sterile syringes with removed tip and incubated at 24-25°C (standard fermentation temperature) ad modum Heir et al. (2010). The pH decline was measured during the fermentation.

Microbial analysis and sampling. To analyse the effect of freezing of the raw meat, 5 g meat was macerated by stomaching in 15 ml 0.9% NaCl for 2 min. Tenfold dilutions were prepared in 0.9% NaCl and colony forming units were determined on Salmonella selective Xylose Lysine Deoxycholate (XLD) agar plates by spotting 3 times 10 µl from appropriate dilutions.

Enumeration of Salmonella during sausage fermentation was performed at day 0 and two times a day the following three days. The plungers push a piece of sausage out of the syringe for each sampling. The first 0.5 cm sausage was discarded. A sample of 5 g of sausage was cut off and transferred into a stomacher bag with 15 ml 0.9% NaCl and treated as in the freezing experiment. All plates were incubated at 37°C for approximately 24 hours.

Results

The effect of freezing (-18°C) is illustrated in figure 1 for the two phage types of S. Typhimurium, DT12 and U292. Exponential cells were noticeable more sensitive than stationary cells and also a difference between the phage types was observed after 7 days. Exponential DT12 cells were reduced approximately 0.6 log10 units whereas exponential U292 cells were reduced approximately 0.9 log10 units. However, after more than 35 days of freezing the no difference could be measured. The stationary cells were marginally affected by the freezing with only approximately 0.1 log10 unit reduction.
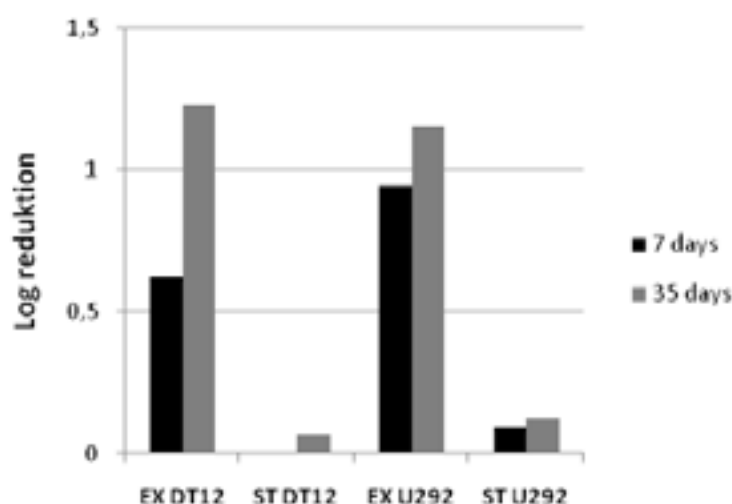
Figure 1. The effect of freezing on stationary and exponential Salmonella Typhimurium cells in minced meat. EX: exponential cells, ST: stationary cells. Black columns: 7 days of freezing, grey columns: more than 35 days of freezing.

The growth/survival of Salmonella Typhimurium DT12 during sausage fermentation at 25°C is outlined in figure 2. Minced meat that was inoculated with Salmonella and then frozen for 7 and < 35 days, respectively, was used for sausage production. Comparison of parameters such as with/without starter culture and exponential/stationary cells on growth/survival of Salmonella were analysed. Sausage batter added starter culture, prevented growth or slightly reduced (max. 1 log10 unit) both exponential and stationary cells during fermentation, independently of the freezing period of the meat.

Freezing the meat for more than 35 days reduced the number of exponential cells with at least 1 log10 unit whereas the number of stationary cells was unchanged. When fermenting batter with no starter culture, the number of exponential cells started more than 1 log10 units lower than the stationary cells, but they grew to the same level during three days of fermentation. The same growth/survival pattern was observed for U292 during sausage fermentation.
Addition of NaNitrite did not affect Salmonella (data not shown).

A.

B.
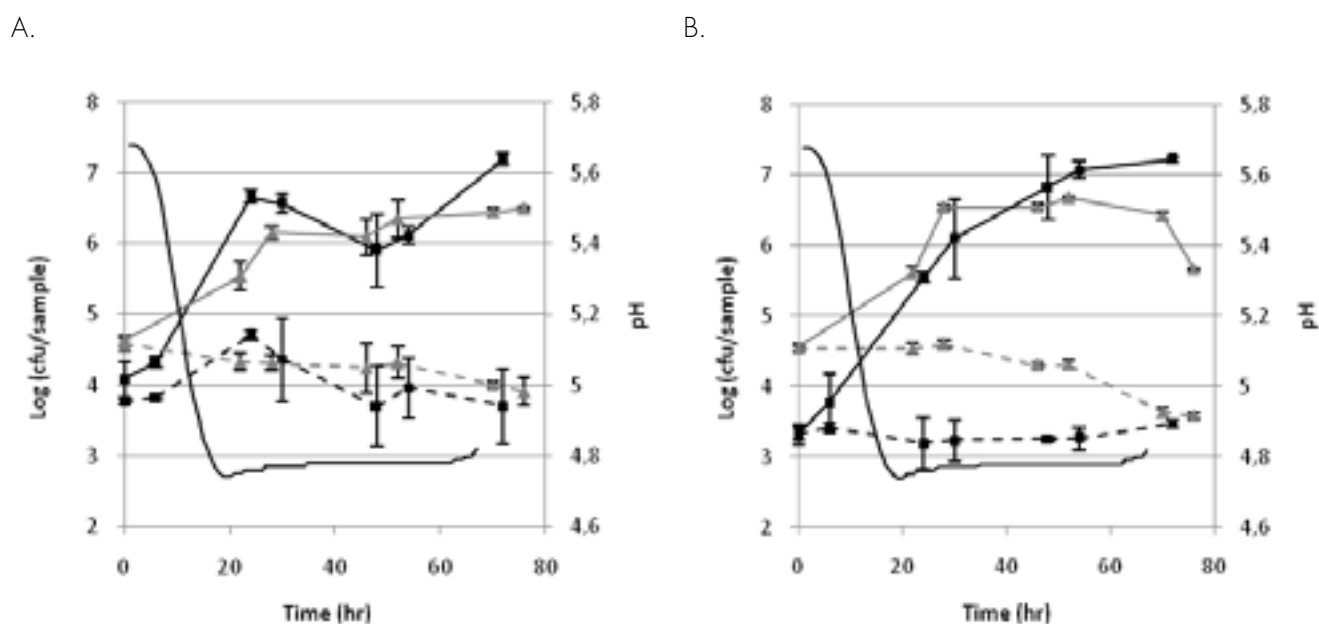
Figure 2. Growth/survival of Salmonella Thyphimurium DT12 during sausage fermentation at 25°C without NaNitrite. Unbroken lines: sausages with no starter culture, dotted lines: sausage with starter culture, black square: exponential cells, grey triangle: stationary cells, black line: pH measurement. A) and B) sausages made of meat that has been frozen for 7 and more than 35, respectively.

Discussion

Exponential cells are more sensitive to freezing than the stationary cells in minced meat. This observation was also found in a previously freeze-thaw treatment of Salmonella in chicken exudates (Obafemi et al, 1986). Freezing minced meat before sausage production has a relatively minimal effect if the bacteria primarily are in the stationary phase. Thus, the freezing of raw material routine used in production of fermented sausage seems ineffective as an intervention.

Fermenting sausages with no starter culture should mimic failure of fermentation. Under these condition Salmonella are able to multiply and grow to a high level during the fermentation at 24-25°C. However, the subsequent drying process of the sausage has shown to reduce the number of Salmonella. A modeling study, calculated that Salmonella can be reduced by 0.3 to 2.4 log10 during the sausage drying process.

Conclusion

It can be concluded that:
* Exponential cells are more sensitive to freezing than stationary cells in a food matrix.
* Growth of starter culture (sausage fermentation) arrest growth of both exponential and stationary cells.
* Failure fermentation can lead to growth of both exponential and stationary cells.

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Evaluation of cleaning and disinfection procedures against Samonella enterica at swine farms, transport and lairage facilities

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Abstract

Evaluation of the cleaning and disinfection protocols effectiveness against Salmonella in three points of the pork production chain: finishing farm, transport and lairage. A 22.2% of the farms, 62.5% of the slaughter trucks and 63.6% of the holding pens tested were positive to Salmonella after cleaning and disinfection procedures. The other samples collected in trucks and lairage shows that there is also contamination before the pigs staying. These results show that the protocols carried out at the farms, trucks and abattoirs included in this survey are not efficient to eliminate Salmonella.

Introduction

Salmonella enterica is one of the most common and widely distributed food-borne pathogens. The bacteria can contaminate almost any food type although raw eggs, poultry and pork are the most common sources of human outbreaks of salmonellosis [1]. Contamination can occur at any point of the food chain including primary production. Due to the ability of Salmonella to survive during large periods of time in the environment, particularly in the presence of organic matter, cleaning protocols are of outstanding importance. This investigation aimed to assert the efficacy of routinely cleaning and disinfection procedures performed at three points of the pork production chain: finishing farms, transport and lairage.

Material and Methods

a) Study design

Finishing farms: Thirty-six pig finishing farms performing a strict all-in/all-out management (AI/AO) were evaluated. Within each farm, twelve samples were collected from pen floors (5 samples), pen walls (5 samples), corridors (1 sample) and dust (1 sample) after cleaning and disinfection procedures, just before the entrance of a new batch of animals.

Transport: Eight slaughter trucks were evaluated in three sampling rounds: (1) before loading the animals at the farm (preload), (2) after unloading the pigs at the abattoir (postload) and (3) after cleaning and disinfection procedures. Each truck included three storeys and therefore three samples, one per floor, were collected in each sampling round. In total, nine samples were recovered from each monitored slaughter truck. Surface samples were collected at preload and after cleaning and disinfection procedures, while floor faeces were collected at postload.

Holding pens: Sixty-six holding pens in two different abattoirs were evaluated in four sampling rounds: (1) before the entry of the pigs, (2) at half of the working day, (3) at the end of the working day and (4) after cleaning procedures. Surface samples were collected in sampling rounds 1 and 3 while faecal samples were collected in the other two sampling rounds (2 and 3) during the staying of pigs.

b) Sample collection

Surface samples were collected using gauzes previously moistened in peptone buffered water (PBW). Each sample was obtained by swabbing five points of 25cm x 25cm.

Floor faecal samples were constituted by five fresh fecal pinches collected at five different locations of the pen or lorry.

c) Isolation methodology

Bacteriological analyses were made according to EN-ISO 6579:2002/Amd 1:2007. Gauzes immersed in 50 ml of PBW or pool faeces were sent to the laboratory immediately after their collection in cooling conditions. One isolate of each positive sample was further serotyped by slide agglutination according to Kaufmann-White scheme using commercial antisera.

Results

Eight farms (22.2%) had at least one Salmonella positive sample after cleaning and disinfection procedures (Table 1). Salmonella was recovered mainly from floor samples (6 out of 8 positive farms were positive in floor samples) followed by pen walls (three farms); it is remarkable that the contamination was only detected in corridors in two of the positive farms. In contrast, Salmonella was not isolated from dust samples in any of the farms included in the present study. S. Typhimurium was identified in seven out of the eight contaminated farms while S. 4,[5],12:i:- was detected in the remaining one. Two different serotypes, S. Typhimurium and S. Rissen, were recovered in one farm.

Results obtained in the evaluation of eight slaughter trucks are included in Table 2. Fifty percent of the samples and 50% of the trucks were positive before the loading of the pigs indicating that lorries were already contaminated by Salmonella. Contamination was also detected in 50% of the trucks after the staying of the pigs. Moreover, five of the trucks (62.5%) were positive after cleaning and disinfection procedures. Altogether, percentage of Salmonella positive samples was 26.1% before loading the pigs, 39.1% immediately after unloading and 34.8% after cleaning and disinfection procedures. Regarding the distribution of contamination within the three storeys of each truck, 12 positive samples were recovered from first floor (50%) and 7 (29%) and 6 (25%), from second and third respectively. Despite that the number of positive samples found in the first floor was twice the other two, no statistically significant differences were demonstrated (χ2=3.8, p= 0.14).

A total of 66 holding pens were evaluated in a total of six different visits to two commercial pig slaughterhouses (Table 3). Salmonella contamination was detected in 39.4 % of the samples collected before the entry of the pigs, 98.5% of the samples collected at half of the working day and 80.3% of the samples collected at the end of the working day. Moreover, Salmonella was recovered in 42 out of the 66 holding pens (63.6%) after cleaning procedures.

Discussion

Herein we present the results of a brief research to determine the effectiveness of the routinely cleaning and disinfection protocols carried out at three different stages of the pork production chain in Spain: farm, transport and lairage. Although our results were obtained from a limited number of farms, slaughter trucks and holding pens, they clearly show that Salmonella can persist in the environment after routinely cleaning and disinfection procedures at these three evaluated points. Taking into account that Salmonella is sensitive to the most commonly used disinfectants, our results indicate that cleaning and disinfection procedures were not performed properly.

At the farm level, strict all-in/all-out management has been pointed as a proper control measure to avoid transmission of the bacteria between batches of animals. However, according to our results even when cleaning procedures were classified as satisfactory by clinicians and a phenol derivate disinfectant was used, Salmonella was still detected in one of each five investigated farms. It has been described that holes in floors and walls make difficult the penetration of disinfectant solutions and what is more, the biofilms created by Salmonella can make the action of the disinfectants difficult [2]. Moreover, particular attention should be paid not only to pens but also to corridors in order to prevent infections between batches.

In a similar way, Salmonella was recovered from fifty percent of the evaluated slaughter trucks before the loading of the pigs at the farm. The fact that more than sixty percent of the trucks sampled after cleaning and disinfection procedures gave at least one positive sample reveals the ineffectiveness of these procedures. A similar result was obtained in the evaluation of holding pens in slaughterhouses. Salmonella was recovered in more than 60% of the samples collected immediately after cleaning and disinfection procedures and in almost 40% of the floor swabs collected at the start of the working day, before the entrance of the pigs. These results are in agreement with previous researches in both slaughter trucks [3] and lairage of pig slaughterhouses [4,5]. Taking into account the short period of time needed by Salmonella to reach and multiply in the intestine of susceptible pigs [5], contamination at both levels is relevant and would explain at least part of the new infections occurring between the farm and the slaughterhouse.

Conclusion

Present research demonstrates that routinely cleaning and disinfection procedures performed at the farm level as well as in the slaughter trucks and the lairage of pig slaughterhouses are not able to eliminate Salmonella properly. According to this, slaughter trucks and holding pens should be taken into consideration as sources of new infections in pigs entering the slaughterhouse. An effort to improve these cleaning and disinfection protocols have to be done as part of the Salmonella control strategies at the slaughter level. On the other hand, cleaning and disinfection procedures should also be improved in pig farms performing all-in/all-out management in order to avoid cross-contamination between batches of animals.

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Table 1. Distribution of Salmonella positive samples in the eight pig finishing farms where Salmonella contamination was demonstrated after cleaning and disinfection procedures. A total of 36 pig farms performing strict all-in/all-out management were evaluated.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8
Pen Floor[1]	1	5	1	0	0	3	3	2
Pen Walls[1]	0	2	2	0	0	0	1	0
Corridors[2]	0	1	0	1	1	0	0	0
Dust[2]	0	0	0	0	0	0	0	0
Total	1	8	3	1	1	3	4	2

1 Five samples were collected in each farm
2 One sample was collected in each farm

Table 2. Distribution of Salmonella positive samples in eight slaughter trucks that were evaluated in three sampling rounds: before loading the animals at the farm, after unloading the pigs at the abattoir and after cleaning and disinfection procedures. Within each sampling round, three samples were collected in each of the three storeys of the track.

	Before loading pigs			After unloading pigs			After cleaning and disinfection procedures		
	First floor	Second floor	Third floor	First floor	Second floor	Third floor	First floor	Second floor	Third floor
Truck 1	+	+	-	+	+	-	+	+	-
Truck 2	-	-	-	-	-	-	-	-	-
Truck 3	-	-	-	+	-	+	-	-	-
Truck 4	-	+	-	+	+	+	+	+	+
Truck 5	-	-	-	+	-	+	+	-	-
Truck 6	+	+	-	-	-	-	+	-	-
Truck 7	+	-	-	-	-	-	+	-	-
Truck 8	-	-	-	-	-	-	-	-	-

+ Salmonella positive sample; - Salmonella negative sample

Table 3. Prevalence of Salmonella contamination in sixty-six holding pens of two commercial slaughterhouses (slaughterhouse A and slaughterhouse B). Each slaughterhouse was visited in three different days (visit I to visit III). Each sampling day, a total of four sampling rounds were performed in each holding pen: before the pig entry, at half of the working day, at the end of the workind day and after cleaning procedures.

	Slaughterhouse A			Slaughterhouse B			
	Visit I	Visit II	Visit III	Visit I	Visit II	Visit III	TOTAL
Before pigs entry	0%	36.4%	54.5%	100%	27.3%	18.2%	39.4%
Half working day	45.5%	72.7%	81.8%	100%	100%	100%	98.5%
End of working day	18.1%	81.8%	90.1%	100%	90.1%	100%	80.3%
After cleaning	27.3%	54.5%	54.5%	90.1%	81.8%	72.7%	63.7%

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Yersinia enterocolitica prevalence and diversity in a pig slaughterhouse

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Abstract

Yersinia enterocolitica is involved in human foodborne infections. Pigs are considered as a major reservoir in many countries. The aim of the study was to contribute to the evaluation of the prevalence of Y. enterocolitica in France in pigs at the slaughterhouse level with optimized detection methods based on ISO 10273-2003. 516 samples of tonsils, feces and carcasses were analyzed from 344 pigs (24 batches) in a single slaughterhouse over 23 consecutive months. Enumeration and isolation were achieved by using CIN agar and a new YeCM chromogenic agar. Strains are characterized by phenotypic and genotypic methods: species identification and bioserotypes by API 20E gallery and biochemical typing confirmed by PCR, targeting the ail virulence, the plasmid (virF), the RfbC and 16SRNA genes. The total prevalence of Yersinia enterocolitica were 26.8%, 18.3% and 0% in tonsils', feces' and carcasses' pigs respectively with 94.5% of pathogenic bioserotype 4 O:3 and 4.5 % of non pathogenic bioserotype 1A (288 typable strains). Moreover, the pigs' contamination in the slaughterhouse is higher in winter (34.3%) than in summer (9.3%) even in batches from the same breeder. 100 strains were analysed and compared by PFGE typing. Only a few different genotypes were obtained: 5 distinct profiles with ApaI, 4 with NotI and 5 combined profiles. More diversity was observed with ApaI than with NotI. The strains of bioserotype 1A have different patterns from the strains of bioserotype 4 O:3. This project will contribute to a better understanding about risks caused by Yersinia enterocolitica. It is the reason why it is important to develop more efficient protocols using classical microbiology and molecular biology methods.

Introduction

Yersinia enterocolitica in human infections is increasing and its prevalence comes just after Salmonella and Campylobacter in Europe with 7595 reported human cases in 2009 (EFSA, 2011). Y. enterocolitica is mainly isolated from pork (tongue and tonsils) and the most encountered bioserotypes are 4 O:3, 2 O:9 and 3 O:5,27, which are pathogenic. The International Standard Organization method for the detection of presumptive pathogenic Y. enterocolitica (ISO 10273:2003) is applied with some optimization and improvement like the use of a new chromogenic media for isolation. We also used a PCR method for the identification of Y. enterocolitica and of pathogenic bioserotypes targeting virulence genes and the 16SRNA gene. The aim of this study was to evaluate the Y. enterocolitica prevalence in the pig's tonsils faeces and carcasses in a single french slaughterhouse. The isolated strains were evaluated for their pathogenicity and genetically compared to some human strains from local clinical cases of yersiniosis.

Material and methods

Bacterial strains

Reference strains of Yersinia CIP 124 (1A), CIP 134 (4 O:3), CIP 383 (2 O:9), CIP 29228 (3 O:5,27) were used as controls for growing on the plate media, presumptive and typing tests.

Pork samples

345 tonsils, 104 faeces and 72 carcasses were analyzed, in a single slaughterhouse, on 24 batches, during 23 consecutive months. One or two batches are taken for each campaign. Tonsils (about 10 cm¬2) and about ¼ forequarter external carcasses are swabbed. About 10g of faeces are taken from intestinal tract of identified animal with r emoved tonsils.

Enrichment

10g from each fecal sample and tonsil were added in a stomacher bag, suspended in 90mL of peptone water (faeces) or PSB (Peptones Sorbitol, Biliary salts) broth (tonsil or carcass), and then mixed in a stomacher for 30 seconds. One

milliliter of the suspension was added to 9mL of ITC (Biorad) broth and was incubated at 25°C for 48h. Swabs are also immerged in 9mL ITC broth.

Enumeration and isolation

Two medium were used: CIN agar (Cefsulodine, Irgasan, Novobiocine, Biorad) and a new modified YeCM (Yersinia enterocolitica Chromogenic medium) (from Weagant, 2008). Before enrichment, 0.1 mL of mixed tonsils and faeces (with dilution 10-2 and 10-4) were spread on and these media are incubated during 24 to 48H at 30°C. After enrichments and isolation, same incubation conditions were applied. A maximum of five colonies were subcultured on TCS (Trypton Casein Soya, Biorad) agar at 30°C for 24h.

Target tests

Y. enterocolitica formed on CIN agar small colonies with deep red center surrounded by a clear colorless zone. On modified YeCM, it formed small colorless colonies surrounded by a yellow halo at 24h. At 48h, colonies center became blue-green with colorless border. Characteristic colonies were subcultured and four presumptive tests were made: Lactose on VRBL medium (Violet crystal, neutral Red, Biliary salts, Lactose, Biorad), oxidase (solution at 1%, VWR). Urea and TDA tests were both carried out on the same plate of 96 wells: After the reading of the urea test, one drop of HCl 1N is added in the wells, before one drop of ferrous chlorate.

Identification

The lactose negative, oxidase negative, urea positive and TDA negative strains were identified by API 20E gallery (Biomérieux).

Biotyping

The biotypes were defined by using 6 tests: esculine hydrolysis (ROSCO discs), pyrazinamidase (pyrazinamide medium, ISO 10273), tween-esterase (Tween-esterase medium, ISO 10273), indole (peptoned water, and revelation with Kovacs reagent, Biorad), xylose and trehalose fermentation (carbohydrate fermentation media at 1% with bromocresol purple). DNA extraction. DNA extraction was performed by boiling at 95°C during 10 minutes some colonies suspended in 200µL of TE 1X (10mM Tris-Hcl-1mM EDTA, Euromedex). After centrifugation at 5000 rpm during 3 minutes, the supernatant was transferred in a new tube.

PCR assay

The PCR contains primers targeting ail (forward, 5'-GTTTATCAATTGCGTCTG TTAATGTGTACG-3'; reverse, 5'-CTATC-GAGTTTGGAGTATTCATATGAAGCG-3'), virF (forward, 5'-AAGGTTGTTGAGCATTCACAAGATGG-3'; reverse, 5'-TTTGAGTGAA ATAAGACTGACTCGAGAACC-3'), rfbC (forward, 5'-CGCATCTGGGACACTAATTCG-3'; reverse, 5'-CCACGAATTCCATCAAAACCACC-3') and 16SRNA (forward, 5'-ATACC GCATAACGTCTTCG-3'; reverse 5'-TTCTTCTGCGAGTAACGTC-3') gene sequences from Y. enterocolitica (Arnold et al, 2004, Thisted Lambertz et Danielsson-Tham, 2005). The sequences were synthesized (Invitrogen) and amplified respectively 454bp, 700bp, 405bp and 345bp DNA fragments. The PCR mixture contained 1X PCR buffer (New England Biolabs), 2mM MgCl2 (New England Biolabs), 0.25mM dNTPs (Invitrogen), respectively 10µM, 15µM, 20µM and 6,4µM of primers, 1U of Taq polymerase (New England Biolabs), and 5µL of DNA template. Thermal cycling conditions were as follows: 94°C for 3min, followed by 30 cycles of 94°C for 30s, 55°C for 1min, and 72°C for 1min. PCR was performed in microtubes, using a thermal cycler (Applied Biosystems). 10µL of PCR products were migrated in a 1,5% agarose gel, at 110V during 1h30. After migration, the gel is stained in an ethidium bromide bath (0,2 µg/mL) during 20 minutes and then rinsed in clear water.

PFGE typing

The genomic DNAs were prepared in agarose plugs, using a protocol described by PulseNet for Listeria (CDC, 2004), with a 2 h at 37°C lysis (solution: 50 mM Tris, pH 8, 50 mM EDTA, pH 8, 1% sarcosyl, 500 µg proteinase K/strain) for. After washes, they were digested with 20 U ApaI or NotI (New England Biolabs) for 5 h at respectively 25°C and 37°C. Pulsed field gel electrophoresis was performed in 1% Seakem Gold agarose (Invitrogen) using a CHEF DR III system (Bio-Rad) in 0.5x TBE (Tris borate EDTA) buffer at 14°C during 18h. Gels were stained with ethidium bromide and digitalized with the Gel Doc 2000 apparatus (Bio-Rad). Salmonella ser. Braenderup strain (H9812) digested with XbaI was used as a reference standard. Similarity is based on 90% by UPGMA method.

Results

The prevalence of Y. enterocolitica in the pigs in a single slaughterhouse was about 31%. 67% of pork's batches are contaminated: the rate varies from 0% (0/20) to 100% (12/12) depending on the batch The prevalence of Y. enterocolitica in the pig's tonsils, whichever breeder or whatever period, was about 26.8% (92 /345) . The prevalence of the bacteria in the faeces was lower than in the tonsils: 18,3% (19 /104). None of the 72 carcasses was detected as contaminated. Some campaigns showed that the modified YeCM medium seemed to be sensitively better than the CIN agar. Besides typical colonies of Y. enterocolitica on this plate are more differentiable compared to those on CIN. The prevalence seemed to be higher in winter/spring than in summer. A difference of prevalence was noted between the batches analyzed according to the pigs' primary producer, indicating the possibility of specific farm factors (see figure 1).

Figure 1: Y. enterocolitica pigs' contamination according to farm origin and season (% positive)
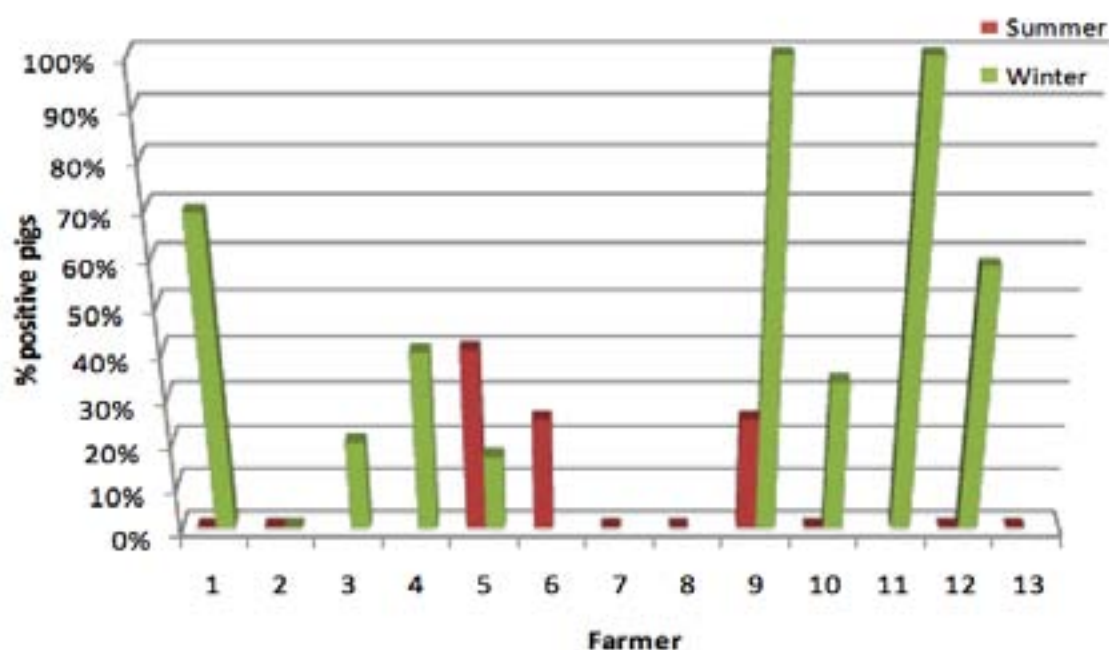
PCR allowed us to confirm the species identified by API gallery and to determine the pathogenic strains and the serotype O:3 of those strains. 94.5% of the 288 analysed strains from pork confirmed that the Y. enterocolitica belonged to the bioserotype 4 O:3 and 4.5 % to the non pathogenic biotype 1A,1% of the positive tonsils contain the bioserotype 4 O:3. One of the tonsils (1.9%) contains Y. enterocolitica of the both bioserotypes 1A and 4 O:3 and another one the 3 O:5,27 bioserotype. The same tendency is observed for faeces with 88.5% of them containing 4 O:3 and 11.5% non pathogenic 1A.

Only a few different genotypes were obtained by PFGE analysis of the 100 isolates of Y. enterocolitica from pork origin (100 with NotI; 69 with ApaI ; 63 with combined profile): 4 distinct NotI patterns; 5 distinct ApaI patterns; 5 distinct combined NotI ApaI profiles) (see figure 2). 53% of pigs' strains showed the same NotI pattern and 45% of pigs' strains showed the same ApaI pattern. The 4 strains of bioserotype 1A present 2 distinct profiles (YN03 and YN04 for NotI; YA04 and YA05 for ApaI) which were clearly different from the 96 strains of bioserotype 4 O:3.
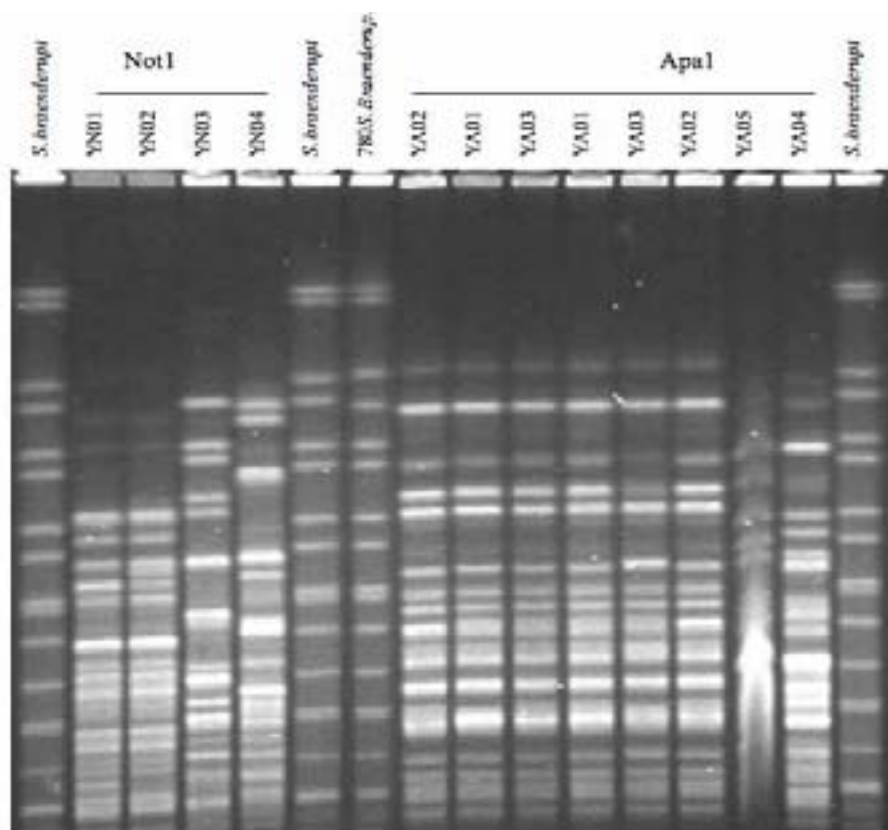
Discussion

The prevalence of Y. enterocolitica in pig's tonsils observed in this slaughterhouse is quite important. The rate of Y. entero-colitica observed in tonsils led us to search the bacteria in the faeces. However, because faeces and tonsils were analyzed on the same animal, it seems that no correlation exists between the presence in tonsils and presence in faeces from the same pork. No carcass was detected positive. The predominance of pathogenic bioserotypes shows that slaugh-tering must be done with precautions to avoid the carcass' contamination. Evisceration and head removing is an important step to avoid the dispersion of Y. enterocolitica. These bioserotypes which were found belong to the pathogenic 4 O:3, in accordance with the trend in Europe (Fredriksson-Ahomaa and Korkeala, 2003). However, the current method of PCR used in this study, cannot differentiate yet two of the major serogroups in Europe (2 O:9 and 3 O:5,27). The prevalence seems to be influenced by the season, because the observed Y. enterocolitica rates more important in winter than in summer kike observed by Milnes et al (2007). However, a difference of prevalence is noted between the batches analyzed, according to the breeder, indicating the possibility of specific farm factors. Characterisation of pork isolates of Y. enterocolitica by PFGE analysis revealed a low genetic diversity, lower than those observed by Fredriksson-Ahomaa and Korkeala (2003). This could be explained by the pork origin: a single slaughterhouse. More diversity was observed with ApaI than with NotI. The strains of bioserotype 1A present clearly different patterns from the strains of bioserotype 4 O:3.

Conclusion

The study of Y. enterocolitica prevalence in a single pigs slaughterhouse, allowed us to establish its prevalence, which was quite important. Indeed, the predominance of pathogenic bioserotypes 4 O:3 found, follows the trend in Europe. The application of a multiplex PCR showed that it was possible to confirm the specie Y. enterocolitica and to determine the pathogenic strains with this technique. The contamination is more important in winter than in summer. These results underline the importance of hygiene in the first steps of slaughtering, because this bacterium seems to take an important place among foodborne diseases, causing a public health issue.

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Proceedings - Posters

Serological characterization of Salmonella spp. infection in finishing pigs from NE Spain

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Abstract

The seroprevalence of Salmonella spp. in finishing pigs in Aragón (NE of Spain) and the potential factors associated with it were assessed. Serum samples were collected directly from the Regional Diagnostic Laboratory (RDL). Only farms submitting a minimum of 30 serum samples to the RDL were included, i.e. exporting and farrow-to-finish farms, and those in the last stages of the Aujezsky´s disease eradication program. Farms were randomly selected and proportionally distributed to the 2008 census. A questionnaire was used to obtain information on selected farms. The HerdCheck ELISA (IDEXX Laboratories) was used for serology. Out of a total of 6,182 sera tested from 217 herds (mean of 28.5 pigs/herd), 2,240 (36.2%) were seropositive when the cutoff used was OD% 20%, and 1,219 (19.7%) at OD% 40%. At least one seropositive animal was found in 91.7% (199) of the herds at OD% 20% and in 71.4% (155) at OD% 40%. The percentage of farms presenting a high within-herd seroprevalence (i.e. 40%) varied from 20% to 40% depending upon the cut-off point used (OD% 40% or 20%). A multivariable random-effect logistic regression showed that seroprevalence (using a cut-off OD% 40%) was significantly lower in winter and positively associated with drinking water sources other than the city supply, higher animal densities, the absence of rodent control programs or all-in/all-out systems, famers being members of pig health protection associations, and non-solid box separation (i.e. bars or similar). The SaTScan software was used to identify potential clusters of Salmonella-infected herds in the area, but no significant clusters were found. Results suggest that Salmonella infection is widely spread in the surveyed area and that some of the factors associated with it could be mitigated through overall hygiene and biosecurity measures.

Salmonellosis in wild birds and its relationship with the infection in finishing pigs

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Abstract

The potential relationship between Salmonella infection in wild birds and pigs was investigated. Feces from pigs, wild birds, and bird droppings or other environmental samples from 25 finishing farms were cultured for Salmonella isolation. In 17 (68%) farms Salmonella was isolated. Out of 57 Salmonella isolates found, 32 (56.1%) were Typhimurium. In 6 (24%) farms the same Salmonella serotype was isolated from samples from different origins and similar AR and PFGE patterns were found, which would support the existence of a transmission cycle of Salmonella infection between birds and pigs in this area. Preventing bird access to farm premises is highly recommended.

Introduction

Pork meat is considered an important source of Salmonella infection for humans in Europe (EFSA, 2008) and the reduction of the prevalence of Salmonella serovars with public health significance in pig herds is considered a major objective in the continent (Regulation (EC) No 2160/2003). The control of pig salmonellosis is difficult. Salmonella spp. are ubiquitous, survive outside the host for months and are able to infect a large variety of wildlife (Murray, 2000). Besides, the Salmonella serovars commonly observed in Europe rarely produce disease, hampering the identification of infected herds. The success of Salmonella control programs relies thus on a good knowledge on the epidemiology of this infection. Factors such as feeding, management, farm design, etc., play a significant role in the spread and maintenance of this infection among pigs (Funk et al, 2004). The role that wildlife may have on the epidemiology of this infection has been suggested many times, but it has been less investigated. With this study we try to gain further insight on the epidemiology of pig salmonellosis in an area of high prevalence in Spain through the search for phenotypic and genotypic similarities among Salmonella isolates from pigs and wild birds that may suggest inter species transmission.

Material and methods

Mist netting was used to humanely trap birds around pig farms. Birds were identified and kept in sterilized dark cages in groups of less than 5 animals of the same species until they defecate. Feces were collected through sterile swabs and birds released after measured and tagged by a licensed bander. Pools of 5 pig feces (from a minimum of 7 pens) and other environmental samples likely contaminated by bird feces were also collected from fattening units. All samples were cultured by triplicate (ISO 6579:2002) and Salmonella isolates serotyped at the National Centre for Animal Salmonellosis (Madrid, Spain) following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).
Salmonella isolates were tested against a panel of 10 antimicrobials (i.e. nalidixic acid, ciprofloxacin, cefotaxime, ampicillin, chloramphenicol, streptomycin, gentamicin, sulphisoxazole, trimethoprim, and tetracycline) using the Kirby-Bauer disk diffusion method (Murray et al, 2003) and following the antimicrobial concentrations recommended by the European Committee on Antimicrobial Susceptibility Testing (Anonymous, 2007) and the Clinical and Laboratory Standards Institute (CLSI) (Anonymous, 2005). Salmonella strains were classified as resistant (R), intermediate (I) or susceptible (S), according to the CLSI guidelines.
Genotyping was carried out by Pulsed-Field Gel Electrophoresis (PFGE) after digestion with XbaI following the CDC standardized laboratory protocol (CDC, 2004). Salmonella Braenderup H9812 was used as molecular size marker (Hunter et al., 2005).
Samples from pigs and wild birds were collected from 25 pig farms. An average of 9.4 (SD=5.6) fecal samples (individual or pools) from wild birds and of 8.3 (SD=3.2) pools of fresh pig feces were collected per farm. In addition, in 23 of these farms environmental samples (i.e. bird feces and samples from aisles, windows or underneath the feed silos) were also collected (an average of 2.7 per farm; SD=1.8).

A total of 234 bird samples, representing 511 wild birds, were collected and 12 (5.1%) were Salmonella positive (Table 1). House sparrows (Passer domesticus) were the most represented species with 44.4% of the samples, followed by European starlings (Sturnus vulgaris) with 6.8%, blackcaps (Sylvia atricapilla) 5.5%, blackbirds (Turdus merula) 3.8%, robyns (Erithacus rubecula) 3.4%, nightingales (Luscinia megarhynchos) 3%, and Sardinian warbler (Sylvia melanocephala) 3%. Other bird species composed less than 30% of the samples. Salmonella was isolated from 4 (33.3%) sparrow samples, 3 (25%) starlings, 1 (8.3%) blackcaps and 1 (8.3%) swallows (Hirundo rustica). Another 3 positive samples belonged to other species.

In 17 (68%) farms Salmonella was recovered from any of the samples taken. In 52% of them it was isolated from pig feces or environmental samples and in 24% from feces from trapped birds. The most common serotype was Typhimurium (56.1%) (Table 1). In 10 (40%) farms Salmonella was recovered from pig feces and bird feces or environmental samples. In 3 farms same Salmonella serotype was isolated from pigs and birds, in 2 from pig and environmental samples, and in one from pig, birds and environmental samples. In 7 out of these 10 (70%) same antimicrobial resistance (AR) pattern was observed between isolates from pig feces and those from bird feces or bird droppings. Multi AR (4 antimicrobial families) was found in 25 (59.5%) out of 42 samples analyzed from these 10 farms. The most common pattern was A(C) SSuT (Table 2). PFGE has been performed on Salmonella isolates from some farms. At least in 3 of them identical PFGE profiles were seen in isolates from birds and pigs (Figure 1).

Discussion

The study was carried out in an area of high Salmonella prevalence (Vico et al, 2011). In almost 70% of the farms Salmonella spp. was isolated. It was most frequently recovered from environmental samples indicating its wide distribution within the farms environment (Table 1).

The capture of wild birds around the pig farms was very variable. In some farms less than 5 birds were trapped (20% of the farms) while in other up to 25 bird samples were collected. In addition, the prevalence of Salmonella infection in healthy birds was expected to be low (Tizard, 2004). Despite of these limitations, in 21% of the farms surveyed infected birds were detected. In another 21% of the farms environmental samples, most of them related to bird droppings, were also positive to Salmonella spp. (Table 2). Although some environmental samples might have been contaminated previously by other sources, overall, these findings support the potential risk of Salmonella contamination carried by birds. Samples from house sparrows and European starlings showed the highest prevalence (33% and 25%, respectively). These bird species were more common in the vicinity of pig farms and therefore captured more often. In 68% of the surveyed farms these bird species or their droppings were usually observed inside the fattening units.

In addition to the observation of similar Salmonella serotypes among samples from pigs and birds within the same farms, identical patterns of AR were observed, suggesting the inter species transmission of Salmonella infection in this area. Preliminary results from PFGE showed important similarities among isolates of some of these farms (Figure 1) supporting this conclusion. This information should serve to persuade farmers to keep birds out of farm premises.

Whether birds were victims or responsible of the infection in the pig farm was however debatable. At least in 6 farms bird isolates presented the multi resistance pattern commonly seen in pigs in the region (Vico et al, 2011) and also observed in isolates from these pigs, suggesting a pig to bird transmission. In contrast, in 2 farms susceptibility to all antimicrobials was seen in all isolates. Absence of AR should be more common in birds than in pigs, suggesting the opposite direction of the infection.

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Table 1. Origin and number of samples collected, positive samples after culturing and serotypes identified.

Sample type	Total samples	No. + samples (%)	No. farms	No. + farms (%)	Serotypes (number of isolates)
Pig feces	208	29 (13.9)	25	13 (52)	Typhimurium (19), 1,4,[5],12: i : - (4), Rissen (3), Brandenburgo (2), Anatum (1)
Wild-bird feces	234	12 (5.1)	25	6 (24)	Typhimurium (9), Arizonae (1), Mikawasima (1), Anatum (1)
Environmental	62	16 (25.8)	23	12 (52.2)	Typhimurium (4), Rissen (3), 1,4,[5],12:i:- (3), Bredeney (1), Anatum (2), Reading (1), Houtonae (1), Kapemba (1)

Figure 1. PFGE patterns from Salmonella isolates from three farms
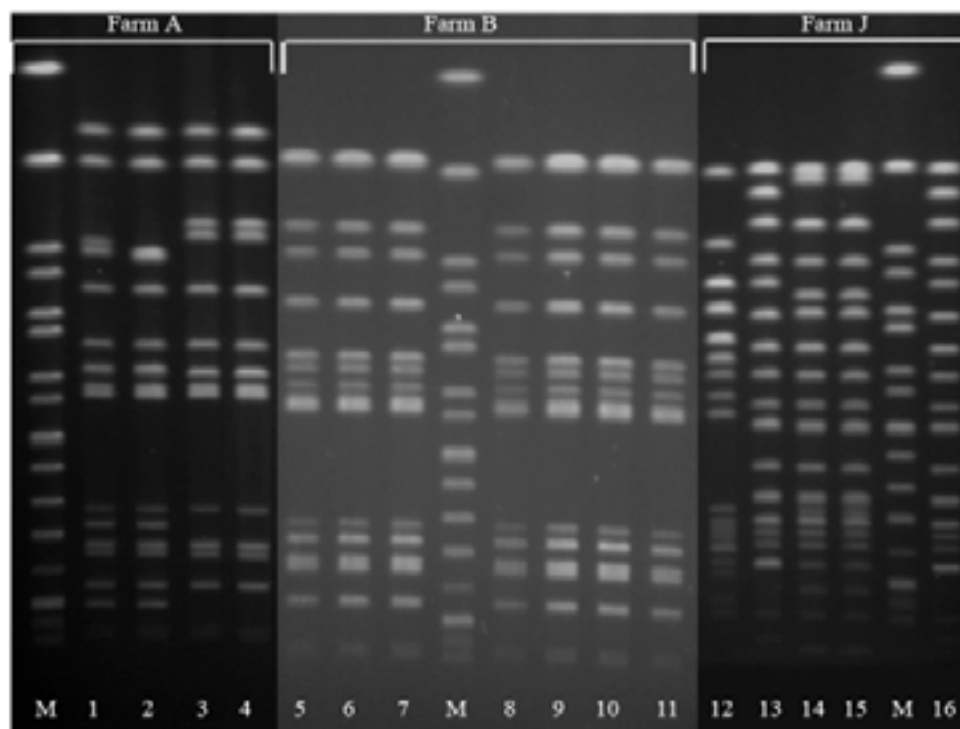
1-11 S. Typhimurium; 12: S. Rissen; 13-16: S. Anatum.
No. 1,2: Pigeon; 3: Blackcap; 4-6, 12 and 13: Pig; 7: European starling;
8: Swallow; 9, 10: House sparrow; 11: bird feces; 14: Feed with bird feces
15: mix of pig and bird feces; 16: House sparrow; M: S. Braenderup H9812

Table 2. List of farms where Salmonella was isolated both from pig feces and other type of samples (bird feces or environmental samples), serotypes identified, and antimicrobial resistance patterns detected.

Farm	Sample type	Origin/description	No. samples	Serotype	AR pattern
A	Pool	Pig	1	Typhimurium	Susceptible
	Individual	Blackcap	1	Typhimurium	Susceptible
	Individual	Rock Pigeon	2	Typhimurium	Susceptible
B	Pool	Pig	2	Typhimurium	Susceptible
	Pool (window)	Bird feces	1	Typhimurium	Susceptible
	Pool	House sparrow	2	Typhimurium	Susceptible
	Pool	Swallow	1	Typhimurium	Susceptible
	Individual	European starling	1	Typhimurium	Susceptible
C	Pool	Pig	1	Typhimurium	ACSSuT-Na
	Individual	European starling	1	Typhimurium	ACSSuT-Na
	Pool (window)	Bird feces	1	4,12:i:-	ASSuT
	Pool	House sparrow	1	Typhimurium	Susceptible
D	Pool	Pig	2	Typhimurium	ASSuT
	Pool (feed silo)	Feed with bird feces	1	Typhimurium	ASSuT-Na*
E	Pool	Pig	1	Rissen	SuT
	Pool (window)	Bird feces	1	Typhimurium	ASSuT
F	Pool	Pig	1	Typhimurium	ASuT-Na
	Pool	Cetti's Warbler	1	Mikawasima	Susceptible
G	Pool	Pig	4	1,4,[5],12: i : -	ASSuT
	Pool (aisle)	Bird feces	1	1,4,[5],12:i:-	ASSuT
H	Pool	Pig	1	Typhimurium	ASuT-Na
	Pool (pen wall)	Bird feces	1	1,4,[5],12:i:-	T
I	Pool	Pig	3	Typhimurium	ASSuT
	Pool	Pig	1	Typhimurium	ACSSuT
	Pool	Pig	2	Typhimurium	ASSuT-Na
	Pool (aisle)	Environment	1	Kapemba	ASSuT-Na
	Pool (window)	Bird feces	1	Rissen	ASSuT
J	Pool	Pig	1	Rissen	ACSuT
	Pool	Pig	1	Anatum	AST
	Pool	House sparrow	1	Anatum	AST
	Pool (aisle)	Pig and bird feces	1	Anatum	AS*SuT
	Pool (feed silo)	Feed with bird feces	1	Anatum	AS*SuT

Antimicrobial families: A (Aminopenicillins), C (Phenicols), S (Aminoglycosides), Su (Sulfonamides), T (TetracyclineS), Na (Nalidixic acid). * Intermediate resistance.

The addition of galacto-oligosaccharides on the feed for the control of salmonellosis in fattening pigs

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Abstract

Prebiotics that block intestinal harmful bacteria and stimulate both the activity of beneficial bacteria and the animal immune system may help in controlling pig salmonellosis. We added a galacto-oligosaccharide (Salmosan®) on the diet of pigs during the whole period of fattening to assess its potential effect on the prevalence of Salmonella spp. In a first trial 56 pigs from a small fattening unit (<200 animals) were fed with a diet where Salmosan® (0.5 kg/Ton of feed) was added, while the rest of the animals were fed with the same feed without the galacto-oligosaccharide. Samples of blood serum were collected after 1 and 2 moths of fattening and previous to slaughter. Individual feces were collected after 1 and 2 moths of fattening. Mesenteric lymph nodes (MLN) were also collected at slaughter. The Herdcheck Salmonella ELISA (IDEXX Laboratories) and the ISO 6579:2002 were used for serological and microbiological analyses, respectively. The prevalence at slaughter was slightly lower in the treated group compared to the control group, but no significant differences were observed (42.9% vs. 54.8%; P=0.25). No significant differences were found in seroprevalence or prevalence between the two groups at any of the collection times either. In a second trial a much higher dose (3 kg/Ton of feed) was used. At this dose significant differences of seroprevalence were observed after 60 days of fattening and at slaughter when a cut-off value of %OD 40% was used (10% vs. 37.5%; P 0.01). Prevalence was also significantly lower at slaughter either on feces (2.6% vs. 57.8%; P 0.01) or MLN (0% vs. 78.7%; P 0.01). These results suggest that the addition of galacto-oligosaccharides on the diet of fattening pigs might be useful to reduce the burden of salmonellosis in fattening pigs. Further research is required to confirm results and optimize the dose and time of treatment.

Meat Juice serology underestimates prevalence of Salmonella in pig herds

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Abstract

Salmonella serology is used for classifying pig herds in risk categories in several national quality programs. Meat juice is used as test matrix in most of these programs. Two studies were done to compare the salmonella ELISA test results from meat juice with blood serum as a reference.

Pig blood and meat samples for these studies were collected in one slaughterhouse. ELISA tests were done with a commonly applied commercial test. In the first study paired blood serum and meat juice samples from 182 pigs were collected and tested in two different laboratories. In the second study meat and blood samples were collected from 470 herds, over 20.000 samples for each matrix.

The first study showed a linear relation between all matrices, but the OD values in meat juice were significantly lower than in blood serum. To obtain comparable outcomes in serum and meat juice, the blood serum OD%-values had to be reduced with 20 to 40%, depending on the lab that applied the test. This underestimation was confirmed in the second study. When the diagnostic cut off, OD10%, was applied on the blood samples, over 57% of the tested pigs showed antibodies and none of the slaughtered herds had fully negative serology, whereas with meat juice and a cut off at OD40% only 7,5% pigs were positive.

It is concluded that meat juice testing for Salmonella antibodies can heavily underestimate the proportion of pigs that have encountered a salmonella infection. Consequently, pigs from herds that are categorised as low risk may be infected with salmonella. These pigs may therefore contaminate the lairage and the slaughter line. Monitoring results based on blood serology can not be compared with results based on meat juice, without taking care of the observed differences.

Introduction

Serology is used to determine the Salmonella status of pig herds as part of control strategies in national control plans in some northern European countries. In the Danish, Dutch and German programmes, pig herds are grouped in three to four distinct risk groups.

To detect the serological response following infection several Salmonella ELISA's are on the market. These are mixed LPS ELISA's that detect Salmonella seroconversion due to infections with O-serogroups B (for example S. Typhimurium, S. Derby, and S. Brandenburg) and C1 (S. Cholerasuis and S. Infantis). The experimental cut off of these tests is at OD 10%. In the national monitoring programmes the cut-off is mostly increased towards 40% to obtain a manageable, i.e. not to high number, of herds that need to take corrective intervention actions (Mousing et al 1997).

In a recent ring trial where meat juice was tested meat juice results where highly variable (Berk, 2008). Berk (2008) concluded that serology on meat juice is therefore not a suited method for target setting in an EU-wide control strategy. More variability may occur in meat juice due to difference in drip loss, which differs depending on the muscle used, the pH of the meat which is partly depending on stress before slaughter, sampling treatment, etc.

The number of scientific publication comparing the performance of commercially available test on blood and meat juice are limited and the number of samples tested are limited. Aim of the present study was to compare results from Salmonella serology in blood serum and meat juice under routine circumstances and see whether outcomes are equivalent. This is a preliminary presentation of the results.

Materials and Methods

In Study I a paired blood and meat sample were collected from the same animal. Pigs were randomly selected from 25 herds at one slaughter day in a German slaughterhouse. In total 182 paired blood and meat juice samples were available.

Meat for meat juice was collected from the neck and from the diaphragm.

The ELISA tests were performed in two commercial labs.

In study II, routine blood samples and neck meat samples were collected. Serum and meat juice were prepared and tested on Salmonella in one lab. The samples were collected with comparable numbers from the same herds, paired on supply level, not on pig level.

Blood was collected before entering the scalding tank. Blood was collected in treated test tubes (12 ml) for serum collection (KABE-Labortechnik), stored and transported at 4 °C and was send to the lab three times a week.

At the lab serum was prepared. In study I the serum was split in two. One sample was send to the other lab, at 4°C.

Meat was collected at the meat inspection platform. The pigs arrive at the sampling place 40 minutes after sticking. Meat (size about 1.5x1.5x1.5 cm) of neck (Study 1 and 2) or diaphragm (only in Study 1) muscle was collected in the SALMOSTORE meat juice container (Labor Diagnostik GmbH Leipzig). The samples were immediately deep-frozen at the slaughterhouse and stored at -20°C up to one week. Serum was harvested in the SALMOSTORE meat juice container after 1-7 days by thawing. After collection of the meat juice the tubes were sent to the laboratory with the blood stored at 4°C. In study 1 the samples were split here and sent to the research lab like the serum samples.

Both labs did the SALMOTYPE® Pig Screen Elisa. In these tests blood serum is diluted 1:100 and meat juice is diluted 1:10 according to the SALMOTYPE® Pig Screen Manual.

Results

In table 1 the number of positive tests at cut-offs OD% 10, 20 and 40 are shown. The tests on meat juice led to lower number of positive samples compared to blood serum at all three cut-offs in both laboratories. The proportion of positives was higher in meat juice from diaphragm than from neck muscle.

The differences between labs was much bigger for meat juice than for blood.

Table 1: Proportion Salmonella positive serological tests at different cut-offs in different matrices in two laboratories

	lab 1			lab 2		
OD%	Blood	Neck meat juice	Diafragm meat juice	Blood	Neck meat juice	Diafragm meat juice
>10	52,7%	51,1%	58,8%	47,8%	24,2%	37,4%
>20	34,6%	23,6%	23,6%	29,7%	15,4%	19,8%
>40	13,7%	8,2%	9,9%	13,7%	7,7%	9,3%

In Study II samples were collected during the routine monitoring at the slaughterhouse where pigs are sampled at random, the blood and the neck-meat-for-juice sampling were running in parallel. The number of tested samples of neck meat juice was higher than of blood serum (respectively 28.182 and 23.021 samples).

The number of ELISA positives in neck meat juice was lower than in blood, at different cut off levels (table 2). The OD value of all blood analyses result was corrected by multiplying the OD with 0,6. In that case meat juice and blood became comparable.

Table 2: proportion positive samples in Salmonella ELISA at three cut-off values tested on meat juice and blood serum (corrected and not corrected) – Study II

OD%	Meat juice	Blood, standard	Blood, corrected*
>10	37,0%	57,7%	44,2%
>20	19,9%	37,9%	21,9%
>40	7,5%	17,0%	7,3%

* OD values were corrected by multiplying with 0.6.

Categorisation of the herds based on the tests with meat juice and blood, and blood after correction of the outcome was done according to the German QS regulations. The results are shown in table 3.

Categorisation of herds based on meat juice leads to an underestimation of category 2 and 3 farms when blood is the reference. Reducing the OD values of blood by correcting the blood OD with a factor 0.6 makes blood and meat juice comparable. A cut-off of OD65% for blood made the results also comparable, although there were still little more herds in category 2.

Table 3: Categorisation of pig herds bases on corrected and non-corrected test results

Cut Off	Meat juice	Blood			
	OD%40	OD% 40	OD% 40	OD%65	OD%10
Correction:	-	-	OD% x 0.6	-	-
cat 1	90,4%	67,9%	90,2%	89,1%	0.4%
cat 2	8,3%	24,3%	8,5%	9.6%	8.7%
cat 3	1,3%	7,9%	1,3%	1,3%	90.9%

Discussion

Serum of blood is in general the standard matrix for serological arrays. However, salmonella serology applying meat juice is standard in many European countries. The present study was done in a slaughterhouse that changed from meat juice to blood serum for the routine monitoring. This raised the question whether farmers supplying pigs to this slaughter house could expect to get other Salmonella testing results and whether more supplier of the slaughterhouse would be classified in a higher risk category. The data definitely show that changing from meat juice to blood will results in more positive tests. The proportion of ELISA positives based on blood serum was significantly higher than the proportion based on meat juice. This would lead to higher proportions of pig herds being classified in high risk categories. The outcomes of serum tests and meat juice test could be made comparable by applying a correction factor of 0.6 for the blood ODs. This indicates that ELISA on meat juice underestimates the antibody concentration with 40%.

The results of diaphragm meat juice were closer to the blood serum value than those of neck meat juice.

The outcomes also confirm earlier studies (e.g. Berk, 2008), which showed that between lab variability is much bigger for meat juice than for blood.

The acquired data show that many of the pig herds had encountered Samonella, about 60% of the samples were positive at a cut off of 10%, which is the diagnostic cut off of commercial tests.

Other studies, with smaller numbers and under experimental conditions, showed much better correlation between meat juice and serum .Therefore no correction was considered in any other study, except Wilhelm (2007). The present results were equal to the normal daily practise in this slaughter house. This underlines that underestimation in meat juice may be sampling (i.e. slaughterhouse) and lab dependent.

Variability, discrepancy and not full correspondence between meat juice and blood serum have been reported before. But a comprehensive understanding of the causes hereof are not given (Wilhelm 2007, Nielsen 1998) Meat juice is intrinsically less robust and vulnerable to sampling variability, physiological changes in the meat of the sampled animal, etc.. Categorisation of pig herds is used to control Salmonella by implementing hygienic measures at primary production level and to steer logistic slaughtering. The present study shows that herd categorisation based on serology has serious limitations that need to be taken into account before taking conclusion from a monitoring programme that applies serology with particular cut offs. For meat juice this even more true than for blood. Serology can not prevent that finally pigs from so called low category herds excrete Salmonella and contaminate lairage and slaughter line (Van der Wolf, 2001). With the uncertainties of test outcomes, and additionally the high levels of infection in slaughter pigs, as well as the evidence that contamination of carcasses is mostly depending on hygienic slaughterhouse (Swanenburg, 2001; Van der Gaag, 2004) logistic slaughtering based on categorisation by means of serology has to be done with care.

Conclusion

Routine testing of meat juice can seriously underestimate the real Salmonella serological prevalence. The study confirms that variability between labs is much higher for meat juice than for blood. Serology on blood is more robust than serology on meat juice as sampling can be better standardized and physiological changes in meat infuences the composition of meat juice stronger.

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Practical experiences with the reduction of prevalence of Salmonella infections in pig herds

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Introduction

Salmonella reduction is an issue with still increasing importance for the swine production. PIC in Germany has already started with specific interventions to reduce Salmonella prevalence in their breeding herds in 2001 – just one year before the "QS Qualität und Sicherheit GmbH" began to set up the serological Salmonella monitoring in German finishing pigs. Including the breeding pig production in programmes implemented for Salmonella reduction is a sometimes underestimated prerequisite for Salmonella reduction in the entire pig and pig meat production chain. PIC herds with enhanced Salmonella prevalence have been identified by a continuous serological monitoring. The results from the serological monitoring were analysed with the help of PIC Health Database (Siebert, 2011) and a thorough on-farm analysis of potential risk factors leading to an individually tailored catalogue of measures for reducing the Salmonella prevalence in the specific herd.

Method

In total 98 herds (grow out units for replacement gilts) were involved. The herds were monitored by a continuous blood sampling performed in market weight pigs (180 days of age). The sample size was 120 pigs per herd and year with a monthly sampling interval. Antibody titres were determined by ELISA according to the manufacturer's instructions. The results of the previous 3 months' serological samples were weighed 0.6:0.3:0.1 (the immediate month counting two times as much as the previous months), and the weighed average was called the "serological Salmonella index" like a modified danish System (Alban et al., 2002). Herds with a Salmonella index of more than 20 were classified as "problem herds" and selected for a thorough on-farm analysis. Epidemiological on-farm investigations were performed by regional veterinarians to evaluate the possible routes of Salmonella introduction into the herd as well as potential risk factors for the internal spread and reasons for the obviously insufficient levels of immunity.

Cross sectional blood sampling was performed in some herds to identify the time point of infection deduced from seroconversion. Samples for further investigations especially from the environment were only collected to substantiate suspicion, not to confirm well known risk factors. Antimicrobials were not used except for treatment of acute salmonellosis.

Results

Already in the first year of action 13 out of 98 herds were classified as problem herds due to enhanced Salmonella seroprevalence. In the majority of the problem herds a number of various risk factors was identified while cases where only one risk factor caused the problem were very rare. The multi-causal genesis of enhanced Salmonella prevalences and the according control measures are demonstrated by the following examples:

1. Herd (800 pigs; 28-110 kg), continuous, slight increase in the number of seropositive pigs within 12 month
   Risk factors analysed:
   a. High pressure cleaning of the central corridor with unclosed door ventilation allowing a direct contact of the pigs to the incoming water spray. >> Closing all door vents before cleaning the central corridor with a high pressure cleaner.
   b. No strict AI-AO regime implemented; pigs failed to reach market weight in time were integrated into groups of younger pigs. >> Implementation of strict AI-AO regime combined with diligent cleaning and disinfection.
   c. Feed ration containing more than 50% wheat and triticale. >> Ration with a minimum of 30% barley.
2. Herd (1.100 pigs; 28-110 kg), distinct increase seropositive pigs 3 months after beginning of reconstruction works:

Risk factors analysed:

   a. Strong rodent infestation. >> Implementation of strict pest control measures.

   b. Pelleted feed with only a spot of barley. >> Amending the physical form (meal instead of pellets) and composition of the diet (30% barley), supplementation of organic acids.

3. Herd (950 pig; 28-110 kg), 20 to 40% seropositive pigs for a long time period in spite of good hygiene and adequate feeding.

Risk factors analysed:

   a. Overcrowding of the pens during the first 6 weeks (10 to 16 weeks of age) due to limited barn capacities. >> Enhancing the barn capacities by construction measures according to the number of pigs that needs to be housed.

   b. Regrouping of the smallest pigs from the overcrowded pens after 6 weeks when an additional unit becomes available.>> Implementation of a strict AI-AO policy without any regrouping of pigs.

4. Outdoor herd (600 pigs; 7-28 kg), more than 40 % seropositive pigs for a longer time.

Risk factors analysed:

   a. Deficiencies in the pig flow in the nursery units. Nursery pigs with obvious growth retardation were kept in the nursery unit for a longer time and mixed with younger pigs weaned from the subsequent farrowing groups. >> Implementation of a strict AI-AO policy without any regrouping of nursery pigs.

   b. Small amounts of faeces visible on the floor of cleaned and empty pens. >> Diligent cleaning of empty units with subsequent control of the cleaning measures.

   c. Strong rodent infestation >> Implementation of strict pest control measures.

In all these herds it was possible to reduce the serological Salmonella index within 12 month to <20 by implementing the measures described.

Discussion

In all cases described the Salmonella seroprevalence could be significantly reduced by the means of a thorough on-farm analysis followed by an intensive instruction of the farmer. Today, all PIC herds are classified in the best possible Salmonella category (I). In contrast, only 83% of the finishing pig herds in Germany have been classified to this category in the first quarter of 2011 (May et al., 2011). The success of the PIC Salmonella monitoring in breeding herds strongly depends on the intensive cooperation between the regional veterinarians and the farmers (grosse Beilage, 2002). Improving biosecurity measures (quarantine, optimised working routines, AI-AO, age segregation, pest control, stress reduction), optimising hygiene conditions (cleaning and disinfection, correct high pressure cleaning, general health management) and adapting the feed (meal, enhanced barley portion, acid additives) could again be approved as the main factors for the reduction of salmonella prevalence. These factors that have already been identified by several studies and their positive effect to reduce Salmonella seroprevalence can be assessed as well evaluated (Hotes et al., 2010). The cases described above emphasise the necessity of a thorough on-farm analysis since the combination of risk factors impairing the course of Salmonella infection in the individual herd is highly variable.

With the Salmonella monitoring in breeding pig herds that has started already in 2001, PIC makes a valuable contribution to reduce Salmonella in the early stages of pig production chain.

Conclusions

With the results of a thorough on-farm analysis followed by an intensive instruction of the farmer, it was possible to reduce the Salmonella seroprevalence in breeding pig herds significantly. Salmonella monitoring in pig breeding herds as the earliest stage of the pig production chain is a prerequisite for the successful reduction of Salmonella in pig meat.

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Monitoring for Salmonella at PIC Deutschland

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In Germany August 2000 a first draft on reducing the salmonella prevalence in slaughter pigs was made. As reaction PIC Deutschland expanded their health monitoring by testing for salmonella on nucleus and multiplier farms.

PIC Deutschland wanted to push and prove with the implementation of the PIC salmonella monitoring program the importance of starting at the highest level in the breeding pyramid. Reduction of the salmonella prevalence in the final product by implementing the program in the top of the pork chain in the nucleus and multiplier farms and then follow the program in the weaner producers and fattening farms.

Our goal was explained by the picture of the breeding pyramid with the nucleus and multiplier farms on the top, with the information how and why to do special measures.
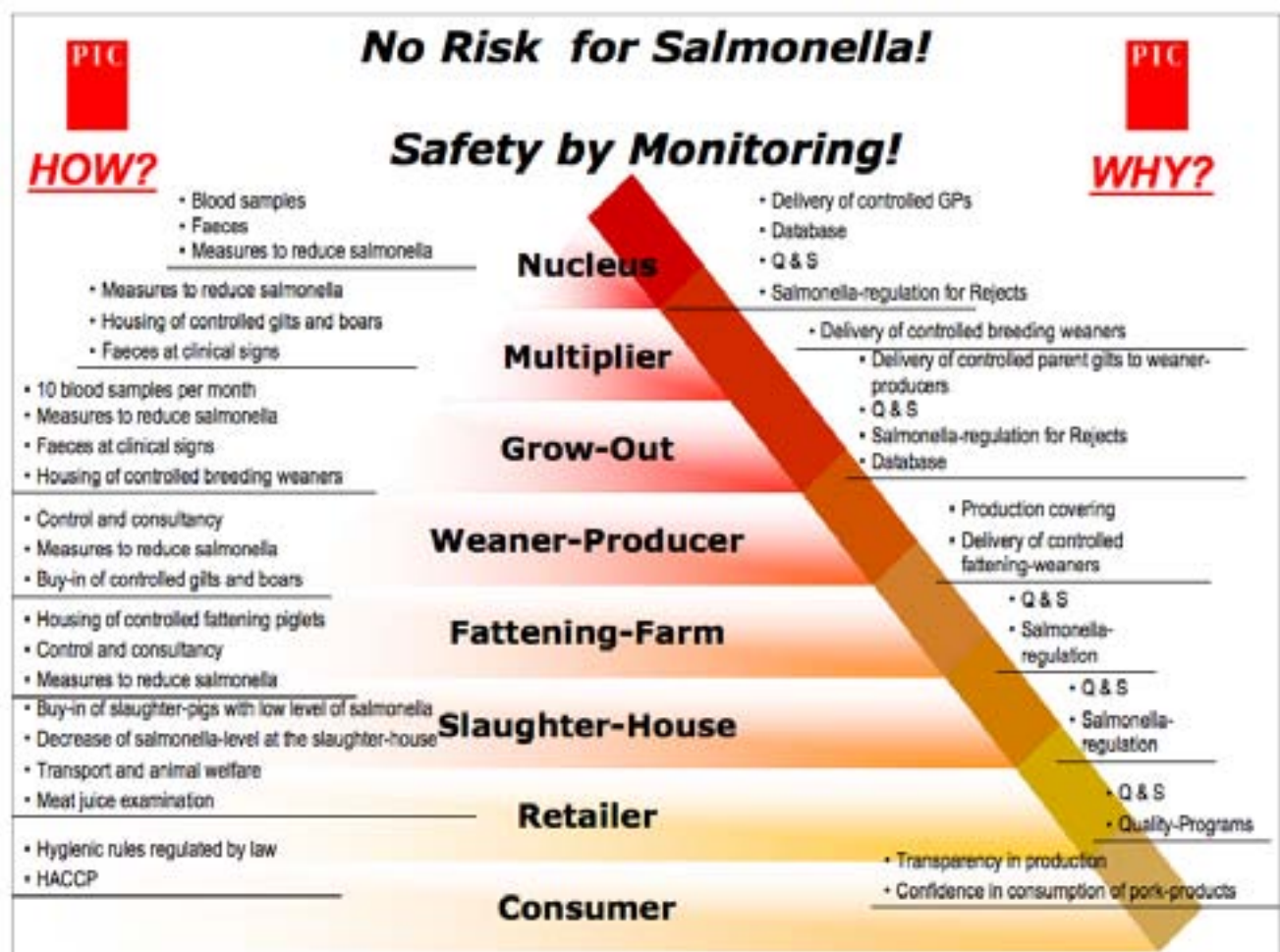
When PIC was founded in 1962 a strict health program immediately became part of its fundaments. The high health statuses of the PIC nucleus and multiplier farms were very important and how to safe, control and keep this.

It is our goal to produce and deliver pigs with high health and a high production capacity to prevent destroying the clients trust (Dr. Tom Alexander). To assure this high health status even more, the intensive and strict extra Salmonella monitor program of the selected gilts was implemented to be able to react quickly when needed.

Monthly 10 samples of the oldest gilts or boars of 98 grow outs were tested for salmonella with ELISA at the Bakum Field Station for Epidemiology. 2 regional vets of PIC were trained in controlling salmonella introduction in the herds, internal spreading of salmonella and improving the situation in the farms to increase immunity and to stabilize the health of the gastrointestinal tract.

The visits of the 2 PIC vets were done together with the herd vet and the PIC production team.
Of every farm-visit a report was made with specific points of action. The PIC vets supported the program especially in farms with higher serological titres or increasing titres on the basis of strict salmonella reduction protocols. A rolling index was calculated according to the Danish system with the average results of the last 3 months. 4 categories were used: Average of the 10 OD values is between 0 and 10% (71% of farms), 10-20% (17%), 20-40% (10%) or higher than 40% (2%). A check list was used to control nursery, pre-test and grow out units. With this list specific actions to reduce the salmonella titres were implemented. These actions were controlled by the herd vet and the PIC production team and always monitored by PIC vet-coordination.

One year later, strict control measures on salmonella reduction decreased the percentage of farms with the indexes above 20% from 12 to 7%.
Main actions were focussed on:
- reduce introduction like using a good isolation for the cool down period or strict rodent control
- reduce spreading on farm like strict hygiene control, all in all out, no mixing of pigs,
- stabilize health of the gastrointestinal tract like increasing the barley in the diet up to 50%, using acids in the feed or using meal feed.

2004 the German QS program (Quality and Safety) started. The experience of the PIC salmonella monitoring program became part of the salmonella program of QS. All datas were transformed to the QS salmonella monitoring. Now according to the QS regulations 10 blood samples are tested every 2 months (minimum 60 samples per year) with the Salmotype Pig Screen, LDL ELISA, OD>10% positive. In QS there are 3 categories: I = <20% positive samples over 1 year, II = 20-40% positive samples and III = >40% positive samples. The PIC farms started the QS with 64% in I, 26% in II and 10% in III.

The difference between the PIC salmonella monitoring and the QS monitoring is that with the QS the farmer got the categorization information one year after starting and then every quarter. In the PIC program there is a permanent monitoring and feedback and actions on increasing titres. Actions can start very quick which is very important for the safety of our products. The control of the salmonella situation in the PIC farms is now part of the regular visits of the PIC regional vets which are done every 3 months.
With the discussions regarding the salmonella monitor and reduction program in the EU the request for information from farmers, traders, slaughter houses and retail is going up. Especially information is requested on the good results of the program. In many presentations, publications and also in a dissertation the experiences and advises were presented.
The information on the PIC and the QS salmonella status is more and more asked for in the health status information regarding f.i. PRRS and Mycoplasma hyopneumoniae, the health monitor and vaccination program of gilts delivered. This information can be found in the health information if the "PIC vetinfothek", accessible by the web.

Making a resume, it can be said that the PIC salmonella program has been very successful. By the efforts and consequent cooperation of the multipliers and farmers of the grow-outs, the inspiring effort of the farm- and regional vets, who immediately reacted and implemented the actions for salmonella reduction and the production people, all our farms are now in category 1. The salmonella monitor and reduction program of 2001 till present resulted in a lot of information, protocols and success stories.

Effect of different treatments on swine carcasses surface contamination with Salmonella Typhimurium

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Abstract

Salmonella is worldwide related to the most cases of food poisoning in humans. The meat contamination may occur from direct or indirect sources during the slaughter and pork processing. The main factors that contribute to pig carcass contamination at the slaughterhouse are the presence of asymptomatic Salmonella shedders and Salmonella transmission during the pre-slaughter transport and lairage. Usual slaughter procedures may be not able to totally avoid the contamination of the surface of carcasses. Therefore, the aim of this study was to test different treatments to reduce Salmonella contamination, which may be adopted for decontamination of pig carcasses. Skin samples from pigs were artificially contaminated with a Salmonella Typhimurium phage type DT144 suspension (106 CFU/mL), and afterwards underwent nine treatments: 1) water, 2) water at 80°C, 3) water at 80°C with an organic acids blend (ascorbic, citric and lactic, Citrex®), 4) chlorinated water at 80°C with acids, 5) chlorinated water at 80°C, 6) water with acids, 7) chlorinated water with acids, 8) chlorinated water and 9) negative control (no treatment). Concentrations of 1,000 ppm and 2 ppm of Citrex® and chlorine, respectively, were used. All treatments were performed in ten repetitions and applied under controlled pressure (3 atm) for 10 seconds. Each skin was sampled, by swabbing a 5cm²-area on three occasions: before, shortly after and 24 hours after treatment. Swabs were placed individually in Buffered Peptone Water, homogenized, and 100 μL were spread on XLD agar for colony-formation unit counting of Salmonella. Data were analyzed using repeated measures model by the MIXED procedure of SAS. The effects of block, treatment, time and the interaction between them were tested. The treatment with chlorinated water at 80°C with organic acids had the best performance immediately after treatment and 24 hours later, followed by the treatments with chlorinated water plus organic acid, and water with organic acid.

Identification of Salmonella clonal groups and enterobacteria quantification in different risk areas of manufacturing process in four Brazilian feed mills

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Abstract

Identification of critical points for contamination of feed and spread of Salmonella may contribute to the development of control plans. A cross-sectional study was conducted to identify Salmonella clonal groups in feed mill facilities. A total of 1,322 samples were collected in four swine feed manufacturing facilities. Samples were taken from ingredients and from dust deposited on the floor and on the inner surface of storage bins, bucket elevators, mills, mixers, scales, pelleting chamber and cooler. Besides, all samples were submitted to enumeration of enterobacteria. Salmonella was isolated from a total of 66 (5.0%) samples; most of positive samples were taken from transportation equipment (bucket elevator and conveyor belt). In two facilities, Salmonella was detected in the end product. Serovars Montevideo, Infantis, Orion, Senftenberg, Agona, Worthington and Tennessee were found in more than one step of the manufacturing process, and they were submitted to molecular typing by pulsed-field gel electrophoresis (PFGE). Results from XbaI and BlnI-digestions revealed from one to nine PFGE-profiles. Pulsotypes analyses indicated that dust deposited on the inner surface of equipments and on the floor is responsible for spreading and persistency of Salmonella in feed mills over time. The highest enterobacteria counts were found in the dosage step in all sampled feed mills, indicating lack of cleanliness in this area. Salmonella was isolated in 8.75% (28/320) of the samples presenting enterobacteria counts >100 cfu.g-1. Dust accumulation on the floor and surface of equipment and a high production flow were identified in all feed mills; these factors may have contributed to the spread of Salmonella clonal groups.

Introduction

High quality feed manufacturing and delivery is essential to success of swine production. Contaminated feed can be the introduction vehicle of Salmonella in farms. Therefore, Salmonella has been considered an important microbiological hazard in animal feed (EFSA, 2006). Among the most important sources of feed contamination are the ingredients from both animal and vegetal origin (Davies & Hinton, 2000; Coma, 2003). Moreover, the spread of Salmonella in the feed mills facilities has been associated to factors such as cross-contamination by dust, presence of vectors and poor hygiene condictions (EFSA, 2008). The flow of ingredients and feed through the machinery also contributes to the level of contamination. Thus, the identification of critical points for contamination of feed and spread of Salmonella clonal groups may contribute to the development of control plans. This study was carried out to determine the frequency of Salmonella isolation and to identify Salmonella clonal groups in different areas of feed mill facilities.

Material and Methods

A cross-sectional study was conducted in four swine feed manufacturing facilities. The production flowchart of each facility was studied and sampling spots were defined. Each facility was visited six times. Feed ingredients and dust deposited on the floor and on the inner surface of storage bins, bucket elevators, mills, mixers, scales, pelleting chamber and cooler were sampled. Five to ten samples (100 g) were aseptically collected from each sampling spot and pooled before the analysis. This sampling method has been proposed to increase the probality of Salmonella detection (Richardson, 2008). A total of 1,322 samples were collected. Salmonella isolation was performed according to a protocol consisted of non-selective pre-enrichment, selective enrichment, and plating onto selective solid medium Xylose Lysine Tergitol 4 agar (XLT4, Merck) and Brillian-green Phenol-Red Lactose Sucrose agar (BPLS, Merck) (Michael et al., 2003). Typical colonies were submitted to biochemical and serological confirmation. Salmonella isolates were serotyped at Fundação Oswaldo Cruz (FIOCRUZ) following the Kauffmann-White scheme. Salmonella isolates were analysed by Pulsed-Field Gel Electro-

phoresis (PFGE) to identify clonal relationships between strains of a same Salmonella serovar. The Pulsenet protocol for molecular subtyping by PFGE was used (Ribot et al., 2006). PFGE was performed on the CHEF DR II system. Gels were stained with ethidium bromide (10mg.ml-1) and photographed under UV illumination with the Kodak 2200 system. Restriction profiles were visually analyzed, and band position was determined. Enterobacteria enumeration was performed in all pooled samples tested for Salmonella. Aliquots (1 mL) from serial dilutions (10-1 to 10-4) were transferred to selective medium for enterobacteria (Violet Red Bile Agar-VRBA, Merck). Typical colonies were counted and the results expressed as colony forming units per gram (cfu.g-1). Enterobacteria enumeration results were categorized into above or below 100 cfu.g-1. Association of enterobacteria count level and Salmonella isolation was tested by chi-square analysis. P values <0.05 were considered significant.

Results

A total of 66 (5.0%) samples were Salmonella-positive, and most of positive samples were originated from the transportation equipment (bucket elevator and conveyor belt). In two feed mills (A and D), Salmonella was also detected in the end product samples (2/78; 2.5%). Among the 66 Salmonella strains isolated, serovars Montevideo (22.7%), followed by Mbandaka (10.6%), Senftenberg (10.6%) and Agona (9.0%) were the most prevalent. Serovars Montevideo, Senftenberg, Agona, Worthington, Infantis, Orion, and Tennessee were found in more than one step of the manufacturing process, and were submitted to PFGE. From one to nine PFGE-profiles were identified in the aforementioned serovars (Table 1). Strains belonging to a common genotype were identified in serovars Orion, Montevideo, Worthington and Agona. Serovar Montevideo presented the highest number of clonal groups, which were distributed among ingredients, dust collected from the floor and equipment, and feed. The highest frequency of samples presenting enterobacteria counts above 100 cfu.g-1 was found in the dosage area followed by crusts formed on equipment surfaces, milling and mixing steps, while end product and storage bins had a low frequency of enterobacteria counts above this limit (Figure 1). Salmonella isolation was significantly (P<0.001) more frequent in samples with enterobacteria counts above 100 cfu.g-1 (28/320; 8.75%) than in samples with counts below this limit (38/1127; 3.26%).

Discussion

Salmonella was detected in ingredients and dust collected from equipment and floor of all feed mill facilities, as well as in end product samples of two feed mills. These results highlight the importance of feed contamination during manufacturing, since the amount of feed produced daily by a feed mill will suply a high number of farms. Dust deposited on the floor and on the inner surface of the bucket elevators were found to be the most important sources of Salmonella isolation. A higher frequency of Salmonella enterica was found in dust compared to ingredients, and dust was pointed as the main risk factor for cross-contamination of feed (Torres et al., 2011). Dust produced during the feed manufacture process may set down on the surface of the equipment and on the floor. Deposited dust on equipment surfaces may absorb moisture and originate crusts that may foster bacteria.

Salmonella Montevideo and S. Agona have been found to persist in biofilms formed in feed mill equipment for several years (Vestly et al., 2009). In our study, S. Montevideo was the most prevalent serovar and presented the highest number of clonal groups. The analyses of pulsotypes demonstrated the cross-contamination during feed processing as well as the persistence of serovar Montevideo strains over time. Pulsotype Mo4 encompassed strains isolated from ingredient, equipment and feed collected in a same sampling event, while pulsotypes Mo2 and Mo5 were found in samples collected in more than one visit. Thus, ingredients, such as bone meal and soybean meal, may introduce Salmonella in the equipment, while dust and crusts accumulated on their surface may facilitate the persistence of clonal groups. As a consequence, ingredients and feed flowing through the equipment may be cross-contaminated.

Enterobacteria enumeration has been considered an indicator of Salmonella presence in feed (Davies & Hinton, 2000). In line with this result, Salmonella isolation was significantly (P<0.001) more frequent in samples with enterobacteria counts above 100 cfu.g-1 in our study. Moreover, the highest frequency of samples above this limit was found in the dust deposited on the inner surface of equipment, which proved also to be a major hazard for Salmonella feed contamination. High counts of enterobacteria indicate uncleanliness of equipment and facilities. In all sampled feed mills a lack of inspection windows and the presence of dead areas and crevices in the equipment were observed. The poor hygienic design of equipment associated to the high flow of production may hinder proper cleaning operation and contribute to the accumulation of dust, enhancing the hazard of cross-contamination of processed feed.

Conclusion

Salmonella clonal groups can persist in feed mills over time and cross-contaminate the processed feed. Dust and crusts accumulated on surface of equipment are the main site of Salmonella persistence. Thus the improvement of hygienic design of equipment and the avoidance of dust accumulation should be targeted in Salmonella control programs in Brazilian feed mills.

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Table 1: Sources of Salmonella pulsotypes isolation at four Brazilian feed mills.

Feed mill	Visit	Serovar	Pulsotype	Source
A	5	Montevideo	Mo7	Bucket elevators*
	5		Mo8	Bone meal
	5		Mo9	Bone meal
	5	Infantis	In1	Crust
	5		In2	Crust (Drag conveyors)
B	1	Orion	Or1	Wheat bran, scale*
C	1	Montevideo	Mo1	Feed truck
	1, 5		Mo2	Bone meal, floor*, feed
D	1	Montevideo	Mo3	Bucket elevators*
	1		Mo4	Rice meal, milk, bone meal, Bucket elevators*, feed
	1, 2, 3		Mo5	Sorghum, bone meal, floor*
	2		Mo6	Mill*
	1	Worthington	Wo1	Floor*, crust
	2	Tennessee	Te1	Mill*
	2		Te2	Floor*
	3	Senftenberg	Se1	Floor*
	2		Se2	Floor*
	2		Se3	Mixing*
	1		Se4	Soybean meal
	1	Agona	Ag1	Wheat bran
	1		Ag2	Floor*
	2		Ag3	Corn gluten, floor*

*Dust collected from the equipment or floor was analyzed.

Figure 1: Frequency (%) of samples presenting enterobacteria counts above 100 cfu.g-1 in four Brazilian feed mills.
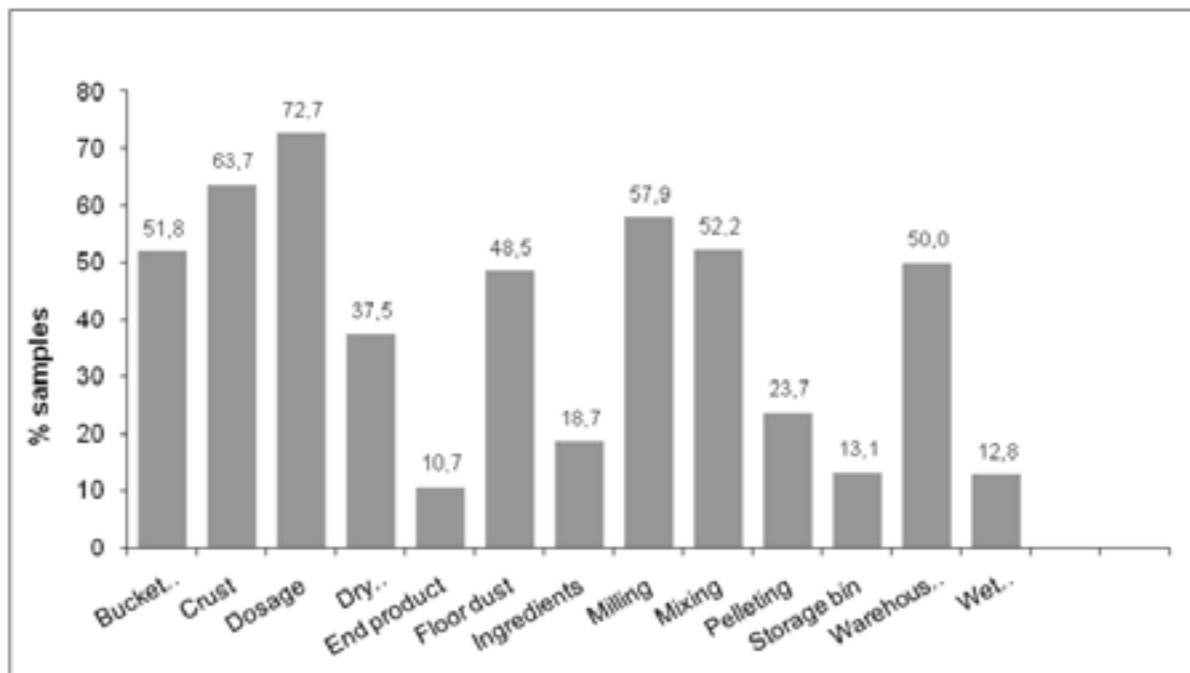
In-vitro experiment of Listeria reduction in ready-to-eat dry cured sausages

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Abstract

The risk of listeriosis associated with ready-to-eat foods is a major concern in United States. The recently published United States regulations require ready-to-eat meat producers to control Listeria monocytogenes, using interventions which may include antimicrobials that reduce post-processing contamination by at least 1 log cycle and that no more than 1 log increase throughout product shelf life. This regulation impact also the Spanish meat producers especially dry cured sausages, which export their products to USA. In this study, we analyzed in vitro, individually and in combinations, the commonly applied antimicrobials to reduce Listeria. Performing in-vitro experiment before applying directly on dry cured sausages offer us the benefits such as time and cost saving. Optimum concentration of each treatment was firstly determined, which is the concentration that kills 50% of population of the two strains used, Listeria monocytogenes and its surrogate, Listeria innnocua (105CFU/ml). The results showed that there are two different affects of treatments, bactericidal effect (obtained with bacteriophage, nisin, and pediocin-producing Pediococcus acidilactici as additives) and bacteriostatic effect (obtained with organic acids, lyzozyme and lactic acid bacteria as additives). The optimum treatment against Listeria is pediocin-producing Pediococcus acidilactici (2.8 mg/ml) which could be applied alone or in combination with bacteriophage or other bacteriocin-producing lactic acid bacteria. The obtained results will be further proceed, to direct application in different types dry cured sausages.

Introduction

In many commercial ready-to-eat (RTE) foods, recontamination with pathogens during post processing leads to outbreaks of food borne diseases. Good handling during post processing has been reported to increase the safety of these products. However, some robust pathogens like L.monocytogenes, may still grow during storage. L.monocytogenes is particularly problematic for food industry, due to it is widespread in the environment, and because of its ability to grow in a wide range of temperatures. Although listeriosis is rare, it is of public health concern, because of its high case fatality (20-30%). In addition, the number of notifications of L.monocytogenes in RTE products was reported to have increased (RASFF, 2006, 2007).

In fermented sausages, a sequence of barriers that appear along the ripening process (e.g. reduction of aw and pH) is applied. In typically Mediterranean sausages, the barriers applied during their production are quite low (e.g.moderate pH decrease). Hence, slightly resistant pathogens such as L.monocytogenes may be able to survive. Hurdle technology, which is a mild preservation technology based on the combination of multiple antimicrobial factors or processes, is considered to be effective to be reduce Listeria in production of dry cured sausages. The principle behind this technology is that when microorganisms are confronted with multiple antimicrobial factors, the probability for survival decreases due to an increase in the energy costs that leads to cell exhaustion and death. The synergy between different factors in addition may permit a decrease in their dose.

Therefore, before applying various antimicrobials in production of Spanish fermented sausages, an in vitro experiment, aiming at studying the inhibition effect of several antimicrobials against Listeria was performed. The antimicrobials used in this study were bacteriophage, nisin, pediocin, organic acids (e.g. sorbic acid, lactic acid), lysozyme, and lactic acid bacteria. The effect of their inhibition against L. innocua and L.monocytogenes under 30℃ and low temperature (10℃) were evaluated.

Material and Methods

A. Microorganisms and growth medium

The microorganisms used in this study are two strains of Listeria innocua (strain C910 which was isolated from meat product and strain CCC05 which was supplied by EBI Food Safety-provider of ListexTMP100) and one strain of Listeria

monocytogenes (isolated from meat product). The medium used to grow these strains are Brain Heart Infusion (BHI) media (Oxoid) adjusted to pH 6 with acetic acid (in order to mimic the conditions of sausages) and ALOA agar (Oxoid) which is used for plate count. The bacterial cultures were prepared by diluting -80°C stored stocks in BHI media, grown at 37°C for overnight. 1 ml of this overnight culture was then transferred into fresh BHI media, grown at 37°C for 4-6 hours.

B. In vitro experiment

The additive used in these experiments are bacteriophage (ListexTM P100, EBI Food Safety), nisin (Larbus SA), sorbate (potassium sorbate E-202, Larbus SA), lactate (Conservador Prolac, Larbus SA), lysozyme (Larbus SA), bacteriocin producing bacterial cultures (pediocin-producing Pediococcus acidolactici in Fargo 37 (Amarex), Lactobacillus plantarum in HoldbagTM Listeria 10 IP (Danisco); Staphylococcus xylosus, Lactobacillus lactis, Lactobacillus plantarum in HoldbagTM 261 (Danisco); Micrococcus varians, Staphylococcus carnosus in Fermitrat N (Larbus SA). The concentration of additives used in this study was 101-1010 CFU/ml for bacteriophage (Listex), 0-400 µg/ml for nisin, 0-5 mg/ml for pediocin (Fargo 37), 0-5 mg/ml for sorbate (E-202), 0-50 mg/ml for lactate (conservador Prolac), 0-0.215 mg/ml for lysozyme, 0-5 mg/ml for culture Holdbag 10, and 0-2.5 mg/ml for culture Holdbag 261. The amount of inoculum used in the study was 105 CFU/ml. The growth inhibition of Listeria was measured spectrophotometrically at 630 nm after incubating the 96 well plate at 37°C for certain period of time.

Results

The in-vitro experiment performed in this study could be divided into two parts, determination of the optimum concentration of each additive and the optimum combination of additives. The result of first part was summarized in Table 1. The results demonstrated that among the additives applied, two general trends exist. Bacteriophage and pediocin-producing Pediococcus acidolactici (P) showed bactericidal effect against Listeria, whereas sorbate, lactate, lysozyme, culture of Lactobacillus plantarum (L), and mixed culture of Staphylococcus xylosus, Lactobacillus lactis, Lactobacillus plantarum (SLL) showed bacteriostatic effect.

Table 1. Determination of optimum concentration of each additive against Listeria

Treatment	Recommended dosage[a]	Effective dosage[b]	Comments
Bacteriophage	10^8 CFU/ml	10^9 or 10^{10} CFU/ml	At 10^8 CFU/ml slightly growth was observed after 48 h of incubation.
Nisin	200 µg/ml	-	At all concentration
Lysozyme	0.215 – 0.035 mg/ml	0.032 – 0.215 mg/ml	At lower concentration (0.032 and 0.043 mg/ml), it worked as bacteriostatic whereas at higher concentration (0.053, 0.081, 0.108, 0.215 mg/ml) it served as bacteriocidal.
culture of *Lactobacillus plantarum* (L)	1 mg/ml	0.5 – 5 mg/ml	At concentration, 0.5, 0.6, 0.8, 1, 2, and 5 mg/ml the growth inhibition was observed.
mixed culture of *Staphylococcus xylosus, Lactobacillus lactis, Lactobacillus plantarum* (SLL)	0.5 mg/ml	0.1 – 2.5 mg/ml	At all concentration applied (0.1 – 2.5 mg/ml), growth inhibition was observed.
Micrococcus varians, Staphylococcus carnosus (MS)	1 mg/ml	-	Not determined

a is the dosage which is suggested by the supplier catalogue, b is the dosage obtained as result of experiment.

After obtaining the optimum concentration of each additives, the in-vitro experiment was proceed with determination of the effective combination. Two different temperature were applied in this experiment, 30°C (as control) and 10°C (which simulates the curing process of dry-cured meat products).

At 30°C, combination of P-bacteriophage, P-MS, P-MS-bacteriophage, and application of bacterial suspension of P and MS (previously grown overnight at 30°C in MRS broth) inhibits better Listeria than P alone. However, for combination of P-MS or P-MS-bacteriophage, lower Listeria growth was obtained when less amount (2.8 mg/ml) of P was applied.

At 10°C, in general similar Listeria inhibition was obtained, irrespective of combination applied. Although the number of remaining Listeria after 2 weeks of incubation was similar, MS inhibition profile against Listeria at lower temperature are quite different in comparison to others. The application of bacterial cell suspension of P and MS gave no difference effects with the one that with P (without pre-incubation). Therefore it might be not necessary to use the bacterial cell suspension, as it will affect the natural fermentation process of meat products. For both temperatures studied, similar results were obtained with L.monocytogenes.

Discussion

Among all additives examined in this study, bacteriophage and pediocin-producing Pediococcus acidolactici (P) are the ones that effectively kills Listeria. Similar results of successful reduction of Listeria using pediocin or pediocin-producing strain in RTE products have been reported (Cosansu S et al, 2010; Nieto-Lozano JC et al, 2010; Olaoye OA and Dodd CER, 2010). The widely known and characterized pediocin reported in literature were pediocin AcH or PA1. This pediocin, produced by Pediococcus acidilactici, is the strain that was used in this study.

Regarding the effective combination of additives against Listeria, combination of pediocin-producing Pediococcus acidolactici (P) with bacteriophage or with other bacteriocin-producing cultures resulted in better inhibition than P alone. This demonstrated that multiple barrier technology is an effective strategy against listeria. The use of more than one bacteriocin producing strain overcomes some of the problems of limiting the effectiveness of bacteriocins in food systems (Kouakou P et al, 2010). Pediocin can also be used with other chemical barrier such as lactate and diacetate (Grosulescu C, 2011).

Conclusion

Our in-vitro experiment showed that the optimum treatment to inhibit Listeria growth is by using pediocin-producing Pediococcus acidolactici, which could be applied alone or in combination with other additives. The combination of multiple treatments should however be optimized as different effect can be encountered (e.g. synergistic, additive, or antagonistic effects). Our result demonstrated that the pediocin-producing Pediococcus acidolactici can be used as alternative for protection against Listeria in Spanish dry cured meat products, especially for the purpose of exportation to demanding countries such as USA.

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Attachment of Salmonella spp. to pork meat

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Abstract

Five strains of Salmonella, one wildtype and four knock-out mutants (the prg, f hDC, yhjH and f iC genes) were investigated based on their probability to attach and subsequently detach from a surface of pork fillet. The attachment followed by detachment was measured and modelled for two different contact times using cells coming from either a planktonic or an immobilized state of growth. The results showed that the probability of detachment generally decreased when the contact time increased and that the highest difference between contact times was achieved when the cells were grown planktonic.

Introduction

Severe infections caused by Salmonella contaminated food products still pose a threat to human health. A critical step in transferring Salmonella from animals to the consumer is usually through the slaughter process where contamination is almost unavoidable. A better understanding of the behaviour of Salmonella in food production environments is needed for optimization of the production and thereby minimizing the contamination. Attachment is an important prerequisite for adhesion and persistence of Salmonella in the food production chain. The adhesion of bacteria to a surface is inf uenced by many factors such as surface composition, roughness, charge and hydrophobicity of both the surface and the cells (Tresse et al. 2007). It is also affected by cell surface structures such as f agella and fimbriae (Dickson and Koohmaraie 1989, Li and Mclandsborough 1999). The surface structure typically results in the formation of colonies due to the immobilization of the bacteria. Previous studies have shown that certain bacteria under stressed conditions grow differently when they are immobilized as colonies, compared to their behaviour during planktonic growth (Brocklehurst et al. 1995, 1997). Using the IFR Gel Cassette System (Brocklehurst et al. 1995), which allows the study of immobilized cells, it is possible to mimic growth on biological surfaces so that growth in food (as immobilized cells) and planktonic growth (cells in solution) can be compared under controlled conditions.

In this study, the Gel Cassette System was used together with a developed meat surface model in order to study whether Salmonella immobilized as colonies reacts differently according to the probability of detachment when transferred to a surface of pork meat compared to cells grown planktonic. From a wildtype Salmonella serotype Typhimurium, four knock-out strains (deletion of the prg and f hDC operon and the yhjH and f iC genes; all involved in the f agellum biosynthesis pathway) were constructed. The knock-out strains were investigated and compared to the wildtype strain with respect to their ability to detach from a pork fillet surface during a blotting series.

Material and Methods

Strains

Strains used in this study are listed in Table 1.The deletion of the prg operon and the yhjH gene were made in Salmonella serotype Typhimurium 4/74 as described by Datsenko and Wanner (2000).

Table 1. List of strains

Salmonella Typhimurium Strain	Origin
4/74	Supplied from Gitte Knudsen (Bowden et *al.* 2010)
4/74::Δ*prg*::kan[R]	This study
4/74::Δ*yhjH*::kan[R]	This study
4/74::Δ*fliC*::chor[R]	Supplied by Maj-Britt Nielsen, University of Copenhagen
4/74::Δ*flhDC*::kan[R]	Supplied by Maj-Britt Nielsen, University of Copenhagen

Preparation of inocula

Bacteria were grown in 8 ml of Luria-Bertani (LB) broth at 25°C for 24 h. An 100× dilution were made in 8 ml LB and the bacteria were grown again at 25°C for 24 h. Inocula for the cassettes were prepared by a 1000× dilution of the culture in maximum recovery diluent (MRD), which contained 1 g peptone (Fluka) and 8.5 g sodium chloride (Sigma) dissolved in 1 litre, pH 7.0. Two different media were used for the gel cassettes; LB media (for planktonic growth) and LB with 29.3% pluronic (for immobilized growth).

Cassettes

The Gel Cassette System for immobilized growth of bacteria was obtained as a kit from IFR Enterprises, Norwich, UK. The gel cassettes were prepared as described previously (Brocklehurst et al. 1995, 1997). An appropriate volume (30 ml) of either LB media or LB media with pluronic containing bacteria culture was transferred into the cassettes by a sterile pipette. The filled cassettes were incubated at 25 °C for either 16 h (LB) or 18.25 h (LB with pluronic).

Sterile meat pieces. Pork fillet was chosen as a model surface and obtained from a local retailer. The packages were sprayed with 70% ethanol, opened and the fillet was scalded with boiling water. Slices of meat were cut from the fillet to a desired thickness (approximately 1 cm) under sterile conditions. Pieces of meat were punched out with a meat stamp with a diameter of 30 mm.

Detachment

After incubation, a sample of the gelled medium (~10g) or 10 ml of the LB medium was removed from the cassettes, mixed with 90 ml cooled MRD and blended in a Stomacher Lab Blender (Seward) for 1 min at high speed. 50 ml of the suspension were spun down at 6500 rpm for 7 min at 10°C and the resulting pellet was dissolved in 6 ml cooled MRD. An 250× dilution were made in MRD and 100 μl of this dilution were spread onto the surface of two meat pieces and incubated for 2 and 60 min at room temperature. After incubation, the meat piece was transferred to a beaker containing 100 ml MRD and was shaken for 1 min at 250 rpm. The meat piece was transferred to an XLD plate (Oxoid, Basingstoke, UK) with the inoculated surface facing down. The meat piece was left on the plate for 1 min and was then transferred to a new plate and so on for a total of 16 plates. After each move, the liquid remaining on the plate surface was spread out. The plates were incubated at 37°C for 24 h and colonies were counted. The detachment rate was calculated as described by Garrood et al. (2004). In short, the log10CFU/plate was plotted against the plate number and the detachment rate calculated from the slope of the resulting linear relationship.

Results

The effect of contact time and preceding growth conditions on the numbers of bacteria detaching from the surface of a pork fillet by a blotting series are shown in Table 2. From the data, the slopes (x) and coefficients (R2) of the straight lines representing the probability of detachment from the surface of a pork fillet were obtained by linear regression (Table 2). All mutant strains, except the fiC strain, demonstrated a detachment probability that decreases when the contact time was increased. The largest differences between 2 and 60 min for all strains (except the wildtype strain, were no difference was seen) was observed when the cells have grown planktonic before being applied to the meat surface. There was a significant difference in the detachment rate for the different contact times for the two knock-out strains prg and fhDC, with the fhDC having the highest difference, when they were grown planktonic and they also differed from the wildtype.

Table 2. Coefficients describing the detachment probability of different Salmonella Typhimurium strains to pork fillet

Strain	Contact time (min)	Planktonic		Immobilized	
		x^a	R^{2b}	x	R^2
4/74	2	0.045 (0.028-0.062)[c]	0.737	0.062 (0.042-0.082)	0.810
	60	0.045 (0.033-0.058)	0.839	0.078 (0.068-0.089)	0.962
4/74::Δprg	2	0.125 (0.104-0.146)	0.933	0.063 (0.052-0.073)	0.931
	60	0.088 (0.077-0.099)	0.963	0.065 (0.053-0.077)	0.922
4/74::ΔyhjH	2	0.063 (0.045-0.081)	0.825	0.090 (0.068-0.113)	0.914
	60	0.056 (0.041-0.070)	0.843	0.075 (0.048-0.102)	0.772
4/74::ΔfliC	2	0.075 (0.053-0.097)	0.883	0.063 (0.039-0.087)	0.752
	60	0.098 (0.063-0.132)	0.839	0.085 (0.066-0.104)	0.886
4/74::ΔflhDC	2	0.177 (0.151-0.203)[d]	0.989	0.058 (0.029-0.087)	0.671
	60	0.089 (0.063-0.115)	0.853	0.054 (0.030-0.078)	0.743

[a] *Probability of detachment of a bacterium from a meat surface during a single blotting event*
[b] *Coefficient of determination of the linear fit to the data, with 1 being perfect fit to the data*
[c] *An uncertainly estimate interval using a 95% confidence interval*
[d] *Data only based on the first 6 plates*

Discussion

In the present study, it has been demonstrated how different strains with deleted attachment genes detach from a pork surface when they have been immobilized in gel compared to planktonic growth before addition to the meat surface using different contact times. When applying cells that has been grown in a planktonic state, an increase in contact time decreased the probability of detachment, indicating an increasing difficulty in detaching the cells from the meat surface, which consists with previous reports. The detachment probability of Listeria monocytogenes from potato decreased over the first 2 min but then remained constant up to 60 min (Garrood et al. 2004). Another experiment with the probability of detachment of Campylobacter jejuni from stainless steel showed that the detachment decreased when the contact time increased (Nguyen et al. 2010).

However, in the experiments with cells being immobilized before contact with the meat, no significant differences were found between the detachment probabilities for the two contact times. Furthermore, the prg, fiC and fhDC strains had lower detachment rates than the one for the planktonic growth, in contrary to what was seen for the wildtype and yhjH. There were no significant differences in the detachment probabilities for the prg, fiC and fhDC strains compared to the wildtype. This result could indicate that other attachment genes have been up-regulated during immobilization in compensation for loss of prg, fiC or fhDC. The up-regulation of other attachment genes might be a process that takes some time, especially for the prg and fhDC strains, which can be noted when comparing to the data for the planktonic growth. The data for the wildtype and yhjH strains suggests that the attachment genes might already be down-regulated when they are applied to the meat surface, and therefore are the following attachment a bit weaker due to a slower start on expression. This correlates with the findings of Wang et al. (2004) that found transcription of genes involved in the fagellum biosynthesis to drop after 4 h, when Salmonella was grown on 0.6% agar. In this study cells have been immobilized for 18.25 h before being applied to the meat.

The planktonic growth of the prg and fhDC strains, both have detachment probabilities that are significant different from the wildtype strain. This finding correlates with previous experiments where it has been shown that the fhDC operon is the activator of the fagellum biosynthesis pathway which includes prg, fiC and yhjH (Frye et al. 2006). However on basis of this knowledge, a larger difference would have been expected for the fhDC strain. The prg operon being dependent on the activation of fhDC, explains the lower detachment probability for the prg strain compared to the fhDC strain. Studies have shown that the entire prg operon is required for the process of assembling the fagella (Kimbrough et al. 2000), which can explain the higher detachment probability seen.

Conclusion

The results indicate that an increase in contact time of the cells to the meat surface result in better attachment. This tendency seems to be of higher impact for planktonic cells compared to immobilized cells. Deletion of the prg and fhDC operons has the highest infuence on the detachment probability compared to the wildtype when grown planktonic. To further investigate these findings, real-time PCR are being used to look at the gene expression of seven selected attachment genes in the knock-out strains. This is to see if there is a change in the gene expression in the knock-out strains compared to what is seen in the wildtype. The findings of changed detachment properties in the prg and fhDC strains can used for further investigations in which factors that can reduce the gene expression. This knowledge can then be transferred to the industry and thereby be used for lowering the contamination of Salmonella in the slaughter process.

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Salmonella in pork – Lessons to be learned from salmonella control in poultry

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Abstract

Based on Reg. (EC) No. 2160/2003, programs to control Salmonella in primary production in poultry have been decided and implemented in Europe. This paper addresses the question of the efficacy of these programs to reduce the incidence of salmonellosis in humans in Germany.

From the available data it becomes clear, that there is a strong reduction by 54 % of the annual salmonellosis incidence in humans from 2007 to 2010 that is mainly attributable to a reduction in cases of S. Enteritidis (-74 %), but also to a reduction in S. Typhimurium (-30 %). Data from the established control programs and from food surveillance at the same time indicate a reduction of positive herds, of positive samples of poultry meat and of the share of S. Enteritidis and S. Typhimurium among the Salmonella isolates from the positive food samples. Overall, this justifies the assumption that the implementation of the Salmonella control programs in poultry has been successful with respect to the aim of reducing human salmonellosis so far. This is encouraging for the discussion on reduction targets in pig production.

Introduction

Based on Reg. (EC) No. 2160/2003, the requirements for programs to control Salmonella in primary production in poultry have been fixed in specific regulations. All Member States had to implement these control programmes. Regulations to control Salmonella in primary production in poultry were released in several steps, starting with breeding flocks (Reg. (EC) No. 1003/2005), followed by laying hens (Reg. (EC) No. 1168/2006), broilers (Reg. (EC) No. 646/2007), and turkeys (Reg. (EC) No. 584/2008). At the same time, eggs from flocks of laying hens that were positive for certain Salmonella serovars were banned from being sold as category A eggs for human consumption (Reg. (EC) No. 1237/2007).

The regulation for laying hens fixes a flexible reduction target to combine public health needs (reduction in human salmonellosis cases) with highly variable baseline prevalences in the respective poultry population in the Member States identified during baseline studies. For breeding flocks, broilers and turkeys a target of one percent of the relevant serovars should be achieved. The control programs focus on serovars that have continuously been associated with high incidence rates in the human population: in Germany, S. Enteritidis is closely associated with Gallus gallus, especially laying hens, and S. Typhimurium is more prevalent in meat production lines of poultry and the predominant serovar in pigs. Baseline studies in breeding and finisher pigs have also been carried out in Europe. However, reduction targets have not yet been defined.

The incidence of salmonellosis in humans in Germany has decreased substantially over the last 20 years. While this looked like a continuous process, the decline has gained speed since the implementation of the new regulations on salmonella control in poultry. The relationship between these regulations and the sharp decrease in human salmonellosis is underlined by the even more prominent decrease in human salmonellosis due to S. Enteritidis, the predominant serovar in laying hens.

Unfortunately, poultry production and pig production differ in many aspects pre and post harvest. Therefore, a simple copy of the control programs in poultry will not be feasible.

Material and Methods

For layers, broilers and turkeys the results of the baseline studies (Käsbohrer et al. 2010) are compared to the results reported in the framework of the control programs.

Data from human infections were taken from the respective reports of Robert Koch-Institute for the years 2001 to 2009

and from the survstat system run by RKI for 2010 (data taken as per 06. May 2011). Datasets without reported serovars were attributed to the known serovars according to their share of the reported serovars.

Results

Results of the baseline studies are shown in figure 1. It is obvious that in layers most (80 %) of the reported positives were S. Enteritidis, while a limited proportion was S. Typhimurium. In contrast, in broilers and turkeys, most isolates were other serovars.
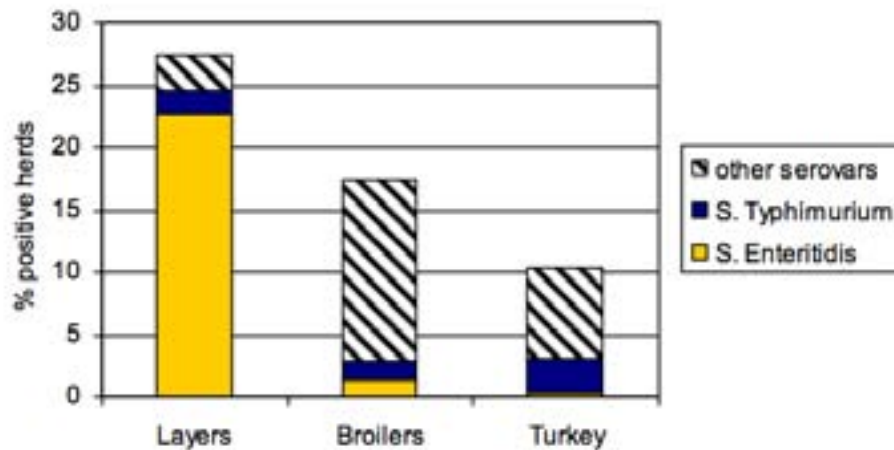
Fig 1: Proportion of positive flocks in Germany within the EU baseline studies on Salmonella in laying hens (2004/2005), broilers (2006/2007) and turkeys (2008).

In 2009, S. Enteritidis was still the predominant serovar in layers accounting for 4.5 of the 6.6 % positive herds reported (68 %) but the proportion of reported positive herds was far lower than in the baseline study. In broilers, in 2009, there were only 0.4 % of herds positive for S. Enteritidis and S. Typhimurium, i.e. the number was reduced by 86 %.

In broiler meat, between 2005 and 2008 the rate of positive samples collected in the framework of official food control was between 8.5% and 11.5% (Fig. 2). In 2009 it dropped to 6.2% and was at 6.6 % in 2010. In turkey meat, the rate was at 5.8 % in 2010 after considerable variation in previous years.
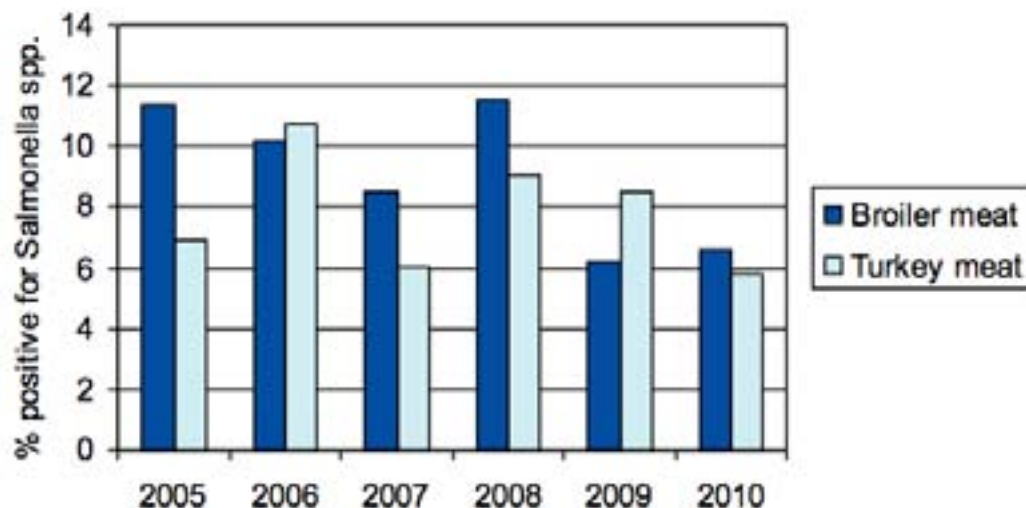
Fig. 2: Proportion of broiler and turkey meat samples collected within official food control and positive for Salmonella spp. between 2005 and 2010.

Among the Salmonella isolates from broiler meat the proportion of S. Enteritidis and S. Typhimurium dropped sharply from 2008 (19.5 and 13.8 %) to 2009 (5.2 and 3.4%). Data for 2010 are currently being generated.

The incidence of reported salmonellosis cases in humans has dramatically decreased by 54 % in Germany from 2007 to 2010 (Fig. 3). The decrease mainly involved the incidence of infections with S. Enteritidis, the predominant cause of salmonellosis in humans in the last decade. Infections with S. Enteritidis were reduced by 74% with a constant annual decrease of more than 30 % for the years 2007 to 2010. Infections with S. Typhimurium did not decrease to this extend. However, the overall decrease from 2007 to 2010 was 30% despite an increase from 2009 to 2010 (Fig. 4). In contrast, there was an overall dramatic increase of infections due to other Salmonella serovars from 2007 to 2008, followed by a decrease by from 2008 to 2010.

Discussion

It is tempting to attribute the constant decrease of S. Enteritidis infections in humans to the application of the regulations based on Reg. (EC) No. 2160/2003. S. Enteritidis infections in the past have mainly been attributed to products from Gallus gallus, i.e. eggs and broiler meat. The combinations of strict regulations on the level of primary production and on the trade of eggs probably contributed a lot to the decrease in S. Enteritidis.
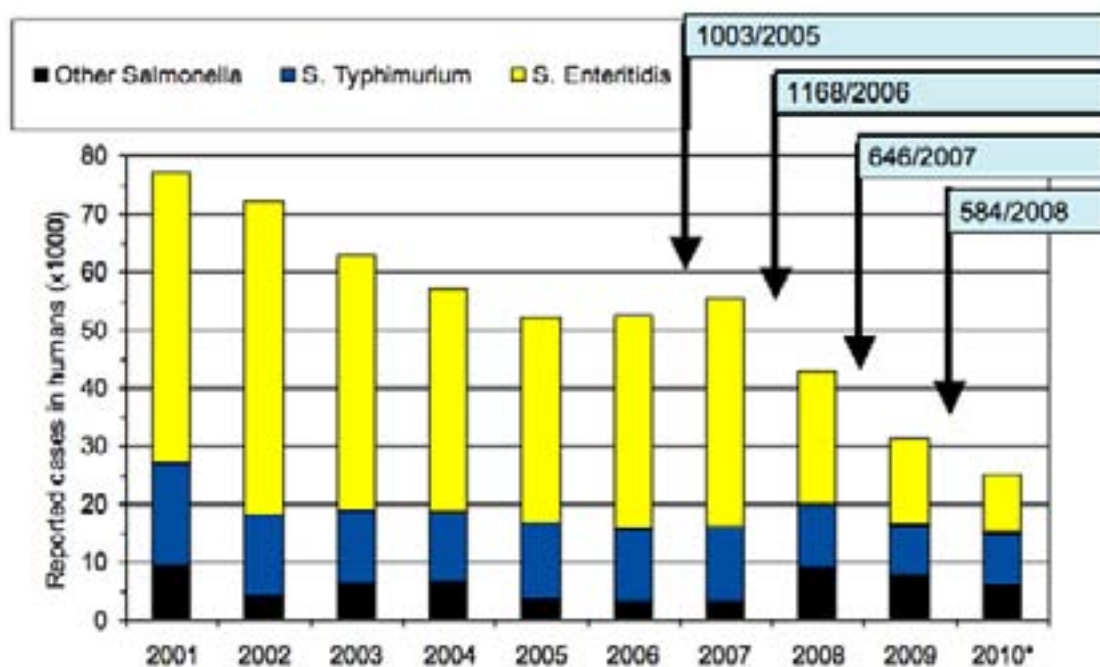
Fig. 3: Human cases reported to Robert Koch-Institute in Germany 2001 to 2010 and implementation of EU regulations of control of Salmonella in primary production in poultry. Arrows indicate beginning of control programs according to the respective EU-regulations.

Interestingly, S. Typhimurium also decreased from 2007 to 2010, however, to a lesser extend and not consistently. It is not clear, whether this reduction is due to the new regulations in place, as there was an actual increase in 2010 compared to 2009. Human infections with S. Typhimurium have been mainly attributed to contaminated meat and it could be expected that the decrease would not be observed before the regulations on broilers and turkeys were put in place. In line with a potential effect of the regulations, contamination rates of meat from broilers with Salmonella spp. were lower in 2009 and 2010 than in the years before. Moreover, the proportion of S. Enteritidis and S. Typhimurium among the positive samples sharply dropped. Likewise, in turkey meat rates were lower in 2010 than the average of 2005 to 2009. However, future surveillance programs will have to prove that this is a permanent effect.
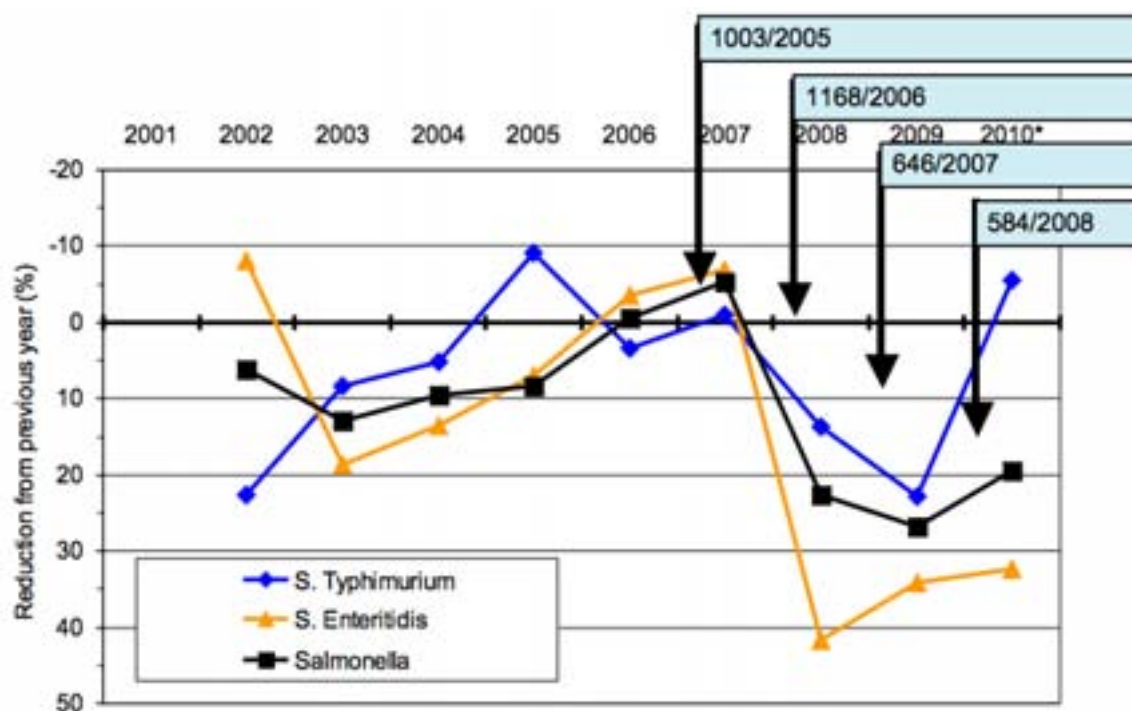
Fig. 4: Annual reduction of salmonellosis cases in humans based on data published by Robert Koch-Institute. Arrows indicate beginning of control programs according to the respective EU-regulations.

For controlling Salmonella in pigs, this is a challenge as infections with S. Typhimurium have been attributed to pig meat and control measures in pigs are expected to reduce the caseload of S. Typhimurium infections in humans.

Conclusion

The development of human cases of salmonellosis indicates that consistent measures to control salmonellosis in livestock can reduce the human burden of disease. This is encouraging for the setting of targets for Salmonella spp., especially S. Typhimurium in pigs. However, due to the differences in pig and poultry production, the procedures cannot be copied one by one but will have to be adapted to the specific situation.

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Pig fecal and tonsil contamination of Yersinia enterocolita in one French slaughterhouse

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Abstract

Pig is considered to be the main animal reservoir of human pathogenic Yersinia enterocolitica strains which is frequently isolated from tonsils, but can also be found in the feces and onto carcasses. In France, while the main pathogenic biotypes are known for humans, few data are available regarding their prevalence in the pork chain production, and generally focus on tonsils contamination.

In 2009, a study was initiated in one slaughterhouse located in Brittany (France), investigating tonsils, feces and carcasses contamination. A total of 278 pigs from 17 batches were followed-up during slaughtering during 3 campaigns: 120 pigs in June-July 2009, 114 in October 2009 - March 2010, and 44 pigs in November - December 2010.

Microbiological methods used were enrichment in ITC broth and streaking on CIN agar plates; typical colonies of Y.enterocolitica were confirmed by using Api strips. Pathogenic and non pathogenic strains biotypes were determined by multiplex PCR.

Results showed a high variability in the pig Yersinia enterocolitica contamination (either positive tonsils or feces): 0%, 14% and 13,6% respectively for the 3 campaigns, confirming the reported seasonality. The farm prevalence was on average 40,6% in campaign 2 and 3 (32 farms, 5 pigs/farm).

On the 22 positive pigs found, 6 (27,3%) and 13 (59%) were respectively positive only in tonsils or feces, and 3 pigs only (13,6%) were positive both in tonsils and feces. Despite this unexpected high detection rate on feces, no carcass was found to be positive for Y.enterocolitica (swabbing of 500 cm²; campaign 2 and 3).

In conclusion, with 14% of positive pigs in the cold period, this study confirms the variability (seasonnality) of Y. enterocolitica contamination. At slaughter level, classical tonsils detection of Y. enterocolitica should be completed by feces sampling, and carcass contamination due to fecal cross-contamination should also be considered.

Identification of plasmids in a Salmonella Typhimurium septicemic isolate without the classical 95 kb virulence plasmid

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Abstract

In this study, we report the characterization of plasmids from a Salmonella Typhimurium strain isolated from a septicemic pig. This isolate did not possess the classical 95 kb plasmid associated with virulence, but contained several low molecular weight plasmids. This isolate was as well one of the most invasive in intestinal epithelial cell lines (58.34 ± 7.32%) and showed no acquire resistance to tested antimicrobial agents. We therefore sequenced these plasmids. The size of the first plasmid, pST36-4-b5, was 3.6 kb and the size of pST36-1-b6, the second one, was 4.9 kb. They contained some open reading frames (ORF) that carry some genetic information for replication and mobilization. These plasmids contained also information for enzymatic functions and some hypothetical proteins also found in various bacterial species. Finally, a third plasmid has been partially characterized. The size of this plasmid is higher than the two other plasmids. These plasmids contained several genes of unknown function that will need to be further studied for their putative role in virulence.

Introduction

S. Typhimurium is an important zoonotic agent and a pathogen in swine. Infections caused by septicemic strains of S. Typhimurium are associated with significant mortalities in mature pigs and therefore with economic losses for the porcine industry. However, in most cases, the majority of affected pigs will become asymptomatic carriers and can be the source of meat contamination during evisceration process at slaughter. It is thus important to better characterize these isolates in order to understand pathogenesis of infection and develop appropriate control measures. The aim of this study was to characterized plasmids present in one isolate of S. Typhimurium associated with septicemia in swine.

Material and Methods

Bacterial strains. A collection of isolates that have been characterized in previous studies was used in the present study (Bergeron et al., 2007; Bergeron et al., 2009; Bergeron et al., 2010). One isolate from septicemic pig was chosen for plasmids characterization. Based on previous findings, this highly virulent isolate did not possess the classical 95 kb plasmid associated with virulence but contained many low molecular weight plasmids. We assumed that this isolate would likely contain new and unknown virulence factors. This isolate was one of the most invasive in intestinal epithelial cell lines (58.34 ± 7.32%) (Bergeron et al., 2009) and showed no acquire resistance to tested antimicrobial agents (Bergeron et al., 2010).

Characterization of plasmids from this isolate. The donor E. coli SM10λpir (asd) strain (Miller and Mekalanos, 1988), containing pLOF/Km, a Tn10-based transposon plasmid (Herrero et al., 1990), was conjugated with the recipient S. Typhimurium isolate. The donor strain was grown in LB broth with DAP (2,6-diaminopimelic acid) (50 μg/ml), kanamycin (50 μg/ml), and ampicillin (100 μg/ml) and the isolate was grown in a LB medium only at 37 °C overnight without agitation. The cultures were centrifugated at low speed and resuspended in LB broth with DAP. The conjugation was made on LB agar with DAP and IPTG (isopropyl-beta-D-1-thiogalactopyranoside) (20 mg/ml) at 37°C for 6 hours and was kept at room temperature overnight. The conjugants were plated on selective media.

The conjugants were pooled and grown overnight for plasmid extraction using the QIAGEN® Plasmid Midi kit (QIAGEN, Mississauga, Ontario, Canada) in accordance with the manufacturer's instructions. Plasmids DNA were visualized on 0.7% (w/v) agarose/EtBr gel. A Supercoiled DNA Ladder (Invitrogen Canada Inc., Burlington, Ontario, Canada) for low molecular weight plasmids was used as marker.

The plasmids were transformed into chemically competent E. coli DH5 cells. The cells were plated on LB agar supplemented with kanamycin. Plasmids from different clones were extracted using the QIAprep® Miniprep Kit (QIAGEN), in accordance with the manufacturer's instructions, and these were visualized on 0.7% (w/v) agarose/EtBr gel.

Sequencing and analysis of plasmids. Plasmids were sequenced using primers specific to the transposon at Université de Montréal's Institute for Research in Immunology and Cancer (IRIC), Montréal, Québec, Canada. The sequences were submitted at the National Center for Biotechnology Information (NCBI) data banks for homology searches.

Results

Plasmids characterization. Two plasmids from the isolate were sequenced (pST36-4-b5 and pST36-1-b6) and a third plasmid is currently being characterized. This strain was chosen for plasmids characterization based on highly invasive criteria and absence of virulence plasmid (approximately 95 kb) and presence of several low molecular weight plasmids. The size of pST36-4-b5 and pST36-1-b6 is 3.6 kb and 4.9 kb respectively. The characterisation of the third plasmid is not ended yet, but it has more than 6.5 kb in size. The smaller plasmid contained 8 open reading frames (ORF) ranging in size from 120 bp to 726 bp. The second one contained 12 ORFs ranging in size from 102 bp to 1554 bp. Plasmids pST36-4-b5 and pST36-1-b6 possess similar functions: plasmid mobilization, plasmid replication, enzymatic functions, but the majority of ORFs are genes with unknown function. Tables I and II show ORFs identified with potential functions in plasmids pST36-4-b5 and pST36-1-b6 in a S. Typhimurium septicemic isolate.

Discussion

In this study, plasmids present in a S. Typhimurium isolate associated with septicemia in swine were characterized in order to analyze the genetic basis of this isolate and found new or unknown virulence factor.

The characterization of pST36-4-b5 and pST36-1-b6 indicates that these plasmids carry some genetic information for replication and mobilization, although it does not seem to possess all the information for a functional plasmid mobilization system. Beta-galactosidase-α protein, a hydrolyse enzyme was found in each plasmid. This enzyme is involved in aerobic glycolyse for digestion of lactose in glucose and galactose. The plasmid pST36-4-b5 possesses the LabA (low-amplitude and bright)-like proteins. These proteins belong to a well conserved group of bacterial proteins with no known function in Enterobacteriaceae. In cyanobacteria, the gene labA modulates the circadian gene expression by the negative feedback regulation of KaiC (Taniguchi et al., 2007). The same plasmid possesses a winged helix-turn-helix (WHTH) DNA-binding domain of the GntR family of transcriptional regulators. The GntR family has many members distributed among almost all bacterial species for the regulation of various biological processes. The plasmid pST36-1-b6 possesses an ORF that code for a histidyl-tRNA synthetase. It is responsible for the attachment of histidine to the 3' OH group of ribose of the appropriate tRNA. This domain is primarily responsible for ATP-dependent formation of the enzyme bound aminoacyl-adenylate. Finally, other ORFs showed similarities with hypothetical proteins found in Enterobacteriacea, Vibrio cholerae, and Citrobacter were found to both plasmids. Despite some technical difficulties, it will be important to continue the characterization of the third plasmid. This plasmid being larger, it can be more likely associated with virulence associated genes.

Conclusion

The S. Typhimurium isolate from septicemic pig characterized in this study possesses plasmids containing several genes of unknown function that should be further studied for their putative role in virulence. Although genes that possess similarities with existing proteins appeared to be involved in housekeeping functions, we cannot exclude participation of these proteins in virulence since some metabolic enzymes have already been shown to participate in both virulence and bacterial metabolism.

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Table I. Open reading frames (ORF) identified in plasmid pST36-4-b5 (3.6 kb) in a *S.* Typhimurium septicemic isolate.

ORF	Length (bp)	Frame from...to (bp)	e-value	Putative product	Source and reference number
1	348	683...1030	$5e^{-61}$	Plasmid mobilization	*Salmonella enterica* subsp. *enterica* serovar Virchow strain SL491YP_002221413
2	213	1187...1399	$7e^{-19}$	Plasmid mobilization	*Salmonella enterica* subsp. *enterica* serovar Virchow strain SL491YP_002221413
3	195	1290...1484	$1e^{-27}$	RNAI modulator protein	*Salmonella enterica* subsp. *enterica* serovar Typhimurium strain G8430 YP_198405
4	120	1795...1914	$8e^{-11}$	Beta-galactosidase alpha protein	Broad host range expression vector pRK415iq AB032164
5	726	2290...3015	$1e^{-66}$	LabA_like proteins	*Escherichia coli* strain B088 ZP_06662273
6	228	3366...3562	$2e^{-18}$	WHTH_GntR	*Escherichia coli* strain B088 ZP_06662274

Table II. Open reading frames (ORF) identified in plasmid pST36-1-b-65 (4.9 kb) in a *S.* Typhimurium septicemic isolate.

ORF	Length (bp)	Frame from...to (bp)	e-value	Putative product	Source and reference number
A	300	218...517	0.13	Histidyl-tRNA synthetase	*Desulfatibacillum alkenivorans* strain AK-01 YP_002431917
B	1554	1605...3158	0.0	Mobilization protein MobA	*Salmonella enterica* strain 79500 NP_203138
C	357	3148...3504	$3e^{-59}$	Mobilisation protein MbeC	*Escherichia coli* strain ETEC H10407 CBJ04269
D	225	3503...3727	$2e^{-36}$	Regulatory protein Rop	*Salmonella enterica* subsp. *enterica* serovar Virchow strain SL491 YP_002221408
E	120	4209...4328	$8e^{-11}$	Beta-galactosidase alpha protein	Broad host range expression vector pRK415iq AB032164

Evaluation of ozonated water as a microbiological risk mitigation option in pork production

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Abstract

Ozone is an oxidative moleculewith a bactericidal effect. This molecule can be solubilized in water and wasproposed as an alternative disinfecting solution to be used in food production plants. Ozone molecule possesses many industrially relevant characteristics, such as the absence of residues following its application, usability at meat industries' room temperature, and applicability during production activities. The objective of this study was to evaluate the benefits of an ozonated water rinse on the wrapping of meat logs at the entrance of a slicing plant. From a single batch, the surface of ten units (meat logs : ML) of cylindrical shape of approximately 7000 cm2 were entirely swabbed before treatment and compared with 3 groups of 10 units that were passed through a curtain of either chlorinated water (20 ppm), ozonated water (3,5 ppm) or tap water only. As part of the bacteriological analysis, total aerobic counts were measured, Salmonella and Listeria monocytogenes detection were individually conducted on each units, and enumeration of E.coli and coliforms were completed. The results obtained from 4 different batches showed a very low aerobic contamination at the entrance of the plant before treatment (2.49 log cfu/ML). The chlorinated water and the ozonated water treatment reduced significantly the bacterial contamination (respective diminution of 0.83 log cfu/ML and 0.63 log cfu/ML), while reduction from the tap water treatment was not significant (0.21 log cfu/ML). All samples were free of the researched pathogens, and coliforms counts were below the technical threshold for numeration. These results show that an ozonated water treatment is an effective tool in reducing aerobic flora contamination before the meat slicing process. It also indicates that ozonoted water could be an alternative to chlorinated water treatments as it represents an effective method to control product wrapping contamination prior to its entrance at the slicing plant.

Introduction

Ready-to-eat production and transformation plants need to take important bacterial control measures due to the nature of their products, which areto be consumed without further treatments by consumers. Plant operators must prevent the introduction and proliferation of Listeria monocytogenesin the plant.This bacteria can produce biofilms that are hard to eliminate and multiplies in the product at a low temperature (4°C) (1). It has already been documented that potentially pathogenic bacteria such as Listeria monocytogenes can enter the plants with the products andbecome established in the processing environment (2). These strains can then contaminate the equipment and the meat during the cutting or slicing operations (3). This reinforces the importance of efficient control measures at the entrance of the product in the plant.

Ozone is an oxidative molecule that has a bactericidal effect. This molecule can be solubilized in water, and its use as a disinfecting solution has been proposed in food production plants. This product possesses many industrially relevant characteristics such as the absence of residues following its application, usability at meat industries room temperature, and applicability during activities (3).

In this study, ozonated water was assessed as a new tool to control product contamination at the entrance of a cold cuts slicing plant.

Material and Methods

Samples : In an industrial cold-cuts slicing and wrapping plant, the entire exterior surface (7000 cm2) of the wrappings of ten randomly chosen units of cold-cut meat logs (ML) were individually sampled with a cotton swab for four different lots

(units of same product and shipped in the same box) to evaluate the initial level of contamination of the products shipped at the plant.

Treatment:
Three groups of ten units from the same lot were passed through a curtain of chlorinated water (20 ppm), ozonated water (3,5 ppm) or tap water at a temperature of 4°C. Each unit passed through the curtain in 30 seconds on a conveyer belt and after removal of water surplus was sampled as explained previously.

Bacteriological procedure:
Right after sampling, each swab was placed in 4 ml of neutralizing buffer at the plant. Total aerobic counts were measured on petrifilm (3M). Salmonella and Listeria monocytogenesdetection were individually conducted on each of the swabs using modified government of Canada methods (MFLP-75 and MFHPB-30) and enumeration of E.coli and coliforms on petrifilm (3M) completed the bacteriological analysis.

Statistics:
The effectiveness of the three treatments was determined by comparing the mean aerobic f ora and coliform contamination (in log cfu / units) before and after the treatments using Student t test (SPSS software license U Montreal). Differences in proportion of Salmonella and Listeria monocytogenespositive samples depending on treatment were considered.

Results

Table no.1: Aerobic f ora contamination before and after treatments

Treatment	Mean (log cfu/units)	Standard deviation	Difference Control/Treated	N
Control	2.49	0.69	-	40
Ozone	1.86	0.34	0.63	30
H$_2$O	2.28	0.46	0.21	20
Chlorine	1.65	0.13	0.83	30

Significantaerobic f ora reduction for the ozonated and chlorinated water treatments (t-test p < 0.05)
No significant reduction for the tap water treatments (t-test p > 0.05)

Pathogens detection:
Salmonella: 0 / 120 ML
Listeria monocytogenes: 0 / 120 ML
Coliforms and E.coli: 120 ML under detection limit (40 cfu / ML)

Discussion

The samples have been collected during four different visits of a plant that receives cold-cut meat in the form a log wrapped in a plastic envelope for the slicing and packaging operations. The aerobic contamination of the wrapping of four different types of meat products has been evaluated. The results show that the initial aerobic contamination of the meat logs wrapping is very low even before treatment with a mean aerobic contamination of 2.49 log cfu / ML. The sampled units were also free of the two researched pathogens (Listeria monocytogenes, Salmonella spp.) and the coliform and E. coli contamination was under the detection limit (40 cfu / ML). These low values demonstrate a good control of the risks of contamination at the end of the transformation procedures and during transportation between the transformation plant and the slicing plant.

After the application of the chlorinated or the ozonated water treatments on the wrapping of the meat logs, the mean aerobic contamination was significantly reduced when compared with the control samples with a mean reduction of 0.83 and 0.63 log cfu / ML respectively. However, the reduction for the chlorinated water treatment could be underestimated here as many of the samples were under the detection limit and were estimated at 39 cfu / ML, which is a conservative value, for the mean calculation. For the tap water treatment the reduction was 0.21 log / units and was not significant. The lack of significant reduction for this treatment demonstratesthat the observed reduction in the two other testedmethods

is not caused by a physical removal of the bacteria by the water during the application but by a real bactericidal effect of the active molecules present in the water (ozone and chlorine). This reduction of the bacterial presence on the wrappings confirms that ozonated water has a bactericidal effect and that the chlorinated water treatment, which is currently used in the plant, is very effective.

The effectiveness of these treatments to reduce the presence of the pathogens could not be evaluated as all the samples before and after treatment did not contain them. However events that could lead to the contamination of the product by these microorganisms during the transport or the preparation can't be excluded.The inactivation of different species of Salmonella, Listeria monocytogenesand E. Coli by ozonated water has already been described in laboratory experiments with mean reduction of more than0.5 log for each of these microorganism (4). These reduction are relevant in the present industrial context were the presence of microorganisms is low as we can assume that in the case of a contamination by pathogenic bacteria it would also be very low. Based on the results of these previous studies and the results obtained on the reduction of the presence of the aerobic f ora, ozonated water treatment could be an effective way to mitigate the microbiological risk on the product wrapping. Hence, using total aerobic counts as indicators, we can assume that this treatment could be an effective way of preventing the introduction of potentially pathogen bacteria in the plant with the product, reducing the risks of contamination of the environment by Listeria and of the final product during the subsequent steps such as the peeling of the wrapping.

Conclusion
The results obtained in this study show that the meat logs entering this plant have a very low microbial charge on their wrapping and are free from Listeria and Salmonella. The study also confirms the effectiveness of the chlorinated water treatment, which is currently used at this plant, to reduce to a minimum the product aerobic f ora wrapping contamination. Furthermore, we showed that ozonated water is effective and can be considered as an interesting alternative to the chlorinated water treatment to control bacterial introduction in the plant by the product.

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Salmonella in Irish pig farms; prevalence, antibiotic resistance and molecular epidemiology

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Abstract

The objective was to examine the prevalence of Salmonella in manure from 30 Irish pig farms and to characterize any recovered isolates in order to assess potential risks and epidemiological relationships. Salmonella was detected in the manure from finisher pigs in 50% of the herds investigated. S. Typhimurium was the predominant serotype recovered and the most common phage types were DT104 and DT104b. Nineteen of the 29 Salmonella isolates recovered were resistant to one or more antibiotics and 15 of these (all Typhimurium) were multi-resistant. Molecular analysis revealed 19 PFGE types and facilitated tracking of isolates across farms. Overall, Salmonella prevalence correlated well with Irish findings from an EU-wide study of pig production holdings conducted in 2008 (47.7%). The high level of antibiotic resistance observed among the porcine isolates is a concern, but not uncommon in S. Typhimurium.

Introduction

Pigs are well recognized as carriers of Salmonella and transmission of the pathogen to humans via carcass contamination at slaughter is a major food safety concern (Boyen et al., 2008). A number of baseline surveys have been conducted to determine the prevalence of Salmonella in pigs. In 2008, a European Food Safety Authority (EFSA) study found that 33.3% of pig production holdings in the EU were positive for Salmonella (EFSA, 2009). However, Ireland had a much higher prevalence (47.7%); in fact it was the third highest in the EU.

Because of the high prevalence of Salmonella in pigs, a number of countries have introduced Salmonella surveillance and control programmes. In Ireland, a monitoring and control programme was first established in 2002. Monitoring is based on determining the Salmonella status of pig herds by serological testing of meat juice at slaughter and not bacteriological testing. In the past, if less than 10% of samples were positive the herd was classed as Category 1; if 11-49% of samples were positive it was categorized as 2 and if greater than 50% of samples were positive it was a category 3 herd. However, a revised national pig Salmonella control programme was implemented in Ireland in January 2010. Monitoring is still based on serological testing of meat juice samples but there are now only two categories; 1 (< 50% prevalence) and 2 (> 50% prevalence).

While some studies have investigated Salmonella carriage in Irish pig herds, none to date have performed molecular as well as phenotypic analysis on the Salmonella recovered. The objectives of this study were (1) to determine the prevalence of Salmonella in finisher pigs from a sample of Irish pigs farms representing different Salmonella categories and (2) to characterize any Salmonella isolates recovered, both phenotypically and genotypically in order to identify predominant isolates and thereby assess potential risks as well as epidemiological relationships.

Materials and Methods

Pig manure sampling

A total of 30 manure samples were collected from the finisher houses of commercial pig farms in Ireland between January 2009 and March 2010. Farms were chosen based on their categorization within the initial (pre-2010) Irish National Salmonella Control Programme. It should therefore be noted that any reference to farm categories in this study refers to this historical categorization system (as outlined above). Ten farms were sampled from each of Categories 1, 2 and 3. Samples (~100 ml) were obtained from manure storage tanks situated directly underneath finisher houses at a depth of

1m below the crust or from a sluice, if present. They were collected into sterile containers and transported on ice to the laboratory where they were stored at 4°C until analysis (within 24 hr).

Microbiological analysis of manure samples

The presence/absence of Salmonella in 25 g samples of manure was determined according to standard procedures (ISO, 2007) with modified brilliant green agar (Merck, Darmstadt, Germany) used for additional selective plating. Based on results of biochemical tests, presumptive Salmonella isolates were tested using a Salmonella latex agglutination kit (Oxoid, Basingstoke, Hampshire, UK). Isolates confirmed as Salmonella were grown in brain heart infusion (BHI) broth overnight at 37 °C and stored at -20 °C in BHI containing 40 % glycerol. Two Salmonella isolates per sample were sero-typed based on O- and H-group antigens according to the White Kaufmann Leminor scheme. Antimicrobial susceptibility testing was performed according to the broth dilution method of the Clinical and Laboratory Standards Institute (formerly NCCLS) (NCCLS, 1999). Salmonella Typhimurium isolates were phage typed by the National Salmonella Reference Laboratory at Galway University Hospital, Ireland. Molecular typing of Salmonella isolates was performed by pulsed field gel electrophoresis (PFGE) using XbaI (New England Biolabs, Hitchin, Herts, UK) according to the standardized protocol of PulseNet (CDC, 2002). PFGE was performed on a CHEF-DRII system (Bio-Rad Laboratories, Hercules, California) using a mid-range II PFGE marker (New England Biolabs) with the following parameters; run time of 19h, initial switch time of 2.2 s, final switch time of 63.8s, 6 V, 14 °C. Gel images were visualized under UV light and saved as TIFF files which were analyzed using BioNumerics software (v3.5, Applied Maths, Sint-Martens-Latem, Belgium). Clustering analysis was performed using Pearson correlations. The similarity coefficient was used to create a dendrogram using the unweighted pair group for arithmetic means (UPGMA).

Results

Salmonella was detected in the manure from finisher pigs in 50% (15/30) of the herds investigated; 30% (3/10) of Category 1 herds and 60% (6/10) of each of Category 2 and 3 herds. Two isolates from each Salmonella-positive manure sample were characterized (except for Farm14 where only one isolate was obtained) and both were identified as the same serotype and had the same antimicrobial resistance profile (Fig. 1). In total, 29 isolates, comprising seven serotypes were recovered. S. Typhimurium predominated, both overall and within each category. It was isolated from 30% (9/30) of herds and accounted for 58.6% (17/29) of all isolates recovered. Within these, six phage types were identified; DT104 and DT104b were the most common and were isolated from two and three herds, respectively, while U288, DT193, U311 and DT17 were each isolated from one herd. The other serotypes recovered were Manhattan, Goldcoast, Bredeney, Brandenburg, Livingstone and Derby, each from one herd. PFGE revealed 19 banding patterns among the 29 Salmonella isolates (Fig.1). Where two isolates from one farm were characterised both had the same PFGE fingerprint, except for isolates from Farms 8, 12, 15, 16, 30 and 38. The 29 Salmonella isolates grouped into three clusters (1–3) based on their PFGE patterns (Fig. 1). However, there was no correlation between clustering of isolates and herd categorization. S. Typhimurium grouped into clusters 2 (which contained phage types DT104, DT104b, U288, DT193 and DT17) and 3 (containing phage types DT104, D104b, U311, an untypable isolate and two Brandenburg isolates). Within cluster 2, two S. Typhimurium DT104b isolates from Farm 8 (WIT 385 and 386) were just less than 80 % similar to a DT104 isolate from Farm 1 (WIT 384) and all had the same antimicrobial resistance profile. Within cluster 3 an untypable Typhimurium isolate from Farm 16 (WIT 412) was highly related (> 90% similar) to a DT104 isolate from Farm 30 (WIT389), although they had slightly different resistance profiles. Furthermore, other isolates with different phage types, albeit from the same farm were highly related e.g. the U288 and DT193 isolates within cluster 2. The other five Salmonella serotypes recovered (Bredeney, Goldcoast, Manhattan, Derby and Livingstone) grouped into cluster 1 (Fig. 1).

% similarity

Isolate No. (PFGE type)	Serotype	Phage Type	Antimicrobial resistance profile	Farm No. (Category)
WTT 406 (H)	Bredeney	-	Fully Sensitive	Farm 31 (2)
WTT 407 (H)	Bredeney	-	Fully Sensitive	Farm 31 (2)
WTT 401 (E)	Goldcoast	-	CpNa	Farm 18 (2)
WTT 400 (E)	Goldcoast	-	CpNa	Farm 18 (2)
WTT 403 (F)	Manhattan	-	Fully Sensitive	Farm 24 (1)
WTT 402 (F)	Manhattan	-	Fully Sensitive	Farm 24 (1)
WTT 411 (L)	Derby	-	Fully Sensitive	Farm 15 (3)
WTT 410 (N)	Derby	-	Fully Sensitive	Farm 15 (3)
WTT 408 (K)	Livingstone	-	Fully Sensitive	Farm 2 (3)
WTT 409 (K)	Livingstone	-	Fully Sensitive	Farm 2 (3)
WTT 385 (A)	Typhimurium	DT104b	ACFSSuT	Farm 8 (3)
WTT 384 (A)	Typhimurium	DT104	ACFSSuT	Farm 1 (1)
WTT 386 (S)	Typhimurium	DT104b	ACFSSuT	Farm 8 (3)
WTT 391 (B)	Typhimurium	U288	ACSSuTTm	Farm 10 (2)
WTT 392 (B)	Typhimurium	DT193	ACSSuTTm	Farm 10 (2)
WTT 383 (A)	Typhimurium	DT104	ACFSSuT	Farm 1 (1)
WTT 399 (C)	Typhimurium	DT17	Fully Sensitive	Farm 12 (3)
WTT 397 (T)	Typhimurium	DT104b	ACCpFNaSSuTTm	Farm 14 (3)
WTT 396 (D)	Typhimurium	DT17	Fully Sensitive	Farm 12 (3)
WTT 412 (M)	Typhimurium	Untyp.	ACFSSuTTm	Farm 16 (3)
WTT 389 (M)	Typhimurium	DT104	ACFSSuT	Farm 30 (1)
WTT 390 (L)	Typhimurium	DT104	ACFSSuT	Farm 30 (1)
WTT 394 (R)	Typhimurium	U311	ACSSuTm	Farm 27 (2)
WTT 395 (R)	Typhimurium	U311	ACSuTm	Farm 27 (2)
WTT 393 (O)	Typhimurium	Untyp.	ACFSSuTTm	Farm 16 (3)
WTT 405 (G)	Brandenburg	-	T	Farm 28 (2)
WTT 404 (G)	Brandenburg	-	T	Farm 28 (2)
WTT 443 (P)	Typhimurium	DT104b	ACFSSuT	Farm 38 (2)
WTT 444 (Q)	Typhimurium	DT104b	ACFSSuT	Farm 38 (2)

Cluster 1 — Cluster 2 — Cluster 3

80 % similarity cut-off

Fig. 1. PFGE patterns (obtained using XbaI) of Salmonella isolates recovered from pig manure samples. aIsolates showing > 80% similarity were assigned the same letter and can be considered highly related. The serotype, phage type, antimicrobial resistance profile and farm of origin are also shown. bFully sensitive to all 13 antibiotics tested. cA, ampicillin; C, chloramphenicol; Cp, Ciprofloxacin; F, florfenicol; Na, Nalidixic acid; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; Tm, Trimethoprim. dUntypable by phage typing

Within this cluster, both Bredeney isolates were highly related (> 90% similar), as were the other isolates of the same serotype, except the two Derby isolates which were only 75% similar. Nineteen of the 29 Salmonella isolates recovered were resistant to one or more antibiotics and 15 of these (i.e. all of the Typhimurium isolates) were multi-drug resistant (resistant to 3 antibiotics from different classes). The most common resistance observed was to tetracycline (89% of isolates), followed by ampicillin, sulfamethoxazole and chloramphenicol (79% each), streptomycin (74%), florfenicol (58%), trimethoprim (37%), ciprofloxacin and nalidixic acid (16% each). Thirteen S. Typhimurium isolates displayed the typical ACSSuT penta-resistance pattern of S. Typhimurium DT104, although they were not all DT104 and they were also resistant to at least one other antibiotic. In fact, one DT104b isolate was resistant to nine antibiotics.

Discussion

Salmonella was detected in manure from finisher pigs on 50% of Irish farms sampled. A lower prevalence was observed in Category 1 herds, demonstrating some correlation between serological and bacteriological data. Seven serotypes were identified; S. Typhimurium predominated and DT104 or DT104b were the most common phage types recovered. Although only a small number of farms were sampled, data from the present study correlates well with Irish findings from an EU-wide study of pooled fecal samples from all production stages. This showed that 47.7% of pig production holdings were Salmonella-positive, with S. Typhimurium the second most commonly isolated serotype(EFSA, 2009). Our findings are also in agreement with those of Rowe et al. (2003) who found that 51% of faecal samples from at least one production stage of Irish pig farms were Salmonella-positive, with S. Typhimurium the most common serotype. However, prevalence in finisher pigs was only 23%. In a Northern Irish survey of slaughter pigs, 31% harbored Salmonella in the cecum, with S. Typhimurium again accounting for the majority of isolates (Mc Dowell et al., (2007).

Increasing antimicrobial resistance has been observed in Salmonella spp. worldwide. In the present study, antimicrobial resistance was most common amongst isolates of S. Typhimurium, in agreement with previous findings (Boyen et al., 2008). Many of these isolates were DT104 or DT104b, which are a common cause of foodborne disease, notoriously multi-drug resistant and frequently isolated from pigs (Boyen et al., 2008). One S. Typhimurium DT104b isolate from the present study was resistant to nine antibiotics, including two fuoroquinolones (nalidixic acid and ciprofoxacin), which is worrying, as these are the drugs of choice used to treat human infections. In addition, molecular typing with PFGE facilitated tracking of isolates across farms and to our knowledge this has not been performed to date on Irish pig farms. This analysis revealed that highly related S. Typhimurium isolates originated on farms in different geographical locations. However, some S. Typhimurium isolates with different phage types and antimicrobial resistance profiles were indistinguishable by PFGE, which is not uncommon.

Conclusion

In this small-scale study of Irish pig farms, Salmonella was detected in the manure from finisher pigs on 50% of farms sampled. In agreement with previous studies S. Typhimurium predominated and the DT104 and DT104b phage types were commonly recovered. A number of multi-resistant S. Typhimurium isolates were recovered, which is a concern, but not uncommon. PFGE analysis revealed the presence of highly related isolates on different farms. However, a more discriminatory method, such as multi-locus variable number tandem repeat analysis is needed to further differentiate S. Typhimurium isolates.

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The Salmonella monitoring programme in The Netherlands

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Abstract

The Salmonella monitoring programme in The Netherlands started in 2005. The programme consists of Salmonella monitoring as well on the pre harvested as the post harvested stage.

The monitoring programme is an obligatory PVV-programme, which includes both the herd level of fattening pigs (pre harvest) and the slaughterhouse level (postharvest). Herd level monitoring is based on testing blood samples for the presence of antibodies against Salmonella. Per period of 4 months, 12 blood samples have to be collected. The blood samples can be taken on the farm or in the slaughterhouse. The samples are tested in the Idexx ELISA or comparable serological tests, with a cut off of 40% OD. The tests are carried out by approved laboratories.

When a total of 36 blood samples per farm is reached, this farm is classified into one of the three Salmonella categories. Besides serological monitoring, bacteriological monitoring is performed on carcasses. Slaughterhouses have to sample 5 carcasses per day and are analysed in approved laboratories as one pooled sample. Slaughterhouses can choose between sampling with the destructive method (cork bore) or the sponge method.

Pre harvest data show that 75% of the herds in the Netherlands are in category 1, 20% in category 2 and 5 % in category 3. At this moment, farms in category 3 are advised tot take measures against Salmonella.
The average Salmonella contamination of the carcasses in the slaughterhouses was less than 2,0% (sponge method).

In the EU-baseline study fattening pigs, the prevalence in the Netherlands of the lymph nodes was 8% while the average 11%. In the EU-baseline study breeding pigs, the prevalence in the Netherlands was 55% while the average was 29%. The prevalence of the carcasses is less than 2,0%. These figures show that there is no linear distribution between the prevalence of the breeding pigs, the fattening pigs and the carcasses. The aim of elimination of Salmonella is to reduce the cases of human Salmonelloses.
We suggest to fix one Salmonella target on carcass level for all the EU memberstates. The different EU memberstates can chose their own control strategies based on the country specific Salmonella prevalence as well as the country specific herd and slaughterhouse structures.

Effect of transportation and mixing with unfamiliar pig on Salmonella susceptibility in market weight pigs

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Abstract

There is increasing evidence that stress can have a significant deleterious effect on food safety through a variety of potential mechanisms. However, there is very little research conducted to determine the potential effects of specific pre-slaughter stressors on Salmonella infection and carriage in pigs. Understanding when pathogen loads are the highest or when animals are most susceptible to infection is critical to determine when intervention strategies for pathogen control may be most effective, and consequently, increase pork safety. Therefore, this study was conducted to determine the effect of two common pre-slaughter stressors, transportation and mixing with unfamiliar pigs, on the susceptibility of market-weight pigs to a low-dose Salmonella challenge. A total of 40 market-weight pigs were randomly assigned to one of the following four treatments: 1) control, 2) mixing with another pig for 6 hours, 3) transportation for 1 hour, and 4) transportation for 1 hour followed by mixing with another pig for 6 hours. Immediately after the transportation treatment, all pigs were individually inoculated with 104 cfu of Salmonella Typhimurium. After 6 hours, the pigs were euthanized and subjected to necropsy for sample collection, including ileal and cecal contents, ileal tissue, and mesenteric lymph node. All samples were processed for the isolation and enumeration of the challenge strain. Even though a low challenge dose was used, infection and shedding were established in all market-weight pigs used in this study. Pigs subjected to any of the stress treatments had higher ($P<0.05$) levels of Salmonella in the ileum, whereas only pigs subjected to both stressors combined (i.e., transportation and mixing) had higher ($P<0.05$) Salmonella levels in their cecum, compared to control pigs. Therefore, it is concluded that pre-slaughter transportation and mixing with unfamiliar pigs increases the susceptibility of market-weight pigs to a low-dose Salmonella challenge.

Introduction

Colonization of swine by Salmonella, and its subsequent dissemination along the pork production and processing chain is a major public health and economic issue for the pork industry worldwide. There is some evidence that stress in farm animals can have a significant deleterious effect on food safety through a variety of potential mechanisms (Rostagno, 2009). However, there is very little research conducted to determine the potential effects of specific pre-slaughter stressors on Salmonella-infected pigs. Moreover, there is almost no research to determine how stress affects the susceptibility of pigs to Salmonella infection/colonization.

The gastrointestinal microbiota may be disturbed by many factors, causing levels of pathogens and shedding from unapparent carriers (i.e., subclinically infected animals) to be affected. For instance, during the process of being transported from production farms to abattoirs, pigs are exposed to a variety of potential stressors before slaughter (Warriss, 2003; Averos et al., 2008). Consequently, many believe that the number of animals carrying and shedding Salmonella, as well as its levels in the gastrointestinal tract will be increased in response to stressors. It is also believed that the pig's susceptibility to new infections will increase. However, although these assumptions are widely accepted, definitive proof still lacks, as most of the current knowledge is based on limited scientific evidence. Therefore, this study was conducted to determine the effect of two common pre-slaughter stressors, transportation and mixing with unfamiliar pigs, on the susceptibility of market-weight pigs to a low-dose Salmonella challenge.

Material and Methods

A total of 40 market-weight pigs were randomly assigned to one of the following four treatments: 1) control, 2) mixing with another pig for 6 hours, 3) transportation for 1 hour, and 4) transportation for 1 hour followed by mixing with another pig for 6 hours. Immediately after the transportation treatment, all pigs were individually inoculated with 104 cfu

of Salmonella Typhimurium. After 6 hours, the pigs were euthanized and subjected to necropsy for sample collection, including ileal and cecal contents, ileal tissue, and mesenteric lymph node. All samples were processed for the isolation and enumeration of the challenge strain.

The study was conducted in 2 replicates of 20 pigs each, including 5 individually housed pigs per treatment (total of 10 individual observations per treatment). All bacteria count data were subjected to transformation, and analyzed as log 10 colony forming units (CFU) per gram (g) of sample. Treatments were compared by ANOVA, and statistical inferences were based on P < 0.05. Data analysis was performed using JMP 9.0.0 (SAS Institute Inc., Cary, NC).

Results

All pigs used in this study were Salmonella-negative prior to the challenge, based on the analysis of multiple individual fecal samples. Even though a low challenge dose was used, infection and shedding were established (verified through recovery of the challenge strain from individual fecal samples) in all market-weight pigs included in this study. No clinical signs of infection were observed during the experiment.

Pigs subjected to any of the stress treatments (i.e., transportation and/or mixing) had higher (P<0.05) levels of Salmonella in the ileum, whereas only pigs subjected to both stressors combined (i.e., transportation and mixing) had higher (P<0.05) Salmonella levels in their cecum, compared to control pigs. There was no difference between pigs subjected to any of the treatments and controls regarding the levels of Salmonella translocating from the intestinal tract to mesenteric lymph nodes (i.e., in ileal wall and lymph node samples).

Discussion

A variety of stressors may cause animals to generate a stress response, based on the activation of the hypothalamo-pituitary-adrenal axis and the autonomic nervous sytem, which results in the release of several mediators, including the catecholamines, norepinephrine and epinephrine (Elenkov and Chrousos, 2006). The gastrointestinal tract has long been known to be sensitive to the effects of stress mediators (Tache and Brunnhuber, 2008). However, although physiological and motor activity disturbances have been frequently shown to result from stress, recent studies have shown that mediators of the stress response can also affect the function of the intestinal mucosa as well as bacterial populations (Lyte et al., 2011). Because of the considerable cross-communication that occurs between the neuroendocrine and immune systems (Ziemssen and Kern, 2007), the ability of stress to influence the pathogenesis of bacterial infections, has mostly been attributed to stress-induced immunosuppression (Butts and Sternberg, 2008; Salak-Johnson and McGlone, 2007). However, the ability of enteric bacteria to directly respond to stress mediators, particularly catecholamines, has been demonstrated (Freestone et al., 2008), offering a new and more direct pathway for the potential effect of stress on bacterial populations. Recently, Toscano et al. (2007) examined the effects of in vitro pre-treatment of Salmonella Typhimurium with norepinephrine before infecting young pigs. Examination of the tissue distribution revealed that norepinephrine-treated bacteria were present in greater numbers and more widely distributed in gastrointestinal tissues than control bacteria.

While being transported from production farms to abattoirs, market pigs are exposed to a variety of stressors immediately before slaughter, including handling, feed withdrawal, transportation, mixing, etc. (Warriss, 2003; Averos et al., 2008). An early small study by Williams and Newell (1970) was the first to suggest that transportation of pigs could lead to increased shedding of Salmonella. Later, Isaacson et al. (1999) and Marg et al.(2001) reported that pigs experimentally infected with Salmonella exhibited increased shedding after transportation. However, in a study conducted with young pigs naturally infected with Salmonella, Rostagno et al. (2005) did not find difference in prevalence estimates, based on pre- and post-transportation fecal samplings. Scherer et al. (2008) also did not observe any effect of transport-induced stress on Salmonella shedding rates in feces. Morrow et al. (2002) observed no feed withdrawal effect on Salmonella prevalence in pigs at slaughter, whereas Martin-Pelaez et al. (2008) reported that increasing pre-slaughter feed withdrawal and lairage lead to increased numbers of Salmonella in market pigs.

Although inconsistent, these reports present some evidence linking stress with pathogen carriage and shedding in swine. However, all these studies focused on the effect of stress on Salmonella-infected pigs. To the best of our knowledge, our study is the first one to determine under controlled conditions the effect of specific pre-slaughter stressors on the susceptibility of market-weight pigs to Salmonella. It is important to know when pathogen levels are the highest in infected pigs. However, it is also important to know when animals are most susceptible to infection. Understanding these two critical points in the pork production chain is key to determine when intervention strategies for pathogen prevention and control may be most effective, and consequently, increase pork safety.

Conclusion

Based on the results of this study, it is concluded that pre-slaughter transportation and mixing with unfamiliar pigs increases the susceptibility of market-weight pigs to a low-dose Salmonella challenge.

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Comparison of two commercial ELISA kits and magnetic stirrer method for detection of Trichinella spp. in a pig slaughterhouse

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Abstract

ELISA represents a useful rapid method to detect the presence of specific antibodies on serum, plasma or meat juice collected at slaughter, however, false- and positive-results may occur depending on the sensitivity and specificity of the test. In this study we compare two commercial ELISA kits for the detection of specific antibodies against Trichinella spp. with respect to the gold standard method (artificial digestion) in a pig slaughterhouse. A total of 709 Iberian pigs belonging to 79 free-range herds were randomly selected and sampled (five to ten animals/herd) (Win Episcope 2.0; 95% confidence level, 8% accepted error). Blood samples were collected at the slaughterhouse, and serum was harvested and frozen at -80 °C until testing. Sera samples were analyzed for the detection of specific anti-Trichinella spp. antibodies by means of two commercial ELISA kits, following manufacturer's instructions (PrioCHECK® Trichinella Ab, Prionics; ID Screen® Trichinella Indirect, IDVet Innovative Diagnostics; cut-off > 15% and > 50%, respectively). Samples from the diaphragm pillar (1 gram/animal) were collected and subjected to artificial digestion method for pooled sample digestion (100 g/pool) following the regulation EC-2075/2005. Specific anti-Trichinella spp. antibodies with PrioCHECK® ELISA were detected in 3 out of 709 animals, belonging to 3 out of 79 herds. Nonetheless, all the sampled animals displayed negative results for both IDScreen® ELISA and artificial digestion. The positive results observed with the first ELISA may be related to a higher sensitivity, being able to detect contact but not infestation with the parasite. Although both ELISA kits are coated with Trichinella E/S antigen, differences in the preparation and purification of the antigen may be related to different sensitivity and specificity. For this reason, serological tests are only recommended for surveillance studies, whereas direct methods should be used for food safety purposes.

Introduction

To date, the gold standard method for the detection of Trichinella is the digestion assay (OIE, 2009). However, there are several methods available for trichinellosis diagnosis, which are not recommended because of their lack of efficiency or reliability. In this sense, a diagnostic assay should be validated and may allow a repetitive measure. Although several serological tests have been developed, the Enzyme-Linked Immunosorbent Assay (ELISA) is considered as the test of choice based on economy, reliability, adaptability to good quality assurance practices, increasing body of validation data and good sensitivity and specificity when conducted under appropriate conditions (OIE, 2009).

ELISA represents a useful method for the rapid detection of specific antibodies in different body fluids, just as serum, plasma or meat juice. It is a useful tool for testing populations and is routinely used for surveillance programmes and out-breaks investigations. In this sense, antigen preparations have been developed to provide a high degree of specificity for Trichinella infection in swine (Gamble et al., 1988). Moreover, the Excretory-Secretory (ES) antigens from T. spiralis has been reported to be conserved in all species and genotypes of Trichinella (Ortega-Pierres et al., 1996), making feasible the detection of specific anti-Trichinella antibodies in pigs infected by any of the eight species of Trichinella.

For these reasons, two commercially available ELISA kits were compared with the artificial digestion method in order to determine the usefulness of each test for trichinellosis diagnosis. In our study ELISA test PrioCHECK® Trichinella Ab (Prionics) for Trichinella spp. showed positive results only in three animals, however, all the animals displayed negative results by the second ELISA test ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics). In addition, all the sampled animals yielded negative results by the reference method of detection (magnetic stirrer method for pooled sample digestion; EC-2075/2005).

Material and Methods

A total of 709 Iberian pigs belonging to 79 free-range herds were randomly selected and sampled during 2008 and 2009. Sample size was assessed by the software Win Episcope version 2.0 on the basis of the number of samples required for a previous unknown prevalence (95% confidence level and 8% accepted error were assumed and confidence intervals of the prevalence were calculated).

Five to ten pigs per herd were randomly sampled. Blood samples were collected at the slaughterhouse into evacuated tubes, allowed to clot at room temperature and centrifuged at 1200 X g for 10-15 minutes at room temperature. The serum was harvested and frozen at - 70 °C until testing.

Sera samples were analyzed for the detection of specific antibodies against Trichinella spp. by means of two different ELISA kits, following manufacturer's instructions (PrioCHECK Trichinella Ab, Prionics; and, ID Screen® Trichinella Indirect, IDVet Innovative Diagnostics). The cut-off value used for discriminating between positive and negative serum samples was 15% and 50% respectively.

Magnetic stirrer method for pooled sample digestion was routinely performed on all the animals sampled following the regulation EC-2075/2005 for the detection of Trichinella spp.

Results

The ELISA PrioCHECK® Trichinella Ab (Prionics) allowed to detect specific anti-Trichinella spp. antibodies only in 3 out of 709 animals (0.42% CI95: 0.14-1.23) and 3 out of 79 herds (3.80% CI95: 0.18-7.42). However, no positive result was obtained when the ELISA test ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics) was used. In addition, all the sampled animals displayed negative results for routine artificial digestion (Table 1).

Table 1. Number of positive free-range herds and pigs against each technique used for the detection of Trichinella spp.

	Techniques		
	PrioCHECK® Trichinella Ab (Prionics)	*ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics)*	*Artificial digestion*
Herds (n=79)	3 (3.80)	0 (0.00)	0 (0.00)
Pigs (n=709)	3 (0.42)	0 (0.00)	0 (0.00)

Discussion

ELISA represents a useful rapid method to detect the presence of specific antibodies on serum, plasma or meat juice collected before or after slaughter. Trichinella infestation levels as low as one larva/100 g of tissue has been detected by ELISA in pigs (Gamble et al., 2004). However, although ELISA has shown a high sensitivity in the detection of specific anti-Trichinella spp. antibodies, false-negative results may be observed due to infected animals do not develop an antibody response until 3–5 weeks post infection (Gamble, 1996). In addition, ELISA may yield a low rate of false-positive results due to the specificity of ELISA for Trichinella infection is variable according to the type and quality of the antigen employed in each test (OIE, 2009).

ELISA tests are able to detect specific antibodies in serum, which may appear just after a contact with a microorganism, despite no efficient infestation. Thus, the positive results obtained with the first ELISA test (Prionics) in our study may be related either to a lack of specificity of the test or to a contact but not infestation with the parasite.

In our study, both ELISA kits were coated with Trichinella E/S antigens, but differences in the preparation and purification of the antigen may be related to different sensitivity and specificity (OIE, 2009). In addition, both tests presented different cut-off points. Whereas ELISA PrioCHECK® Trichinella Ab (Prionics), which yielded three positive animals and herds, had a cut-off < 15%, the ELISA test ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics), which generated only

negative results, presented a cut-off < 50%. Therefore, the differences observed in the cut-off values for each test may be involved in the discrepancy observed in sensitivity and specificity of each test.

Conclusion

The differences observed between the different methods used in this study point to serological tests may be only recommended for surveillance studies, whereas direct methods should be used to individual carcass inspection, as recommended by the OIE (2009).

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Enrichment or maceration influence post harvest isolation of Salmonella from mesenteric lymph nodes

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Abstract

Two enhanced microbiological methods were evaluated for recovery of Salmonella species from samples collected at slaughter, with a focus on ileocecal lymph nodes. Samples from one hundred and sixty two animals (vaccinated = 79, non-vaccinated = 83) were collected along with 25 pooled environmental samples (pen, truck, lairage). Animal sample types included ileocecal lymph nodes, peritoneal sponges and shoulder sponges. Initially, swabs from all samples were used to directly inoculate hektoen enteric (HE) plates. Additionally all samples were set up for enrichment in Tetrathionate (Tet) only (Method 1). Two additional methods were utilized on samples previously frozen to attempt to isolate Salmonella species after the initial swab-only culture process yielded all negative results. Lymph nodes were thawed in equal numbers from each group on several occasions, homogenized in Phosphate Buffered Saline (PBS) and enriched using one of the two additional methods:

- Tet and Rappaport-Vassiliadis (RV) (Method 2), or
- Buffered Peptone Water (BPW) + novobiocin and RV (Method 3).

Enriched samples were plated onto brilliant green and XLT4 differential media. Up to three suspect colonies were restreaked onto HE and tested with several biochemical reactions (Kligler's, urease, indole, lysine, oxidase). Positive Salmonella isolates were confirmed by Salmonella serogrouping and serotyping. Salmonella was not isolated from peritoneal and shoulder sponges or from direct lymph node swabs. Salmonella Anatum and S. Muenchen were isolated from two environmental pen samples. Salmonella serogroup C1 was isolated from homogenized lymph nodes using both enrichment methods. Five samples were positive with the BPW + novobiocin and RV method, and 3 samples were positive using the Tet and RV method. All 5 Salmonella positive samples were from animals that were not previously vaccinated (p-value = 0.03, Fisher's Exact Test). Maceration of lymph nodes and use of sample specific culture methods may influence results of food safety investigations.

Progress in salmonella isolation and their serovar composition in pigs

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Abstract

Salmonellosis monitoring and control in farm animals in the RF are performed by several state services and according to some national programmes. One of the trends in salmonellosis control is the "Programme for risk identification and assessment in the context of targeted veterinary monitoring of animal product safety in the RF territory". The work was aimed at the analysis of data of monitoring of salmonellosis outbreaks in pigs, which occurred in the RF during 2005 – 2009. Within this period 38 712 diseased animals and 22 631 (58.46%) dead animals were reported in the salmonellosis outbreaks among pig population. The mortality was due to salmonella infection. According to the laboratory diagnostic data, the RF veterinary laboratories tested 107 996 samples of pathological materials during this period and salmonella of various serovariants were detected in 4174 (38%) samples. During the observation period Salmonella enterica was the most often to isolate: S. Choleraesuis (from 71.8% to 81.5%), S. Typhimurium (from 2.0% to 16.5%), S. Dublin (from 2.2% to 5.7%), S. Enteritidis (from 2.2% to 2.7%). Other salmonella serovariants (S. Hamburg, S. London, S. Muenchen, S. paratyphi B, S. Lagos, S. Nancy, S. Anatum, S. Adamstua, S. Veddel, non-typed and others) amounted to 19.6%. During animal food safety monitoring, pork samples amounted to 13.4% of the total amount of samples. Salmonella were detected in 1.6% of samples. The dominating serovariants were S. Typhimurium (29.8), S. Choleraesuis (19.7), S. Enteritidis (7.7), S. Lagos (5.8).

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1st National ring trial on detection of antibodies to trichinella in pigs

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Abstract

Regulation (EC) 2075/2005 ensures official inspection of food of animal origin with specific rules on official controls for Trichinella in meat. Regarding certification of Trichinella-free farms, this regulation recommends serological methods for monitoring. The aim of the ring trial was to evaluate a new ELISA regarding test accuracy and practical usage. The participants of the ring trial tested 22 sera prepared by the German National Reference Laboratory for Trichinellosis and additionally 22 field samples from their own sample collection using the commercial ELISA kit. This ELISA demonstrated a very good diagnostic sensitivity and robustness in the ring trial.

Introduction

Trichinellosis is a food-borne disease caused by nematodes of the genus Trichinella, which occurs seldom in Germany. Humans are infected by consuming raw or insufficiently heated meat or raw sausages containing parasitic larvae.
According to the Regulation (EC) 2075/2005 different methods of artificial digestion have been approved for the detection of Trichinella in fresh meat. Serological tests such as the ELISA are useful for monitoring purposes and may be implemented in surveillance programs for farms or regions with a negligible risk of infection with Trichinella. ELISAs for the diagnosis of infection with Trichinella in swine are well established (Gamble et al. 1983, Nöckler et al. 1995).
To evaluate a new ELISA a ring trial was organised by the German National Reference Laboratory for Trichinellosis at the BfR with 21 participants in September 2009.

Material and Methods

During the ring trial, 21 laboratories from 11 states of Germany tested 22 serum samples from experimentally infected pigs, as well as 212 serum, 33 plasma and 169 meat juice samples from hogs and 26 sera from wild boars, respectively, from their routine submission. The samples were tested using the BfR in-house ELISA and the PIGTYPE® Trichinella Ab ELISA (Labor Diagnostik GmbH Leipzig; officially approved in Germany in 2009). The number of correct, false positive and false negative results per laboratory was compared to the sample status obtained by the German National Reference Laboratory for Trichinellosis. Furthermore the repeatability of the assay was analysed for the OD-values obtained by the ring trial participants by calculation of the variation coefficient. Additionally z-scores were calculated to determine the deviation of the laboratory mean from the overall mean and the result variance of the labs was compared to the mean variation by Mandel´s k (DIN ISO 5725-2:2002-12 [2002], ISO 13528:2005 [2005]). At last the Pearson correlation coefficient was determined in comparison to the results determined at BfR to evaluate the reproducibility of the test results in different laboratories.

Results

14 of 21 participants reported all results for the reference samples as expected. Incorrect result calculation and testing performed by two different lab technicians were identified as one major cause of laboratories reporting false positive or false negative results. All tested field samples but four wild boar samples scored negative. The ELISA-kit demonstrated very good diagnostic sensitivity, specificity and robustness. None of the laboratories experienced major problems implementing the assay in their daily testing routine, but thorough reading and following the manufacturer´s instructions is crucial for good test results.

Discussion

The main objective of the ring trial comprising 21 laboratories was to evaluate the PIGTYPE® Trichinella Ab ELISA regarding test accuracy and practical usage. Overall the ELISA results for laboratories demonstrate a good sensitivity and specificity of ELISA with 98.93% and 95.39%, respectively. Most of the borderline sera were identified as positive indicating that the diagnostic sensitivity of the evaluated ELISA was higher than the in-house ELISA. Variation coefficients were used to assess the repeatability of the ELISA. As only 6.8 % of the sera showed a variation coefficient above 30 %, the repeatability of the ELISA was good for the participating laboratories (OIE [2009]). Z-scores and Mandel´s k were calculated to analyse the variability of the test results in more detail and also demonstrated that the test results of both tests were reproduced by most laboratories.

Taken together the PIGTYPE® Trichinella Ab showed a stable performance in both repeatability and reproducibility in this ring trial. The close correlation for S/P ratios between participants and the reference laboratory also demonstrate a good performance of the ELISA.

Conclusion

In conclusion monitoring of pigs determined for human consumption for Trichinella by serological examination using ELISA seems to be a suitable tool, since the method is well established and standardized.

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Combined serology and antibiotic residue detection in a Luminex assay

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Abstract

Serum of slaughter pigs is being used for routine testing of antibodies against pathogens and can also be used for detection of antibiotic residues. Since the assay formats differ (direct detection of antibodies vs. inhibition assays for residues), these tests are performed in parallel, not in a multiplex set-up. For this study, a protocol for testing both antibodies and residues in one sample was investigated using the dedicated multiplex xMAP platform of Luminex. The results suggest that direct detection of antibodies combined with an inhibition assay for antibiotic residues is possible. Although the required sensitivities are not yet met, the results are promising and further optimizations to successfully combine the two detection methods in one multiplex assay are ongoing.

Introduction

Slaughter pigs are tested for several types of hazards, such as zoonotic pathogens like Salmonella or Trichinella and antibiotic residues. Pathogen detection can be done by the detection of serum antibodies with immunoassays like ELISA. For the detection of antibiotic residues, a microbial inhibition test in renal pelvis fluid (pre-urine) is being used. As a faster alternative, these (small) residues can also be detected in serum with an immunoassay, i.e. an inhibition test. The purpose of the research described here is to combine these different assay principles (Fig. 1) in one protocol using the bead-based multiplex technology of Luminex.
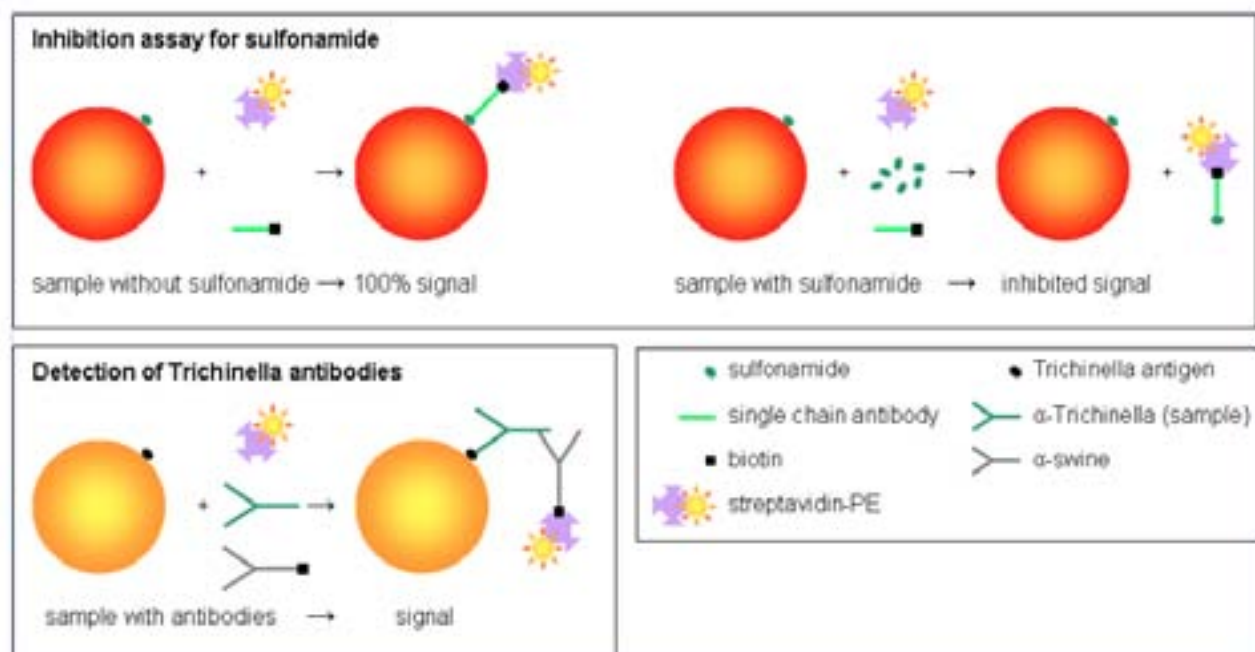

Figure 1 Assay principles. For each type of assay Luminex beads are depicted that are either conjugated with sulfonamide (top) or Trichinella antigen (bottom). All ingredients required for the respective assays are described in the legend. Abbreviations: PE: phycoerythrin.

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Material and Methods

A previously developed Trichinella Luminex assay (1) was chosen as representative serological assay, while the detection of sulfonamides was chosen as a representative inhibition assay, with sulfa-methoxazole as model compound (2). For setting up the protocols, well-described swine serum samples with and without the addition of sulfamethoxazole were used. Sera were spiked with 100ppb and 20ppb, respectively the maximum residue limit (MRL) and the minimum detection limit.

Results

To combine the serological assay with the residue inhibition assay, the effects of various conditions were investigated. Differences between the two assays were the number of incubation steps, the required serum dilutions and the sample pre-treatment (Fig. 2). As the standard protocols of the two assays require different serum dilutions, an effort was made to reduce the background in the serology assay and to increase the sensitivity of the inhibition assay by evaluating the effects of sample pre-treatment and sample dilution.
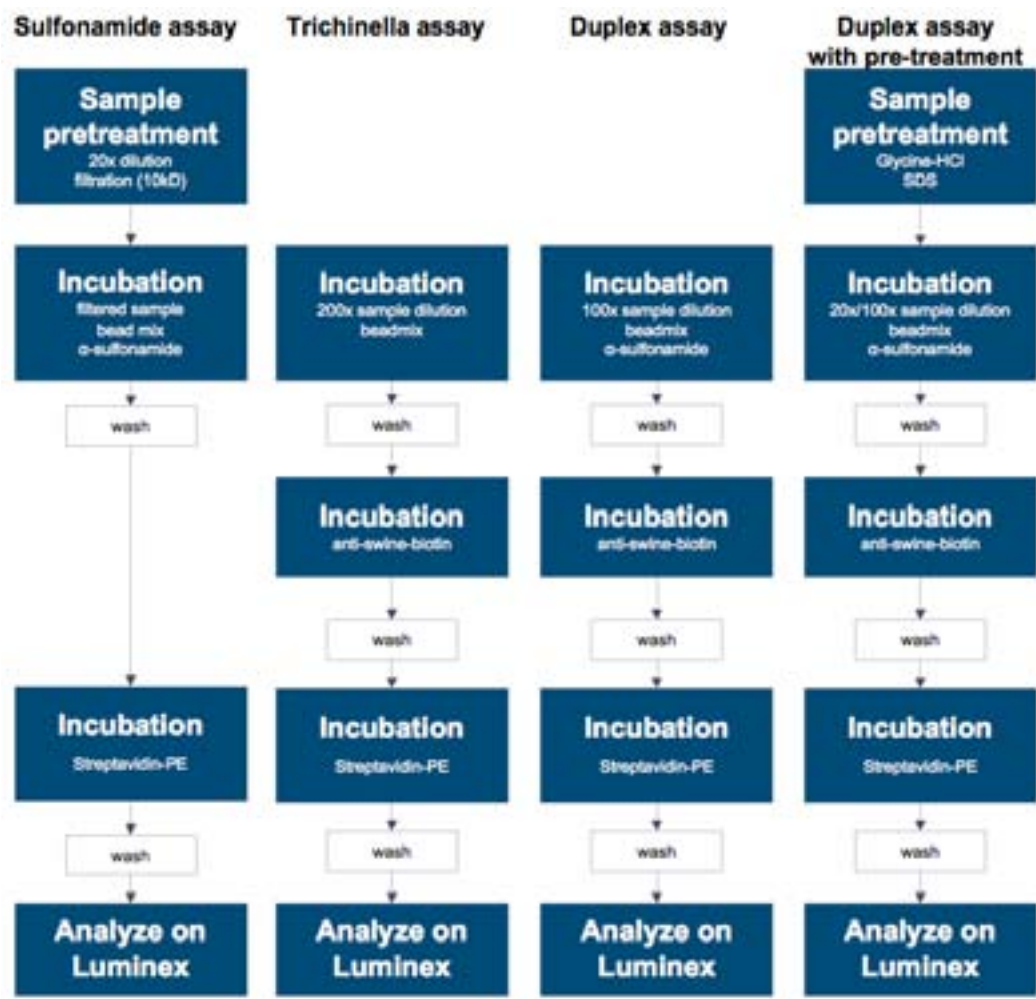
In the sulfonamide assay, a 10 kDa filtration step is performed after sample dilution in order to remove proteins and other large compounds. Since this includes removal of antibodies, this is not appropriate for the Trichinella assay. Therefore, this step was omitted in the combined duplex assay format that was established. When spiked swine serum samples were tested in this duplex assay, the sulfonamide assay did not detect the required MRL (Fig. 3). When unfiltered pre-treated samples (acid glycine and SDS)(3) were diluted 20x (optimal for sulfonamide), both assays showed modest differences between positive and negative samples. When these pre-treated samples were diluted 100x, the Trichinella assay was re-established and the signal of the sulfonamide assay increased, albeit with a high standard deviation.
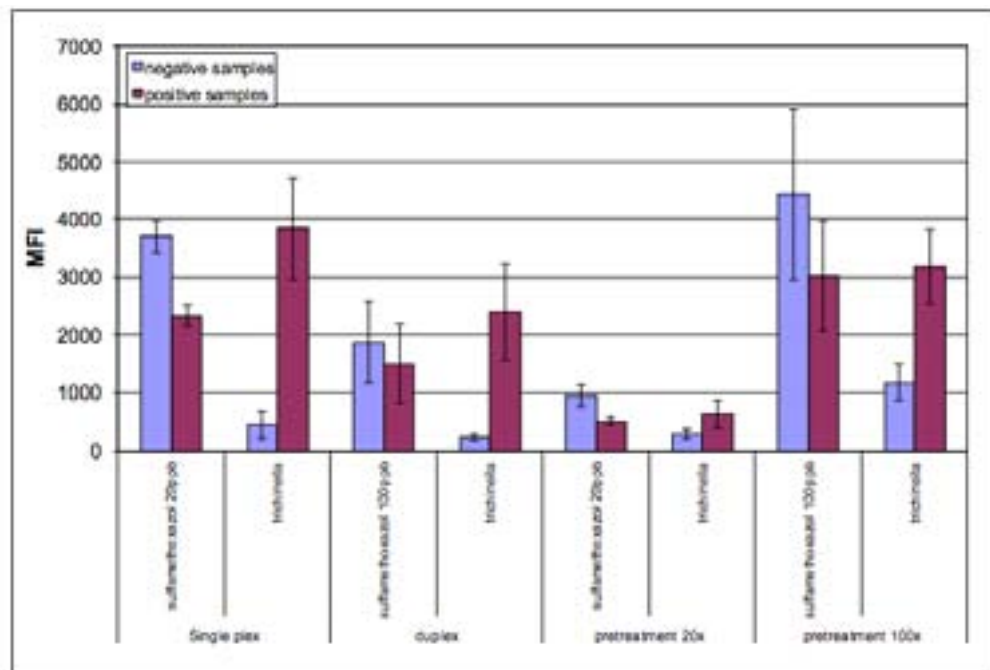
Figure 3 Results of combined assays. Mean results of the 20 swine sera with and without sulfamethoxazole are presented.

It is clear that filtering and only modest serum dilution are crucial for the sulfonamide assay, whereas a higher sample dilution is important for the Trichinella assay and filtration is absolute impossible as it depletes the serum of antibodies. In order to solve these problems an alternative 'split-and-pool' method was designed. In this alternative procedure, serum is split in two and treated according to the requirements of the respective assay. These treated samples are then pooled in a microplate well. In detail: after making a 10x dilution the sample is split in two. One half is filtered to remove anything larger than 10kDa. The other half is further diluted to a 100x dilution. The filtered 10x diluted sample is pooled with the 100x diluted sample, resulting in a combined sample with 20x diluted residues and 200x diluted antibodies (Fig 4). Early results with this split-and-pool procedure show that both sulfonamide residues (i.e. sulfa¬chloro¬pyridazine) and Trichinella antibodies can be detected in this combined assay format.
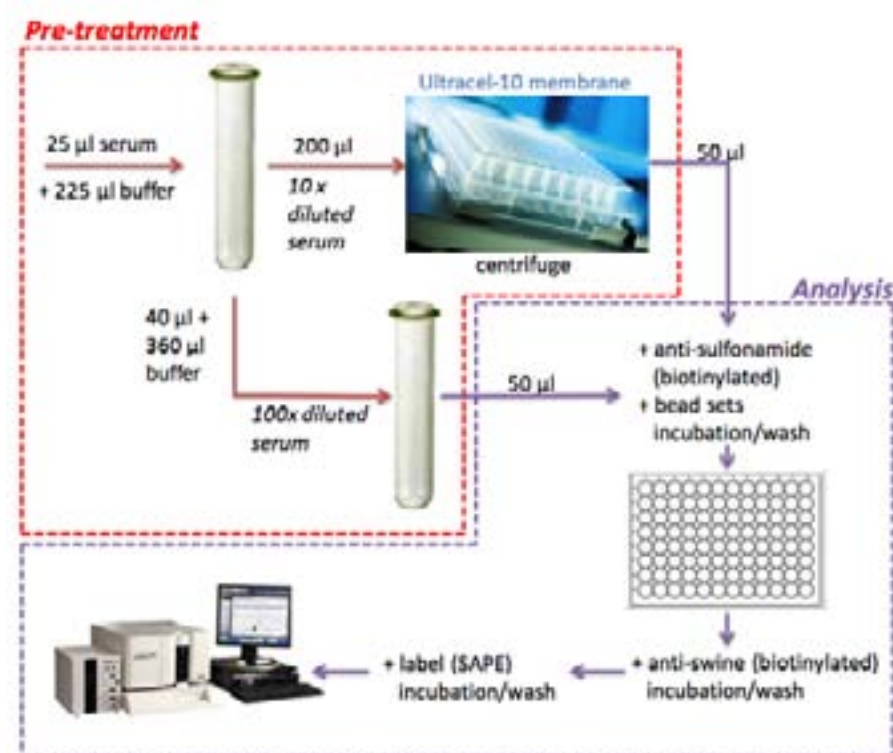
Figure 4 Split-and-pool assay. Schematic representation of the proposed split-and-pool method to detect residues and anti¬bodies in a Luminex assay.

Discussion and conclusion

The results demonstrate that it is possible to combine a serological assay and an inhibition assay in one Luminex protocol. Since the used representative assays are optimized for their respective goals, the resulting duplex assay needs to be further optimized for variables like bead production, buffer composition and labelling. The split-and-pool protocol is promising but needs further investigation and optimization. This type of assay could find its use in cost-reducing monitoring systems in a production chain where several pathogens and small compounds like antibiotic residues are being monitored.

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Comparison of different enrichment media for the isolation of Salmonella from naturally infected slaughter pigs

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Abstract

The present study aimed to assess the impact of different enrichment media, Rappaport-Vassiliadis (RV) broth, Rappaport-Vassiliades Soya (RVS) broth, Diagnostic semi-solid Salmonella (DIA) agar, Simple Method Salmonella (SMS) agar, Modified Semisolid Rappaport Vassiliadis (MSRV) agar and Mueller Kauffmann Tetrathionate novobiocin (MKTTn) broth, on the detection of Salmonella as well as on the isolated serotype and genotype. Up to 3 suspected colonies per medium were examined.

In total, duodenal contents of 458 slaughtered pigs were examined for the presence of Salmonella. In 14.8% (68/458), Salmonella was isolated by at least one of the used techniques. MSRV showed the highest detection rate (86.8%), followed by DIA and SMS (both 85.3%), RV (58.8%), RVS (54.4%) and MKTTn (50.0%). Of the 8 identified serotypes, S. Typhimurium (67.9%) was the predominant serotype, followed by S. Derby (17.3%). In the isolates of 9 pigs (13.6%) multiple serotypes were identified between (1 pig), within (4 pigs), and between and within (4 pigs) the different media used. Genotyping by pulsed field gel electrophoresis (PFGE) was performed on isolates of 38 from 60 pigs that were Salmonella positive on at least two enrichment media types. Within the same serotype, similar genotypes were found except for the isolates deriving from 3 pigs, showing different genotypes within the same medium. In isolates of 2 pigs, the PFGE fingerprint showed a difference in only one band, while in isolates of the last pig a total different genotype was identified.

The results show that testing multiple media and multiple colonies per medium increase the number of serotypes and genotypes found in the duodenal content. This may be important to consider in epidemiological studies.

Performance of four different diagnostic tests for C. difficile infection in piglets

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Abstract

Clostridium difficile is emerging as pathogen in man as well as in animals. In 2000 it was described as a cause of neonatal enteritis in piglets and it is now the most common cause of neonatal diarrhoea in the USA. In Europe, C. difficile infection (CDI) in neonatal piglets has also been reported. Diagnosis of this infection is based on detection of the bacterium or its toxins A and B. Most detection methods, however, are only validated for diagnosing human infections. In this study three commercially available Enzyme Immuno Assays and a commercial RT-PCR were evaluated by testing 172 pig faecal specimens. The results of each test were compared with cytotoxicity assays (CTA) and toxigenic culture as gold standards. Compared with CTA, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were for RT-PCR respectively 91.6%, 37.1%, 57.6%, and 82.5%, and for Premier Toxin A+B (Meridian): 83.1%, 31.5%, 53.1% and 66.7%, and for Immunocard tox A/B (Meridian) 86.6%, 56.8%, 66.9%, and 80.7%, and for VIDAS (BioMérieux): 54.8%, 92.6%, 85.0%, and 72.8%.

Compared with toxigenic culture sensitivity, specificity, PPV and NPV were; for RT-PCR 93.0%, 34.7%, 50.0%, and 87.5%, and for Premier Toxin A+B: 80.3%, 27.7%, 43.8%, and 66.7%, and for Immunocard tox A/B: 80.0%, 46.2%, 52.8% and 75.4%, and for VIDAS: 56.4%, 89.8%, 77.5%, and 76.7%.

We conclude that all tests had an unacceptable low performance as a single test for detection of C. difficile in pig herds and that a two step algorithm is necessary. Of all assays, the RT-PCR had the highest NPV compared to both reference methods and is therefore the most appropriate test to screen for absence of C. difficile in pigs as a first step in the algorithm. The second step would be a confirmation of the positive results by toxigenic culture.

Risk assessment of MRSA isolates from swine using a diagnostic DNA microarray

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Abstract

The aim of this study was to support a risk assessment of MRSA-isolates from swine using a diagnostic DNA-microarray to detect virulence-, toxin- and resistance related genes. In comparison with other species like poultry, cattle and humans there were only few isolates with virulence genes.

Introduction

Methicillin- resistant Staphylococcus aureus (MRSA) is an important pathogenic agent causing nosocomial infections. The clonal lineage ST398-MRSA-V dominates in swine, in humans with occupational exposure to swine and in regions with intensive swine production (Tenhagen, B.-A. et al. 2009). Investigation of their antimicrobial resistance and SCCmec typing produced multiple analogue results. The aim of this study was to detect virulence-, toxin- and resistance related genes of swine isolates to support a risk assessment, in particular with respect to the transmission to humans.

Material and Methods

We used DNA-chip-technology based on the STAPHY TYPE Kit (Alere Technologies GmbH, Jena) which identifies 333 clinically relevant markers for resistance and virulence in a single test.

After a linear PCR amplification the resulting single stranded and biotin labelled amplicons are stringently hybridised to a set of highly discriminative probes that are covalently bound onto the microarrays. Additionally, the phenotypic resistance of 71 isolates was investigated by broth microdilution to compare the genotypic and phenotypic resistance profiles. 83 isolates from swine and 48 isolates from other species were included in this genotype characterisation.

Results and Discussion

The tests showed a good correlation between genotype and phenotype resistance. The array provides information about genes for PVL, toxic shock syndrome, exfoliativ toxins and enterotoxins and in addition resistance genes for example pleuromutiline and streptothricin. 10 of 131 investigated isolates contained virulence genes; only 2 of them were isolated from pigs. Within one spa-type we can discriminate a lot of different resistance gene patterns. For example in 39 isolates of the Spa-type t011 we investigated 16 different resistance gene patterns.

This microarray-technique is useful for risk assessment in veterinary diagnostic like it is in human medicine (Monecke et al, 2008) and it also provides information for epidemiological studies.

Conclusion

Only 2,4% of the investigated isolates from swine showed virulence genes for enterotoxin, and none was positive for PVL, toxic shock syndrome or exfoliativ toxins. The isolates showed good correlation between phenotypic and genotypic resistance. The DNA-Chip-technology provides essential information for epidemiological studies in a one step analysis.

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A longitudinal study on the persistence of Livestock Associated-MRSA in swine herds

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Abstract

In recent years, a new type of MRSA, now called livestock-associated MRSA (LA-MRSA), belonging to the clonal complex (CC) 398, has globally emerged in swine world wide. Aim of this study was to gain more insight into the persistence of LA-MRSA in different types of pig farms over a period of two years. To investigate this, 15 MRSA-positive herds from a previous study were selected; an additional pig farm was selected because this one was found to be positive with a human MRSA-strain. Starting in September 2009, five dust samples were collected every two months. Samples were analysed for MRSA and spa-typing was done to confirm that MRSA isolates belonged to CC398 and to gain insight into persistence of strains within a farm.

Three herds were positive on all sampling occasions and were consistently contaminated with LA-MRSA. In the remaining herds, occasionally no positive dust samples were found at some sampling moments. The predominating spa -types were t011 and t108. A maximum of 5 different spa-types were found in two herds, with 3 different spa-types present in one sampling, indicating multiple introductions.

These results show that LA-MRSA remains present on a pig farm over a long period. Most likely, transmission within the herd occurs after initial introduction, and an endemic situation seems to be the endpoint. The relatively low sensitivity of dust sampling compared to sampling of animals, the small sample size and lack of strict standardization of dust sampling might explain occasional negative samplings in overall positive herds. However, a true change of a positive MRSA-status to a negative status, followed by re-introduction cannot be ruled out in our study design.

Introduction

Since the first detection of a new type of MRSA in pigs in 2004 (Voss et al., 2005) belonging to the clonal complex (CC) 398, as determined by multilocus sequence typing (MLST), this MRSA clone has globally emerged in swine and other livestock worldwide (Broens et al., 2008) (Livestock-associated MRSA, LA-MRSA)Observational studies have been limited to single observations at farm level so far, and information on the transmission and persistence of LA-MRSA within a farm is very limited. To develop effective intervention strategies, more insight is needed into persistence of LA-MRSA within a pig farm over a longer time period. Persistence of LA-MRSA might differ depending on management strategies on a farm, which are very different between farm types. For example, the all in-all out production system is often applied on finishing farms, whereas a continuous production system is usually applied on farrowing farms.

The study objective was to gain more insight into the persistence of LA-MRSA in different types of pig farms over a period of two years.

Material and Methods

Fifteen pig farms which were found positive for MRSA CC398 in a previous prevalence study on Dutch pig farms (Broens et al., submitted), were selected comprising five finishing, farrow-to-finish and farrowing farms, respectively. One particular pig farm was added, because this one was found to be positive with a human MRSA-strain (spa-type t002/ST5). On all farms, five dust samples (s1 kit ringer solution, Sodibox) were collected every two months from five different farm sections to sample all age categories present. No more than one sample was collected in each compartment. At the start of the study, samples were taken by an employee of the Animal Health Service and the farmer was instructed how to take the samples. Information on farm characteristics was collected by a questionnaire. After that, a parcel containing sampling materials, a submission form, a short questionnaire, a billing form and materials for return posting was sent to the farmers

every two months. The farmers received payment for each sampling submitted. Questionnaires were checked for completeness at receipt. Sampling started in September 2009 and will run until September 2011; results from samplings till March 2011 are analysed and presented in this paper.

MRSA isolation was performed on single dust samples according to the protocol described by Broens et al. (2011a) at the Animal Health Service, The Netherlands. One suspected colony per sample was confirmed to be MRSA by 2 PCR tests for the S. aureus specific DNA-fragment (de Neeling et al., 1998) and the mecA gene (Martineau et al., 1998). For all MRSA isolates spa typing was performed at the Institute for Hygiene, Germany (Harmsen et al., 2003). So far, spa-typing was only done on samples taken before November 2010. A farms was classified MRSA-positive if at least one dust sample tested MRSA-positive at a sampling.

Results

So far, 4-10 samplings were received and analysed per farm, resulting in 677 samples. Farm 7 stopped farming during the study, so no samples were received from this farm after sampling 4. Farm 8 did not comply with the sampling scheme and sent in samples very irregularly. One sampling from farm 5 was lost in the mail. One dust sample was missing in samplings from farm 8, farm 12 and farm 13. Average time between samplings was 9 weeks (SD=3.7 weeks), varied from less than a week (farm 8) to 24 weeks (farm 5) and was not different for the different herd types (P=0.86). Of all samples, 220 were positive (32.5%), and was lower for farrow-to-finish farms (25.0%; P=0.01) than for farrowing farms (35.8%) and finishing farms (36.6%). The average percentage of positive dust samples per farm over the study period was 34%, ranging between 10 and 62%, and was not different per herd type, being resp. 37.2% for farrowing, 26.4 for farrow-to-finish and 37.0% for finishing farms (P=0.49). In only 6 out of 136 samplings, all 5 dust samples were positive. At least one dust sample was MRSA-positive in 72.1% (98/136) of samplings moments. Per farm, on average 74% of sampling moments was found positive, varying from 38% (3 out 8 samplings) to 100% (farm 1, 7 and 15) where at all samplings MRSA-positive samples were found. This percentage was 80.4%, 68.9% and 66.7% for farrowing, farrow-to-finish and finishing farms (P=0.30), respectively.

So far, spa-typing was done on 181 isolates out of 220 positive samples. Fourteen spa-types were identified; 3 formerly confirmed to be non-CC398 (t002, t127 and t2164), 9 formerly confirmed to be CC398 (EFSA, 2009; Reischl et al., 2009; Wagenaar et al., 2009), and 2 'new' spa-types most probably belonging to CC398 (t6320 and t7621; Table 1). Spa-type t011 and t108 accounted for 73.5% of all spa-types. On five farms, only 1 spa-type was isolated over the whole study period, these were spa-type t108 in 3 farms and spa-types t899 and t011 in one farm each. On the remaining 11 farms, 2 to 5 different spa-types were found over the study period and up to 3 different spa-types at one sampling moment. Farm 16, which was selected for the presence of a human MRSA-strain (spa-type t002), was MRSA-positive in sampling 1 – 4 and 8. In sampling 3, 4 and 8, spa-type t002 was again found and in sampling 1, spa-type t2164 was found; typing results of sampling 2 were missing.

Table 1. Spa-types, their repeat succession and their frequencies (number and percentage) found on 16 pig herds.

spa -type	repeat succession	frequency	
		n	%
t011	08-16-02-25-34-24-25	86	47.5
t108	08-16-02-25-24-25	47	26
t899	07-16-23-02-34	18	9.9
t127*	07-23-21-16-34-33-13	6	3.3
t6320#	08-16-02-25-34-24-34-24-25	5	2.8
t034	08-16-02-25-02-25-34-24-25	4	2.2
t7621#	08-16-02-25-34-02-25-34-24-25-25	4	2.2
t002*	26-23-17-34-17-20-17-12-17-16	3	1.7
t2346	08-16-02-25-34-24-24-25	3	1.7
t1184	08-16-02-25-25	1	0.6
t1451	08-16-02-25-34-25	1	0.6
t1606	08-16-34-34-24-25	1	0.6
t2164*	26-23-17-34-17-82-17-12-17-16	1	0.6
t2330	08-16-02-25-34-24-25-25	1	0.6

*non-CC398; #'new' spa-type, unknown CC

Discussion

These data show that pig farms can remain MRSA-positive for over a year, and that the same spa-type can be found on a farm during this period. This holds for farrowing, farrow-to-finish and finishing farms. Whether the discriminatory power of spa-typing method is sufficient to indicate continuous contamination of a farm by the same MRSA-strain, needs confirmation by further genetic typing of the isolates using whole genome techniques. For spa-typing, a single locus, the staphylococcal protein A, is sequenced. Despite a high concordance of results from spa-typing with other typing methods such as MLST, the spa-type only indicates that isolates belong to a certain Clonal Complex. Consequently, selected isolates will have to be typed by MLST to confirm that the strains belong to CC398. Selecting more than one suspected colony per sample will also contribute to getting more insight in continuous contamination or re-introduction. Most likely, transmission within the herd occurs after initial introduction, and an endemic situation seems to be the endpoint.

The relatively low sensitivity of dust sampling compared to sampling of animals, the small sample size and lack of strict standardization of dust sampling might explain the, occasionally found, negative samplings in overall positive herds (Broens et al., 2011a). However, a true change of positive MRSA-status to a negative status, followed by re-introduction cannot be ruled out in our study design. On the other hand, repeatedly isolating the same spa-type suggests that the herd is still contaminated with the same MRSA-strain, but sometimes at levels below the detection limit of the method we used. The repeated isolation of spa-type t002 in one herd shows that also human MRSA strains can remain in a herd for a period of more than a year.

The occurrence of more than one spa-type in some herds might indicate that more than one introduction has taken place in that herd. This can be caused by buying MRSA-positive replacement stock from different sources or by a lacking external biosecurity of the farm (Broens et al., 2011b). Identification and sampling of contact farms in the national identification and registration system might help to confirm the introduction of MRSA by the purchase of pigs.

Conclusions

Pig herds can remain MRSA-positive for over a year, not only with livestock-associated MRSA-strains, but also with a human MRSA-strain. It can be concluded that spontaneous extinction of MRSA from a pig population does not occur frequently. This indicates that control strategies should focus on preventing introduction of MRSA into a farm and that active intervention measures are needed to eradicate MRSA from pig farms. Further research in needed to determine which intervention measures are effective.

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Antimicrobial resistance patterns of Salmonella enterica subsp. enterica serovar Derby and Typhimurium isolated from pigs slaughtered in southern Brazil

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Abstract

Salmonella enterica subsp. enterica serovar (S.) Derby and S. Typhimurium were commonly isolated from slaughter pigs and pork. Thus, the monitoring of the resistance profile exhibited by strains of both serovars should be regularly conducted. This study aimed to assess the antimicrobial resistance pattern of strains isolated form pig carcasses and to investigate the genetic relatedness with isolates from intestinal content and lairage environment. Thirty-four S. Derby and seventeen S. Typhimurium strains isolated from carcasses (n=30), intestinal contents (n=16), and lairage environment (n=3) were tested. The antimicrobial resistance was determined by the agar disk diffusion test according to the document M31-A2 of the CLSI using twelve antimicrobials. Strains were also genotyped by pulsed-field gel electrophoresis, and the presence of class 1 integrons was investigated. The isolates were resistant to tetracycline (96%), sulfonamides (78.4%), streptomycin (76.5%), ampicilin (35.3%), gentamicin (29.4%), kanamycin (27.5%), nalidixic acid (23.5%), chloramphenicol (17.6%), and ceftazidime (1.9%). Only two isolates from intestinal content were susceptible at all antimicrobials. No resistance to cefotaxime and ciprofloxacin was detected. Five resistance patterns were found among S. Derby isolates, and the most prevalent (TE-S-S3) was detected in 26 strains (76.5%). Among strains of S. Typhimurium, eight patterns were found, and the most prevalent (K-TE-CN-AMP) was detected in four (23.5%) isolates. All S. Derby strains with the TE-S-S3 pattern belonged to a common pulsotype (De1) and harbored Class 1 integrons. Resistant strains of S. Derby and S. Typhimurium isolated from carcasses presented resistance patterns in common with strains from intestinal content and lairage environment. Results indicate that resistant clonal groups originated from pig feces and lairage environment are able to contaminate pig carcasses and enter the pork processing chain.

Introduction

Salmonella enterica is recognized as the most important cause of food-borne illness in southern Brazil. Although chicken and eggs are the foods most often involved in outbreaks, salmonellosis associated with pork consumption is also reported (COSTALUNGA and TONDO, 2002). Serovars Typhimurium and Derby figure among the most prevalent in pigs and pork in southern Brazil (BESSA et al., 2007; MÜRMANN et al., 2009). Multiresistant strains from both serovars have been frequently isolated from pigs at slaughter (MICHAEL et al., 2006b; BESSA et al., 2007), however the resistance pattern of strains originated from carcasses was not determined. The emergence of antimicrobial resistance in Salmonella strains isolated from food has been an increasing concern. Thus, this study aimed to assess the antimicrobial resistance pattern of S. Derby and S. Typhimurium strains isolated from pig carcasses and to investigate the genetic relatedness with isolates from intestinal content and lairage environment.

Material and Methods

Thirty Salmonella strains isolated from carcasses sampled in southern Brazil were included in this study. Moreover, strains isolated from intestinal content (n=18) of pigs belonging to the same slaughter batch as the carcasses, and strains (n=3) isolated from the holding environment were also tested. Among all tested strains, 34 belonged to serovar Derby and 17 to serovar Typhimurium.

The antimicrobial resistance was determined by the agar disk diffusion test according to the document M31-A2 of the CLSI using the following disks: thrimetoprim (W; 5 µg), kanamycin (K; 30 µg), tetracycline (TE, 30 µg), ceftazidime (CAZ; 30 µg), sulfonamides (S3; 300 µg), chloramphenicol (C; 30 µg), gentamicin (CN; 10 µg), streptomycin (S; 10 µg), nalidixic acid (NA; 30 µg), ampicillin (AMP; 10 µg), cefotaxime (CTX; 30 µg), ciprofloxacin (CIP; 5 µg). Escherichia coli ATCC 25922 was used as quality control of disk diffusion tests.

Salmonella isolates were genotyped by the macro-restriction of total DNA, followed by pulsed field gel electrophoresis (PFGE). The macro-restriction analysis was performed following the PulseNet protocol (http://www.cdc.gov/PulseNet/protocols.htm). Whole DNA was digested with XbaI (20U) and BlnI (10U). Moreover, all isolates were submitted to investigation of intI1 integrase gene from class 1 integrons by PCR assays, as previously described (FRENCH et al., 2003).

Results

Salmonella isolates were resistant to tetracycline (96%), sulfonamides (78.4%), streptomycin (76.5%), ampicillin (35.3%), gentamicin (29.4%), kanamycin (27.5%), nalidixic acid (23.5%), chloramphenicol (17.6%), and ceftazidime (1.9%). Only two strains (S. Derby and S. Typhimurium), obtained from intestinal contents, were susceptible at all antimicrobials. No resistance to thrimetoprim, cefotaxime and ciprofoxacin was detected. Five resistance patterns were found in S. Derby isolates, and TE-S-S3 was the most prevalent (76.5%), as depicted in Table 1. The most common pulsotype (De1) encompassed 26 strains isolated from carcass (20), feces (4) and lairage (2). Most strain of pulsotype De1 showed the resistance pattern TE-S-S3, and this pattern was found in isolates from carcasses, feces and lairage. Class 1 integrons were detected in 32 (94.1%) S. Derby strains.

Among the six S. Typhimurium strains isolated from carcass, one was resistant only to tetracycline. The most frequent multi-resistance pattern among carcass isolates was K-TE-CN-AMP, detected in four isolates. The pattern K-TE-CN-NA-AMP was detected in one carcass strain and in one isolate from the lairage. Ten strains isolated from feces exhibited a high diversity of resistance profiles. The pattern TE-S-C-CN-S3-AMP was the most common, followed by K-TE-S-C-CN-S3-AMP, TE-S3-NA-AMP, K-TE-S-C-S3-AMP and K-TE-S-CN-NA-AMP. Strains from carcass, feces and lairage presented no common PFGE profile, and in only seven (41.8%) of them were class 1 integrons detected.

Discussion

In the present study, most strains showed resistance patterns that included at least tetracycline, streptomycin or sulfonamide. While S. Typhimurium strains presented a great diversity in resistance profiles and unrelated pulsotype patterns, S. Derby showed mostly common phenotypic and genotypic patterns. The most frequent resistance phenotype (TE-S-S3) in our study was previously reported as the most prevalent in S. Derby strains originated from the same region (Michael et al., 2006b). This study demonstrated that strains exhibiting the TE-S-S3 pattern also belonged to a common pulsotype and carried class 1 integrons. In our study, strains of S. Derby isolated seven years later in the same region exhibited the same common genotypic and phenotypic profile. Thus, clonal groups with the TE-S-S3 pattern seem to circulate over time in pig herds of the region and can contaminate carcasses, as observed in our study. The resistance to antimicrobials has been associated with their use for extended time periods in farm animals (WEGENER, 2003). However, streptomycin and sulfonamide are not administered to pigs anymore, and the use of tetracycline has been steadily declining in the last years. In spite of that, resistance determinants are likely to persist in the Salmonella population.

 Class 1 integrons carry frequently the aadA cassette gene, which is associated to streptomycin/ spectinomycin resistance (MICHAEL et al., 2006a). In our study class1 integrons were present in all strains of pulsotype De1 that presented de TE-S-S3 profile. Moreover, carcass strains presenting this profile with additional markers, such as nalidixic acid resistance, also harbored class 1 integrons. It may indicate that other gene cassettes have been integrated in the variable region of the integrons. This may be an additional concern, since nalidixic acid resistance determinants can be responsible for diminished susceptibility to f uoroquinolone (GORMAN and ADLEY, 2004).

In spite of the low number of strains investigated in our study, common resistance profiles were observed in S. Typhimurium and S. Derby isolated from carcass, feces and lairage environment. It highlights that multi-resistant Salmonella strains, which colonize the pig gut or are found in the lairage environment, are able to enter the pork processing chain.

Conclusion

Salmonella Derby clonal groups originated from pig feces and lairage environment contaminate pig carcasses and enter the pork processing chain. Multi-resistance profiles and class 1 integrons are observed in all strains of the most prevalent clonal groups.

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Table 1. Antimicrobial resistance patterns and pulsed-field electrophoresis (PFGE) profiles of S. Derby strains isolated from carcass, intestinal content and lairage environment.

Antimicrobial resistance pattern	Number of S. Derby isolates (PFGE pulsotype)			
	Lairage	Intestinal content	Carcass	Total
TE-S-S3	2 (De1)	5 (De1;De4)	19 (De1; De2; De4)	26
TE-S-S3-NA		1 (De1)	3 (De1)	4
TE-CAZ-S-C-CN-S3-NA-AMP			1 (De1)	1
K-TE-C-CN-S3-AMP		1 (De3)		1
K-TE-S-C-S3-AMP			1 (De4)	1
Total	2	7	24	33

PFGE, pulsed-field gel electrophoresis; TE, tetracycline; S, streptomycin; S3, sulfonamides; NA, nalidixic acid; CAZ, ceftazidime; C, chloramphenicol; CN, gentamicin; AMP, ampicillin.

Methicillin resistant S. aureus (MRSA) in the pork food chain in Germany

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Abstract

This paper gives an overview on studies carried out in Germany on the prevalence of MRSA on different stages of the pork food chain.

Prevalence studies were carried out on herd level for breeding (201 herds) and fattening pigs (290 herds), at abattoirs (n=1026 pigs), in a pork processing facility and in pig meat at retail. MRSA were characterized using spa-typing, SCCmec-typing and testing for antimicrobial resistance.

The highest proportion of positive samples was determined in pigs at slaughter (58% of the pigs, 98% of slaughter batches), followed by fattening herds (52%) and breeding herds (42%). MRSA in primary production was associated with larger pig herds and purchasing pigs of different origins. MRSA were isolated from all stages of the post-harvest production chain. Meat samples at retail were positive for MRSA in both years considered, namely 2008 and 2009 (8.4% and 15.8%). Most isolates were of spa-types associated with the multi-locus sequence type ST398 (t011, t034 and t108). Regional differences in the contribution of different spa-types to the overall burden of MRSA in primary production were identified. Non ST398 strains were isolated from breeding herds (7%) but not from fattening herds or slaughter pigs. However, such strains were also isolated from fresh pork. Most isolates carried SCCmec-types V, III and IVa. Antimicrobial resistance was predominantly observed for beta-lactams, tetracycline, lincosamides, and macrolides. Resistance to fuoroquinolones was present at varying levels. Resistance to vancomycin, fusidic acid and linezolid was only observed in exceptional cases.

The results of the studies indicate that MRSA is widespread in pig production and that it is readily transmitted to pork via the food chain. Further studies are needed to elucidate the potential to control MRSA in primary production, at harvest and in the post harvest food chain.

Prevalence studies

Methicillin resistant Staphylococcus aureus has been recognized as a nosocomial pathogen for decades. Triggered by findings in the Netherlands, Germany has extensively investigated the prevalence of MRSA in pigs and pig products during recent years.

It was determined, that MRSA have been prevalent in the German pig population at least since 2004 (Fig 1) (Meemken et al. 2010)

Fig 1. S. aureus in pigs at necropsy. Retrospective analysis of isolates from 2004 to 2007 (Meemken et al. 2010)
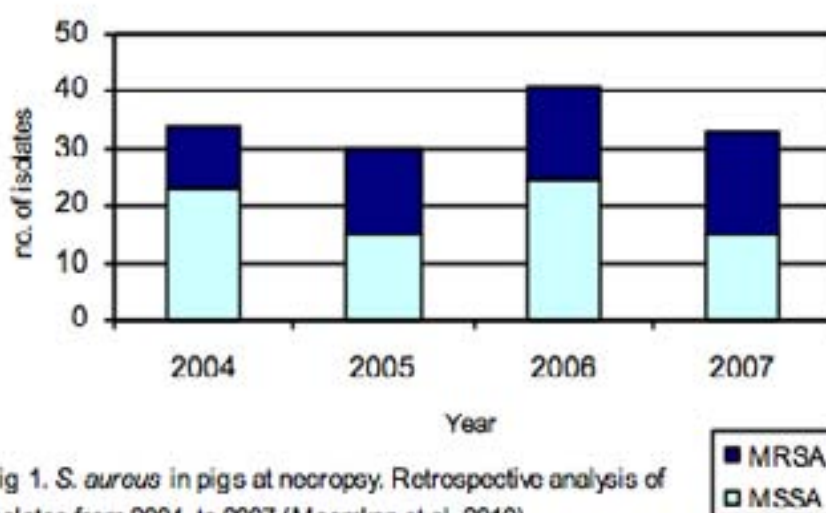
However, older samples were not available. Therefore, it is not clear, when the first strains of MRSA occurred in the German pig population.

In 2008 and 2009 surveys were carried out to determine the prevalence of MRSA in the breeding and fattening pig population (EFSA 2009; Tenhagen et al. 2009a). Results indicated that MRSA are widespread in the German pig population (Fig 2). In a study at 5 large abattoirs during winter 2007/2008, 1026 pigs nose swabs were investigated. Between 49% and 80% of the pigs were positive per abattoir and most slaughter batches included at least 1 positive pig out of 10 tested. However, it has been pointed out that transport and lairage contribute to the contamination of pigs examined at stunning (Broens et al. 2010).

The distribution of MRSA along the production chain was studied in a slaughterhouse with a connected processing unit producing fresh pork (Beneke et al. 2011). Results indicated that MRSA were frequently found in the noses of pigs (65 %), but less frequently on carcasses (16 %) and in products ready for marketing (3%).
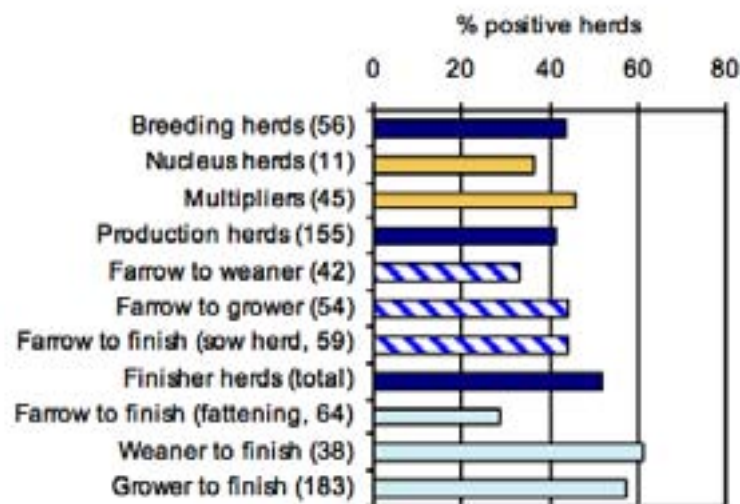
Fig. 2 MRSA in pig herds in Germany 2008/2009

Monitoring at retail level indicated that pork is regularly contaminated with MRSA (Fig 3) with minced meat showing a substantially higher contamination rate than other fresh pork or fresh pork preparations (Tenhagen et al. 2011). The difference in the results of the monitoring and the study in one processing unit point to a potential variation between abattoirs and processing units that needs to be elucidated. Investigations into the quantification of MRSA in food items indicated, that the number of germs (colony forming units) in the food items is low (van Loo et al. 2007; Weese et al. 2010). Currently there is no evidence of spread of MRSA into the human population via food. MRSA ST 398 found in humans were nearly always associated with persons directly or indirectly exposed to livestock, especially pigs (Köck et al. 2009). This includes livestock professionals such as farmers, veterinarians and slaughterhouse workers and their families (Meemken et al. 2008). However, among the latter, prevalence rates were substantially lower than among those directly exposed to pigs (Cuny et al. 2009).
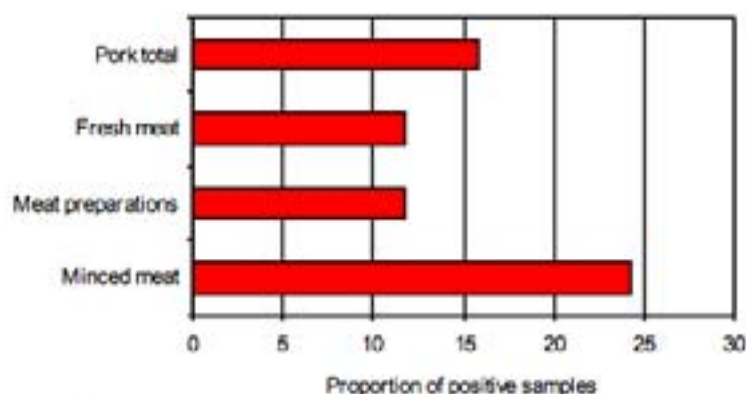
Fig 3. MRSA in pork in Germany 2009 (Tenhagen et al. 2011)

Typing results

Most of the MRSA isolated in the pork production chain were from spa-types associated with the clonal complex CC398 that is typically addressed as livestock associated MRSA. Among those, spa-types t011 and t034 clearly dominated with some regional differences concerning the prevalence of the two.

However, while in fattening pig herds and pigs at slaughter all isolates were from CC398, other spa-types were found occasionally in isolates from some breeding pig herds and from meat at retail. These types were also typed by multi-locus sequence typing (MLST) and assigned to the MLST-types ST39, ST9, etc. While in isolates from meat this finding might result from secondary contamination by workers carrying MRSA it is not clear, why MRSA of non CC398 types were identified in breeding pig herds.

Concerning the SCCmeccassettes identified some association between spa-types and SCCmec types were observed. Isolates from spa-type t011 nearly always carried the SCCmec type V. In contrast, isolates of spa-type t034 more frequently carried the SCCmec type which was assigned to type III by the method of Zhang (Zhang et al. 2005). This association could be observed in the fattening pig and the slaughter pig study.

Virulence factors and antimicrobial resistance

Investigations of virulence factors and resistance patterns have shown that MRSA ST398 from the pig production chain invariably carried only few known virulence factors (Argudin et al. 2011). In line with this, most isolates were derived from apparently healthy pigs and no association between disease occurrence and contamination of the herd with MRSA could be shown in a study in fattening pigs. In contrast, LA-MRSA have also been found as the only identifiable pathogen in lesions of pigs at necropsy. However, this was rarely observed and in some instances MRSA may have been a secondary pathogen rather than the causative agent. MRSA have also been identified in lymphnodes and internal organs of experimentally colonized pigs pointing out that they are not necessarily restricted to mucosal surfaces of the animals (Szabo et al. 2011).
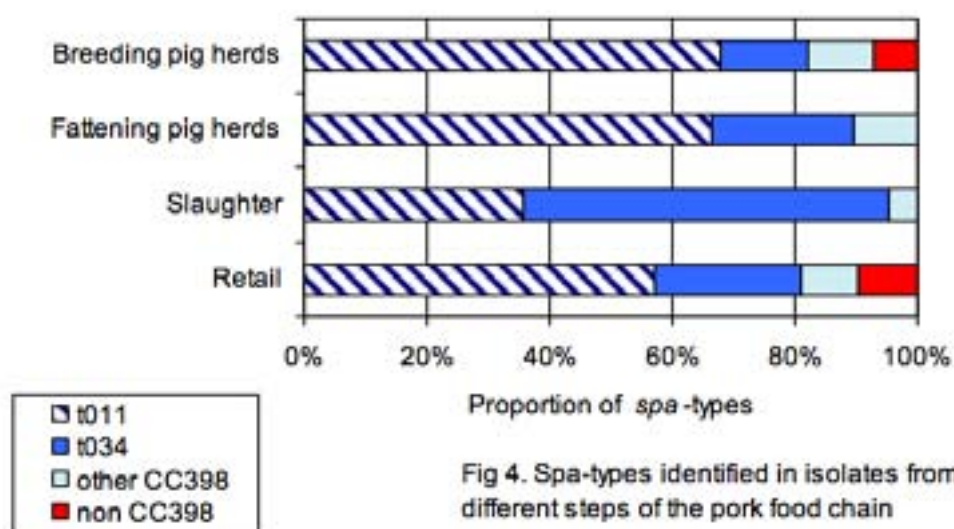
Fig 4. Spa-types identified in isolates from different steps of the pork food chain

In addition to the resistance to beta lactams, resistance to tetracycline, lincosamides, and macrolides are common (Tenhagen et al. 2009b). Remarkably, the resistance pattern is quite different from those observed in hospital associated strains in Germany (Robert Koch Institut 2007). While resistance to fuoroquinolones is common in the latter it is still rare in strains from pigs. Resistance to tetracycline on the other hand, is rare in human hospital acquired strains in Germany, while it is carried by basically all pig strains.

Conclusions

MRSA are widespread in the pig production chain in Germany. Most of the isolates are from the clonal complex 398 and do not carry major virulence factors. Resistance patterns differ substantially from isolates of MRSA-types frequently observed in the hospital setting in Germany.

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Reduction of antibiotics after implementing PCV2 vaccination on 460 sow Dutch pigfarm

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Abstract

The antibiotic use in the food producing business in the Netherlands is one of the highest in the EU and has great governmental attention. Vaccination can play a vital role in the reduction of the use of antibiotics and at the same time improve the technical performance. Production data of a 460 sow farm was retrospectively reviewed from January 2008 until December 2009. Begin 2008 the farm expanded their fattening unit from 1900 to 3500 places. At the same time with this expansion, big health problems were seen in the fattening unit, resulting in a high number of runts, mortality, lung problems and big difference in uniformity. These problems did not resolve although a lot of antibiotics were used. In August 2008 PCV2 was diagnosed as primary agent by multiple necropsies in pigs of 12 to 17 weeks of age and positive convalescent serology. Ingelvac CircoFLEX® vaccination (1 ml) was implemented (pigs from 15 weeks backwards to 5 weeks of age were vaccinated at start; from that point pigs from 5 weeks of age for convenience reasons). Continuous flow data was used for evaluation, on monthly basis: 8 months before vaccination- transition period of 4 months – 12 months of vaccinated pigs. A clear improvement was seen in growth (654 vs 747 vs 834 g/d) and a reduction of mortality (4,39 vs 4,9 vs 2,20 %). The use of antibiotics was measured by Defined Daily Dosages (DDD), the standard method used in the Netherlands to compare antibiotic use in time, and between farms. At the same time the production parameters improved, the amount of antibiotics used reduced strongly by -39% in the fattening unit (49,87 vs 45,12 vs 30,27 DDD). The vaccinated pigs of the last 8 months had a further improvement in growth, mortality and antibiotic use (18 DDD).

Introduction

The antibiotic use in the food producing business in the Netherlands is one of the highest in the EU (1,2) and has come under greater governmental attention the last years (3). Vaccination against different diseases can play a vital role in the reduction of the use of antibiotics and at the same time improve the technical performance, resulting in a better economical payoff for the primary producer (4, 5). For PCV2 it is known it can have an immunosuppressive effect (6) and as a result of this secondary (bacterial) infections can have a bigger impact. This study looks at the technical performance and the antibiotic use in the fattening unit following the introduction of a PCV2 vaccine in the nursery.

Material and Methods

Production data of a 460 sow farm was retrospectively reviewed for the period January 2008 until December 2009. Begin 2008 the farm expanded their fattening unit from 1500 to 3500 places (closed herd). At the same time with this expansion, big health problems were seen in the fattening unit, resulting in a high number of runts, mortality, lung problems and big difference in uniformity. These problems did not resolve although a lot of antibiotics were used. In August 2008 PCV2 was diagnosed as primary agent by multiple necropsies in pigs of 12 to 17 weeks of age (with high viral load) and positive convalescent serology. Immediately Ingelvac CircoFLEX® vaccination (1 ml) was implemented (pigs from 15 weeks backwards to 5 weeks of age were vaccinated at start; from that point pigs from 5 weeks of age for convenience reasons). Continuous flow technical data was used for evaluation, on monthly data basis: 8 months before vaccination-transition period of 4 months – 12 months of vaccinated pigs. The following parameters were evaluated over time: average daily gain (ADG), mortality and antibiotic use, over the 3 defined periods. The use of antibiotics was measured by Defined Daily Dosages (DDD), the standard method used now in the Netherlands to compare the antibiotic use in time, and between farms (2, 7, 8).

Results

For the 3 consecutive periods there was an increase of the growth (654 vs 747 vs 834 g/d) and a reduction of mortality (4,39 vs 4,9 vs 2,20 %; Figure 1). This was also reflected in clinical healthier pigs, with improved uniformity at time of slaughter. At the same time the production parameters improved, the amount of antibiotics used reduced strongly by -39% (49,87 vs 45,12 vs 30,27 DDD; figure 2). The vaccinated pigs of the last 8 months had a further improvement in the antibiotic use (18 DDD) compared with the first batch of vaccinated pigs after start.

Figure 1: ADG (g/day) and % mortality on monthly basis before and after implementation of CircoFLEX®
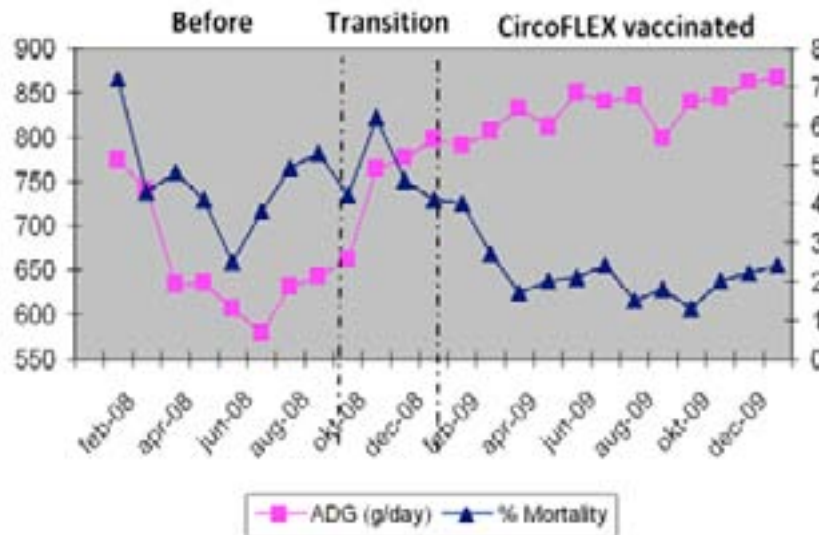
Figure 2: Antibiotic use (DDD) on 4 months period before and after implementation of CircoFLEX®
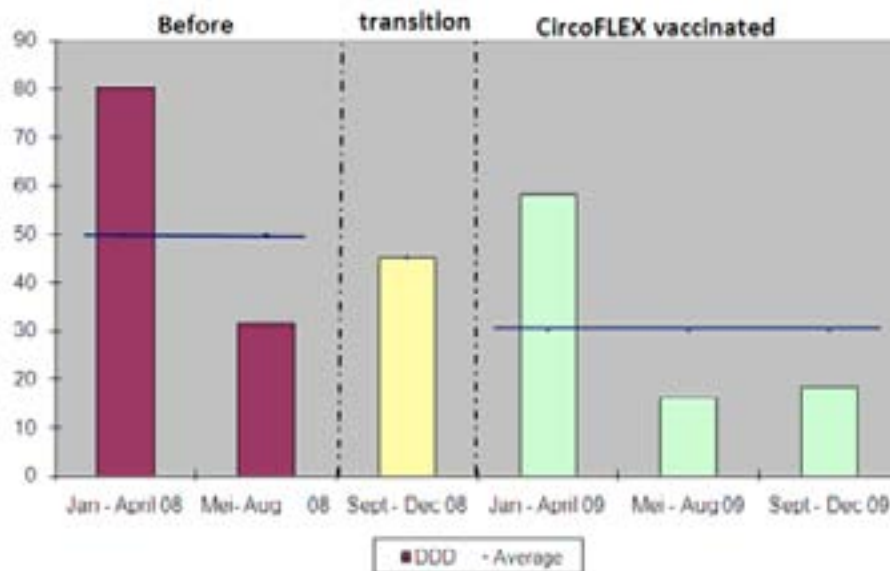
Discussion and conclusion

For PCV2 it is known it can have an direct impact on the technical performance of the pig but also, as it can have an immunosuppressive effect (6) and as a result of this, secondary (bacterial) infections can have a bigger impact. The introduction of PCV2 piglet vaccination with Ingelvac CircoFLEX resulted in a clear improvement of the technical results, improving average daily gain and reducing mortality. At the same time, comparing the 8 month before vaccination with 12 month after vaccination was implemented, antibiotic usage was reduced by 39%. This decline in antibiotic usage might be explained by a reduced impact of secondary bacterial infections in PCV2 vaccinated pigs. Including the transition period, data was reported for 24 month. The antibiotic use fluctuates over time, being related to a seasonal effect affecting this farm, with a higher incidence of coughing (mainly due to APP) beginning of the year (January to April). For

each 4 month period observed the use of antibiotics was lower in vaccinated animals compared to the period before, demonstrating the consistent efficacy of vaccination independent of the seasonal effect. The results on this farm are in line with other reports (9,10) showing that by controlling PCV2 with vaccines can help in reducing the use of antibiotics and are a useful tool for the farmer to produce safe food, demanded by the consumers and politics.

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Implementing PCV2 vaccination resulting in reduction of antibiotic use on Dutch farrow-to-finish farm

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Abstract

The antibiotic use in the food producing animals is of a growing concern for consumers, human health care, politicians and retail. Also the food producing sector itself is looking for (economical) alternatives for these treatments. One of the tools of reducing antibiotics are vaccinations. Production data of a 500 sow farm with 1900 fattening places was retrospectively reviewed for the period January 2009 till December 2010. The fattening unit had a history of diarrhea (Salmonella and Brachyspira negative, Lawsonia positive). Other clinical signs included an increased number of runts, pigs growing apart, and a high mortality (including euthanasia). There were no lung problems involved. The general treatment was to medicate with tiamulin on a regular basis in the fattening unit. Investigation on blood samples from several runts (mid fattening), showed high levels of PCV2 virus load. In July 2009 the farm started with vaccinating Ingelvac CircoFLEX® (1ml) at 3 weeks of age. Continuous flow data of the fatteners was used for evaluation: 8 months before vaccination (total of 2869 pigs) were compared to 12 months in which only vaccinated pigs were present on the farm (5933 pigs). The transition period lasted from September to December (1944 pigs) with vaccinated and non-vaccinated being present in the finishing unit at the same time.. The mortality was reduced by 46 % (4,03 vs 2,15%), comparing non-vaccinated versus Ingelvac CircoFLEX® vaccinated pigs; the health status and uniformity was improved (less runts), so less pigs needed to be transferred to another (younger) compartment. Also very evident was the reduction in antibiotic use by 85 % (40,61 vs 6,47 Defined Daily Dosage). These results suggest that there are situations where PCV2 vaccination can decrease the use of antibiotics and improve the production and economical performance.

Introduction

The antibiotic use in the food producing animals is of a growing concern for consumers, human health care, politicians and retail (1, 2). Also the food producing sector itself is looking for (economical) alternatives for these treatments. The Netherlands is among the countries with the highest antibiotic use in food producing animals in the EU (3, 4). Recently the Dutch government issued the goal of a 50% reduction on the use of antibiotics by 2013 compared to 2009 (5). One of the tools of reducing the usage of antibiotics are preventive measurements such as vaccinations. The objective of this study was to evaluate the effect of a PCV2 vaccination on the antibiotic use under field conditions.

Material and Methods

Production data of a 500 sow farm with 1900 fattening places was retrospectively reviewed for the period January 2009 till December 2010. The fattening unit had a history of diarrhea (Salmonella and Brachyspira negative, Lawsonia positive). Other clinical signs included an in increased number of runts, pigs growing apart, and a high mortality (including euthanasia). There were no lung problems involved. The general treatment was to medicate with tiamulin on a regular basis in the fattening unit. Investigation on blood samples from several runts (mid fattening), showed high levels of PCV2 virus load. In July 2009 the farm started with vaccinating Ingelvac CircoFLEX® (1 ml) at 3 weeks of age. Continuous flow data of the fatteners was used for evaluation. 8 months before vaccination (total of 2869 pigs) were compared to 12 months in which only vaccinated pigs were present on the farm (5933 pigs). The transition period lasted from September to December (1944 pigs) with vaccinated and non-vaccinated being present in the finishing unit at the same time. The parameters mortality and antibiotic use were monitored. For evaluation and comparison of the antibiotic usage in time, the standardized method of Defined Daily Dosage (DDD) of antibiotics used per animal year was applied (4, 6, 7).

Results

The mortality was reduced by 46 % (4,03 vs 2,15%; fig 1), comparing non-vaccinated versus Ingelvac CircoFLEX® vaccinated pigs. For the vaccinated pigs it was not needed to treat them with antibiotics for diarrhea anymore and as a result of this there was a reduction in antibiotic use by 85 % (40,61 vs 6,47 DDD per animal per year; fig 1).

Figure 1: Mortality (%) and average DDD per animal year in the fattening unit for the 6 periods of 4 months
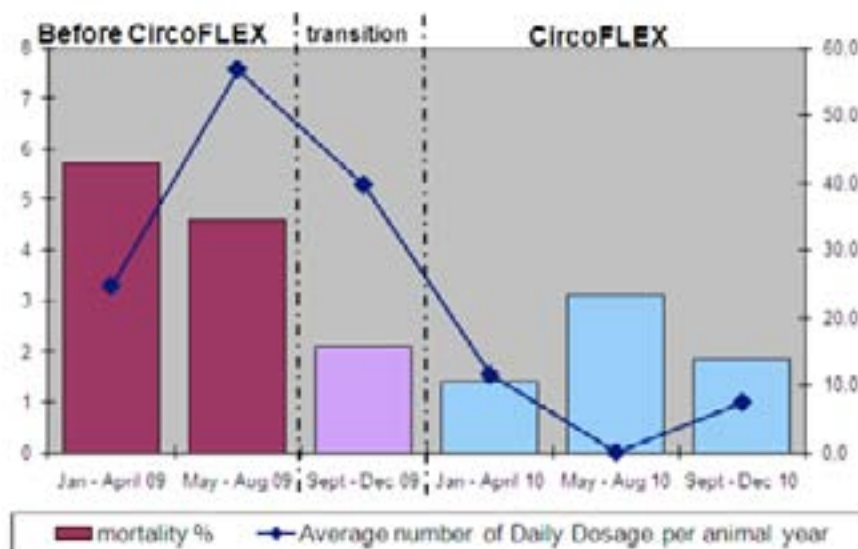
Discussion and conclusion

This retrospective analysis of a Dutch pigfarm demonstrates that the use of a 1 dose PCV2 vaccine around weaning can improve performance and improve animal welfare. The health status of the farm and the uniformity of the pigs improved (less runts), so less pigs needed to be transferred to another (younger) compartment or euthanized. These results suggest that there are situations where PCV2 vaccination decreases the use of antibiotics and improve the production and economical performance. Similar findings are confirmed in other reports (8,9).

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Significantly reduced use of antimicrobials with PCV2 and ileitis vaccination in a Danish herd

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Abstract

The present study evaluated, whether vaccination against both PCV2 and Lawsonia intracellularis in the same herd could be economically beneficial for the farmer and at the same time reduce the antibiotic consumption. The study was carried out in a wean-to-finish herd receiving 1000 weaned pigs every 7th week. The herd experienced lowered average daily weight gain (ADWG), increased feed conversion rate (FCR), and high mortality in the finishers, and use of group medication with antibiotics for treatment of clinical disease was frequently neccesary. Based on analysis of blood samples from several age groups of pigs, vaccination against PCV2 and ileitis (Lawsonia intracellularis) was initiated. Data for antibiotic use and performance was collected for one year before and after vaccination. The data showed that the antibiotic consumption, given as daily doses per produced pig, was reduced by 39% in the weaners and by 59% in the finishers. In the finishing unit, ADWG was increased with 44 g/day, FCR was reduced with 0.21 feeding units/kg gain, mortality was reduced with 1.79%, and antibiotic expenses was reduced with 1.15€/produced pig (p-values ANOVA 0.0133, 0.0005, <0.0001, and 0.0156). Economic evaluation showed a return on investment of 1:2.5. Thus, vaccination against PCV2 and ileitis was economically beneficial and reduced antibiotic consumption significantly.

Introduction

The typical way to evaluate the value of pig vaccinations is to calculate the impact on the gross margin for the producer, but other parameters might influence the choice of disease control strategy. In Denmark, reduced use of antibiotics for pigs has high political priority, and even though Danish pig producers use a low amount of antibiotics compared to many other countries (1), Danish producers experience pressure from the public to reduce this amount. The use of vaccines to prevent disease before the pigs need treatment with antibiotics is a way to comply with the political demands, providing that the use of vaccines does not lower the gross margin for the producer.

The use of antibiotics are significantly lowered in herds vaccinating against ileitis (2) or PCV2 (3), and vaccination against either ileitis (4) or PCV2-virus (5) has proven to be efficient tools to increase performance. This study was designed to examine whether vaccination against ileitis and PCV2 in the same herd could significantly reduce the use of antibiotics and still be economically beneficial.

Figure 1. Before vaccination: 1-2 pigs per pen started to waste in the end of finishing Materials and methods

Materials and methods

The study herd was a wean-to-finish herd. The herd received 1000 4 week old SPF weaners every 7th week, and they were moved to a finishing unit 7 weeks later. Antibiotics were used only when indicated by the clinical status of the pigs.

The study was a before-after study comparing selected parameters for the herd before and after initiation of vaccinations against PCV2 and ileitis. The vaccines were implemented based on clinical observations in the herd (fig. 1) and analyses of blood samples. The analyses showed moderate to high values for PCV2 in PCR analysis and seroconversion for Lawsonia intracellularis in the finishing period.

For description of the herd before and after, production parameters and the amount of antibiotics prescribed was collected. Efficacy reports was prepared every 7th week after emptying of the nursery, and data for antibiotic prescriptions were obtained from the Vetstat database. Vetstat data were collected 1 year before and 1 year after start of vaccination, leaving out 2 months as transition period, where both vaccinated and non-vaccinated pigs were present in the barn. Comparison of before and after was done with ANOVA (ADWG, FCR and antibiotics) or Fishers Exact test (mortality), using p 0.05 as significance level. Key values from the Danish Pig Producers (6) were used for economical calculations.

Results

The number of daily doses of antimicrobials for vaccinated pigs was reduced with 39% in the weaning unit and with 52% in the finishing unit after start of vaccination. Details regarding the choice of antibiotics showed that the reduction mainly was seen in oral medication, both in the weaning unit and in the fattening unit. The products used for the finishers before and after vaccination is shown in figure 2.

Comparison of performance before and after vaccination showed no differences in the weaning unit, but in the finishing unit, a significant improvement was seen regarding ADWG, FCR and mortality (table 1). The development in performance of the finishers is illustrated in fig. 3 and 4. The return on investment was 1:2.5, meaning that one € spent on vaccine was paid back 2.5 times in improved production and saved antibiotic expences.

Mean/period	Non-vacc	Vacc.	Diff.	Value (€/pig)
ADWG (g/day)	928	972	+44*	0.59
FCR (FE/kg)	2.85	2.72	-0.13*	2.43
Mortality (%)	3.4	1.6	-1.79*	1.34
Antibiotics (€/pig)	2.18	1.02	-1.15*	1.15
Total value		5.50 €/pig		

*= Values with a significant difference (p≤0.05).
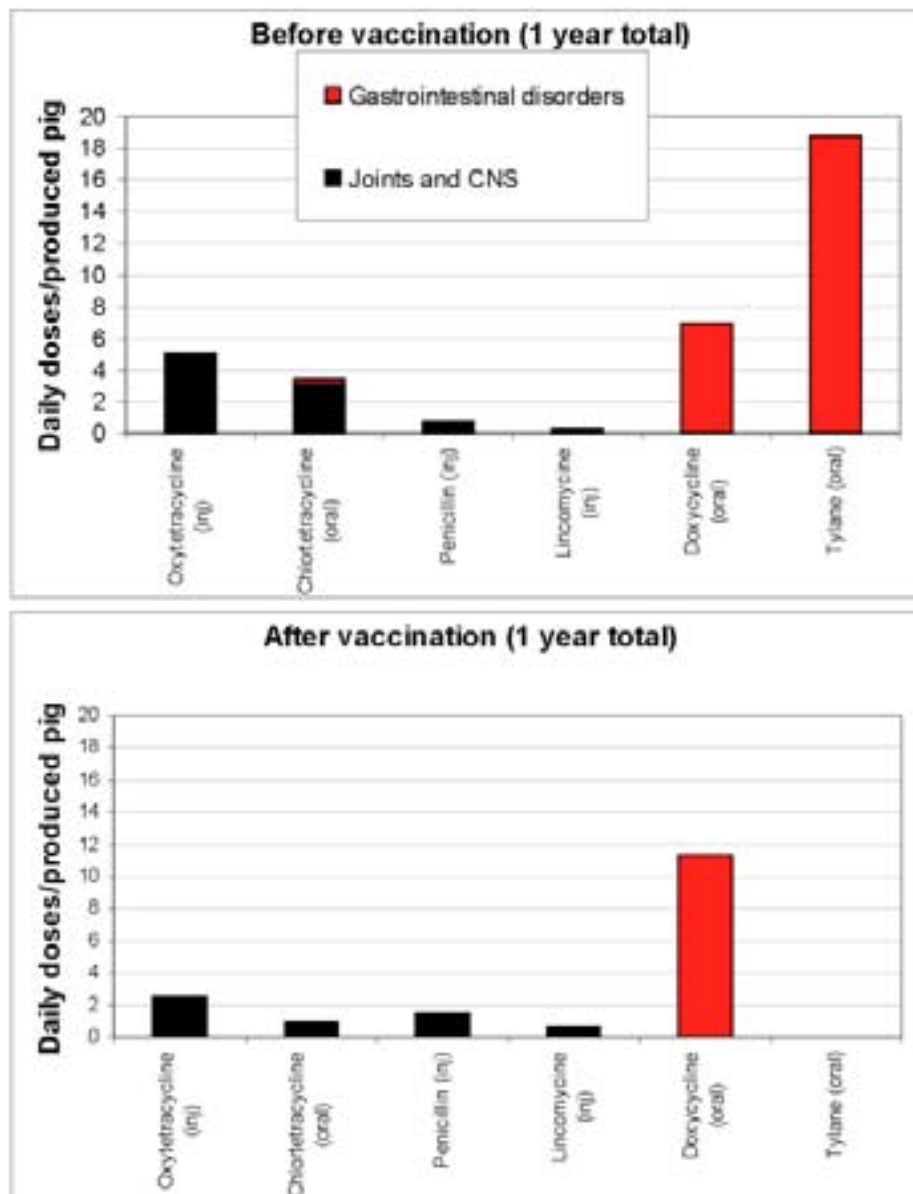
Figure 1: Antibiotic use in finishers without or with vaccination against PCV2 and ileitis
The total amount was reduced by 59% in the year before vaccination compared to the year after.

Discussion

A large part of the antibiotics used to treat herds infected with PCV2 and ileitis will be given to all pigs in the herd. This was reflected in the results of this study, where the main reduction in the number of daily doses per produced pigs was seen in oral medication. However, injections given to individual pigs were also reduced. Though this will affect the overall antibiotic use to a lower extent, it is still important to the farmer, because it will reduce the daily workload for identifying and treating diseased pigs.

For the production parameters, an error in the feed composition disturbed the calculations. In a short period after the transition period, the pigs were fed 13% less protein than expected, and this had a negative influence on the average daily gain (ADWG) and the feed conversion rate (FCR) for the first two periods with vaccinated pigs, as seen in figure 3. Therefore, these two observations were excluded from the statistical calculations regarding these two parameters. The mortality was not affected by the erroneous feed composition, but decreased immidiately as a response to vaccination against PCV2 and ileitis (fig. 4).
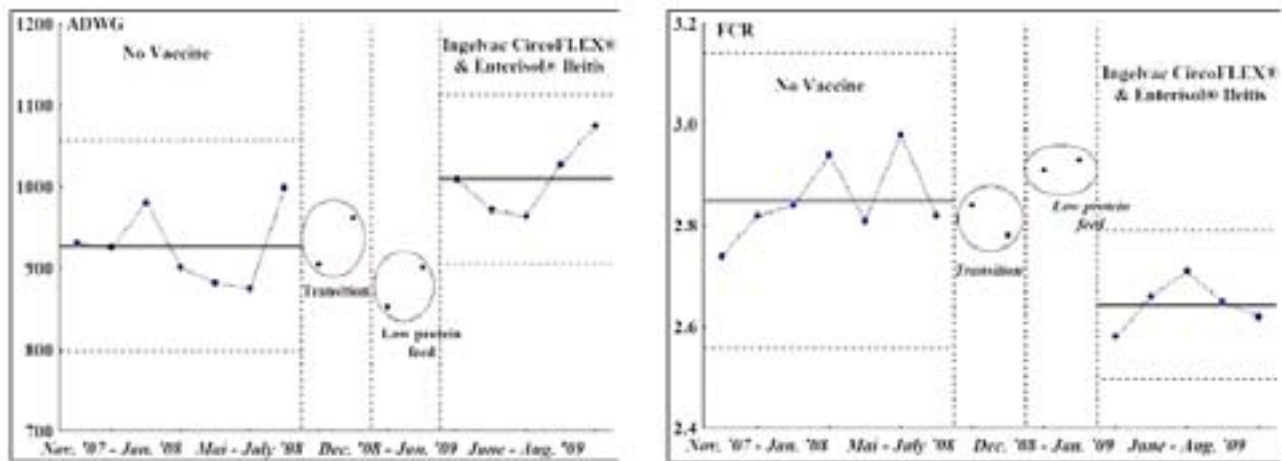
Figure 3: ADWG and feed conversion rate in finishing unit before and after vaccination against PCV2 and ileitis. Each point on the graph represents one efficacy report covering 2000 finishers
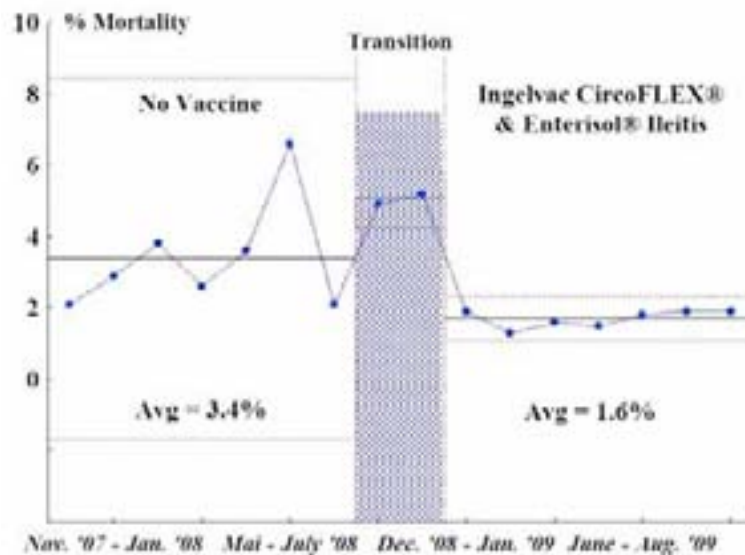
Figure 4: Mortality in finishing unit before and after vaccination against PCV2 and ileitis.
Each point on the graph represents one efficacy report covering 2000 finishers

Conclusion

Vaccination against ileitis and PCV2 in the same herd successfully decreased the need for antibiotic treatment, especially regarding oral medications. With this reduced use of antibiotics, the vaccinated pigs grew faster and had a better FCR than non-vaccinated pigs getting more antibiotics, thus demonstrating, that prevention (vaccination) is better than cure (antibiotics), also from an economical point of view.

Hence, the use of vaccines can help pig producers to maintain a sustainable production system, by improving the gross margin per pig at the same time as the use of antibiotics is decreased to meet the increasingly higher demands from politicians and consumers.

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Activity of Sangrovit® against Lawsonia intracellularis in grower pigs and its impact on gut physiology and host immunity

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Abstract

Sanguinarine, a quaternary benzophenanthridine alkaloid plant extract of Macleya cordata, has demonstrated to have anti-inflammatory, antimicrobial and immunomodulatory effect. It increases the availability of aromatic amino acids and decreases the levels of toxic biogenic amines. This study was aimed to evaluate the effect of Sangrovit® supplementation as compared to tylosin on growth performance, feed efficiency and Lawsonia intracellularis shedding in pigs, and to determine the effect of Sangrovit® on the immune system. A total of 24 pigs, four weeks-old challenged with Lawsonia intracellularis were randomly allocated to a treatment group (control non-supplemented, 40 g Sangrovit®/mton, 75 g Sangrovit®/mton, and 22g /kg tylosin). Pigs were weighed weekly and average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were calculated. Fecal samples were collected weekly for isolation and quantification of Lawsonia intracellularis using qPCR as well as blood samples for determination of IgA and IgG levels. After 21 days (acute phase), three pigs from each treatment group were euthanized for recording of lesions of the acute phase of the disease; the remaining 12 pigs were euthanized 90 days after challenge (chronic phase). Results showed that ADG and ADFI was higher for pigs receiving tylosin as compared to the other groups (p>0.05). Pigs receiving 75 gr. Sangrovit®/mton showed a higher G:F ratio as compared to the other groups (p>0.05). None of the treatment groups showed significant differences in Lawsonia shedding level based on quantitative PCR. Only control group presented characteristic lesions of Lawsonia infection at the acute stage of the disease (21 days). At the chronic stage, the highest ileum thickness score was observed in pigs receiving tylosin. Findings suggest that Sangrovit® supplementation is effective for improving growth performance and reducing pathognomonic lesions. Further studies are needed to determine the effect of Sangrovit® on the immune system.

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The effect of the application of mono-lauric acid with glycerol mono-laurate in weaned piglets, on the use of antimicrobials in sow herds

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Abstract

The Dutch government has obliged the pig industry to reduce the use of antimicrobials at farm level with 50% by 2013. The search for alternatives for antimicrobials and other tools which can improve the health status of the farm is intensified. One example of an alternative for antimicrobials is Daafit, a combination of lauric acid and glycerol-mono-laurate, produced by the firm Daavision B.V.. Daafit is used by Veterinary practice Lintjeshof to increase the health of pigs, specifically weaned piglets at a dose of 1 kg per ton dry feed. The weaned piglets are supplemented with this additive during the entire weaning period (7 – 25 kg body weight).

Veterinary Practice Lintjeshof has compiled a dataset with the DD/AY (Daily Dose per Animal Year) of 33 test farms which used the additive Daafit and 29 control farms which did not use the product. Data analysis by the Veterinary practice Lintjeshof showed that the DD/AY of antimicrobials on sow farms who used Daafit was lower when this product was used compared to other sow farms within Veterinary Practice Lintjeshof. To investigate whether this effect was statistically significant, the Animal Health Service was asked to analyze this dataset. The change in the DD/AY from the period before and during the use of Daafit was calculated for both test and control farms (delta-DD/AY). The dataset showed a significant difference between the delta-DD/AY for the sow farms that used Daafit in the weaned piglet feed in comparison with farms where Daafit was not used. The DD/AY was reduced with approximately 8 days on the test farms while the DD/AY on the control farms remained the same. These results indicate that Daafit might help reduce the use of antimicrobials in sow herds.

Introduction

After years of an increasing use of antimicrobials on pig farms the Dutch government obliged the pig industry to reduce the use of antimicrobials by 20% in 2011 and by 50% by 2013. This obligation triggered the increasing interest in alternatives for antimicrobials such as mono-laurate. Mono-laurate is a medium chain fatty acid with antimicrobial properties against a wide range of microbes (http://en.wikipedia.org/wiki/Glyceryl_laurate). The Dutch firm Daavision B.V. (www.daavision.com) produces a product called Daafit which is a mixture of lauric acid and glycerol-mono-laurate. This product is used in a dose of 1 kilogram product per 1000 kilogram of compound feed in weaned piglets. Aim of this additive is to reduce the number of bacteria circulating among these weaned piglets, especially Streptococcus suis. As a result of a lower number of circulating bacteria fewer treatments with antimicrobials are necessary, resulting in a smaller number of Daily Doses per Animal Year (DD/AY) per herd.

Material and methods

Veterinary Practice Lintjeshof has compiled a dataset where the DD/AY (Daily Dose per Animal Year) of 32 test farms and 29 control farms is calculated.

The DD/AY was calculated according to the guideline given by the Veterinary Pharmacy of the Faculty of Veterinary Medicine at Utrecht University as described in the MARAN report 2009.

Quote page 14 of MARAN 2009: "For example, a farm with 150 fattening pigs with an average weight of 70.2 kg used 2 litres of antibiotic preparation X during the course of one year (X contains 40% = 400 mg/ml active ingredient a) and 20 kg of antibiotic preparation Y (Y contains 25% = 250 mg/g active ingredient b). Antibiotic preparation X: the defined daily dosage of active ingredient is 10 mg per kg animal weight per day. Antibiotic preparation Y: the defined daily dosage of active ingredient is 50 mg per kg animal weight per day.

Antibiotic X can be used to treat (2,000 * 400)/10 = 80,000 kg animal weight. Antibiotic Y can be used to treat

(20,000 * 250)/50 = 100,000 kg animal weight. Consequently, the farm has used antibiotics for treatment of a total of 180,000 kg animal weight. The farm has an average of 150 fattening pigs per year, with a total weight of 10,530 kg. 180,000 kg were treated in that year, equivalent to 180,000/10,530 = 17.1 daily dosages. Consequently, an average fattening pig on the farm in that year was administered a prescribed dosage of antibiotics on 17.1 days. In this example the farm uses 17.1 daily dosages per animal year of antibiotic preparation X plus Y."

For two swine categories standard body weights have been set: for sows and for fatteners/finishers. To calculate the number of kilograms animal present at a farm one needs to know the number of sows, weaned piglets, maiden gilts and boars and multiply by the respective standard body weight (www.antibioticawijzer.nl).

The DD/AY has been calculated for the category sows during the period that Daafit was being supplied and a period before that had the same length. The period during which the supplement was added varied from 4 to 6 months. The DD/AY was calculated with the help of a calculating module developed by the Agricultural Economic Institute (LEI), based on the rules of the pharmacy from the Faculty for Veterinary Medicine as described above.

The dataset supplied to the Animal Health Service contained the following fields: farm identification, number of sows, piglets, gilts and fattening pigs , whether there was being vaccinated or not against PCV2, PRRS, Mycoplasma Hyopneumoniae or with a autogenous vaccine against Streptococcus Suis and also the DD/AY in the period before and during the use of Daafit . De change in DD/AY was calculated by subtracting the DD/AY-before from the DD/AY-during. A reduction in DD/AY would show as a negative value. Statistical analysis was done using Statistix 8.0 and Stata/SA 11.2 for Windows.

Results

The control farms had overall more sows than the test farms , the median for the test farms was 440 sows and for the control farms 685 sows. This difference is statistically significant (Statistix 8.0, Median test, P=0.03). The DD/AY of the periods before the use of Daafit in the test and control farms were comparable (Statistix 8.0 , Wilcoxon rank sum test , P=0,97).

The descriptive statistics of Delta DD are presented in table 1 and figure 1.

Initial inoculum CFU/10gr	Method	1		2		3		4	
	Name	InviMag Stool DNA		QIAmp DNA Stool		Lysis reagent		Extraction DNA mix	
	Pretreatment	*	**	*	**	*	**	*	**
24 CFU/10gr		148	102	8	2,2	200	200	200	200
81 CFU/10gr		82	110	5	2,3	200	195	200	199
136 CFU/10gr		184	91	4	1,8	200	185	200	199

Table 1. Descriptive statistics of DD/AY before and during use of Daafit and delta-DD/AY for test and control farms
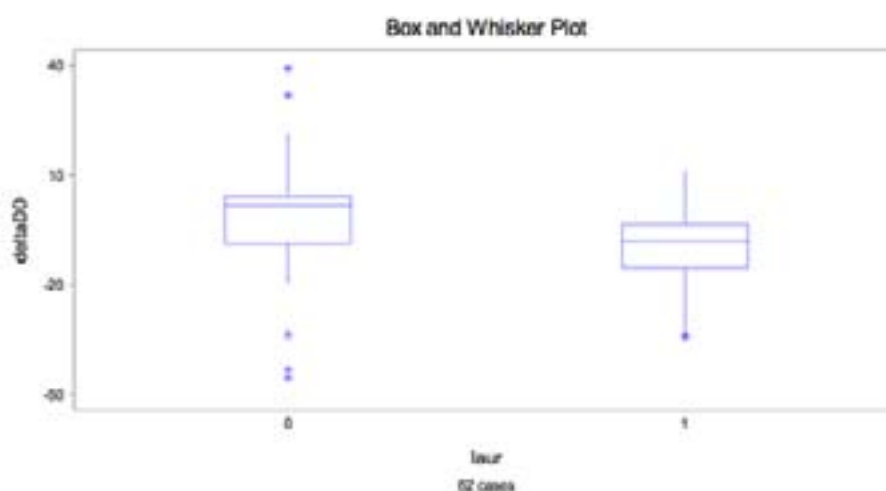
Figure 1. Box and Whisker plot for the delta-DD/AY for test (1) and control (0) farms.

The Two sample Wilcoxon rank-sum (Mann-Whitney) test of delta-DD/AY for test and control herds showed a significant difference (z=2.674, P=0.0075).

Discussion
This method of data-analysis in which antimicrobial use in the periods before and during the application of a certain product in test and control farms are being compared with one another, can only be seen as an indication of the efficacy of this product. In the test design and the statistical analysis we did not correct for the many factors which might have an influence on the change of DD/AY e.g. herd size. Also, the test design was not randomized and blind so a "placebo effect" can not be ruled out in this study. This is why a definite conclusion on the causality between the use of Daafit and the improvement of the DD/AY based on these data is not possible. However, these data show that the effect of Daafit on the reduction of DD/AY in the test herds can not be ruled out and that Daafit might help in the reduction of the use of antimicrobials on sow farms.

Conclusion
The data show a significant reduction in the use of antimicrobials (daily dosage per animal year) in the herd category sows in the period before and during the use of Daafit in the feed of the weaned piglets , in comparison with farms were Daafit was not used where this reduction did not occur. The daily dose per animal year was reduced by approximately 8 days in the test herds.

Acknowledgements

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Administration of drinking water supplement containing organic acids and medium chain fatty acids to sows significantly reduced incidence of Clostridium-associated diarrhoea in neonatal piglets: A case study

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Abstract

Neonatal diarrhoea in newborn piglets is an important problem in pig production that is frequently diagnosed as being the result of Clostridium perfringens infections. During parturition and in the first hours of life, the sow transmits the pathogen to its offspring. The objective of this study was to examine the possible prophylactic effect of a drinking water supplement containing organic acids and medium chain fatty acids (Selko-4-Health©), administered to sows on prevalence of neonatal diarrhoea in piglets during early lactation. The study was carried out at a farm with 1300 sows with a high incidence of neonatal diarrhoea. Gestating sows received the water supplement (0.1% per litre) daily from day 35 to end of gestation and during the lactation phase for 2 days a week. Excreta of sows were collected at day 0, 35, 56, 91 of gestation and at day 21 of lactation for microbial examination counting the numbers of Lactobacilli and Clostridium spp.. The number and type of veterinary treatments were recorded during the trial period. The numbers of Clostridium spp. in faecal samples of sows decreased progressively from day 0 to 91 in gestation from log 6 to log 4 cfu/g. Counts in faecal samples of lactating sows (day 21) decreased from log 6 to log 3 cfu/g. There was a pronounced decrease in the ratio Clostridium spp to Lactobacilli spp., indicating a more specific effect of the water supplements towards lowering Clostridium spp. counts whilst maintaining higher levels of Lactobacilli. Although there was no effect on mortality of piglets, the number of veterinary treatments of newborn piglets decreased during the trial period, leading to a total reduction in antibiotic usage of 60%. The improved health status of neonatal piglets was also associated with a reduction of meningitis incidence.

Drinking water supplement containing organic acids and medium chain fatty acids induces significant changes in the intestinal microbiota and lowers incidence of diarrhoea of piglets post-weaning

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Abstract

Antibiotic treatment of piglets post-weaning may lead to re-occurring diarrhoea after stopping the antibiotic treatment. The objective of the study was to test the efficacy of a commercial drinking water supplement containing organic acids and medium chain fatty acids (Selko-4-Health©) on diarrhoea control in piglets weaned at 26 days of age. In total 244 piglets were allocated at weaning to 4 treatments in a 2x2 experimental design for the duration of 4 weeks. Piglets received either a non-medicated feed, oxytetracycline medicated feed (400 ppm) during the first week post weaning and thereafter no medication, a drinking water supplement during the whole experimental period or the combination of the two treatments. Jejunal samples were taken of 4 piglets from each treatment at 2 and 4 weeks post-weaning to examine the intestinal microbiota with 16SRNA gene-targeted Denaturant Gradient Gel Electrophoresis, quantitative PCR and the Pig Intestinal Tract Chip (PITChip), a diagnostic microarray custom-designed for the profiling of porcine intestinal microbiota. None of the treatments significantly affected performance. Both, the antibiotic treatment as the water supplement treatment significantly lowered the incidence of diarrhoea in week 2 and 3 post-weaning and in the overall experimental period (p<0.05), with no significant interaction between these treatments. The microbiota assays revealed a shift of the microbial profiles in time. Overall, the organic acid blend as well as oxytretracyclin had a significant effect on weaned piglet gut microbiota, with observed changes in Lactobacilli spp. composition (DGGE) and microbial profiles analysed with the PITChip. The results demonstrate that drinking water supplements containing organic acids and medium chain fatty acids can be applied in strategies to establish a prudent use of antibiotics in control of diarrhoea in piglets. More information is needed to understand the impact of intestinal microbiota changes in relation to occurrence of diarrhoea in piglets.

Improvement in pig growth and feed conversion due to knowledge transfer about disease prevention and improving immune response

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Abstract

The ability of pigs to avoid illness can be influenced by pig farmers via management measures which prevent stress and introduction and spread of diseases. A training program consisting of three meetings, was developed to achieve more consciousness at pig farmers about their influence on disease prevention and the immune response of their pigs. We tested the effect of the training program on the average daily gain in weight, feed conversion, the mean percentage of lymphocytes and I-FABP values.

After one year, the farmers of the test group who joined three meetings, indicated that they achieved more insight in the points of action to improve the immune response of their pigs and prevent diseases as a result of the meetings. They also undertook significantly more improvements than farmers from the control group. On average the pigs of the farms in the test group had a better gut health than the pigs of the farms in the control group. The average daily gain in weight and the feed conversion of the pigs were significantly better from farms in the test group compared to the pigs from the farms of the test group, but only during the first three months after the meetings.

Introduction

The ability of pigs to avoid illness is affected by intrinsic factors (e.g. genetics) and by extrinsic factors (housing situation, feed, hygiene, temperatures, handling). By optimizing the extrinsic factors, less adaptive capacity is required to withstand health challenges, which means that more energy is left for growth and development. Many of the extrinsic factors are management factors, and can be controlled directly or indirectly by farmers (Boersma et al., 2005). A training program should give farmers more insight in their influence on the immune response of the pig and in disease prevention. This newly gained knowledge should also be taken over by the farmers to achieve effect. We tested the effect of a farmers training program on pig health, average daily gain in weight, feed conversion, the mean percentage of lymphocytes and I-FABP values.

Material and Methods

For one year seventeen pig farmers, the test group, attended a test which consisted of three meetings in which the farmers, together with their veterinarian, achieved knowledge about their possibilities to influence the immune response of the pig and to prevent diseases. Eighteen pig farmers, the control group, did not attend these meetings. The aim for the meetings of the training program was application of management measures for a better immune response of the pigs and to minimize disease introduction into the farm and transfer of disease within the farm. Before the first meeting, researchers visited the farms and filled in a questionnaire together with the farmers, to identify the points of improvement. The first meeting consisted of a) knowledge transfer and b) farmers discussing their farms points of improvement with other farmers at five themes (animal handling, feed and water, climate, pathogen burden/ hygiene/ vaccination, care of sow and piglet) to achieve more insight in the factors to be optimized at their own farm. Hereby, farmers knew the strong points of their farm and the points for improvement. During the second meeting, farmers answered their own formulated questions together with other farmers, veterinarians and pig researchers, to improve their farms. A plan of action was made with set deadlines during the third meeting. Farmers carried out this plan for at least half a year. The three meetings were held every four to six weeks.

Before the first meeting and six months after the third meeting (one year after the first visit to the farm), the percentage of lymphocytes and I-FABP (Intestinal Fatty Acid Binding Protein) was measured in blood taken from 30 fattening pigs of 50 kilogram. The percentages of lymphocytes indicates the disease resistance in general or the state of health at a certain

moment. I-FABP can be measured in blood when leakage of the intestine is present for example due to stress or changes in feed (Niewold et al., 2004). Average daily gain in weight of the pigs (ADG) and feed conversion ratio (FC) were measured every three months. An evaluation of the training program and the number of improvements taken by the farmer were achieved by means of a questionnaire. The difference on the number of improvements during the test period taken by the farmer was tested with a generalized linear regression model. The difference in increase or decrease of the percentage of lymphocytes and I-FABP between the test and control group was analysed with a linear regression model (Genstat8, 2005). The farms with changes in breeding strategy were excluded from the analyses. The difference in the development of ADG and FC was tested with a regression model.

Results

After one year, the farmers of the test group indicated that they improved their farm and that they achieved more insight in the points of action to improve the immune response of their pigs and prevent diseases as a result of the training program (figure 1).

Figure 1 Farm evaluation of the training program

The average number of improvements was significantly higher at farms of the test group (table 1).

Table 1 Average number of improvements per farm and per theme.

Theme	Test Group (n=16)	Control Group (n=17)
Average number of improvements per farm	16.4	7.4*
Improvements per farm		
Others (farm size, breed,..)	0.5	0.3 (n.s.)
Pig management	4.1	1.7 *
Feed and water	3.6	1.4 *
Climate	2.1	1.1 (n.s.)
Pathogen burden/ hygiene/ vaccination	4.4	2.2 *
Care of sow and piglet	1.1	0.8 (n.s.)

*: significance: $P<0.01$; n.s. = not significant ($p>0.05$)

No significant difference was found between the groups for the mean percentage of lymphocytes and the median of I-FABP value per farm (table 2). However, when analysing the 75%-quartiel of I-FABP, the pigs of the farms in the trail group showed a significant lower increase of I-FABP than the pigs of the farms in the control group ($p<0.05$).

Table 2 Mean percentage of lymphocytes and Median of the category I-FABP > 40 of farms in the test group and control group.

	Mean percentage of lymphocytes		Median of the category I-FABP > 40	
	Test group (n=9)	Control group (n=13)	Test group (n=9)	Control group (n=13)
Before first meeting	61.55	60.87	110%	100%
After one year	62.02	61.73	139%	173%
Development during one year	+ 0.47	+ 0.86	+28% (•)	+73%

*: significance: p-value < 0.10 (p=0.055)

The ADG (p<0.05) and the FC (p= 0.001) were significantly better for the test group compared to the control group, but only during the first three months after the meeting, 4-6 month after the first visit (table 3).

Table 3 Average Daily Gain and Feed Conversion Ratio for the test and control group during four periods of three months.

Period	Average daily weight gain (n=17)		Feed conversion ratio (n=17)	
	Test group (n=9)	Control group (n=8)	Test group (n=9)	Control group (n=8)
Month 1-3 (after first farm visit)	0	0	0	0
Month 4-6	30.2 (•)	0	-0.24(•)	0
Month 7-9	14.9	0	-0.18	0
Month 10-12	0	0	0.06	0
Significance (treatment x period)	P=0.18		P<0.001	

*: significance: P-value <0.05

Discussion

The meetings, the content and the frequencies were effective and useful. Farmers of the test group applied more management measures than the farmers of the control group, however farmers also changed type of antibiotics used and applied extra vaccination where we advised to find the cause leading to the use of antibiotics. This can be more emphasized during the meetings.

There was no difference between the test group and the control group concerning the percentage of lymphocytes. Possibly, the effects of management measures on pathogen burden can be expected after a longer period than one year. The pigs from farms in the test group showed a lower increase of I-FABP values than the pigs from farms in the test group which might indicate a better gut health of the pigs from farms in the test group.

The effect of the knowledge transfer on ADG and FC are only seen in the first 3 months after the last meeting. This can be the effect of the measurements taken just after the first meeting. Emphasize on management during the meeting period possibly resulted in improvement of ADG and FC, however after the first three months after the last meeting, habituation and less attention diminished the attention of the farmer and therefore the difference diminished.

Conclusion

A training program consisting of three meetings, gave farmers more insight in their influence on the immune response of the pig and in disease prevention. In this training program farmers achieved knowledge about their possibilities to nfuence the immune response of the pig and to prevent introduction and spread of diseases. This awareness resulted in more measures on management to improve their farms, a better average daily gain in weight and a better feed conversion, but only during the first three months after the meeting. No difference was found in the mean percentages of lymphocytes. The I-FABP level of the pigs from the test farms increased less than the I-FABP level of the pigs from the control farms, indicating a better gut health in pigs from the test farms.

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Antibacterial and antioxidant activity of oregano essential oil

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Abstract

The swine industry is investigating phytonutrients like oregano essential oil (OEO) because of its potent antimicrobial and antioxidant activity. These activities are attributed to OEO's most abundant polyphenols, carvacrol and thymol. Carvacrol and thymol have been shown to permeabilize and depolarize the bacterial cytoplasmic membrane, resulting in cell death. The objective of this study was to quantify the antimicrobial and antioxidant activity of OEO. Antibacterial activity was determined by testing for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of OEO for common livestock pathogens. A standardized microtiter protocol was used. Several bacteria were tested including Salmonella enteritidis, S. typhimurium, S. choleraesuis, Escherichia coli, Klebsiella pnemoniae, Streptococcus suis, and Staphylococcus aureus. Results showed that MICs for both gram-positive and gram-negative bacteria ranged from 1.25 to 10.0 µg/ml. MBCs were identical to the MIC showing bactericidal activity. Antioxidant activity of OEO and vitamin E (positive control) was determined by the oxygen radical absorption capacity (ORAC) value against five oxygen radicals: peroxyl radical, hydroxyl radical, peroxynitrite, superoxide anion, and singlet oxygen. Antioxidant testing showed that OEO had much higher level of total antioxidant activity (2,520,600 trolox equivalents/100g) than natural vitamin E (39,200). These results demonstrate that OEO has high antimicrobial activity for pathogens that cause swine disease. The very high level of antioxidant activity of OEO may protect enterocytes against inflammatory damage caused by reactive oxygen molecules that are released during immune system activation. OEO has several benefits for the swine industry: it is a safe and accepted feed ingredient, it has potent activity against gram-negative and gram-positive bacteria, and it does not leave residues in the environment. Synergistic activity has been demonstrated between OEO and common antibiotics. OEO when used alone or in combination with antibiotics will allow the producer to reduce antibiotic use.

Introduction

Oregano essential oil (OEO) is well known for its antimicrobial properties, as well as its antifungal and antioxidant actions. When harvested at the proper growth stage and steam extracted, oregano essential oil is a mixture of >30 different compounds. The major constituents, carvacrol (55-85%) and thymol (0-5-10%), have the most potent antimicrobial activity due to their phenolic structure. Mechanism studies have shown that carvacrol and thymol kill bacterial cells by altering the permeability of the cell membrane causing leakage of essential cations (1). Selectivity against Gram-negative bacteria but with lesser activity against Gram-positive Lactobacillus and Bifidobacterium has been observed (2). Antimicrobial activity of OEO has been demonstrated in different ways. Activity varies depending on the assay method, the source of oregano essential oil, and bacterial isolates tested.

Antioxidants are compounds that prevent damage to cells and tissues in the body. This is particularly important in the gut, which is continuously exposed to dietary and environmental challenges that can cause tissue damage. The gut is the first line of defense against enteric pathogens. When bacteria or viruses invade gut cells, the body responds with inflammation. Inflammation produces "reactive oxygen intermediates" or ROI's, which destroy disease-producing organisms. However, these ROI's are quite toxic and often cause unintended tissue damage. Antioxidants neutralize ROI's and other molecules that damage host tissues.

Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacities in biological samples. A wide variety of foods have been tested using this methodology, with certain spices, berries and legumes rated very highly. Correlation between the high antioxidant capacity of fruits and vegetables, and the positive impact of diets high in fruits and vegetables, is believed to play an important role in defense against many health conditions.

The objective of this study was to determine MIC and MBC of OEO for several swine and livestock pathogens using a standardized microtiter protocol. In addition, the antioxidant activity of OEO was determined by ORAC value and compared with vitamin E, a well known antioxidant which is routinely added to livestock feeds and topical products.

Materials and Methods
MIC and MBC were performed for a wide range of livestock and poultry pathogens at the University of Minnesota Udder Health Laboratory using a microtiter assay method following Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines (3).

OEO (Ralco Nutrition, Inc., Marshall, MN) was prepared as a stock solution and serially diluted by two-fold dilutions in Mueller Hinton broth from 20 μg/ml to 0.039 μg/ml. Positive (no oregano oil) and negative (no bacteria) controls were included in each microtiter plate. Pure cultures of each bacterium were prepared on agar plates. A standard bacterial suspension was prepared (i.e. 0.5 McFarland standard), bacteria were added to microtiter plates, and plates were sealed and incubated at 37°C. Each microtiter plate was run in duplicate. After 18h, wells were scored for bacterial growth (i.e. turbidity). The MIC was the lowest concentration of OEO showing no bacterial growth. After scoring for growth, one loopful of broth from each clear well was streaked onto a blood agar plate. Plates were incubated at 37°C and examined for bacterial growth after 18h. The MBC was determined as the lowest oregano essential oil dilution showing no growth on plates.

Antioxidant activity of OEO and vitamin E, a well known antioxidant, was assessed using the oxygen radical absorptive capacity (ORAC). ORAC values are expressed as trolox equivalents (TE). Trolox is a vitamin E analog (Hoffman LaRoche) with high antioxidant activity. ORAC values are given as micromoles TE/g or micromoles TE/100 g. Anti-oxidant activity was measured against five (5) oxygen radicals: (1) peroxyl radical; (2) hydroxyl radical; (3) peroxynitrite; (4) superoxide anion; and (5) singlet oxygen. Results are given as a sum of the activity against these five ROI's (total ORAC).

Results
MIC and MBC
Table 1 shows that MIC for all pathogens ranged from 1.25-10.0 μg/ml. MBC ranged from 1.25-10 μg/ml, confirming prior reports that OEO is bactericidal for the bacteria tested. The killing activity of OEO has been attributed to the action of carvacrol on the bacterial membrane (1).

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oregano essential oil for common livestock pathogens.

Bacteria	Source	MIC (μg/ml)	MBC (μg/ml)
E. coli K88	Swine	2.5	2.5
E. coli F88	Swine	2.5	2.5
E. coli O157:H7	Cattle	2.5	2.5
E. coli	Mastitis (cow)	2.5	2.5
E. coli	Mastitis (cow)	5	5
Klebsiella pneumoniae	Mastitis (cow)	5	5
Klebsiella pneumoniae	Mastitis (cow)	2.5	2.5
Streptococcus suis	Swine	5	5
Listeria monocytogenes	Cow	5	5
Salmonella typhimurium	Swine	5	5
Salmonella choleraesuis	Swine	5	5
Salmonella enteritidis	Poultry	5	5
Staphylococcus aureus	Mastitis (cow)	5	5
Streptococcus agalactiae	Mastitis (cow)	5	5
Streptococcus uberis	Mastitis (cow)	5	5
Pseudomonas aeruginosa	Human	10	10
Bacillus cereus	Environment	1.25	1.25

Total ORAC values for Ralco's OEO oil and common high antioxidant foods are given in Table 2. Results are given as ORAC value per 100 grams. Results showed that OEO has the highest ORAC value of the tested ingredients as well as one of the highest ORAC values in the databases listed.

Table 2. Antioxidant activity for oregano essential oil, vitamin E and other foods with known antioxidant activity.

Substance	Total ORAC (μmoles TE/100 g)
Red grapes – raw[a]	1,206
Raspberries - raw[a]	4,882
Red wine[a]	5,034
Cranberries – raw[a]	9,584
Dark chocolate candy[a]	20,823
Natural vitamin E	39,200
BHT[a]	72,000
Cinnamon[a]	267,536
Oregano essential oil	2,520,600

[a] ORAC values were obtained from one of these websites: http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/ORAC/ORAC07.pdf
[b] http://www.nutritiondata.com

Discussion

In this study, we used a validated microtiter assay to investigate the antibacterial activity of OEO. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for several swine and other livestock pathogens, including Escherichia coli, Salmonella enteritidis, S. typhimurium, Klebsiella pnemoniae, and Staphylococcus aureus. Results showed that MIC and MBC ranged from 1.25-10.0 μg/ml, with the MBC equal to the MIC in all cases demonstrating bactericidal activity. Similar bactericidal action of OEO for Clostridium perfringens and Candida albicans has been shown previously.

Several of the bacteria in this study are zoonotic pathogens (Salmonella spp., E. coli O157:H7, L. monocytogenes) or have zoonotic potential (4). S. aureus is a difficult pathogen to eliminate, whether it causes mastitis in dairy cows or skin, bone or systemic infections in humans. Staphylococci sequester in fibrin-like clots, making elimination by antibiotics difficult. Methicillin-resistant S. aureus (MRSA) has been in the popular press recently because of its increasing spread in humans outside of the hospital setting and possible association with swine, poultry and other livestock. Infections caused by MRSA are difficult to cure because of its resistance to oral antibiotics. Results from this studies and others (5) show that S. aureus is effectively killed by OEO at concentrations similar to other pathogens.

These results demonstrate that OEO has high antimicrobial activity for pathogens that cause swine disease. The very high level of antioxidant activity of OEO may protect enterocytes against inflammatory damage caused by reactive oxygen molecules that are released during immune system activation. OEO has several benefits for the swine industry: it is a safe and accepted feed ingredient, it has potent activity against gram-negative and gram-positive bacteria, and it does not leave residues in the environment. Synergistic activity has been demonstrated between OEO and common antibiotics. OEO when used alone or in combination with antibiotics will allow the producer to reduce antibiotic use while maintaining protective gut health.

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Emerging Hepatitis E viruses from swine in Europe

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Abstract

Hepatitis E virus (HEV) is endemic in much of the developing world. Infections in humans can result in acute hepatitis and especially in pregnant women the infection may cause serious complications. The most important route of transmission is faecal-orally, and HEV disease outbreaks are often associated with contaminated drinking water or poor hygienic conditions. Of four HEV genotypes, genotype 3 is responsible for indigenous infections in industrialized countries worldwide. Genotype 4 is observed in sporadic cases in developed as well as developing countries in Asia, while genotype 1 is dominant in the endemic countries in the developing world. In the industrialised countries of Europe, seroprevalence is rather low (1-5%) but in recent years there has been an increasing number of diagnoses of HEV infection due to locally acquired strains. Since HEV genotype 3 and 4 are zoonoses involving several comestible animals, in low-endemicity areas special groups such as farmers, veterinarians, butchers and persons handling animal meat or consumers of undercooked swine, wild boar or deer meat present with a considerably higher seroprevalence than the general population. There may still be an underdiagnosis of HEV infections in Europe; however tens of infections are reported yearly in all countries in North West Europe. In almost all cases this involves HEV genotype 3 strains closely related to HEVs detected in domestic pig, wild boar or deer from the same geographical region. Recently a HEV genotype 4 strain was first isolated from swine in Europe and a closely related HEV sequence was reported from an autochthonous case in Germany. These observations indicate that "new" HEV strains, including genotype 4 strains, may be emerging in Europe. In future HEV genotype 3 and 4 infections might even evolve from a zoonosis to an established human infection.

HEV in the pork food chain in United Kingdom

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Abstract

Hepatitis E virus (HEV) is responsible of acute viral hepatitis in people and it is endemic in developing countries where it is transmitted mainly through faecal contamination of drinking water. Some of the cases in developed countries are autochthonous. The presence of an animal reservoir was hypothesized and HEV strains closely related to the ones circulating in human were found in pigs, wild boars and deer. Foodborne transmission of HEV via consumption of contaminated meat and presence of viable virus in pork products were demonstrated.

The European FP7 project VITAL (Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains) aims to gather data on virus contamination of food and environmental sources, for quantitative viral risk assessment. In the UK the contamination level of HEV in the pork food chain was investigated. Three phases of the chain were investigated: production (slaughterhouse), processing (meat processing plant) and points of sale. Different sample types were collected: faeces and livers (production), muscle samples (processing) and sausages (point of sale). Additional samples (mainly surface swabs) were collected in the premises in areas where viral contamination was considered more likely. All sample types were tested with standardized protocols (real-time PCR) for the detection of HEV (target virus) and Porcine Adenovirus (PAdV; indicator of faecal contamination). HEV was detected at different levels in samples from the production phase and from the point of sale (testing of some samples has still to be completed). Further studies are ongoing to determine the viability of HEV detected by real-time PCR. PAdV was detected only in the production phase, both from pig samples and from environmental swabs.

The results of the investigation conducted in the UK within the VITAL project underline the possible public health risk associated with consumption of undercooked pig meat or liver. Information on the viability of the virus will be indispensable to assess this risk. Viral contamination of surface swabs underlines that viruses as well as bacteria should be monitored in environmental samples.

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Human health risk of residues in Danish pork – in theory and practice

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Abstract

Residues of pharmacological active substances or their metabolites might be found in food products from food-producing animals posing a potential risk to human health. Maximum Residue Limits (MRLs) for pharmacological active substances in foodstuffs of animal origin are established to assure high food safety standards, and residue surveillance programmes are required to verify compliance with legislation, export requirements and consumer confidence.

A residue surveillance programme in Danish pigs and pork has been in place since 1972. A qualitative risk assessment was conducted to evaluate the human health risk of residues in Danish pork. The hazard identification step identified the residues that could potentially be found in Danish pork. The release assessment evaluated the probability of release of antibacterial residues in Danish pork, based on antibacterial consumption data in 2008. The exposure assessment estimated the probability of human exposure, based on findings of residues in Danish pork in the surveillance that involved approximately 20.000 samples annually (2005-2009). Finally, the consequence assessment evaluated the potential public health consequences and likelihood of its occurrence, based on a literature search.

The risk associated with antibacterial residues was estimated to be low to negligible in sows (low risk associated with penicillin residues) and negligible in slaughter pigs. To further reduce the already very low prevalence of residues in Danish sows, increased focus on good management practices regarding antibacterial use and education of farmers and farm workers should be promoted to increase awareness regarding the impact of potential detection of residues. Although the probability is low, residues are found occasionally. Experience with recent findings of residues show that there is a need for risk-based control implying quick risk assessments in each case covering among others the purpose of the meat and the risk for humans related to consumption of such meat.

Introduction

Use of veterinary medicinal products in food-producing animals might result in presence of residues of pharmacological active substances or their metabolites in food products from these animals. Hereby, humans might be exposed to residues via animal products which might have harmful human health consequences. To assure a high level of consumer protection at the EU level, specific legislation regarding surveillance of residues and contaminants in food of animal origin establishes the group of substances to be tested, including the sampling criteria (Council Directive 96/23/EC). Under this legislation, member states are required to have in place national residue surveillance plans, assuring the implementation of specific actions to detect and minimise the recurrence of residues in food of animal origin. Each year, more than 20,000 samples are analysed for presence of residues in Danish pork and the prevalence of residues in Danish pork are found at a very low prevalence (Baptista et al., 2010). The main part of the samples is taken as a part of the slaughterhouses own-check programmes.

This study aimed at evaluating the human health risk posed by residues in Danish pork.

Material and Methods

A qualitative risk assessment was conducted to evaluate the likelihood and the human health consequences of residues in Danish pork, according to international guidelines (Vose et al., 2001):
1) Hazard identification – based on Danish residue surveillance data from 2005-2009;
2) Release assessment – based on antibacterial consumption data obtained in Vetstat database;

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3) Exposure assessment – based on residue surveillance data from 2005-2009;
4) Consequence assessment –based on a literature search.

The literature search was conducted in May 2010 to identify reported cases of adverse reactions in humans to antibacterial residues in meat products via PubMed database. Abstracts obtained were screened to ensure they were original reports of adverse reactions to antibacterial residues in meat products.

The steps 1-4 were combined into a risk estimate, where the outcome of each step was expressed in the following qualitative terms: high (event occurs very often), medium (event occurs regularly), low (event is rare), very low (event is very rare but it cannot be excluded) and negligible (event is so rare that is not worth considering) (adapted from OIE, 2004). Final risk estimates were obtained by combining the qualitative outcomes.

Table 1. Qualitative risk assessment of human health risk of antibacterial residues possible found in Danish pigs, 2005-2009

| Antibacterial | Assessment of risk[1] | | | | | | | |
| | Release[2] | | Exposure[3] | | Consequences | | Risk estimate | |
	Sows	Finisher pigs	Sows	Finisher pigs	Sows	Finisher pigs	Sows	Finisher pigs
Aminoglycosides	L	VL	N	N	N	N	N	N
Amphenicols	N	N	N	N	N	N	N	N
Cephalosporins	VL	N	N	N	N	N	N	N
Fluoroquinolones	N	N	N	N	N	N	N	N
Lincosamide/spectino-mycin combinations	L	M	N	N	N	N	N	N
Macrolides (primarily tylosin)	L	H	N	N	N	N	N	N
Penicillins, β–lactamase sensitive	H	H	L	N	VL	VL	L	N
Penicillins, other	M	L	N	N	VL	VL	N	N
Pleuromutilins (primarily tiamulin)	M	H	N	N	N	N	N	N
Sulfonamides/ Trimethoprim	H	VL	N	N	N	N	N	N
Tetracyclines	M	H	VL	N.	N	N	N	N
Other	N	N	N	N	N	N	N	N

[1] Qualitative risk terms: H = high (event occurs very often), M = medium (event occurs regularly), L = low (event is rare but the occurrence is possible), VL = very low (event is very rare but it cannot be excluded) and N = negligible (event is so rare that is not worth considering).

[2] Probability of release was evaluated based on the estimated number of antibacterial doses per pig per year: H = high (≥ 3.0), M = medium (≥1.0 and <3.0), L = low (≥0.5 and <1.0), VL = very low (≥0.1 and <0.5) and N = negligible (<0.1).

[3] Probability of exposure was evaluated based on the maximum proportion of antibacterial residues (%) above maximum residue limits: H = high (≥1.00), M = medium (≥0.50 and <1.00), L = low (≥0.10 and <0.50), VL = very low (≥0.01 and <0.10) and N = negligible (<0.01).

Results

The probability of occurrence of residues other than antibacterials was considered insignificant and therefore excluded from the analysis. Although found at a low prevalence, antibacterial residues in Danish pork of sow origin might – if judged very conservative – be considered a potential hazard for human health for people that are allergic to penicillin. Table 1 presents an overview of the risk assessment and the risk estimated for the most commonly used antibacterials in Danish pig production.

Discussion

It is generally accepted that "zero risk" is impossible to achieve in the context of food safety (FAO, 1998). However, in this study the definition of negligible could not be differentiated from zero and hence, according to the classification matrix used, the product of negligible probability implied that the risk was negligible, indicating that the event was "so rare that it was not worth considering".

Antibacterial residue surveillance in Danish pigs includes 0.1% of the total slaughter pig population and more than 1% of the sows slaughtered in the previous year, exceeding the 0.03% level required by EU authorities. Antibacterial residue prevalence in Danish pigs has been consistently very low. Human cases of allergic reactions to penicillin residues in food are few and not that well-documented. The best documented case deals with a German butcher who was known to be allergic to penicillin. The butcher ingested pork originating from a pig that he had slaughtered himself. However, the pig had been treated with penicillin 3 days prior to being slaughtered. The butcher developed skin symptoms which declined after treatment with corticosteroids (Tscheuschner, 1972).

Overall, the results show that the human health risk associated with antibacterial residues in Danish pork is low to negligible in sows and negligible in slaughter pigs. The difference between sows and finishers might be explained by different practices regarding antibacterial use and management. The most common causes of positive findings were related to poor keeping of treatment records and/or inadequate identification of treated animals (data not shown). This is more likely to occur in sows, where individual management is used (against batch management in slaughter pigs) and time of slaughter is not defined. Still, the number of sows represents only a minor proportion of the total pork consumed in Denmark, which further reduces the already low overall risk posed to Danish consumers.

Study findings presented here are in agreement with previous studies assessing the human health risk of antibacterial residues in food products (Berends et al., 2001; Dayan, 1993; Dewdney et al., 1991). Accordingly, in the Netherlands, the human health risk associated with presence of tetracycline residues was estimated to be 80,000 times lower than the risk of human salmonellosis through pork products (Berends et al., 2001). Moreover, the small concentrations of antibacterial residues through which humans are exposed through food represent a negligible proportion of the total amount of antibacterials consumed by humans (Cerniglia and Kotarski, 2005). Hence, long-term effects are also not likely to be expected from consumption of Danish pork as human exposure to antibacterial residues is very low to negligible and below the average daily intake (ADI) for lifetime exposure. This is in agreement with previous studies (Paige et al., 1997). Furthermore, at the EU level, very conservative assumptions are used for determining ADIs, MRLs and withdrawal times, assuring a very high level of protection to consumers. Accordingly, it has been shown that when MRLs for tetracyclines in meat are exceeded by a factor of 400, the risk of an adverse reaction in humans is estimated to be 1 in 3 millions exposed consumers (Berends et al., 2001).

To further reduce the very low prevalence, increased focus on good management practices regarding antibacterial use and compliance with withdrawal periods should be advocated for. Awareness should be increased regarding the impact of potential detection of antibacterial residues above the MRLs on industry reputation and exports. Moreover, experience with recent findings of residues show that there is a need for risk-based control implying quick risk assessments in each case covering among others the purpose of the meat and the risk for humans related to consumption of such meat. A reporting system should be in place that will motivate farmers to make immediate notification if animals by mistake are sent to slaughter to enable correct action. The human health risk associated with residues in Danish pork is negligible in general. However, reasons other than food safety might apply and require that residue surveillance activities are in place. Residue data might be used as an indicator of animal health and welfare, use of veterinary drugs and meat quality. In line, consumers in the EU have residues of antibacterials and other similar substances high on the agenda (Anon., 2010). Above all, residue surveillance is required to document fulfilment of regulations and export requirements.

Denmark has several risk mitigating initiatives in place regarding use of antibacterials in pig production, which further contributes to mitigate the human health risk of antibacterial residues in pork. These are described in detail in Andreasen et al. (2011) and will only be listed here in brief: Vetstat database recording antibacterial use data, official guidelines for antibacterial treatment of food-producing animals, no use of fuoroquinolones in livestock, and in 2010 a 2-year ban on use of cephalosporins in Danish pigs was put in place by the industry. Furthermore, the industry has extended the withdrawal period for tetracyclines to 30 days and banned use of sulfadimidine in sows and slaughter pigs. Regardless of the substance used, withdrawal periods of less than 5 days are not accepted.

Conclusion
Risk assessment results showed that the human health risk associated with antibacterial residues in Danish pork is low to negligible in sows and negligible in slaughter pigs.

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Quantifying the effect of natural microflora on growth of salmonellae in fresh pork

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Abstract

This study was undertaken to provide predictive models to help prevent health problems in relation to salmonellae in fresh pork. The models consider different time and temperature of storage as well as microbial interaction with the natural microflora in the meat. At six temperatures between 4 and 20°C, duplicate growth curves of Salmonella Typhimurium DT104 and Salmonella Derby were established in both sterile (irradiated) minced pork as well as in minced pork with a natural microflora. The inoculated meat was incubated in aerobic atmosphere. Each growth curve was fitted to the exponential growth model to obtain estimates of maximum specific growth rate, µmax. The effect of storage temperature on µmax was modelled using a square-root type model. Faster growth of both Salmonella serovars was observed in sterile meat at 8 to 16°C as compared to meat with a natural microflora. Around 20°C, growth was, however, independent on type of meat. Besides testing of Salmonella, appearance and odour of all meat samples, having a natural microflora, was evaluated throughout the incubation periods. These observations determined for how long the meat was acceptable for consumption at different temperatures. Above 10°C, both Salmonella serovars started to grow before the meat was rejected for consumption. This indicated that safety, rather than spoilage, could be the shelf-life limiting factor of fresh pork at temperatures from 12 to 20°C. This interaction between Salmonella and the natural microflora should be included in risk assessment models regarding salmonellae in fresh pork.

Introduction

Mathematical models that predict growth of pathogens in food are usually developed in laboratory broth (McClure et al., 1994). However, these models do not always provide relevant predictions of pathogen growth in non-sterile and non-homogeneous food (Ross, 1996), because interaction or competition between the pathogen and the background flora may occur. Thus, there is a need to develop growth models for pathogens in food with the natural microflora present (Oscar, 2007). The strategy of the present work was to build scenarios for baseline temperature abuse as well as for the presence of a natural microflora in fresh pork in order to predict the growth potential of Salmonella in fresh minced pork.

Material and Methods

Meat. Packages of approx. 500 g modified atmosphere packaged minced lean pork meat were obtained from local retailers. The packages used for preparing sterile meat were mixed manually in a sterile bag for 10 min. Portions of 100 ± 3 g were vacuum packaged and frozen at -18°C. Sterilization of volumes of 5 times 100 g was done by irradiation at a dose of 5 kGy for 523 min, followed by freezing at -18°C. In the beginning of every new test round meat packages were defrosted in water at a temperature of approx. 40°C for 30 min. Packages of meat with a natural microflora were obtained from local retailers one day before each test round and divided into 100 g portions at the following day.

Preparation of Salmonella cultures. A cocktail of Salmonella Typhimurium DT104, carrying resistance to ampicillin, chloramphenicol, florfenicol, streptomycin and sulfa, and Salmonella Derby, carrying resistance to gentamycin, streptomycin, sulfa and spectinomycin, were used. Both Salmonella serovars had been isolated from pigs. One loop of a stock culture (−80°C) of each isolate was cultured separately in 10 ml LB-broth by overnight shaking at 37°C. Subsequently, the tubes were stored at 5°C for 3 days. Prior to inoculation, the cultures were diluted in phosphate buffered saline to obtain a concentration of approx. 106 CFU ml-1 and equal volumes of each was mixed and used as the inoculation cocktail.

Storage experiments. Each 100-g-meat sample was aseptically transferred to separate sterile stomacher bags and inoculated with 1 ml of the Salmonella cocktail to a final concentration 104 CFU g-1. To ensure even distribution of the cocktail in the whole meat sample, it was mixed by stomaching for 2 times 1 min. The inoculated samples were stored in normal atmosphere at selected temperatures between 4°C and 20°C. At appropriate time intervals, the whole meat sample was removed from the incubator and mixed in a stomacher for 2 times 1 min. Subsequently, 5 g meat was sampled aseptically and transferred to a sterile filter bag and the remaining meat sample was returned to the incubator within 5 min. The samples were diluted in 45 ml of buffered peptone water and mixed in a stomacher for 2 min. For bacterial enumeration, further 10-fold dilutions were performed using isotonic saline solution and appropriate dilutions were drop-plated (10 μl) onto XLD+ampicillin and XLD+gentamycin agars to enumerate S. Typhimurium DT104 and S. Derby, respectively. The plates were incubated at 37°C for 16 to 24 h.

Sensory evaluation. Throughout the incubation periods, appearance and odour of all meat samples, having a natural microflora, was evaluated by a four-member expert panel. Besides describing appearance and odour, the panel was also asked to evaluate whether they found the meat acceptable for consumption.

Data analysis. The growth model described by Baranyi and Roberts (1994) was fitted to the experimental growth curves and estimates of the maximum specific growth rate, μ_{max}, were obtained for each growth curve using the freeware DMFit web edition. For the description of the effect of temperature on μ_{max}, a square-root-type model (Eq. 1) was applied separately for meat with and without a natural microflora.

$$(\mu_{max})^{\frac{1}{2}} = b \cdot (T - T_{min})$$ (Eq. 1)

where b is a constant to be estimated, T is the temperature in °C and where the estimated value of Tmin is the intercept between the model and the temperature axis.

Results and discussion
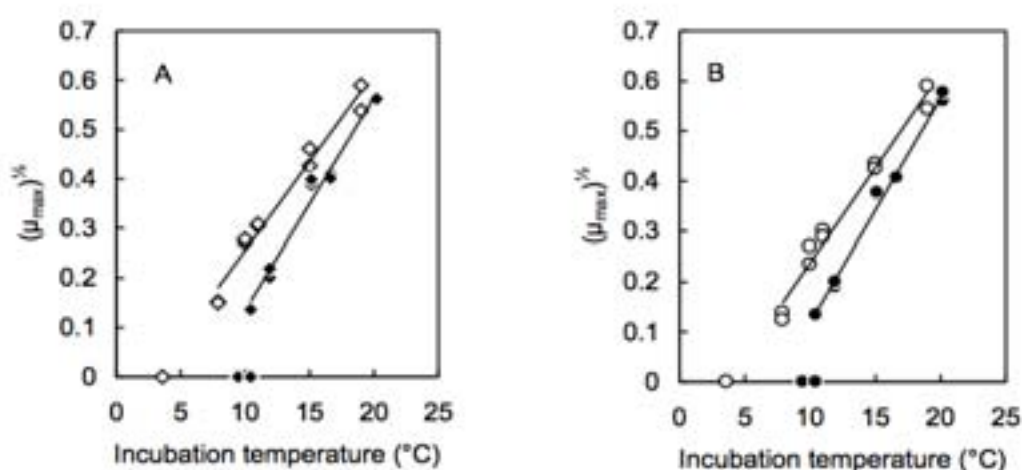
Figure 1. Influence of storage temperature (°C) on growth rates (μ_{max}, 1/h) of A) Salmonella Derby and B) Salmonella Typhimurium DT104 in sterile minced pork (open symbols) and minced pork with a natural microflora (closed symbols).

Faster growth of both S. Typhimurium DT104 and S. Derby was observed in the sterile meat at the temperatures below 20°C (Figure 1). The growth rate for each growth curve was estimated by fitting the Baranyi & Roberts growth model (1994) to observed data. The results confirmed the ability of competitive microflora to suppress growth of Salmonella during incubation of food samples in isolation broths as reported by Beckers et al. (1987). Oscar (2006) detected a similar effect when studying growth of S. Typhimurium DT104 in ground chicken breast with a competitive microflora. Figure 1 compares the effects of storage temperature and natural microflora in fresh minced pork on the growth rate of S. Typhimurium DT104 and S. Derby. The growth rates in minced pork could not be predicted satisfactory from literature models developed in chicken meat (Oscar, 2006; Oscar 2007), and it was necessary to develop new predictive models

specifically for Salmonella in fresh pork with a natural microfora in order to improve accuracy of predictions. Therefore, secondary predictive models, following a square-root-type equation, were developed for describing the effect of temperature on the maximum specific growth rates, μ_{max} found in this study (Table 1). The T_{min} estimates of the fitted models suggested that the minimum growth temperature for both Salmonella serovars was lower in sterile meat as compared to meat with a natural microfora. Predictions from the models also showed that below 15°C, the natural microfora of fresh pork meat slowed down the generation time of both Salmonella serovars with more than 2-fold. When evaluating the consumer risk from Salmonella in pork these observations suggest that different growth models have to be considered in situations where where the background fora has been inactivated by decontamination as opposed to traditionally slaughtered pork. The observed differences between growth rates of S. Derby and S. Typhimurium DT104 were of minor importance, in this respect.

Table 1. Predictive models describing the effect of temperature on growth rates (μ_{max}, 1/h) of Salmonella Derby and Salmonella Typhimurium DT104 in minced pork with and without a natural microfora present.

Salmonella	Pork type	Secondary model	R^2
S. Derby	Sterile (irradiated)	$(\mu_{max})^{\frac{1}{2}} = 0.0357 \cdot (T - 2.84)$	0.974
	With a natural microflora	$(\mu_{max})^{\frac{1}{2}} = 0.0429 \cdot (T - 6.86)$	0.978
S. Typhimurium DT104	Sterile (irradiated)	$(\mu_{max})^{\frac{1}{2}} = 0.0377 \cdot (T - 3.70)$	0.979
	With a natural microflora	$(\mu_{max})^{\frac{1}{2}} = 0.0446 \cdot (T - 7.34)$	0.990

Besides the quantification of Salmonella, appearance and odour of the meat samples, having a natural microfora, were assessed throughout the incubation periods. These observations determined for how long the meat was acceptable for consumption at different storage temperatures. As shown in Figure 2, the shelf-life of minced pork was found to be 4 days at 9.5°C and below 4 hours at 20°C. At all storage temperatures above 10°C, both S. Typhimurium DT104 as well as S. Derby started to grow before the meat was rejected for consumption (Figure 2). Growth was most pronounced around 15°C, where an increase of more than 1 log-unit was found, but also at 12°C and 20°C Salmonella was observed to initiate growth before the meat was spoiled (Figure 2). This indicated that safety, rather than spoilage, could be the shelf-life limiting factor of fresh pork at temperatures from 12 to 20°C.
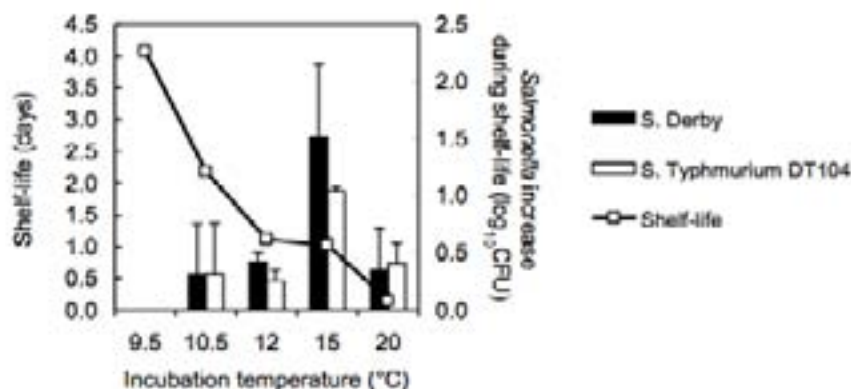
Figure 2. Average increase (log10CFU) of Salmonella Derby and Salmonella Typhimurium DT104 in minced pork with a natural microflora measured on the final day of shelf-life.

Conclusion

The present study found that the natural background fora in pork slowed down growth of salmonellae considerably at temperatures below 20°C. Risk assessment models have to consider this. It was also observed that temperature abuse, even in the chilled temperature area, may induce critical Salmonella growth before spoilage occur. This is important in relation to the setting of critical limits in the cold chain, i.e. for temperature shifts during handling.

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VITAL, Monitoring and Control for Virus Safe Pork

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Abstract

VITAL is an ongoing (2008-2011) EU funded project on monitoring and control of food-borne viruses. The concept of VITAL is the integrated risk assessment and management of contamination of the European farm to market food chain by pathogenic viruses, such as norovirus and hepatitis E virus. The project's focus is on the production and processing phase, moving away from the concept of endpoint monitoring towards input monitoring. The project's objectives include: 1) The acquisition of data on virus contamination of food and environmental sources, 2) The assessment of foodborne viral risks for determining high risk situations and the efficacy of interventions along the food supply chains, 3) To develop new measures to prevent virus contamination of foods and the environment, 4) To develop and assess measures for virus reduction and control in case of virus contamination.

VITAL development and testing of standard operating procedures includes SOPs for the analysis of samples from the pork production chains which are most at risk from foodborne virus contamination, in particular hepatitis E virus. Specific points of sampling along the production chain of pork have been identified and the developed methodology was used to gather data in the various phases of this food supply chain. In addition VITAL works on studies on the survival and elimination of hepatitis E virus in the pork production setting. The data from monitoring of raw as well as processed pork will be used with Modular Process Risk Models (MPRM) to build up specific hazard analysis critical control point (HACCP) recommendations, also using the results of hepatitis E virus survival studies. Measures for virus reduction and control developed and assessed by VITAL must be of value to Europe and beyond and therefore will finally be recommended and published in guidance manuals.

Improvements in processing hygiene indicator and microbial hazard levels in Australian finisher pigs from 1996 to 2010

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Abstract

In 2010 a national pig carcase baseline survey was undertaken to help determine the impact of regulation on processing hygiene and hazard levels and to inform national standard setting. The results are compared to those from a 1996 baseline survey of Australian finisher pigs. For E. coli a reduction in prevalence of around 10% (29.3% in 1996 to 20.7% in 2010) and an average reduction in concentration of 1.5 log10 cfu/cm2 were observed. Similarly, average concentration of TVC reduced by 1.9 log10 cfu/cm2 over the same period. Despite more stringent sampling techniques utilised in the 2010 survey, the carcase hygiene indicator data indicates a substantial improvement in dressing hygiene compared to the 1996 survey. Salmonella levels remain low (0.4%; 95% CI: 0.0 – 2.1%) by international standards. This substantial improvement in dressing hygiene over the past 14 years is most likely a result of the mandating of HACCP in Australian abattoirs in the late 1990s that has in part driven investment in processing effectiveness.

Introduction

In 1996/97 a national microbiological baseline survey of 680 Australian pig carcases was conducted. This involved swab sampling pig carcases on three x 20 cm2 areas (Coates et al 1997). The study found low levels of Salmonella and Y. enterocollitica (1% and 0.15% respectively), no detections of C. jejuni, L. monocytogenes and E. coli O157 and relatively high levels of S. aureus (14.9% with a carcase toxigenic strain prevalence of 7%).

Since that time, international guidelines have been developed for determining carcase microbiological levels, the so-called ESAM (E. coli and Salmonella Monitoring) system (Anon 2000). Given that the original survey was conducted 14 years prior, and utilised a different methodology, the results could no longer be relied upon to demonstrate the quality of Australian pig meat in the event of a food borne disease outbreak or satisfy new and existing export market requirements.

Materials and Methods

A total of 294 finisher pig carcases were sampled by swabbing at 6 major pig abattoirs in Australia. Carcases were sampled on 2 occasions approximately 6 months apart (March/April and August/September) using the standard ESAM sponge method and a single sampler. The number of samples allocated to each abattoir was in proportion to that abattoirs throughput. Overall, the 6 abattoirs represented approximately 60% of the national finisher pig kill.

For each abattoir a similar number of their available ESAM Salmonella test results, which straddled both sampling periods, were assessed. If there was sufficient ESAM data around each survey sampling date, half the ESAM sample results were taken prior to each survey date and half after. Otherwise, for the first survey sampling period more ESAM data was taken prior to the date and for the second survey sampling period more was taken after the sampling date (to avoid crossover of ESAM data).

Carcases were aseptically sampled after a minimum of 12 hours chilling in accordance with AQIS Meat Notice 2003/6 (Anon 2003), using the sponge method and stainless steel 100 cm2 template. Swabs were placed immediately into an esky with ice packs and shipped overnight to the laboratory for testing within 24 hours.

Total Viable Count (TVC) and E. coli

The sponge swabs were massaged in a stomacher for 60 seconds. Ten-fold serial dilutions of the swab diluent were

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prepared in Peptone Saline Solution (Oxoid, Thebarton, South Australia). Aliquots (1 mL) from each serial dilution were inoculated onto 3M™ Petrifilm™ Aerobic Plate Count Plates and 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M Corporation, St Paul, Minnesota) and incubated at 35°C for 48 hours for the aerobic count and 35°C for 24 hours for E. coli. Colonies were identified and counted as per manufacturer's instructions with the limit of detection being 10 cfu/mL.

E. coli O157:H7

After removing aliquots of the diluent for Total Aerobic and E coli counts, carcass swabs were tested for the presence of E coli O157:H7 using the BIOCONTROL VIP® Gold for EHEC single step immunoassay (BIOCONTROL). 50 mL of pre-warmed modified Tryptone Soya Broth was prepared as per the manufacturer's instructions and added to the sponge swab. This was incubated for 18 to 28 h at 35 - 37°C. The culture (1 mL) was transferred to the sample addition well of a VIP Gold unit. This was the incubated at room temperature for 10 min and examined as per the manufacturer's instructions. Provisionally positive isolates were sent to the Melbourne Diagnostic Unit (MDU) for confirmation.

Results

The microbiological results for this survey are compared with the 1996 survey in Table 1. For log10 TVC cfu/cm2 there was a significant difference between abattoirs (p<0.001), but no consistent seasonal effect. For E. coli Abattoir 2 had only 1 positive result and the prevalence estimate was significantly lower than all other establishments (p=0.02). Excluding Abattoir 2, there were significant differences in the mean log10 E. coli cfu/cm2 between all abattoirs (p=0.02). For Salmonella, routine ESAM sampling for the 6 abattoirs for a similar number of samples over the survey period detected Salmonella in 1/266 (0.4%) samples (Table 1)

Table 1. Comparison of the finisher carcase microbiological survey results 1996 (Coates et al 1997) versus 2010

Year	# abattoirs[a]	# samples	Mean log$_{10}$ TVC/cm^2 (SD)	# E. coli pos (%)	Mean log$_{10}$ E. coli/ cm^2 (SD)	# Salmonella pos (%)
1996	18	680	3.8	(29.3)	0.71	(1)
2010	6	294	1.93 (0.7)	61 (20.8)	-0.79 (0.4)	1 (0.4)[b]

[a] there has been significant rationalisation in the pig processing industry since 1996
[b] from ESAM records for corresponding time period. Serovar = S. infantis. SD = standard deviation

None of the 294 samples in 2010 were positive for E. coli O157:H7 (prevalence < 1.3%). Five samples were identified as suspect following screening with BIOCONTROL VIP®, however on confirmation were determined to be negative.

Discussion

Comparisons with other studies, both in Australia and overseas are difficult as they often use variable methodologies (eg sponge vs. swabs; pre- vs. post-chill) and swabbed areas (eg ESAM 300cm2 vs. EU 400 cm2) and target different organisms (eg. ESAM E. coli vs. EU Enterobacteriaceae). Increasing the swabbed area can be expected to increase the detection (prevalence) of an organism, and counter intuitively with high counts it can decrease the estimate of cfu/cm2 (Miraglia et al, 2005).

In 1996 the Australian pork industry funded a microbiological benchmarking study of 680 finisher carcases at 18 Australian abattoirs as part of a Pig Meat Hygiene Program. Care must be exercised, however, in comparing the results of that first National Pig Carcass Microbiology Survey (Coates et al 1997) with this present study. At the time (pre-USA Megaregs) there was no internationally accepted carcase swabbing protocol so there were a number of differences methodologies between the 2 surveys, vis à vis

- The previous study utilised a wet-dry swab method (Kitchel et al 1973) on 3 x 20cm^2 areas. This contrasts with the present study using the internationally accepted ESAM sponge method on 3 x 100cm^2 areas (Anon 2000).
- Carcases were sampled 30 minutes after the final wash (rather than after 12 hours chilling).
- The previous study relied on company employees (compared with a single survey sampler).
- The previous study sampled randomly over a 12 month period (compared with just 2 intensive sampling periods 6 months apart).

Despite these caveats, overall, the present survey can be considered to have been carried out under more stringent conditions, so any improvements can be considered real. The differences in the results between the 2 studies are summarised in Table 1.

Total Viable Count (TVC)
A high TVC is an indication of poor general hygiene and/or poor temperature control. In this study the overall mean log10 TVC was 1.93 cfu/cm2 for finishers. There was no consistent sampling period effect between abattoirs, suggesting that temporal differences are is simply a reflection of day-to-day processing variability.

The results compare favourably with those found in previous studies in Australia and overseas. Since 1996 there has been substantial improvement in dressing hygiene as indicated by a 1.9 reduction in the mean log10 TVC (Table 1, Fig 1). In 1996 the worst 10% of finisher carcases fell in the TVC range of 3.8 to 5.4 log10 cfu/cm2 (Coates et al 1997). In the present study only the worst 2/294 (0.7%) of carcases were found in a similar range.
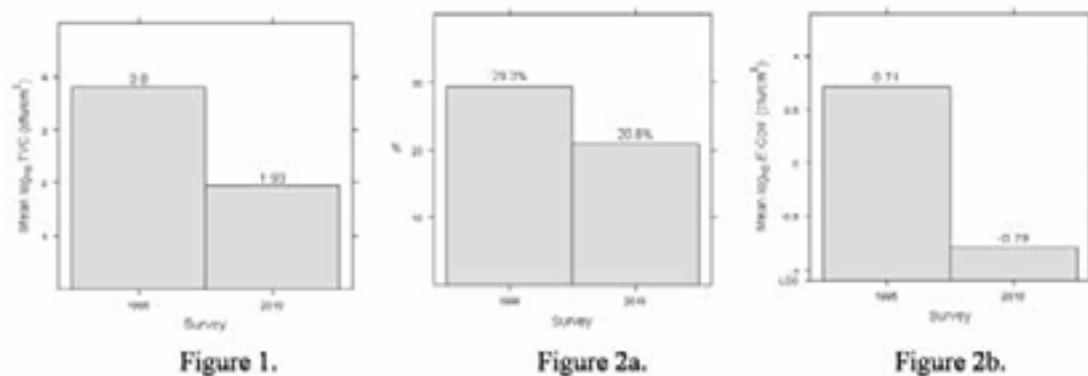
Figure 1. Figure 2a. Figure 2b.

Figure 1. Comparison of mean log10 TVC cfu.cm2 between the 1996 (Coates et al 1997) and 2010 carcase benchmarking surveys. Figure 2a & 2b. Comparison of mean E. coli percent positive and mean log10 E. coli cfu.cm2 between the 1996 and 2010 carcase benchmarking surveys

E. coli
In the US, Australia and Asia, E. coli is tested for as an indication of faecal contamination and poor general hygiene. In this study the overall prevalence for finishers was 20.8%, and the mean log10 cfu/cm2 was low at -0.79 compared with 29.3% and 0.71 in 1996 (Table 1, Fig 2a, Fig 2b). A 13 month Swedish baseline survey of 541 pig carcases at the 10 largest abattoirs in Sweden estimated an

E. coli prevalence of 57% with a mean log10 of 0.05 (Lindblad et al 2007) and a Taiwanese 2003 national baseline survey of 1650 pigs from 39 abattoirs reported a prevalence of 87.5% (Yeh et al, 2005).

E. coli O157:H7
E. coli O157:H7 was not detected in this study. Recently, a New Zealand study isolated E. coli O157:H7 both from 1% of 100 NZ domestic pig carcases and from 3.1% of 65 imported Australian pig meat samples (Wong et al 2009). The sample numbers in this study, however, would have been able to detect this organism at lower prevalence than the NZ study and hence previous NZ results are not supported by the results of this national survey.

Salmonella
A total of 266 Salmonella ESAM test results from the survey abattoirs were examined covering the survey period, of which only 1 was positive giving a prevalence of 0.4%. This reflects the 1996 survey in which Salmonella was isolated from 1% of 680 carcases (Coates et al 1997) and a 7 year summation of the pig carcase National ESAM database from 2000 to 2006 which reported an overall Salmonella carcase prevalence of 1.88% (Hamilton et al 2007) that continues to decline significantly to 2010 (A Kiermeier pers comm.).

In the late 1990s HACCP was mandated in pig abattoirs in Australia (Anon 1996/38). Subsequent investment to improve processing effectiveness and efficiency are inextricably linked with these dramatically enhanced regulatory requirements.

Conclusion

- There have been substantial improvements in the indicators of carcase hygiene between 1996 and 2010 as measured by TVC and E. coli prevalence.
- The relatively good Salmonella status of Australian finisher carcases was confirmed.
- E. coli O157:H7 contamination does not appear to be an issue with Australian pig carcases and the findings do not support the 3.1% prevalence reported by NZ.

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The cost-benefit of salmonella control in Swedish pigs

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Introduction

Analysis of the expected costs and benefits of salmonella control pre-harvest in the pork production has been performed on EU level (1). As optimal measures to begin salmonella control in pig production in a high prevalence situation are not known, estimates of the costs for initiating such a control include large uncertainties. However the costs for running a salmonella control program can be estimated in countries where such programs are in place. In Sweden, where approximately 3 million pigs are slaughtered yearly and the prevalence of salmonella is low, the cost of the control is shared by the tax payers and the producers.

The goal of the program is that animals sent to slaughter should be free of salmonella and it includes (2)
- surveillance of feed production according to HACCP principles and heat treatment of feed
- a voluntary preventive hygienic herd program
- compulsory sampling of breeding herds once a year and sow pools twice a year
- compulsory measures following suspicion or detection of salmonella in pig herds
- surveillance of live animals by sampling of lymph nodes from fatteners and adult pigs at slaughter
- surveillance of carcasses by swab samples at slaughterhouses
- a control program for food
- compulsory notification of human cases of salmonella infection
- surveillance of antibiotic resistance in isolates of salmonella in animals.

A thorough analysis of the cost-benefit of this program has been requested by various stakeholders. Pending this, a quick calculation based on previously published and unpublished data was made. The calculated costs of the program were compared to the costs of two different what-if scenarios without a compulsory control program. The analysis indicates that the saved costs exceed the cost of salmonella control in Swedish pigs.

Material and Methods

Estimated costs of the present control program in Sweden

The costs for the part of the Swedish control program that covers pig production were obtained from published reports and from authorities (Swedish Board of Agriculture; National Food Administration as well as stakeholders (Swedish Animal Health Service; Swedish Dairy Association) engaged in the program.
The data included the yearly cost of:
i)   surveillance in pigs and pig products
ii)   eradication of salmonella from infected pig farms
iii) preventive measures in the feed sector (3).

Estimated costs of human salmonellosis caused by pork

The cost of human illness due to salmonella from Swedish pigs was calculated using an indirect friction method including costs of reactive arthritis, inflammatory bowel disease and the value of statistical life (4). Based on a previous study, the proportion of Swedish salmonella cases caused by domestic pork was set to 0.08% (5).

Expected costs for the control using two different what-if scenarios

The salmonella situations in Denmark and the Netherlands were used as scenarios for a possible Swedish salmonella situation without a compulsory control program.

Information about sampling strategies used in the ongoing surveillance of salmonella in pigs and pig products in these countries were retrieved from Danish official reports (6) and from Peter van der Wolf , GD Animal Health, Deventer, The Netherlands (Pers com, 2011). From these data, probable costs for sampling in the Swedish production chain were calculated.

Expected increase in costs of human salmonellosis under the two what-if scenarios

Estimates of the expected increased number of human cases under the two scenarios were calculated using seroincidence data for Denmark and the Netherlands respectively (7; pers com G Falkenhorst, 2009). The cost of these human cases was calculated with the indirect friction method described above. The proportion of the total cost caused by domestic pork and pork products was estimated using source attribution data for Denmark (8) and the Netherlands (Pers com Wilfred van Pelt, RIVM, The Netherlands, 2011).

The calculated costs of the present Swedish program in pigs/pork were compared to the costs of different surveillance strategies and the expected increased costs for human cases in two different scenarios without a compulsory control program.

Results

The cost of the Swedish salmonella control program for pigs was estimated to 840 000 € (7 600 000 SEK) and the cost of human salmonella cases caused by domestic pork was estimated to 24 000 € (220 000 SEK). The costs of the Swedish control program were similar to the estimated costs when applying the Dutch surveillance strategy under Swedish conditions (figure 1). However, when applying the Danish surveillance strategy under Swedish conditions the costs increased considerably (figure 1).

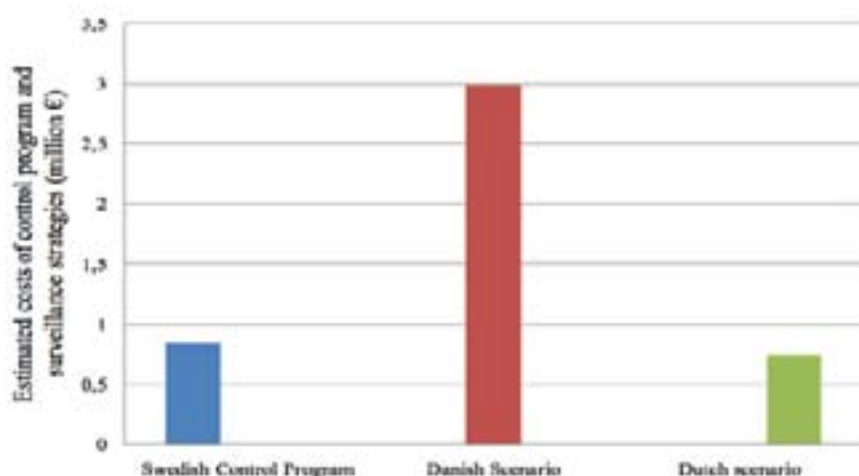*Figure 1: The costs (million €) of the Swedish salmonella control program for pigs compared to estimated costs of salmonella surveillance when applying the Danish and Dutch surveillances systems under Swedish conditions.*

The expected increase in yearly costs for human salmonellosis due to domestic pork when applying the Danish and Dutch surveillances systems under Swedish conditions are presented in figure 2.
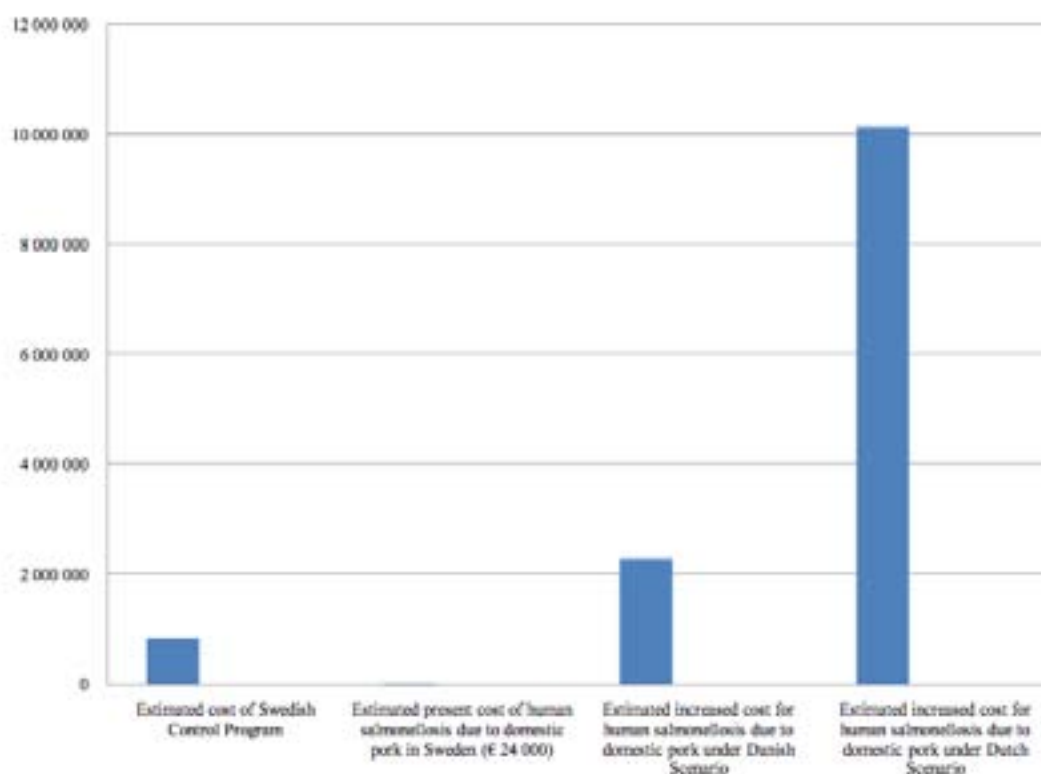
Figure 2: Increase in yearly costs (€) for human salmonella caused by domestic pork in Sweden under two possible scenarios without a control program compared to the cost of the present program and the estimated cost of present human cases caused by domestic pork in Sweden.

Discussion

Although the compulsory Swedish control program for salmonella keeps the costs of human salmonellosis due to pork on a very low level, the costs for the compulsory Swedish program were surprisingly low when compared to the expected cost when applying the Danish and Dutch surveillance systems under Swedish conditions. This may be partly due to the fact that indirect costs for eradication were not included in the calculation. However, the number of farms where interventions are needed is very low as the prevalence in Swedish pigs is very low.

Costs of the control program in Sweden and the surveillance programs in the scenarios are calculated only using direct costs whereas the estimations of costs of human illness are made including indirect costs to some extent. This makes it hard to make a direct comparison between programs and costs of human illness. Furthermore, the calculation of expected number of human cases can be done using several different methods, in this study a method based on seroincidence data was used. However, comparisons between the different scenarios and the present Swedish situation can be done if it is appreciated that the estimations include several assumptions and that the costs should be viewed as relative estimates and not absolute figures.

In this study an attempt was made to estimate the cost-benefit of the Swedish Salmonella Control Program. Under the assumptions made in this study, the calculated saved costs for avoiding the additional human salmonella cases exceed the cost of the present salmonella control in Swedish pigs/pork. Under exceptional circumstances, such as the large feed-borne outbreak in 2003, costs may however exceed the benefits.

Conclusion

In conclusion, the analysis presented in this paper indicates that the saved costs exceed the cost of salmonella control in Swedish pigs.

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Identification of control strategies to manage microbiological risks in typical pork products

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Abstract

Starting from 2009 a pilot project has been implemented by a local veterinary service of the Veneto region of Italy (AZ-ULSS 8) in collaboration with IZSVe (Istituto Zooprofilattico Sperimentale delle Venezie) with the aim of identifying control measures based on own-checks and official controls in order to manage microbiological risks related to traditional pork fermented sausages (Italian salami end soppresse) consumption.

According to the data obtained a control strategy based on microbiological tests performed by the Competent Authority (CA) and the monitoring of the weight decrease in sausages by the food business operator (FBO) has been implemented for 2010-2011 production season.

Introduction

In the Veneto region of Italy the production of traditional pork fermented sausages, salami and soppresse in particular, has significantly increased in the last four years, after specific regional legislation has been issued in this field starting form 2007. In particular in 2008 a specific regional legislation has entered into force mainly focused in acquiring detailed information on technical and sanitary aspects of the production processes with the aim of identifying, within a two years period, control strategies to reduce the potential risks due to the consumption of salami and soppresse at acceptable level. Although traditional processing techniques generally appear to be effective in pathogens control, a preliminary monitoring campaign showed that sausages ready to be marketed may in some exceptional circumstances be contaminated with foodborne pathogens, thus posing a potential risk for the consumers.

Additionally, data from a literary review demonstrate that both traditional and industrial sausages may be contaminated at the end of the fermentation period in particular with Salmonella spp., Listeria monocytogenes and E.coli O157 (Normanno G., et al., 2004; De Cesare et al. 2007; Bianchi D.M. et al., 2007). Furthermore food borne outbreaks have been associated to the consumption of traditional raw pork products typically submitted to a short ripening period (Pontello M., et al. 1998; Luzzi I., et al. 2007).

Thus with the aim of avoiding the marketing of potentially at-risk salami and soppresse produced within the Veneto region a study has been performed focused at identifying control measures easily applicable directly by the producers with the supervision and control of the CA.

According to the information obtained a control strategy based on official controls at the early stages of the production process and on the monitoring of the weight decrease in sausages by FBOs has been implemented. Results from this study have been used to update the legislation issued on this field (DRGV n.2280/2010).

Material and Methods

In order to collect detailed information on microbiological contamination of salami and soppresse at different point of the production process, from farm to fork samples have been collected in 2009-2010 production season according to two different sampling schemes "A" and "B" described in table 1. 32 producers registered according to relevant Veneto region legislation were included in the study.

Briefy the A sampling scheme was applied to all the producers both aimed at estimating the prevalence of selected food-borne pathogens (Salmonella spp., Campylobacter spp., E. coli O157, Listeria monocytogenes and spp.) in samples collected at different points along the production chain (animal, minced meat, products during ripening) and at collect-

ing information on the most inf uent parameters of drying and fermentation (pH, a$_w$, environmental conditions such as temperature and humidity).

The more intensive sampling scheme B was applied in a selection of four producers and was aimed at collecting additional data to evaluate also a possible correlation between the aw and the weight decrease of the sausages during maturation.

Table 1: sampling scheme A and B description

SAMPLING STAGE	SAMPLE TYPE	SAMPLING SCHEME A	LABORATORY ANALYSIS
ABBATTOIR	FAECES	1 pooled faecal sample all pigs per batch (1 batch includes a maximum of 3 pigs)	Campylobacter spp. and E.coli O157
	LYMPHNODES	1 pooled of lymphnodes all pigs per batch	Salmonella spp.
PRODUCTIVE UNIT	MINCE	250 grams of minced raw meat ready for stuffing	Campylobacter spp. , Salmonella spp. E.coli O157, Listeria spp. and Listeria monocytogenes; pH and aw
	SALAMI	2 salami (1selected batch) / productive unit both identified and weighted the first day of ripening and then sampled at 20 and 40 days of ripening . In case of positive batches sampling of 1 salame every 15 days up to two negative results for the identified pathogen	Campylobacter spp. , Salmonella spp. E.coli O157, Listeria spp. and Listeria monocytogenes; pH and aw
	SOPPRESSE	2 soppresse (1 selected batch) / productive unit both identified and weighted the first day of ripening and then sampled at 90 and 130 days of ripening. In case of positive batches sampling of 1 soppressa every 15 days up to two negative results for the identified pathogen	Campylobacter spp. , Salmonella spp. E.coli O157, Listeria spp. and Listeria monocytogenes; pH and aw
SAMPLING STAGE	SAMPLE TYPE	SAMPLING SCHEME B	LABORATORY ANALYSIS
PRODUCTIVE UNIT	SALAME FOR PRODUCTIVE UNIT A AND B; SOPPRESSA FOR PRODUCTIVE UNIT C AND D	identification and weight determination of all salami/soppresse the stuffing day Every Monday =aw, pH and weight determination + microbiological analysis Every Thursday= aw, pH and weight determination Two times a week weight determination of 4 identified salami/soppresse for all the ripening period	Campylobacter spp. , Salmonella spp. E.coli O157, Listeria spp. and Listeria monocytogenes; pH and aw

The two datasets deriving from A and B monitoring schemes were validated before the statistical analysis.

Results

Sampling scheme A

128 pigs batches were slaughtered in 2009-2010 corresponding to a number of estimated sausages of 10.240 salami and 3.840 soppresse.

58 out of the 534 samples collected and analysed resulted positive to one or more foodborne pathogens as described in the table 2.

Table 2: Foodborne pathogens distribution, sampling scheme A

Sample	Salmonella spp.	E. Coli O157	Campylobacter spp.	Listeria monocytogenes	Listeria spp.
Faeces	6	0	7	0	0
Lymphnodes	2	0	0	0	0
Mince	1	0	0	17	16
Salame	2	0	0	8	4
Soppressa	0	0	0	5	1

Out of the 30 soppresse sampled at the end of the ripening period (130 days of ripening,) two resulted positive for Listeria monocytogenes, both <10 ufc/gr and aw < 0.92. Out of the 56 salami at the end of the ripening period (40 days of ripening) 4 resulted positive for Listeria monocytogenes, all <10 ufc/gr and aw > 0.92.

In figure 1 the distribution of aw values registered for salami and soppresse (all the batches) respectively are reported according to the day of ripening.

Figure 1: aw distribution for salami and soppresse
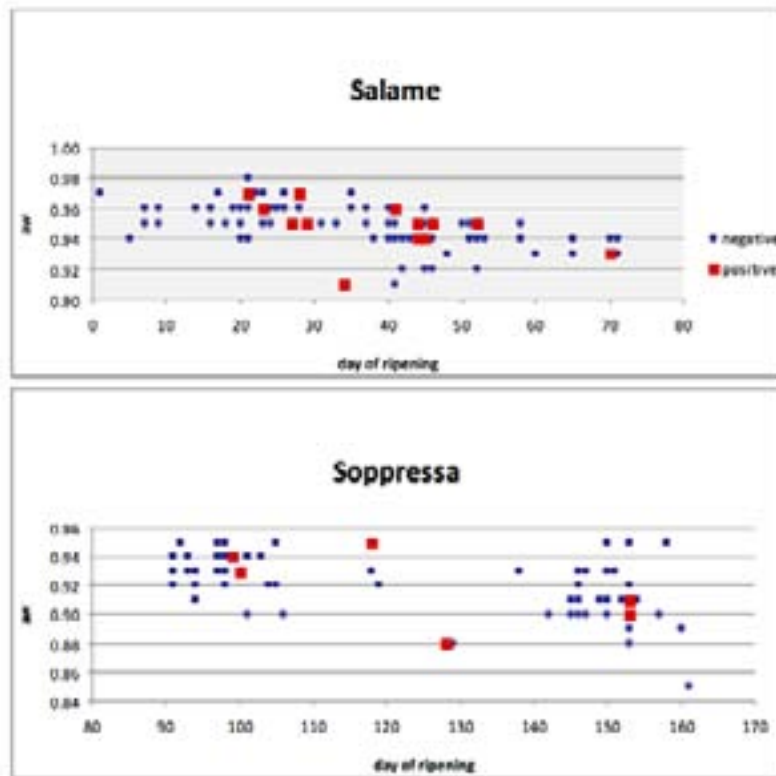
Sampling scheme B

Data from this intensive sampling scheme were analysed in order to evaluate possible correlation between the aw and the weight decrease of the sausages during ripening.

In figure 2 a graph describing the decrease in aw and weight according to the day of ripening for productive unit C is reported.

Figure 2: Sampling scheme B, productive unit C: aw and weight trend
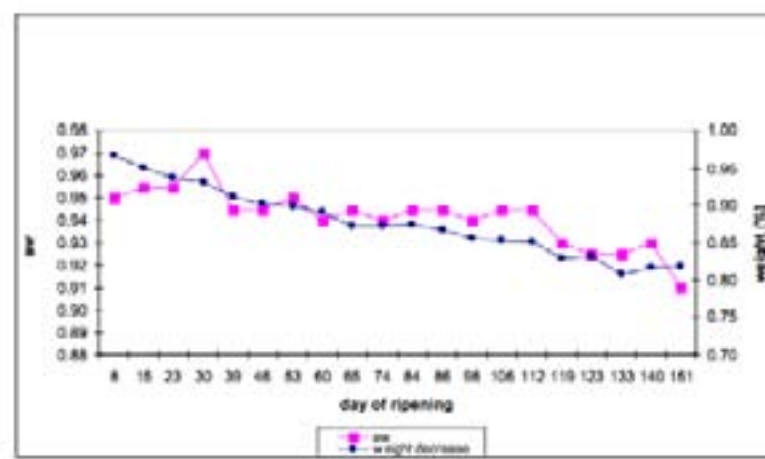
Data related to pH and environmental conditions were analysed but are not reported.

Discussion

Data from sampling scheme A revealed a very low percentage of positive sausages tested positive for Listeria monocytogenes at the end of the ripening period, moreover the level of contamination was always below 10 ufc/gr. aw value seems to be very critical particularly for salami and consistently dependent on the productive unit: the aw value was below 0.92 (not favourable to microbiological growth of Listeria monocytogenes according to Regulation CE 2073/2005) only in a small proportion of salami at the end of the ripening period. Data from sampling scheme B allowed to correlate with a good approximation a weight decrease of at least 25% to an aw decrease equal or below 0.92 both for salami and soppresse.

Conclusion

In 2010 a regional legislation has been published defining that only salami and soppresse, intended to be eaten raw, with an aw value below 0.92 may be marketed to the final consumer.

According to the data obtained a control strategy was defined based on the identification of positive/negative batches by the CA and the monitoring of the weight decrease in sausages by FBO.

CA takes faecal samples form pigs at farm level and mince ready for stuffing for microbiological tests, in case of positive results further samples of sausages are analysed until negative finding; in fact only negative batches may be marketed. FBO monitors all the batches for the weight decrease and once the 25% weight loss is obtained one sausage is submitted to an official control in order to verify the compliance of aw value with regional legislation.

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Assessing the effect of on-farm and abattoir interventions in reducing human salmonellosis from pig meat consumption in the EU

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Abstract

Pigs are commonly infected with Salmonella spp. at the slaughterhouse, and the consumption of pig meat is hypothesised to be an important contributor to human salmonellosis. The European Union (EU) will shortly set targets for the reduction of Salmonella in pigs at slaughter for each Member State (MS), and each MS is expected to put in place a National Control Plan (NCP) in order to achieve their targets. If MSs are to realise their targets then practical interventions that consistently work must be identified.

As part of the evidence base for the development of NCPs, a Quantitative Microbiological Risk Assessment (QMRA) was funded to support the scientific opinion required by the EC from the European Food Safety Authority, which was subsequently adopted by the BIOHAZ panel. Here we describe how the baseline model was modified to simulate the effect of both on-farm and abattoir interventions, and the resultant reductions on the predicted number of human Salmonella cases attributable to pig meat consumption in an EU MS. We present the results from two case study MSs with differing slaughter pig Salmonella prevalence and production systems to exemplify the differences that interventions have between MSs.

In the two MSs, both on-farm and abattoir interventions were predicted to be able to produce a significant reduction in salmonellosis attributable to pig meat consumption. Anal bunging of the carcass during processing was shown to be the most effective intervention mechanism in reducing human illness. Some intervention mechanisms were ineffective in reducing human illness, including increased cleaning and disinfection (C&D) at the farm and logistic slaughter.

Introduction

Pigs are commonly infected with Salmonella spp. upon entrance to the slaughterhouse and the consumption of pig meat is hypothesised to be an important contributor to human salmonellosis in the EU. The EU is predicted to set targets for the reduction of Salmonella in pigs at slaughter for each EU MS in 2013, and each MS is expected to put in place a NCP in order to achieve their targets. Control programs in several EU MSs are already underway (Blaha 2004; Mousing et al. 1997); however, the success of these programs is varied (Nielsen et al. 2001, BPEx 2009), In addition, it is not a easy task to attribute reductions in human cases to a control program due to the natural variation in foodborne cases that would occur regardless of any intervention.

If MSs are to meet their targets, and the EU is to realise its aim of reducing human salmonellosis attributable to pig meat consumption, then practical interventions (across the food chain) that work consistently and efficiently must be identified. The most effective interventions are likely to differ between MSs, due to factors such as differing management practices or production processes involved in producing a MS's commonly consumed pig meat products. Therefore, as part of the evidence base for the development of NCPs in individual MSs, a QMRA was funded to support the scientific opinion required by the EC from the European Food Safety Authority. As such, the main aim of this QMRA was to assess the effectiveness of interventions implemented on-farm and at the abattoir and how this may vary across EU MSs. Therefore the baseline model (EFSA 2010) was modified to describe the effect of both on-farm and abattoir interventions and the resultant reductions (if any) on the predicted number of human Salmonella cases in an EU MS attributable to the consumption of 3 pig meat products (pork chops, minced meat and fermented sausage). We present the results from two case

study MSs (one "low prevalence" MS (MS1) and one "high prevalence" MS (MS2)) to exemplify the differences that may occur in the effectiveness of interventions between MSs.

Material and Methods

In general, one of the main benefits of producing a QMRA is the ability to investigate the relative effect of interventions. These relative reductions can be investigated by comparing the baseline results (in this case, the number of human cases attributable to pork chop, minced meat and fermented sausage consumption) with the percentage reductions in the number of cases observed for each intervention. Not all farm and abattoir interventions investigated in the QMRA are presented here for reasons of space and clarity. The interventions discussed here, and a brief description of how the baseline model was modified for each intervention, are listed in Table 1.

Results

No effect in reducing human cases of Salmonella infection was seen for either MS1 or MS2 for increased C&D (both farm and transport) and logistic slaughter interventions.

The effect of reducing slaughter pig prevalence is shown in Figure 1. Reducing slaughter pig prevalence appears to be effective in reducing the number of human cases per year for each case study MS. Breeding herd prevalence was established as a significant factor within the farm model, via investigation of the main sources of infection (see accompanying conference paper on farm transmission model by Hill et al.). Broadly speaking, low breeding herd prevalence (low number of positive piglets) equates to low slaughter pig prevalence and vice versa. The intervention of reducing breeding herd prevalence produces a similar result as for reducing slaughter pig prevalence, where broadly speaking there is a proportional percentage reduction in human cases for a given percentage reduction in breeding herd prevalence (analysis not shown).

Modifying the resistance of the pig, such that a tenfold increase in dose is needed to cause the same probability of infection as for the baseline model, produced a large reduction (~90%) in the number of human cases in both MS1 and MS2. Increasing that resistance, to a 100-fold increase in dose to cause the same probability of infection, virtually eliminates human infection.

The effect of a 1, 2 or 3 log reduction in contamination of the carcass pre-chill was investigated and the results are also presented in Figure 1. A reduction of carcass contamination level of between 1 and 2 logs is sufficient to produce a large (>80%) percentage decrease in the number of human cases within both MS1 and MS2. The majority of contamination on the carcass post-singe originates from faecal leakage. Preventing this faecal leakage within the model resulted in an average reduction across all carcasses at pre-chill of roughly 1 log. This resulted in an 80-99% reduction in human cases in MS1 and MS2 (equivalent to a 1-log reduction as shown in Figure 1).

Discussion

The results of this analysis suggest that interventions (both on farm and at the abattoir) that can achieve the level assumed in the analysis could lead to large reductions (up to 99%) in the number of human cases per year in both MS1 and MS2. However, to produce these large reductions the slaughter pig prevalence and/or the level of contamination at pre-chill must be reduced by approximately ten-fold. It is unlikely that on-farm interventions, implemented on a nationwide scale, can consistently produce such large (~90%) reductions in slaughter pig prevalence (at least in the short to medium term). There is no evidence that interventions that can modify the resistance of the pig to infection

Table 1: List of interventions and the modifications made to the baseline model to assess their effect

Table 1: List of interventions and the modifications made to the baseline model to assess their effect

Stage	Intervention	Intervention analysis parameter estimate
Farm	Reduce breeding herd prevalence, p_{herd}, within farm model.	MS2 model ran with $p_{herd} =$ {0,0.01,0.05,0.1,0.2,0.3,0.4,0.5}.
	Reduce slaughter pig prevalence	Reduce slaughter pig prevalence to 10, 20, 30, 50, 70, 90, 99% of baseline result.
	Increased effectiveness of cleaning and disinfection (C&D)	An extra 1 or 2-log reduction (over and above the reduction in the baseline model) in *Salmonella* numbers present in pen after C&D.
	Increased resistance of pigs to *Salmonella* infection by using e.g. wet feed, vaccination or organic acids	Dose response parameters modified such that it takes an extra 1 or 2-log increase in dose to produce baseline model dose-response curve.
	All farms use wet feed.	Dose-response curve set to wet feed parameter estimation for all farms.
Transport	Increased cleaning	Additional reduction over and above baseline C&D effect of 0.5, 1 or 2 logs.
Slaughterhouse	Reducing/preventing faecal leakage	Set CFUs in gut to zero.
	Logistic slaughter (process high-risk pigs at end of day)	Model randomly selected batches for one day's processing, and then sorts these batches in ascending order of within-batch prevalence.
	Decontamination step pre-chill	Reduce carcass contamination pre-chill by 1,2 and 3 logs.
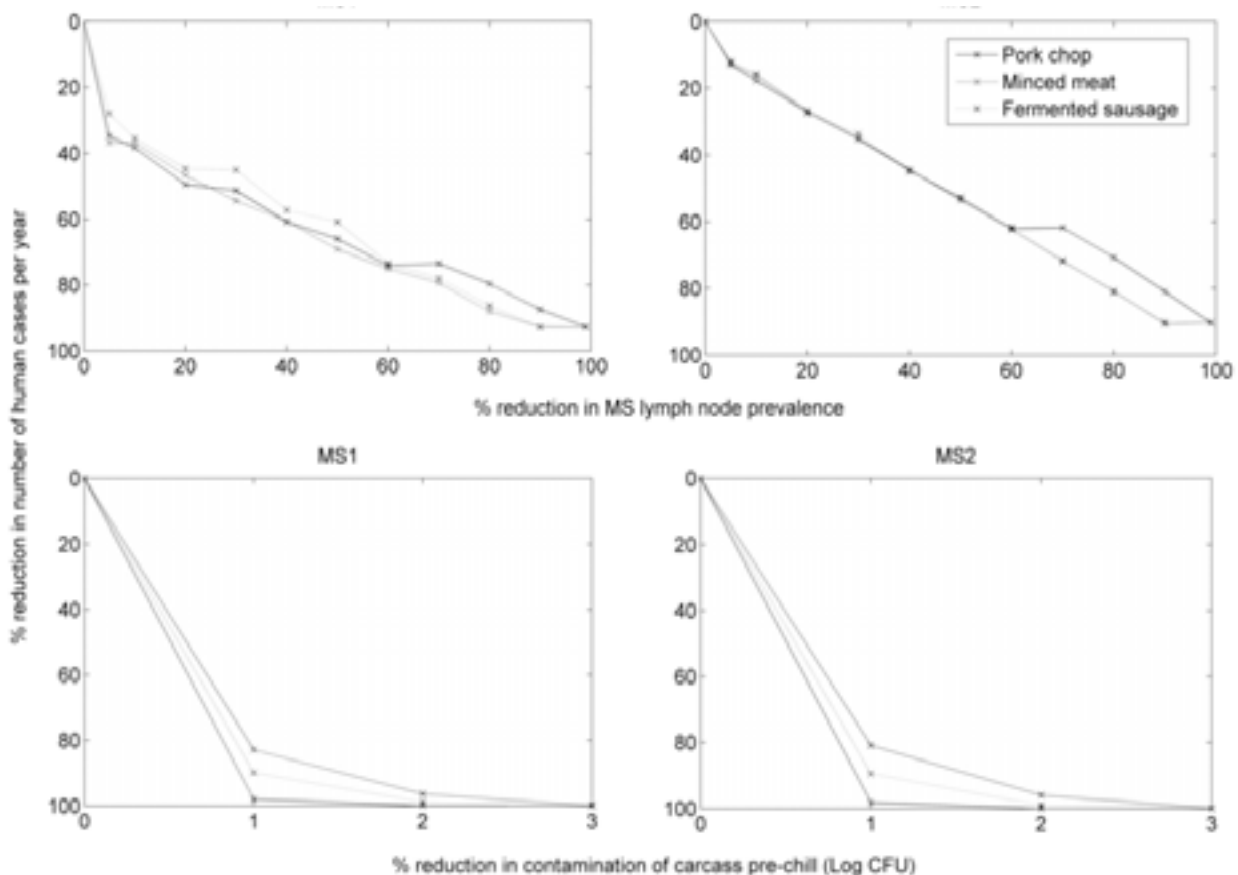
Figure 1: Percentage reduction in human Salmonella cases due to reductions in baseline slaughter pig prevalence (top) or contamination of carcass pre-chill (bottom), for MS1 and MS2.

(e.g. vaccination, organic acids, fermented feed) can induce a ten-fold increase in resistance. However, there are abattoir interventions such as anal bunging that have been shown to consistently reduce the average contamination level of pre-chill carcasses by a log or more (Christiansen et al. 2009; James 2009).

It is difficult to validate such intervention results as they are necessarily predictive. However, it is possible to validate the breeding herd prevalence reduction, by comparing the breeding herd prevalence and slaughter pig prevalence estimates for each MS from the two EFSA baseline surveys (EFSA 2008; 2009). While geographical, farm management and slaughterhouse differences are undoubtedly an issue, comparing the two studies there does seem to be a broadly linear relationship between breeding herd prevalence and slaughter pig prevalence, which is in agreement with the model result. This supports the conclusion that the sow is a major source of infection for slaughter-age pigs.

Conclusion

Theoretically, we predict that intervention at either the farm or abattoir can be effective in significantly reducing human cases. The reductions achieved are strongly dependent on the mechanism of the intervention. Decontamination of the carcass and prevention of carcass contamination are promising interventions that have evidence of consistent efficacy. The only farm mechanism investigated that was estimated to reduce human cases was modifying the resistance of the pig, through interventions such as vaccination and organic acids. However, there is no consistent evidence for the true efficacy of these resistance interventions. Increased C&D and logistic slaughter were predicted to have a negligible effect in reducing human cases.

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A Transport & Lairage model for Salmonella transmission in pigs for individual EU Member States

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Abstract

A model for the transmission of salmonella in finisher pigs during the transport and lairage stages of the farm-to-consumption chain has been developed, specifically designed with the aim of modelling potentially important risk factors and interventions. As such, the model includes factors such as environmental contamination and the effect of stress. The model forms part of a Quantitative Microbiological Risk Assessment, funded by EFSA as part of the evidence base for the development of National Control Plans for control of Salmonella in pigs, to support the scientific opinion requested by the EC and adopted by the EFSA BIOHAZ Panel. This poster describes the modelling methodology and demonstrates the parameterisation of the model for two case-study member states (MSs). For both MSs, the model predicts a small increase in the average lymph node positive batch prevalence during both transport and lairage. While the average change in prevalence over all batches is small, closer analysis shows that there is wide variation in the change in prevalence in individual batches, with some batches showing an increase of up to 70%. Sensitivity analysis (variation inherent in the baseline model) of the model suggests that stress is the most important factor during transport, while a number of parameters including the rate of carryover between batches of pigs and one of the dose-response parameters are important during lairage. This model suggests that the transport and lairage stages of the farm-to-consumption chain can have a large effect on an individual level, potentially being the cause of a large increase in the prevalence within a batch of pigs and also providing an opportunity for previously uninfected batches of pigs to become infected. However, large individual changes at a batch level are infrequent enough to not cause a similarly large change in the average national prevalence between farm and the point of slaughter.

Modelling preparation and consumption of pork products

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Abstract

This poster describes the retail and consumer phase of the EFSA Salmonella in Pork Quantitative Microbiological Risk Assessment (QMRA), which was funded under an Article 36 grant to support the scientific opinion required by the EC from the European Food Safety Authority (EFSA) and andopted by the BIOHAZ panel.

The food chain is modelled from retail to ingestion by the consumer. Three types of pork are considered: minced meat, pork cuts and dry cured sausages. This particular choice was made because each product represents a clear distinct hazard. Pork cuts are usually cooked well, but there is a chance of cross contamination during cutting and handling of the meat. Minced meat is thoroughly mixed, and Salmonellae may be present in the interior of hamburger patties, undercooking may occur, and Salmonellae may survive. Dry cured sausages, including all variations therein like chorizo, salami, etc., are eaten uncooked.

Food preparation habits are highly variable and accurate data on daily life food handling practices are hard to obtain. We performed a literature survey and parametrised the model including the inherent variability in consumer behaviour.

The output is the number of Salmonellae ingested per person per day, for each pig meat product. This output will in feed into the final model, where the risk of illness is modelled using a dose-response relation.

A risk assessment for visual only meat inspection of both indoor and outdoor pigs within the UK

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Abstract

The current system of post-mortem inspection using the typical macroscopic inspection techniques is ineffective in identifying the most common foodborne illness risks, e.g. Salmonella or Campylobacter. Therefore, there is a need to adopt a more appropriate, risk-based approach to meat inspection. One specific example of modifying traditional inspection techniques to represent a more cost-effective approach to meat inspection is the allowance in EC Regulation 854/2004 for only visual inspection of pigs that have been reared under controlled housing conditions since weaning. However, the definition of controlled housing excludes outdoor pig production from visual-only meat inspection, and hence so far the UK has yet to introduce this method of meat inspection into abattoirs because of the associated complications of having a large outdoor pig herd. We have therefore conducted a qualitative risk assessment to assess the comparative risks to public and animal health from allowing visual-only inspection of both indoor and outdoor pigs.

In order for visual-only inspection to be of higher risk than traditional meat inspection, the sensitivity of detection of a condition must significantly decrease for visual-only inspection. In addition, in order for outdoor pigs to pose a greater risk than indoor pigs, then the condition must be more prevalent in the former than the latter. From a large number of diseases/conditions originally identified as worthy of investigation, only two (TB and endocarditis) were considered to be of public or animal health risk and would be less likely to be spotted through visual-only inspection. It was determined that prevalence of TB in outdoor pigs was higher than in indoor pigs; however, endocarditis prevalence was higher in indoor pigs than outdoor pigs. Despite higher rates of TB in outdoor pigs, there was no discernable risk to public or animal health from TB-infected pigs. It was therefore concluded that visual-only inspection of both indoor and outdoor pigs in the UK posed a negligible risk to public or animal health.

Introduction

The current system of post-mortem inspection using the typical macroscopic inspection techniques is ineffective in identifying the most common foodborne illness risks, e.g. Salmonella and Campylobacter. Therefore, there is a need to adopt a more appropriate, risk-based approach to meat inspection. One specific example of modifying traditional inspection techniques to represent a more cost-effective approach to meat inspection is the allowance in EC Regulation 854/2004 for only visual inspection of pigs that have been reared under controlled housing conditions since weaning. However, the definition of controlled housing excludes outdoor pig production from visual-only meat inspection, and hence so far the UK has yet to introduce this method of meat inspection into abattoirs because of the associated complications of having a large outdoor pig herd. We have therefore conducted a qualitative risk assessment to assess the comparative risks to public and animal health from allowing visual-only inspection of both indoor and outdoor pigs.

Material and Methods

Pigs that are raised under controlled conditions since weaning may be visually-only inspected post-mortem under current regulations; among other criteria the two major, relevant conditions that define "controlled" are whether the pigs were raised indoors and in an integrated system (EC 854/2004). Expert opinion from the UK pig industry suggests that all quality-assured farms, regardless of indoor or outdoor production type, would meet the criteria for a fully integrated system given the traceability between farm and abattoir provided by Food Chain Information (FCI) and the Animal Movement Licence Systems (AMLs). Therefore, for the purpose of this document and the UK situation, we define indoor and outdoor respectively:
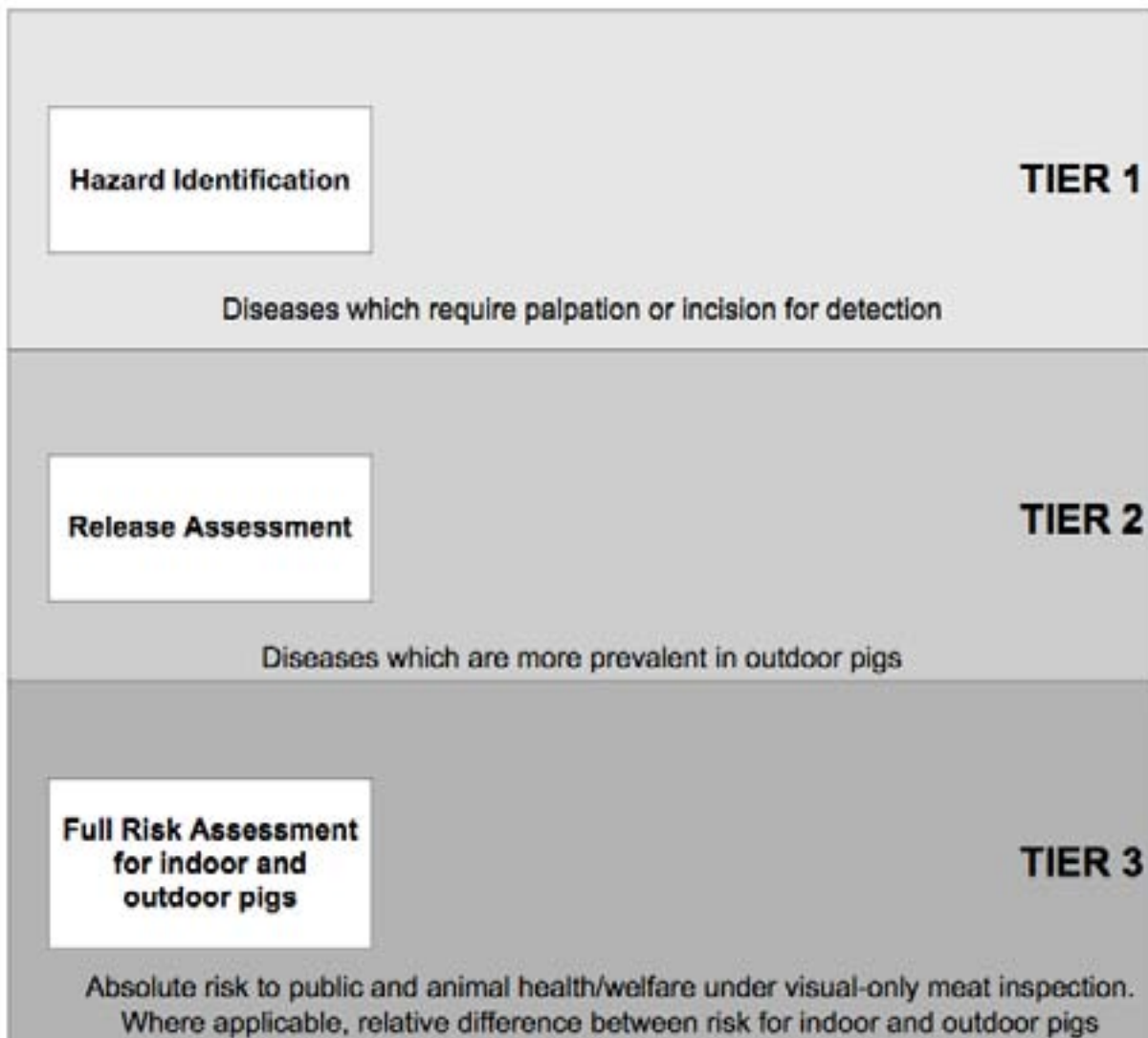
Indoor: Pigs raised indoors since weaning on a quality-assured farm.

Outdoor: Pigs raised outdoors since weaning on a quality-assured farm.

Given that pigs reared indoors since weaning (from integrated production systems) are allowed to be visually inspected, then it would be reasonable to assume that these pigs pose an acceptable risk to public health, animal health and animal welfare. We are therefore interested in whether there is any increased risk, relative to indoor pigs, from visual-only inspection of outdoor pigs, and subsequently what this change in risk means to the absolute risk to public and/or animal health. There are two main criteria that determine whether the risk will change. One, whether the sensitivity of detecting a condition is affected by switching from traditional to visual-only inspection (if not, there is no change in risk). Two, whether a condition of concern is more prevalent in outdoor pigs than indoor pigs (if not, then outdoor pigs pose no greater risk than indoor pigs). The absolute risk to public health is determined by the relationship between the burden of contaminated meat entering the food chain and the rates of human illness attributable to that contaminated pig meat. The absolute risk to animal health is assessed according to whether a potential decrease in the feedback of meat inspection information to indoor or outdoor farmers occurs. In addition to the risk assessment, we also consider, the benefits (if any) of switching to the proposed visual inspection method. Risk-benefit methods are still under development, but we have applied, as far as practically possible, the methods proposed in two recent publications (Hoekstra et al., 2008).

The two frameworks for risk and benefit assessment are shown in detail in Figure 1. Within Tier 1, a comprehensive list of distinct infectious agents and post-mortem conditions was taken from the Veterinary Laboratories Agency's own protocol for post-mortem inspection of submitted carcasses. A number of conditions were eliminated at each stage based on whether or not the risk they posed to public or animal health would be affected by two main criteria stated above. The risk assessment stage is conducted using a modified version of methods described by the OIE for import risk analysis (OIE, 2004). Under traditional OIE guidelines, there are three components: release assessment, exposure assessment and consequence assessment. Under release assessment we first assess the additional rate of carcasses (from indoor or outdoor pigs) that will enter the food chain given visual inspection of pigs with diseases of human or animal importance. Exposure and consequence assessment are treated as one, where we assess for both indoor and outdoor pigs the absolute risk to public or animal health from moving to a visual-only system of meat inspection protocol in UK abattoirs (compared to current traditional meat inspection methods). Finally we conclude with risk estimates, assessing the relative difference in risk between indoor and outdoor pigs based on the burden of relevant conditions in each type of production, and the likely human/animal consequences that occur because of the potentially increased flux of contaminated meat into the food chain, or the decreased rate of reporting of conditions back to farmers.

RISK ASSESSMENT

Hazard Identification		TIER 1

Diseases which require palpation or incision for detection

Release Assessment		TIER 2

Diseases which are more prevalent in outdoor pigs

Full Risk Assessment for indoor and outdoor pigs		TIER 3

Absolute risk to public and animal health/welfare under visual-only meat inspection. Where applicable, relative difference between risk for indoor and outdoor pigs

BENEFIT ASSESSMENT

Benefit Assessment		TIER 4

Public health benefits of visual-only meat inspection
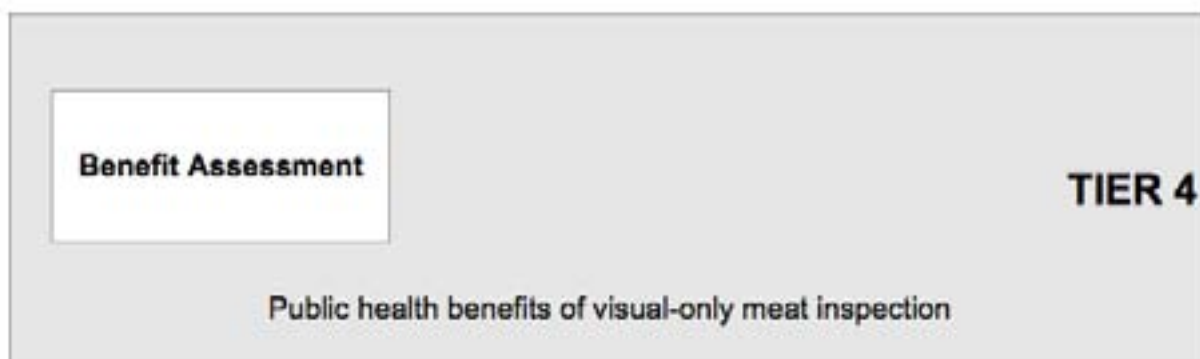
Figure 1: Tiered approach to risk and benefit assessment. Conditions that are unlikely to change in risk given visual inspection methods are identified in Hazard Identification (Tier 1). We conduct a preliminary risk assessment on conditions where risk may potentially change. Given further review, those conditions where risk will probably change are assessed in Tier 3. Benefit assessment is conducted only on those conditions identified for Tier 3 assessment.

Results

Two conditions were fully assessed under Tier 3, porcine Tuberculosis (pTb) and endocarditis, as the sensitivity of detection of these conditions was judged to be reduced under visual-only meat inspection relative to traditional meat inspection. Using data collected for conditions noted by meat inspectors, and linking that data to the batch's origin (indoor or outdoors), we determined that endocarditis was less prevalent in outdoor pigs than indoor pigs. Hence, the risk to public or animal health is no greater from outdoor pig production than indoor pig production.

Using the same meat inspection data, pTb was identified in 40 pigs (4 from outdoor production) in the period 2007-2010. It was also determined to be more prevalent in outdoor production than indoor production (up to double the incidence rate). Outdoor production is of relatively higher risk than indoor production. However, if visual-only inspection had have been implemented for outdoor pigs in this period, we would have missed only around 4 extra pigs that would have subsequently entered the food chain. Within the UK in the region of 50-100 cases of non M. tuberculosis in humans are recorded every year, and the majority of these (mostly M. bovis) cases will be epidemiologically linked to raw milk consumption in older persons. The contribution of pig meat consumption to human Tb infection is therefore judged to be negligible, regardless of the meat inspection method used.

We were unable to collect enough evidence to state the effect on animal health and welfare by the reduction in the detection of pTb using visual-only meat inspection, but it is unlikely the non-detection of around 40 pigs in four years would make a huge impact on animal health and welfare. However, given there is no other surveillance method for Tb in pigs than meat inspection, we gave a conservative assessment of risk as very low, rather than negligible. We did not find any conclusive evidence that visual-only meat inspection benefited public health by the reduction of microbiological cross-contamination (because of eliminating incision of the lymph node or heart, for example).

Discussion

The results of this qualitative risk assessment agree with those produced in a similar Danish risk assessment (Anon., 2008); however, we have also extended the result to outdoor herds as well, and also assessed the effect on animal health/welfare.

There were large data gaps for some crucial aspects of the risk assessment, particularly for animal health and welfare. A major data limitation that would have some bearing on the results of the risk assessment was the meat inspection data used to assess the relative incidence of pTB/endocarditis in indoor and outdoor pigs respectively. We used data collected from an electronic system being trialled in pig slaughterhouses. The results of this data analysis are preliminary, and better, more targeted surveillance is needed to confirm the results. The UK Food Standards Agency is now in the process of funding a study to collect data to fill the gaps identified by the risk assessment.

Conclusion

We conclude that the public health risk of moving from a traditional post-mortem meat inspection method to a visual-only system is negligible, for both indoor and outdoor production systems. The only non-negligible (very low) risk is that posed to animal health/welfare because of pTb, where meat inspection is the only form of animal surveillance. Moving to a visual-only system would reduce the sensitivity of pTb detection to almost zero. However, the risk was still judged to be virtually negligible because of the small numbers of positive identifications of pTb-pigs.

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Sanitary status of 47 pig manures in Brittany: comparison of the effectiveness of manure treatments on the levels of indicator bacteria and two pathogenic bacteria

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Abstract

The hygienic performance of three manure treatment systems (simple storage, biological treatment or thermal treatment) was evaluated for effuents collected from 47 piggeries across Brittany, France. Microbial analyses were carried out on raw manure, on the sludge stored in a tank after biological treatment and on the liquid phase stored in a lagoon after sludge settling or after thermal treatment. The effect of the treatments on E. coli, enterococci, Salmonella and Listeria monocytogenes was evaluated. The concentrations of indicator bacteria were highly variable regardless of the farm or the manure management. The biological treatment had only a small effect on E. coli and enterococci (average reduction between raw manure and sludge £ 2 log10). Salmonella were present in 50% of the raw manures, 14.8% of the sludges and in 7.4% of the lagoons. Despite their high prevalence in raw manure, their concentrations remained low and did not exceed 11 bacteria per gram of manure. The prevalence of L. monocytogenes was lower. However, this pathogenic bacteria was detected in 21% of the raw manures, in 15.4% of the sludges and in 28.6% of the lagoons. Salmonella Derby and L. monocytogenes serotype 4b each accounted for 50% of the serotypes identified in the samples. Although the biological treatments make it possible to decrease the level of E. coli and enterococci, they do not achieve complete sanitisation of the by-products. As a consequence, there remains a significant risk of spreading the pathogenic bacteria during the land application operation.

The Role of Proactive Risk Assessments in Ensuring Business Continuity in the Swine Industry during an FMD Outbreak

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Abstract

Emerging and reemerging pathogens of food animals, such as Foot and Mouth Disease (FMD) and H5N1 highly pathogenic avian influenza (HPAI), have the potential to disrupt the supply of food commodities. In the event of an FMD outbreak in the United States, permit requests to move pigs and pork products must be supported by risk assessments (3). To prevent disruptions in business continuity, we developed a novel approach toward improving veterinary emergency-response preparedness. Through a public private collaboration that involves industry, USDA APHIS and academia representatives, we assess the routes of exposures and transmission pathways of infectious diseases prior to the occurrence of an outbreak. This approach allows us to develop appropriate and applicable mitigation measures and biosecurity practices that will help control or lessen the impact of a disease outbreak. This methodology could also be employed for specific food borne pathogens

Introduction

Emerging and reemerging pathogens of food animals, such as Foot and Mouth Disease (FMD) and H5N1 highly pathogenic avian influenza (HPAI), have the potential to disrupt the supply of food commodities. A brief disruption in product or animal movements could result in devastating impacts to the industry, as well as serious animal welfare concerns. Proactively evaluating the risks before an outbreak occurs enables timely movement permitting decisions to be made and supports continuity of business.

FMD is a disease of cloven hoofed animals that is highly contagious with a pan continental distribution, evident by the recent outbreaks in Southeast Asia and Africa. Infected animals usually develop blister-like lesions in the oral cavity, mammary gland and inter-digital areas, resulting in lameness and excessive salivation. Susceptible pigs become infected primarily by coming in contact with infected animals and animal products. FMD is not recognized as a zoonotic disease (5). The economic impact of this disease is usually devastating to the agricultural industry and to other closely associated industries. A study by the U.K. National Audit Office estimated the direct cost of the 2001 FMD outbreak to the public and private sector at over $15.22 billion, and the number of animals slaughtered at six million. (1). Foot-and-mouth disease was last reported in the U.S. in 1929, thus there is an existing large pool of naïve animals in the U.S. In addition to the ravaging effects of the disease, the emergency response could potentially have a great impact on the US food system. The need to prevent disruptions in business continuity prompted the development of the proactive risk assessment approach, which would help inform decisions makers prior to and during an FAD incursion in the US livestock industry.

Risk assessment is one of the four steps in the process of risk analysis following the OIE framework. The other components include the hazard identification, risk management and risk communication. Hazard identification focuses on providing an understanding of the biology and epidemiology of the agents of concern. Risk assessment is focused on determination of the probability of entry, establishment and spread of a disease, in conjunction with the associated potential of biological, economic and public health ramifications (4). Risk is defined as a product of the likelihood of occurrence of the event and the magnitude of the consequences.

Risk assessment is broken down to two main components, release and exposure assessment. The release assessment seeks to identify the potential pathways for disease introduction (6).The exposure assessment determines the potential pathways leading to exposure of susceptible animals. In the event of a foot-and-mouth disease (FMD) outbreak in the U.S. livestock industries, local, State, and Federal authorities will implement a foreign animal disease emergency response. This

response consists of a control and eradication strategy that will utilize quarantine and movement control measures to prevent further spread of FMD virus. The authorities will issue official permits to allow movement of susceptible animals, and their products, from premises identified in a quarantine order during an outbreak. A request for a movement permit must be supported by a risk assessment (or some scientifically-based logical argument) to demonstrate that the risk associated with the movement of the product in question is acceptable (3).

The concept of proactive risk assessment (PRA) is based on traditional risk assessment tenets. It is a new process which was developed to support the recognition by government and industry for the need to allow business continuity planning prior to a disease outbreak. The risk assessment is done in advance of the outbreak in order to minimize disruptions to industry and consumer markets. The evaluation addressed the current industry practices, such as the just in time product delivery systems. It also identifies potential areas of risk, which can further be reduced by mitigation procedures (3). The goal of the process is to identify the most worrisome commodity and pathogen combinations, evaluate the associated risk, and develop mitigation strategies that are acceptable to all stake holders.

Methods

This PRA was developed as a public-private partnership between government, academic and industry stakeholders including USDA Center for Epidemiology and Animal Health and USDA VS Center for Animal Health and Emergency Management, University of Minnesota Center for Animal Health and Food Safety, industry stakeholders and other subject matter experts. The process follows the OIE risk analysis guidance and uses both qualitative and quantitative methods as applicable.

This PRA focused on the risk associated with the movement of FMD infected, but undetected pigs to a slaughter facility and the processing of these animals into RTE products, during an FMD outbreak. The estimation of this risk involved the evaluation of multiple pathways — from the introduction of a virus or a latently infected pig on-farm through the slaughtering and processing of the pig into RTE product. The likelihoods of virus moving successfully from farm to processing plant were evaluated using varying levels of surveillance. The assessment took into consideration all applicable regulations, including preventive measures already in place, as well as additional preventive measures that will be implemented during an outbreak.

The assessment evaluated the risk that Ready-to-Eat (RTE) pork could be contaminated with FMD virus from potentially infected but undetected pigs, shipped to a FSIS inspected slaughter facility during an FMD outbreak. It involved (1) Determination of the likelihood that animals from which the product was derived were infected with FMD at the time of slaughter and (2) the likelihood of FMD surviving RTE product preparation steps. A SLIR (susceptible, latent, infectious, and recovered) disease model for on-farm spread of disease was built, based on the Reed Frost epidemic disease spread model. Figure 1 shows the differential equations and input values used in modelling the disease stages. The results from the SLIR model were used to simulate the number of animals that could be shipped to slaughter using different surveillance protocols. Estimation of the risk of infected, but undetected pigs not being detected at the slaughter facility and subsequently processed into RTE product was performed using a qualitative approach.

[1] *During an outbreak, APHIS conducts numerous product-specific risk assessments taking into consideration all permit requirements and preventive measures currently in place.*

[2] *Normal day-to-day operations and preventive measures are in place via FMD Response and Preparedness plans, HACCP plans, Good Manufacturing Practices (GMP), State regulations and Federal regulations as required by FSIS, FDA, and APHIS*

Results

The results indicated that regardless of the surveillance strategy used, it was likely that a small number of infected, but undetected animals will be processed into finished RTE products. The number of pigs will be small, but the range of pigs that could be processed can be quite large if multiple farms are infected, but undetected. For an infected farm of 1,000 pigs, assuming no movement of newly susceptible pigs onto the farm during the 30 day period, the results of the SLIR model indicated that it would take a minimum of 3.12 days after an introduction of virus (or latently infected pig) to observe the first clinically infected pig with a 95% detection probability, when the surveillance method was based on testing 30 randomly selected pigs with rRT-PCR. At this time, there are already 262 infected pigs in the herd (168 latent, 62 pre-clinical and 32 clinically infected pigs). All susceptible pigs would be infected by 5.5 days after the introduction of virus.

The probability of receiving at least one or more infected, but undetected pigs at a processing facility ranges is greater than 85%, but the specific number received depends on the surveillance method employed. This percentage is based on shipping pigs from one farm and up to 20 farms that contain 1,000 pigs/farm. On average, the number of infected/viremic pigs that will be shipped is small, but the issue is that the range of pigs that could be infected or viremic and not detected can be quite large.

Data gaps

An important output from the PRA process is the determination of areas of uncertainty (data gaps) that need to be addressed in order to more fully evaluate the risks during an outbreak. This helps prioritize research activities to obtain answers to these areas of data paucity before the occurrence of an outbreak.

Discussion

A number of worldwide FMD responses have resulted in undesirable impacts to the business and livestock industry they seek to protect. Development of such proactive risk assessments prior to an FMD outbreak provide a novel approach towards achieving adequate movement control and bio-security without compromising business continuity.

This process serves as a great example of how public-private partnerships that involve industry, academia and government can work together to achieve better preparedness and develop effective response plans that address the needs of disease control and eradication as well as continuity of business needs during an FMD incursion or any animal health emergency in the United States.

Figure 1: Schematic model of a disease stages in a farm.
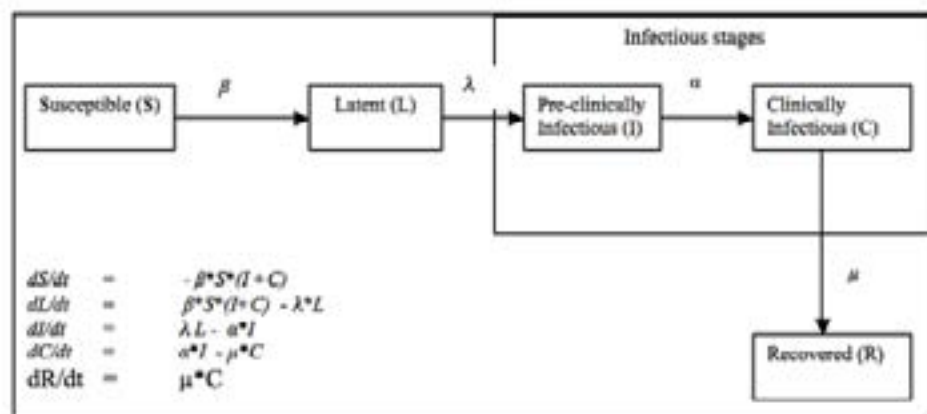
Legend:

Susceptible (S) = animal susceptible to infection.

Latent (L) = pigs have been exposed, but have not shed virus or developed clinical signs.

Pre-clinically Infected (I) = viremic, but have not developed clinical signs.

Clinical (I) = animal is currently infected and showing clinical signs of disease.

Recovered (R) = Animal has recovered from disease.

B, λ, α, μ = transmission coefficients for transitions from one stage to the next stage.

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Determine the room for improvement of processes within the management of crisis and their prevention – the maturity model

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Abstract

Crisis within the meat sector usually causes high economic losses for the affected sector and frequently for other sectors, too. Interrupted or poor communication channels are weak points in management-systems, especially in the management of crisis situations or of the prevention of crisis. In a consequence necessary information for a proper decision making is missing or not available in time. Therefore, processes that provide a sufficient and fast exchange of information between all private and public actors play a crucial role. Against this background the idea of the Engage-Exchange-Model (EEM) was developed to optimize and provide processes to exchange different information between public and private organizations in crises and regular operations. Further on specific information were defined that are necessary to support processes to prevent crisis or to support the crises management. To assess existing or new processes which support an EEM, the maturity model (ISO/IEC 15504) was successfully applied. Even if it was developed for the IT-sector, it also could be used within the meat sector by adapting its main inputs towards the specific requirements. The main advantages of the maturity model are the categorizations of the processes in capabilities-levels. This leads to an absolute assessment of the single processes on a given scale from 0 to 5 instead of a relative assessment in comparison to other methods, like benchmarking or auditing. This will support the decision-making whether to improve a process or not. In this study the inner and inter-organizational processes of public and private actors within the meat sector are investigated. Missing or poor processes in order to prevent crisis or to support the crisis management could be identified and build the basis to determine a specific EEM for the investigated meat sector as a public private partnership.

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Impact of the thermal treatment of pig slurry on vegetative and spore forming bacteria

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Abstract

Microbiological risk from pig slurry is considered a major public health problem, as pathogenic microorganisms can be spread from land application of manure. Furthermore, with growing demand of water quality for domestic and industrial use, it is becoming necessary to find reliable methods for sanitisation that are economically acceptable. In this context, the aim of this study was to establish the effectiveness of thermal sanitation of pig slurry. The continuous pilot plant (115 litres/hour) used in this study, comprised two tubular heat exchangers followed by hot liquid retention set at 10 minutes. The first exchanger recovered up to 70% of heat from the returning hot liquid to pre-warm the feed to an intermediate temperature. External heat was used in the second unit to reach the target temperature set as 70, 80 and 96°C. The effect of the thermal treatment was evaluated on E. coli, Salmonella, enteroccoci, C. perfringens and on Total Culturable Bacteria (TCB), all naturally occurring in the pig slurry. Colonies present after heat treatment on medium used for TCB counts were identified using molecular methods based on 16S rRNA gene analysis. Heating at 70°C was sufficient to inactivate mesophilic vegetative bacteria. Holding for 10 min at 80°C inactivated vegetative forms of all indicators tested but not the related spores. The identification of the colonies revealed the presence of C. botulinum, C. sporogenes and C. perfringens. When held for 10 min at 96°C, we observed a reduction of spore forms by less than 2 log10 for TCB and by 4 log10 for C. perfringens which was still present at around 20 CFU/ g of slurry. A longer retention of 20-30min may be sufficient to ensure its absence in 1 gram. However, a complete removal of risk could not be assured because of the presence of more resistant spore formers such as C. botulinum. Despite the reduction, more than 103 CFU/g of TCB still remained possibly including pathogens. Temperatures over 96°C are thus needed if the target is the complete inactivation of all spores.

Prevalence of MRSA CC398 in pig holdings

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of healthcare- and community-associated infections worldwide.

MRSA has emerged in pigs. Within the framework of the Euregio project SafeGuard VetMed-net, dust samples and nasal swabs were collected in the Euregio in 2009 and 2010. Mostly found are CC 398 Livestock-associated (LA)-MRSA. The prevalence of MRSA on pig farms in the Euregio is higher, than the overall

prevalence in Germany as indicated by a recent EFSA report. Spa types t011and t034 are still predominant.

Using a dust sampling method, 59% of all pig holdings were affected. Among 103 MRSA isolates seven different spa types were found, including t011, t034, t2510,, t1456 and t108, t588, t1606. All MRSA found were associated with CC398.

During the admission of patients in german hospitals located in an area with a high density of pig-production colonisations of MRSA CC398 are frequently found. Nevertheless invasive human infections due to MRSA CC398 are rare until now. The risk of nosocomial spread of MRSA CC398 within the human healthcare setting is undetermined.

The most dangerous component, the Panton-Valentine leukocidin (PVL), which often causes serious human diseases , has not been found in the studied animal-associated (LA) MRSA. It was also found that the MRSA strains in pigs are lacking the gene that is responsible for the development of resistance against effective antibiotics. Therefore it is still possible to prevent infections although the life associatd MRSA pathogen extents in animals and humans.

The future activities of the safeguard project will be to educate the pig farmers how to prevent infections through informations on web sites and folders. The target will be to reduce the colonisation in humans. The MRSA prevalence in the livestock should also be reduced. An identification of virulence markers and an early warning system for epidemic strains will be developed.

Trends in antimicrobial resistance of E. coli isolated from pigs at slaughter

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Abstract

Countrywide, harmonized microbiological resistance monitoring of indicator Escherichia coli isolated from healthy pigs at slaughter wasrun in 2009 and 2010. Resistance to each of the 13 tested antimicrobials was found, reaching a maximum value of 40%in the case of streptomycin and tetracycline. Cephalosporin resistance was sparse andless than 10% of strains possessed quinolone resistance. Numerous resistance profiles comprised up to 11 compounds from 7 antimicrobial classes. Year-to-year trendsin resistance were not significant, with the exception of the decrease of the number of non-resistant strains (P 0,05). The most frequent resistances correlate with the consumption of antimicrobials used for swine treatment. The results justified the need for continuous resistance monitoring followed by study on genetic background of the resistance. The attention is also drawn to the public health impact including possible therapeutic failures with drugs critically important for human medicine.

Introduction

Public health concerns have lead numerous countries to monitoring of antimicrobial resistance in indicator bacteria. The results provide a baseline needed for the assessment of public health threats due to possible therapeutic failures and control of emerging resistances and resistance mechanisms. Furthermore, they are a measurement of antibiotic consumption in animal production leading also to the issue of residues that might compromise consumers' health. European Food Safety Authority recommended a harmonized antimicrobial resistance monitoring scheme of Escherichia (E.) coliaiming at comprehensive and comparable data at the EU level. Pigs are one of the target populations that should be covered by randomized sampling at slaughter to obtain a reasonable number of 170 isolates guarantying a predefined sensitivity of the testing. E. coli isolation and identification procedures, resistance testing and reporting are also addressed in the guidelines[1].

Duringthe study period 36,9mln of pigs were slaughteredat approximately 700 abattoirs in Poland (Tab. 1). The aim of the study was to assess the level of microbiological resistance in indicator E. coli strains isolated from random, healthy pigs at slaughter and the identifyresistance trends in 2009 – 2010.

Material and Methods

The abattoirs contributing to 70% of annual pig slaughter capacity were chosen to perform randomised sampling by veterinary officers (Tab. 1). The randomisation of sampling was validated using index of diversity (D) [2]on the following variables: farm of origin of sampled animal (farm ID), geographical location of farm (county area code), and sampling date (Tab. 2.). Mapping of farm locationswas used to visualise overall coverage of the country territory (data not shown). Each sampling contained rectal swabs collected from 3 consecutive animals from slaughter line. The sampleswere referred to the laboratory in transportation medium in ambient conditions by acourier service. Theywere directly streaked on MacConkey agar the firstisolate showing typical E. colimorphology was used for biochemical confirmation followed by antimicrobial resistance testing with microbroth dilution method (Sensititre, TREK D.S.). MIC values of 13 antimicrobials tested (Tabl. 1) were interpreted according to EUCAST epidemiological criteria tosplit bacterial population into wild-type (WT) and non-wild type (NWT) strains. The latter group was considered as microbiological resistant due to the possible presence of resistance mechanism against given antimicrobial.

Results

A sufficient number of samples was collected evenly over the study period from pigs originated from a number of farms located all over the country. The D index close to the maximum value of 1confirmedsatisfactory randomisation of sampling (Tab. 2).

Tab. 1. Annual pigslaughter data

|	2009	2010
Number of slaughtered pigs (Veterinary Service data)	17420288	19488804
Number of involved slaughterhouses (Veterinary Service data)	706	694
Number (%) of slaughterhouses selected for sampling	109 (15%)	94 (14%)
Contribution of selected slaughterhouses to overall annual slaughter	70%	70%

Tab. 2. Randomization of sampling

	2009	2010
Number of collected samples	181	175
Number of sampled farms (D)	108 (0,988)*	92 (0,985)*
Number of counties – geographical location of farms (D)	70 (0,974)*	90 (0,993)*
Number of sampling days (D)	65 (0,980)*	92 (0,990)*
Number of tested strains (*E. coli* recovery rate)	178 (98,3%)	170 (97,1%)

* data were not available for all sample sets

The frequency of resistance in tested E. coli strains were shown in Tab. 3. Microbiological resistance to each of compounds tested was found and in the case of streptomycin and tetracycline it was reaching 40%. Cephalosporin resistance was sparse in both study periods whereas up to 10% of strains possessed quinolone resistance. Year-to-year changes in resistance level were not significant (Fig. 1). They tend to increase in cephalosporins, phenicols, and streptomycin but decline in other compounds, reaching with maximum decrease in ampicillin (7,5%) and ciprofoxacin (5,8%).

Tab. 3. Microbiological resistance in E. coli

antimicrobial	NWT > mg/L	Microbiological resistance			
		2009 (N=178)		2010 (N=170)	
		N	%	N	%
Ampicillin (AMP)	8	49	27,5%	34	20,0%
Ceftazidime (CAZ)	0,25	2	1,1%	2	1,2%
Cefotaxime (CTX)	0,5	1	0,6%	2	1,2%
Gentamicin (GEN)	2	6	3,4%	4	2,4%
Kanamycin (KAN)	8	9	5,1%	8	4,7%
Streptomycin (STR)	16	71	39,9%	72	42,4%
Nalidixic acid (NAL)	16	18	10,1%	9	5,3%
Ciprofloxacin (CIP)	0,032	24	13,5%	13	7,6%
Sulfamethoxazole (SMX)	256*	60	33,7%	52	30,6%
Trimethoprim (TMP)	2	34	19,1%	24	14,1%
Chloramphenicol (CHL)	16	11	6,2%	15	8,8%
Florfenicol (FLR)	16	2	1,1%	6	3,5%
Tetracycline (TCY)	8	74	41,6%	66	38,8%

Figure 1. Trends in microbiological resistance 2009-2010 (N.S.; P>0.05)
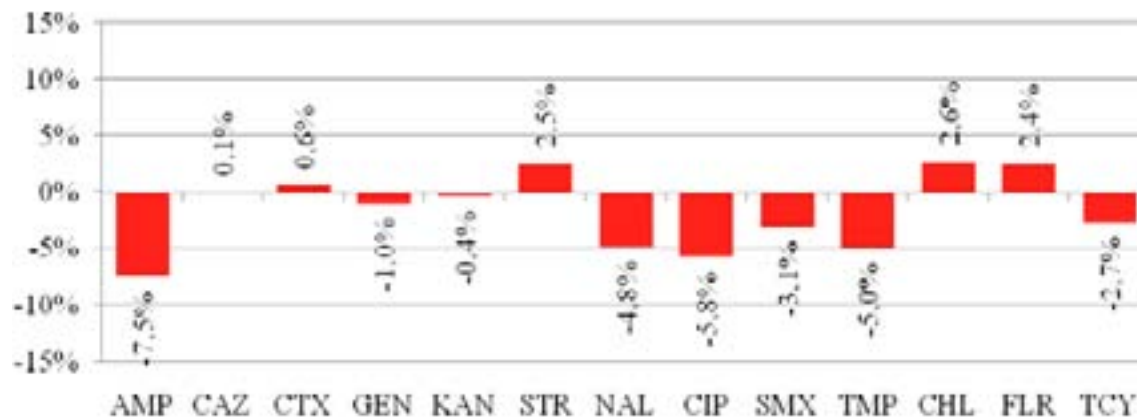
The percentage of WT strains with no resistance mechanisms increased from 34,3% to 44,7% (P 0.05).Fifty-five resistance profiles were observed in 2009 compared to 45 in the next year. The most complex profile comprised 11 antimicrobials from 7 antimicrobial classes (AmpNalCipChlFlrTcyStrGenKanSmxTmp). The shift in the number of strains resistant to one to seven antimicrobial classes was not significant, although occurrence of resistance to 2 classes decreased by 6.1% from 27,4% in 2009. The opposite trend (6,5%) was noted in strains resistant to 3 classes reaching the value of 24,5% in 2010.

Figure 2. Trends in resistance profiles 2009-2010
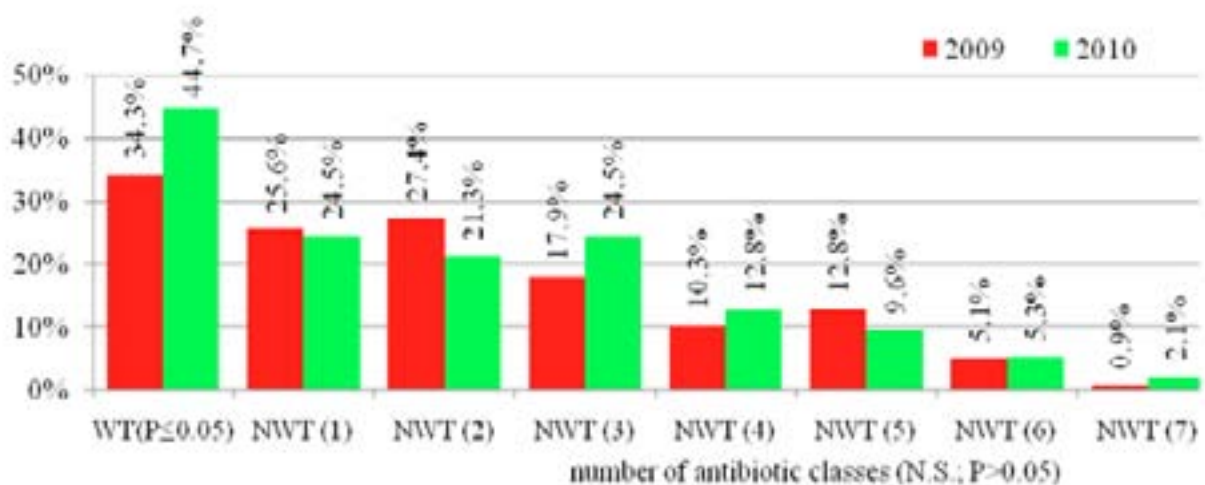
Discussion

Human infections with antibiotic resistant bacteria are often traced back to animal reservoirs of the pathogen or resistance mechanisms[3, 4]. We believe the present study is one of the first reports of the harmonised resistance monitoring as recommended in the EU [1]. Tetracycline, penicillins, sulphonamides and aminoglycosides constitute 83,7% of the compounds used in swine treatment in Poland [5] and the resistances observed in our study demonstrate the correlation with antimicrobial consumption resulting in the selection pressure[6, 7].The observed resistance levels are in concordance with the other studies [7].

Anassociation between E. coli resistance from animal, human, and environmental sources has been reported[3, 7], but resistance mechanisms may significantly differ [8]. Besides the compounds widely used in pig production, our results draw the attention to the antimicrobials showing less frequent resistances, but considered "critically important" for human medicine, such as cephalosporins and fluoroquinolones[9].Our previous studies demonstrated that pigs are often colonised with E. coli resistant to extended spectrum cephalosporinases (ESC), mostly CTX-M1 and CMY-2 [8, 10]. The

higher number of ciprofoxacin compared to nalidixic acid resistant strains proved the presence of plasmid mediated quinolone resistance (PMQR). A subset of those strains were included in the international collaborative study in several European countries and carried qnrS1 and qnrB15 genes [11]. Both PMQR and ESC resistance mechanism may spread horizontally compromising public health safety [3, 4, 6].

Conclusion

We conclude that countrywide, randomized, and harmonized monitoring introduced in pigs at slaughter revealed comparable data on the most crucial microbiological resistances in indicator E. coli, that are correlated with antibiotics used for pig treatment. Some variations were observed over two years, but only a decrease in number of resistant isolates was statistically significant. The trends in E. coliresistance and the complex resistance profiles justify the need for continuous monitoring followed by the identification of genetic backgrounds of the resistance mechanisms to reduce public health risk due to the transmission of resistant pathogens of animal origin.

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Development of a serological Luminex assay for Trichinella and Salmonella in swine

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Abstract

In order to develop veterinary serological multiplex assays, a singleplex bead-based array on the Luminex platform was developed, and with this experience the study was expanded by building a multiplex serological assay. First a serological Luminex assay was developed for Trichinella in swine. As the developed assay performed comparable to commercial ELISA's, work on this platform was continued by developing a serological multiplex assay for Salmonella in swine. This assay is based on five LPS variants of the most important serogroups occurring in pigs. The serological multiplex assay for Salmonella performed comparable to a commercial ELISA. The results from this study demonstrate the feasibility of the Luminex platform for multiplex serology. Ultimately, this type of assay can be used for routine screening of porcine serum samples for immune responses against multiple pathogens in one assay.

Introduction

To combat infections and increase food safety, slaughter pigs are monitored for the presence of pathogens, amongst others zoonotic pathogens like Trichinella and Salmonella. Here we describe the development of serological suspension arrays for Trichinella and Salmonella in swine on the Luminex platform.

Trichinellosis is a parasitic zoonosis affecting at least 11 million people all over the world (1). The disease is caused by nematodes of the genus Trichinella, in humans most prevalently Trichinella spiralis, by ingestion of raw or undercooked meat. As trichinellosis is an OIE notifiable disease, in the European Union meat from over 167 million pigs is inspected each year (2) with an artificial digestion method using pooled material. For positive pools individual samples are investigated microscopically, altogether a labour-intensive and costly procedure. In 2006 the EU approved new legislation on risk-based Trichinella control using serology (SANCO/2537/2005); ELISA based on excretory/secretory (E/S) antigens of T. spiralis larvae has been described (3) but is not common practice in Europe.

Salmonella, the second zoonotic pathogen that is subject of this study, is considered a major public health hazard with more than 29.000 cases of gastroenteritis in the Netherlands alone. About 26% of these cases is attributed to the consumption of pork (4). In contrast to serology for Trichinella monitoring, serology is a widely accepted tool for Salmonella monitoring. In countries like Denmark and the Netherlands ELISA-based serology is used for monitoring the Salmonella status of pig herds (5). Commonly the major antigenic component lipopolysaccharide (LPS) is used for Salmonella serology, and various ELISA's are commercially available.

The Luminex technology enables simultaneous testing of multiple serological components within one sample, i.e. multiplex ELISA. The technology is based on flow cytometry and uses polystyrene microspheres (beads), available in different colours. The bead surface is carboxylated to allow covalent coupling of compounds, i.e. antigens in case of a serological assay (Fig. 1). By using combinations of differently coloured beads, where each set carries a distinctive antigen, multiplex serology is possible. The Luminex platform has successfully been used for multiplexed serology in human serum (6) and veterinary assays that detect antibodies against multiple pathogens have now been published (7).

In order to develop a bead-based serological assay that can serve as a scaffold for expansion with similar assays, we first focused on developing a Luminex assay for Trichinella serology in swine. As an evaluation showed that this assay performed comparable to commercial ELISA's, we continued to develop an assay with multiple Salmonella antigens for serology in pig serum. This developed assay also performed as good as a commercial ELISA. These results showed that the developed assays adequately detect serum antibodies in pig serum, and demonstrate the feasibility of the Luminex platform for multiplex serology in veterinary applications.
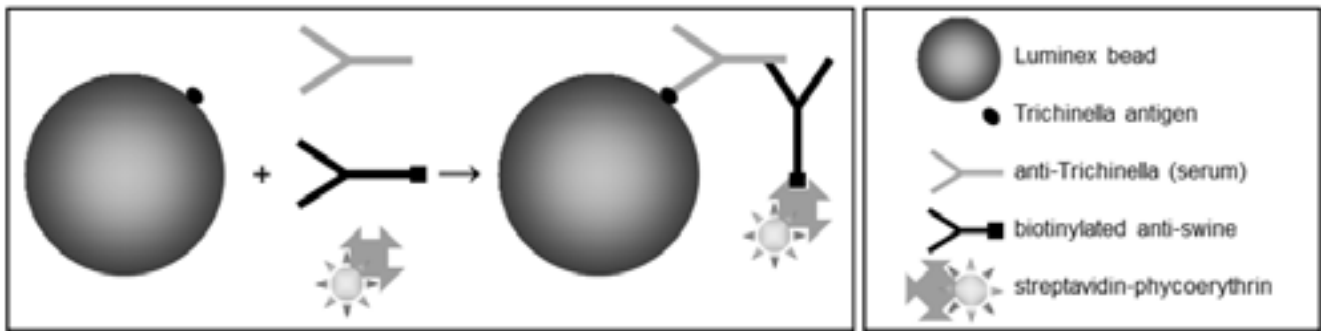
Figure 1 Principle of a serological Luminex assay. In this example Trichinella serology is depicted. A bead with covalently bound antigen is used to catch specific serum antibodies. These are sandwiched using biotinylated anti-swine antibodies and the complex is fluorescently labelled via biotin using streptavidin-phycoerythrin. With two lasers the Luminex system investigates the colour of beads (and thereby the bioassay) and the presence of fluorescent phycoerythrin, where the amount of fluorescence detected reflects the amount of antibodies caught from the serum sample.

Material and Methods

Trichinella excretory-secretory (E/S) antigens (J. van der Giessen, RIVM) and five Salmonella LPS's were purchased (Sigma) or isolated using standard protocols. Antigens were coupled to 2.5×10^6 carboxylated paramagnetic beads (Magplex microspheres, Luminex). Bead-based assays were performed with a flow cytometry-based Luminex 200, essentially as recommended by Luminex, using 1000 beads per assay. Assays were performed in phosphate buffered saline; for Salmonella assays a high salt concentration was used to reduce background. For antibody detection in pig and rabbit sera biotinylated anti-swine or anti-rabbit antibody (Jackson ImmunoResearch) was used in combination with streptavidin conjugated phycoerythrin (Invitrogen). Using xPONENT 3.0 software (Luminex) samples were analysed by measuring the fluorescence of minimal 100 beads per sample at default settings. Results were expressed as median fluorescence intensity (MFI). For evaluation purposes, results previously obtained with commercial ELISA's were used (two for Trichinella, one for Salmonella; C.B.M. Maassen, manuscripts in preparation). To compare results of suspension arrays with ELISA, diagnostic sensitivity (positives in test A / positives in B), diagnostic specificity (negatives in A / negatives in B), and overall agreement (positives and negatives in common / total number) are given. As a measure of agreement between assays Cohen's kappa was calculated using the online Graphpad Quickcalc software.
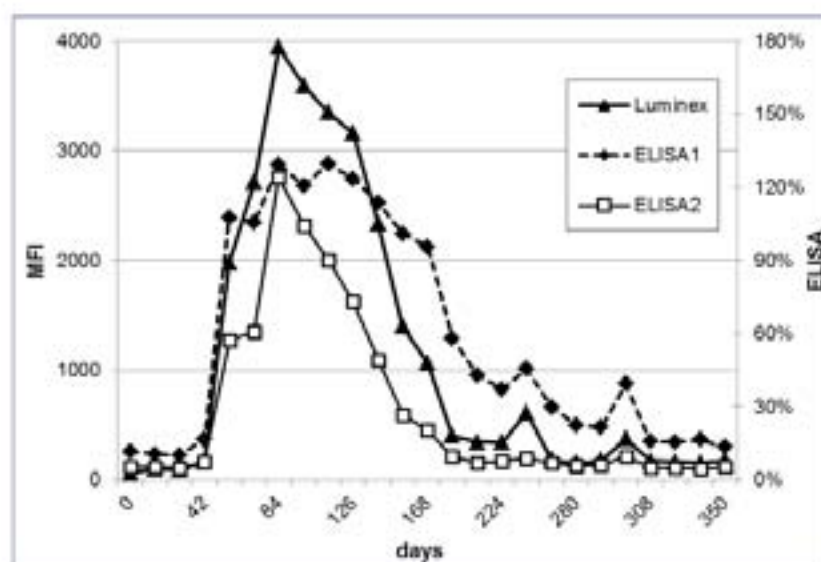
Figure 2 Comparison of the Luminex Trichinella assay with two ELISA's.
Longitudinal sera of an experimentally infected pig were tested with the Luminex assay (MFI, left y-axis) and ELISA (right y-axis). Results were plotted against days after infection.

Results

Longitudinal sera from an experimentally infected pig (R. Gamble) were tested with the Luminex Trichinella assay and the results were compared with results of two commercial ELISA's. With the Luminex assay an IgG response against Trichinella was detected that changed over time; the resulting curve was in essence similar to results obtained with commercial ELISA's (Fig. 2). For this animal, the Luminex assay even gave positive results at time points where the ELISA's had returned below their respective cut-offs. The Luminex assay was further evaluated by testing 150 porcine serum samples collected in Argentina were Trichinella is endemic. The Luminex assay showed an overall agreement of 93% with ELISA, with a Cohen's kappa of 0.82, demonstrating good agreement between the two assays. These results suggest that the developed Luminex assay is suitable for Trichinella serology.

		ELISA 1		
		pos	neg	total
Luminex	pos	39	7	46
	neg	4	100	104
	total	43	107	150

se 91% o.a. 93%
sp 93% c.k. 0,82

Table 1 Comparison of the Luminex Trichinella assay with ELISA. Field sera from an endemic area were tested in the Luminex assay using a cut-off of 1100 MFI. Results were compared with ELISA in a so-called 2x2 table. Diagnostic sensitivity (se) and specificity (sp), overall agreement (o.a.) and Cohen's kappa (c.k.) are given.

Using a similar approach a Salmonella fiveplex assay was developed with five LPS variants covering the most important Salmonella serogroups occurring in pigs (Table 2). The LPS beads were individually tested with O-antigen specific agglutination sera, routinely used for serotyping Salmonella isolates. Signals were generated on the beads in concordance with the LPS present (Fig. 3, cf. Table 2). Also in a multiplex set-up using 14 experimentally Salmonella-infected pigs the porcine sera reacted with the LPS beads in agreement with the serogroup of the strain used for infection (not shown).
Finally, the Luminex Salmonella fiveplex assay was evaluated by testing 150 porcine Dutch serum samples (Table 3). Using separate cut-offs for each bead (resp. 1700, 1500, 1500, 6000, and 4000 MFI), the Luminex assay showed an overall agreement of 91% with ELISA, with a Cohen's kappa of 0.82, demonstrating good agreement between the assays. These results suggest that the developed Salmonella fiveplex assay is suitable for Salmonella serology.

serogroup	subsp/serovar	O-antigens
B	S. Typhimurium	O4, O5, O12
C1	S. Choleraesuis	O6, O7
C1	S. Livingstone	O6, O7
C2	S. Newport	O6, O8
D	S. Enteritidis	O9, O12

Table 2 LPS used for the Luminex Salmonella assay. The O-antigens of each LPS used for coupling to Luminex beads are presented.
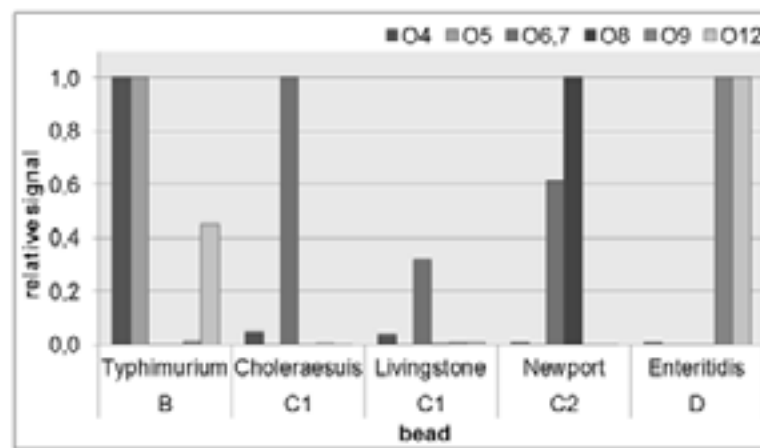
Figure 3 Luminex Salmonella LPS assay tested with agglutination sera.
Relative signal intensities (y-axis) are given for five Salmonella LPS beads tested with agglutination sera against O4, O5, O6/7, O8, O9 and O12. LPS donor strains are listed (x-axis).

		ELISA		
		pos	neg	total
Luminex	pos	54	7	61
	neg	6	83	89
	total	60	90	150

se 90% o.a. 91%
sp 92% c.k. 0.82

Table 3 Comparison of the Luminex Salmonella assay with ELISA. Dutch field sera were tested in the Luminex assay and compared with ELISA in a 2x2 table. For abbreviations see the legend to Table 1.

Discussion and Conclusion

During development of the serological Luminex assays many variables were tested (not shown) and it is possible that the assays presented here can be further improved. However, as these assays are meant as scaffold for expansion with similar assays, optimal conditions for the assays described here are not necessarily the optimal conditions for added tests; this would have to be evaluated upon each addition. In our experience for many variables a broad working range is possible. Variables that can have a profound effect on P/N-ratio (signal strength expressed as signal of a positive sample over signal of a well-defined negative sample (or pool of samples)) are sample dilution and salt concentration, and to a lesser extend incubation time, all very similar to ELISA. The assays presented here will have to be tested with large sample sets for validation before commercial application is possible. Topics that will need attention are the time required to perform assays, and the possibilities of automation to achieve the required sample throughput.

Early results showed that the Trichinella assay, developed to 'test drive' the Luminex platform, can successfully be added to the Salmonella fiveplex, even though initial assay conditions differ. We will use these assays as a scaffold for expansion with serological assays for zoonotic pathogens like Toxoplasma and pig pathogens like SVDV, Aujeszky disease virus and Mycobacterium avium subsp. avium.

Taken together the results show that the developed Trichinella and Salmonella assays can adequately detect antibodies in pig serum, thereby demonstrating the feasibility of the Luminex platform for multiplex serology in veterinary applications.

Acknowledgments

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Control of E. coli and Salmonella in growing-finishing pigs through the use of potassium diformate (KDF) – European case studies

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Abstract

Control of pathogenic bacteria has a high priority in European pork production. They can be a significant cause of zoonotic diseases and cause major economic losses in the pork production chain, through reduced productivity, increased veterinary and hygiene control costs. Preventing the spread of E. coli and Salmonella to the consumer requires special control measures during slaughter and processing. The extra cost of these controls is increasingly being transferred back to the producer in the form of financial penalties or the loss of the market for contaminated pigs. Improving gut health has been shown to be effective against intestinal pathogens, a strategy that has only really been made possible through the removal of antibiotic growth promoters in feed. Creating and maintaining a healthy intestinal environment has become essential to productivity and food safety programmes alike.

While biosecurity and hygiene in the feed mill and on farm are essential, the acidification of feed ingredients or finished feeds with organic acids also offers considerable benefits in E. coli and Salmonella control. Feed acidification is not only effective within the feed; possibly its biggest benefit occurs within the pig itself. Research trials in the UK, Denmark, France and Ireland with 0.6% dietary potassium diformate (KDF) have shown significantly reduced Salmonella count in the feed as well as in the gut of pigs. Further studies have proved that KDF exerts significant effects against E. coli at dosages between 0.6% and 1.2% in the duodenum, jejunum, colon and rectum of growing-finishing pigs. The authors concluded that a reduction in the number of coliforms will result in a better health status of pigs.

Introduction

Salmonella ranks among the world's biggest threats to health. In the United States alone, it is thought to be responsible for around 378 deaths and an estimated 19,336 hospitalisations each year. And these are just the reported cases. Salmonella ranks second on the food poisoning leader-board in the US where it's the premier bacterial food-borne disease. It's certainly serious enough to merit significant attention from the WHO and the US-based Center for Disease Control. Developing and implementing effective Salmonella monitoring, reporting and control systems has been prioritised in many countries. Salmonella is often associated with poultry products – chicken and eggs, but it would be a mistake to assume that these are the only food sources that can transmit the disease. Birds are a major source, true; strains can also be transmitted through pork and processed pork products, but it's also been spread through salad vegetables and peanuts. If we can eat it, there's a good chance that the bacteria can live on it, or at least survive long enough to be carried into an animal (or person)'s digestive tract. Since its discovery in the late 19th Century, more than 2,500 different types (serovars) have been discovered. All of these can cause disease in humans, which is most commonly associated with acute gastroenteritis – fever, abdominal pain, diarrhoea and nausea, sometimes with vomiting. More often than not, this clears up within a week of infection without treatment. However, if the pathogen enters the bloodstream or the disease leads to dehydration, effective antibiotic treatment can be a lifesaver. Children and the elderly are particularly vulnerable, as are people with weakened immune systems. The health statistics also make for some sobering economic facts. In the US, for instance, the Centers for Disease Control recently estimated a total annual cost of US$3 billion associated with Salmonella. Similar calculations from Denmark in 2001 took this further, suggesting that spending the equivalent of US$14.1 million implementing a salmonella control programme actually resulted in a net saving of US$25.5 million to the national economy. Like other foodborne bugs, Salmonella is developing resistance to the drugs we use to treat it. Much of the blame has fallen with animal production. Preventing or treating diarrhoeal diseases in livestock has played a major part in the development of multi-drug resistant strains. The two most commonly seen serovars in human salmonellosis epidemics, Salmonella enteritidis and S. typhimurium have emerged over the past 30 years in parallel with intensive animal husbandry. Now, we find bacteria, including these two, with worryingly high levels of resistance against the

antibiotics we use to treat them. Antibiotic resistance in Salmonella has two major consequences that cause the medical profession so much concern. Firstly, patients taking an antibiotic for unrelated infections, for example a chest infection, are more risk of contracting antibiotic-resistant Salmonella infections. Secondly, treatment for salmonella fails more frequently, causing prolonged or more severe illness, increased hospitalisations and more deaths. A recent US review estimated that antimicrobial resistance in Salmonella may result in 30,000 more infections each year, leading to 300 more hospitalisations and 10 deaths. Salmonella is a big risk to the world's economies. Looking into E.coli, the picture is not changing much. Also this gram-negative pathogenic bacterium is able to seriously impair animal and human health. Known to be a major vector for post weaning diarrhoea in piglets it is also affecting humans, especially in developing countries. Infections due to pathogenic strains of E.coli are probably the commonest source of diarrhoea in developing countries; they may be responsible of up to 25% of all diarrhoeal case in infants and children. But also the Western world" is no exception, as the recent outbreak of enterohaemorrhagic E.coli in Germany shows.

But the risks of pathogenic bacteria can be reduced, also without the prophylactic dosage of antibiotics. All along the food chain, experts have identified points at which intervention can help reduce the risk of Salmonella and E.coli infections. While Salmonella cannot be eradicated in pig units, it can be controlled to minimise the risk to consumers. Biosecurity plays a significant role in Salmonella control. In feed compounding, although heat treatment is effective in reducing contamination of feed leaving the feed mill, this effect does not persist during transport, storage and subsequent outfeeding. When conditions within the feed are less conducive to bacterial infection, Salmonella contamination can be reduced. The next critical control point is within the pig's gut itself, where conditions for bacterial growth may once again be optimal. Salmonella growth requires warmth (35-37°C is optimal), a moisture content greater than 12% and a pH between 4.5-9.0. It is no coincidence that the pig gut can provide Salmonella everything it needs to thrive.

Review of data

While biosecurity and hygiene in the feed mill and on farm are essential, the acidification of feed ingredients or finished feeds with organic acids also offers considerable benefits to Salmonella control. Feed acidification is not only effective within the feed; possibly its biggest benefit occurs within the pig itself. Research trials in the UK with potassium diformate (KDF) shows significantly reduced Salmonella count in the feed as well as in the gut of pigs. This effect is particularly well illustrated by data collected on 12 farms in Ireland as part of a joint study undertaken by Teagasc, University College, Dublin and the Department of Agriculture and Food in Co. Kildare (Lynch et al., 2007). The main objective of this investigation was to evaluate the efficacy of Salmonella control measures on highly infected farms. Salmonella control has been compulsory under Irish law since 2002 and farm status is categorised by the percentage of positive pigs in a herd according to the Danish mix-ELISA test. Category 3 (>50% positive) farrow-to-finish farms and their associated fattening units were selected for the study. The effects of including KDF over the 24 months of the study are given in Table 1. All the farms that were treated with the additive alone; or a combination of KDF with improved hygiene and biosecurity measures (farms J and L) had notable improvements in both bacteriological and serological prevalence of Salmonella spp. All but one farm in which KDF was used ended the trial with a much improved Salmonella status, with bacteriological prevalence also low on most farms. Using improved hygiene and biosecurity measures alone also improved Salmonella status, but to a much lesser extent. The reduction in prevalence obtained by potassium diformate alone, compared to the two farms which also implemented additional hygiene and biosecurity, demonstrates the additive's efficacy.

Table 1. Bacteriological and serological prevalence of Salmonella on 7 farms with or without potassium diformate (KDF) inclusion in the feed. Percentage of positive samples. From Lynch et al. (2007).

	Controls		With KDF	
	Bacteriological prevalence	Serological prevalence	Bacteriological prevalence	Serological prevalence
Farm F	100	88	0	50
Farm G	77	63	3	0
Farm H	32	17	3	4
Farm I	71	54	4	4
Farm J*	15	42	15	0
Farm K	37	96	14	4
Farm L*	4	96	0	0

*Farms J & L employed improved hygiene and biosecurity measures as well as KDF

These findings are not unique, however. Studies by the Danish feed company KFK, a decade ago (Olsen, 1999) and, more recently, in a commercial pig unit in the UK (Dennis and Blanchard, 2004), both concluded potassium diformate, to be an effective tool in a salmonella control strategy in commercial farms, reducing the percentage of salmonella positive pigs by 50% and in pork meat juice ELISA scores by 46%, respectively in grower finisher pigs (Dennis and Blanchard, 2004). The UK trial also showed an improvement in daily gain of 7.7%, reduced mortality and a reduction in medicinal intervention compared to the rolling average for that unit. The economic benefit of implementing salmonella control, as detailed in the Salinpork 2000 research trial, was also evaluated. Excluding reductions in veterinary bills and culling rates that accompany Salmonella outbreaks, improvements in FCR of 3% and in daily live weight gain of 5%, potassium diformate addition to grower/finisher diets and improved hygiene management practices resulted in an estimated net benefit of GB£5850 in an average herd (Blanchard and Burch, 2004).

Visscher et al. (2009) demonstrated the potential of potassium diformate together with coarse feed particle size in supporting a healthy gut while reducing Salmonella prevalence. The study proved that production of the short chain fatty acids propionate and butyrate was stimulated by KDF inclusion in the diet. Higher butyrate concentrations in the distal part of the digestive tract, especially in the colon, also support the growth and development of epithelial cells in the gut, increasing villus length and crypt depth - factors that induce healthy gut function. KDF also contributes to an optimal gut microflora in the last part of the gastro-intestinal tract of the pig via the promotion of butyrate production.

Most recently in France (Correge et al. 2010) demonstrated in a large scale commercial project, that the inclusion of 06.% KDF into the diet of fattening pigs in herds with a high infection level with Salmonella spp. resulted in a significant reduction of Salmonella prevalence at slaughter.

The Danish study (Olsen, 1999), also showed a sustained effect of potassium diformate, even after removal of the additive from the feed. However, as Blanchard and Burch (2004) concluded that even though KDF use can be removed for 3-4 months without detrimental effect to Salmonella status, removal of the additive only proves effective once the original vector animals have been removed from the unit.

Finally, a study by Øverland et al. (1998) proved that KDF exerts significant effects against E. coli at dosages between 0.6% and 1.2% in the duodenum, jejunum, colon and rectum of growing-finishing pigs. The reduction of coliform bacteria is in agreement with several other studies using dietary formic acid. The authors concluded that a reduction in the number of coliforms will result in a better health status of pigs.

Conclusion

The published results described above prove irrefutably how cost-effective Salmonella and E.coli control, a healthy gut and food safety can be secured by dietary means.

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Risk-based Meat inspection: "Meat Juice Multiserology" for improving the food chain information

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Abstract

The new European food safety strategy has three main goals: increasing the food safety, optimizing animal health and improving animal welfare. To achieve all three goals by means of a process control, the intensity of the official control is based on a risk assessment by analyzing the so-called "relevant food chain information" from pig herds. This food chain information consists of seven criteria, which are listed in the EU-Regulation No. 853/2004. One of them is taking into consideration existing bacteriological and serological laboratory results. So far, except of the serological salmonella monitoring in some European countries, there is no systematic serological monitoring for any other pathogen from pig herds. The presented paper describes the concepts of a meat juice based "multi-serology" and shows its usefulness as part of the food-chain information.

Introduction

The traditional meat inspection procedures at slaughter, focussing at removing health risks for humans by condemning carcasses and organs that show pathological signs, has resulted in the eradication and/or control of most "classical" food-borne threats to human health such as tuberculosis, brucellosis and tape worms. The high number of food-borne diseases in humans such as salmonellosis, yersinioses and the health risks due to pharmaceutical residues, which do not cause any pathological lesions in pigs, proves that the traditional post-mortem inspection of single carcasses as end product inspection is not able to prevent and control the risks of today.

The new EU legislation is not any longer prescribing exactly the inspection procedure, but defines the common food safety goals. Thus, each EU-Member State has to develop its own and specific risk profiles and the ways of controlling and managing the risks in question to reach these goals. The paper describes the general concept and first results of a meat juice based "multi-serological" monitoring system by continuously testing random samples of meat juice from different pig herds for antibodies against zoonotic as well as production disease pathogens (Blaha et al., 2010).

Material and Methods

Taking into consideration the needs for a meaningful serological monitoring using random samples per pig herd for identifying serological herd profiles, a set of ELISA tests was selected, which provides results with relevance for human health (measuring antibodies of zoonotic pathogens) as well as for pig herd health (measuring antibodies of infectious pathogens for pigs). For the presented study, it was decided to start with detecting antibodies against the infectious agents that are of interest for following stakeholders:

1) For the food business operators and the official veterinarians: Salmonellla, Trichinella, Toxoplasma and Yersinia,
2) For the pig producers and their veterinary practitioners: Mycoplasma hyopneumoniae as well as Inf uenza A (subtype H1N1 and H3N2).

Altogether, from 291 pigs a blood serum sample (taken at the point of bleeding the animals after stunning) and a meat sample from the diaphragm pillar of exactly the same pigs/carcasses (taken at the point of meat inspection) were collected. It was assured by additional tattooing of the pigs at bleeding that there were 291 specimen pairs (serum and meat juice) that both samples were unmistakably from the same animal.

After freezing and thawing of the meat pieces for producing the meat juice, all samples were tested with seven different ELISA-tests. In several test runs, a ten times lower dilution of meat juice than of blood serum for the ELISA tests turned out to

produce the most comparable results. This dilution ratio was finally used for the presented study.

After twelve months, meat juice random samples from pigs of the same six herds were tested again with the same tests under the same laboratory conditions to look into potential changes of the serological herd profiles over time.

Results

As for the test results (Tab. 1), the tested herds had highly heterogeneous serological profiles that allow for targeted herd health and food safety improvement measures. The repeated testing (Tab. 2) showed that several serological profiles change over time, which proves that the monitoring needs to be permanent, and that such a serological monitoring allows for an early detection of changes in the bacterial and viral burden of pig herds.

Tab. 1: ELISA test results from blood serum and meat juice of 291 slaughter pigs in 2009 and the agreement of the results

relevant for:	ELISA test for:	blood serum: proportion of positive samples (n/N)	meat juice: proportion of positive samples (n/N)	Sensitivity meat juice vs. serum	Specificity meat juice vs. serum
food safety (zoonotic diseases)	Salmonella spp.	13% (38/291)	12% (36/291)	87%	99%
	Yersinia enterocolitica	69% (202/291)	72% (210/291)	100%	91%
	Toxoplasma gondii*	2% (6/291)	2% (6/291)	100%	100%
	Trichinella spp.*	0% (0/291)	0% (0/291)	100%	100%
animal health (production diseases)	Mycoplasma hyopneumoniae	51% (149/291)	48% (141/291)	91%	96%
	Influenza A (H1N1)	32% (93/291)	20% (59/291)	61%	99%
	Influenza A (H3N2)	11% (31/291)	7% (19/291)	55%	99%

*all confirmed Trichinella and Toxoplasma positive control sera and meat juices were clearly identified as "positive"

Tab. 2: Comparison of the proportion of positive meat juices per herd in 2009 and 2010

Herd	Year	Salmonella (n/N) [0-80%]	Yersinia (n/N) [0-100%]	Toxopl. (n/N) [0-20%]	Trichinella (n/N) [0%]	M. hyo (n/N) [0-90%]	SIV H1N1 (n/N) [0-37%]	SIV H3N2 (n/N) [0-90%]
A	2009	11% (12/108)	69% (75/108)	3% (3/108)	0% (0/108)	45% (49/108)	26% (29/108)	10% (11/108)
	2010	10% (8/80)	61% (49/80)	9% (7/80)	0% (0/80)	45% (36/80)	24% (19/80)	0% (0/80)
B	2009	6% (2/31)	58% (18/31)	3% (1/31)	0% (0/31)	39% (12/31)	26% (8/31)	3% (1/31)
	2010	0% (0/10)	100% (10/10)	0% (0/10)	0% (0/10)	30% (3/10)	30% (3/10)	0% (0/10)
C	2009	10% (2/20)	20% (4/20)	10% (2/20)	0% (0/20)	90% (18/20)	0% (0/20)	0% (0/20)
	2010	80% (8/10)	0% (0/10)	20% (2/10)	0% (0/10)	60% (6/10)	10% (1/10)	90% (9/10)
D	2009	0% (0/28)	86% (24/28)	0% (0/28)	0% (0/28)	32% (9/28)	25% (7/28)	25% (7/28)
	2010	5% (1/20)	35% (7/20)	0% (0/20)	0% (0/20)	0% (0/20)	25% (5/20)	5% (1/20)
E	2009	14% (9/63)	82% (52/63)	0% (0/63)	0% (0/63)	51% (32/63)	0% (0/63)	0% (0/63)
	2010	5% (1/20)	25% (5/20)	0% (0/20)	0% (0/20)	0% (0/20)	10% (2/20)	0% (0/20)
F	2009	27% (11/41)	90% (37/41)	0% (0/41)	0% (0/41)	51% (21/41)	37% (15/41)	0% (0/41)
	2010	50% (10/20)	60% (12/20)	5% (1/20)	0% (0/20)	60% (12/20)	20% (4/20)	0% (0/20)
Total	2009	12% (36/291)	72% (210/291)	2% (6/291)	0% (0/291)	48% (141/291)	20% (59/291)	7% (19/291)
	2010	18% (28/160)	52% (83/160)	6% (10/160)	0% (0/160)	36% (57/160)	21% (34/160)	6% (10/160)

Discussion

The results suggest that the closeness of agreement between the measured serum and meat juice antibody concentrations is sufficient for further pursuing the development of a multi-diagnostic "meat juice serology" for pig herds. Creating a flexible system of serological herd profiles for the most important infections (any pathogen can be added to the test panel) provides the opportunity for introducing benchmarking systems. Such systems are the basis for targeted decisions on a) risk-based meat inspection procedures, and b) on herd health and food safety improvement measures along the food chain. Apart from these opportunities, a major advantage of the suggested approach is that this kind of multi-diagnostic monitoring addresses three groups of stakeholders: the food business operators, the veterinary authorities, and the pig producers. Offering all three groups continuous information that serves their specific interests will provide the benefit to share the costs of such monitoring systems.

Conclusions

The tested serological meat juice monitoring of multiple zoonotic and pig health pathogens is highly valuable in terms of improving the meaningfulness of the food chain information, and is very cost effective, if added to an existing salmonella monitoring programme.

The proposed "meat juice multi-serology concept" provides a useful tool for improving food safety and animal health if implemented as part of the risk-based meat inspection.

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Internet based checklist for the risk assessment of Salmonella contamination in finishing pig herds, abattoirs and cutting plants

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Abstract

Monitoring systems are in place to categorize pig finishing herds, abattoirs and cutting plants for their level of Salmonella contamination. In order to improve their status, if necessary, the companies and their advisors need to implement an improvement plan. This can be based on a strengths and weaknesses analysis in relation to all factors that contribute to the Salmonella contamination level. To do this analysis in a uniform, structured and repeatable way, internet based checklists were developed.

In the checklist for herds questions relating to the Salmonella status of introduced piglets, transport hygiene of piglets, housing, management, cleaning and disinfection, feeding, disease status, rodent and fly control, and unloading practices are listed. Standard operating procedures (SOP's) will be available on the website for references on, for example, cleaning and disinfection, rodent and fly control, sampling and testing of weaned piglets / growers as well as hygiene checks. In the checklist for abattoirs questions relating to transport, holding area, slaughter, chilling, cleaning, disinfection and hygiene are listed.

For cutting plants questions relating to the quality of received goods, chilling, cutting, packaging, transport, hygiene, documentation, and cleaning and disinfection are listed.

Expert opinion is and will be gathered to weigh the different chapters and sub-questions in the checklists. The final score allows comparison to previous checks and to peers. Scores per chapter allow a ranking of most urgent points to be remedied in order to improve the Salmonella status. The internet application allows access to the checklist from any location at any time; however, pdf-documents of blank or completed checklists can be printed when desired. Logins provide sufficient privacy protection. Storage of the data in a central database provides data security. The checklists will be available in German, Dutch and English. User feedback will be used to improve all aspects of this tool continuously.

Introduction

Monitoring systems are in place to categorize pig finishing herds, abattoirs and cutting plants for their level of Salmonella contamination. In order to improve their status, if necessary, the companies and their advisors need to implement an improvement plan. This can be based on a strengths and weaknesses analysis in relation to all factors that contribute to the Salmonella contamination level. To do this analysis in a uniform, structured and repeatable way, three internet based checklists were developed. One checklist is intended for the use on finishing farms. The other two are intended for abattoirs and cutting plants respectively.

Material and Methods

Within the SafeGuard project a working group was put together consisting of representatives from an abattoir, animal health services both from Germany and The Netherlands, a private laboratory working in the field of food hygiene, a German state inspection service, the Dutch Product Board for Livestock and Meat and a computer programmer.

Project steps were:

- to list and evaluate the available analysis tools;
- to create three checklists, one for farms, one for abattoirs and one for cutting plants, by considering available stand ard operating procedures (SOP's) and including documentation and scoring systems;
- to carry out trial analyses of high and low prevalence herds and abattoirs and cutting plants to evaluate the scoring system and user friendliness;
- to gather opinions from potential users and other experts;
- to make the checklists available in three languages (German, Dutch and English) through a website.

Results

To evaluate available tools checklists from The Netherlands (in house checklist for Salmonella on farms and http://www.verantwoordeveehouderij.nl/index.asp?producten/netwerken2008/18/spfcheck/index.asp), Germany (in house checklist for Salmonella on farms), Belgium (www.biocheck.ugent.be/2/pages/) and the United Kingdom (www.ukmeat.org/FSAMeat/NewMethod.aspx) were gathered. The evaluation showed that none of the available tools fulfilled our criteria so it was decided to create our own checklists.

To limit the length of the checklists SOP's which were already available were used to refer to in the checklists. For example SOP's for sampling weaners for the detection of Salmonella, cleaning and disinfection of stables, rodent and f y control and a hygiene check were implemented.

The checklist of the three questionnaires consists of the chapters shown in table 1:

Tab. 1:   Chapters of the three created checklists

Farm	Slaughtering	Cutting and deboning
Salmonella status of the weaned pigs on the supply farm(s) of origin of the finishing herd;	Transport	Check on incoming goods
Transport conditions of the weaners / growers	Unloading and inspection of the pigs;	Cooling and storage
Health status at entry of the barn of the weaners / growers	General slaughter conditions	Deboning
Feed and water	Dirty slaughter area	Vacuum and packaging
	Clean slaughter area	
	Official meat inspection	
Hygiene and environmental conditions of the barn at entry of the weaners / growers	Process hygiene control	Process hygiene checks
	Chilling	
Hygiene procedures at marketing	Inspection and shipping	Commissioning and shipment
Management practices	Management;	Management
	Cleaning and disinfection	Cleaning and disinfection
External and internal bio-security	Personnel hygiene	Personnel hygiene
	Construction issues	
	Water;	Water
Rodent and fly control	Rodent control.	Rodent control

A scoring system was set up with general scores per chapter and with individual scores for questions within chapters. The individual score can either be positive or negative for resp. desired and unwanted answers. The scores are based on the expert opinion within the development team and from external experts. Trials will be conducted to fine tune the scoring system and the user friendliness. After an individual checklist is finished a total score is calculated which can be used to compare to previous lists or to peers. Short comings which are identified by the checklist are listed in a report which can be downloaded in pdf, stored and printed. The online development makes it possible to make changes at any moment by team members.

The internet platform and the online-questionnaires were developed by using the scripting language PHP and a MySQL database. Login and password protection ensure privacy of the entered data. Advisers and veterinarians assisting herds or abattoirs can register online to get an individual account with a password. When logged in the user may choose the wanted kind of checklist. After that a new list can be started by entering the unique herd or abattoir number, a name and a date. Alternatively a previously started or finished checklist can be edited or printed. The possibility to create more than one checklist for a herd or an abattoir allows the advisor to monitor the progress.

During the use of the checklist clarification of the question at hand is given on the right hand side of the screen to prevent miss interpretation of the question. If applicable the SOP to which the question is referring can be opened, stored and printed.

Discussion

Salmonella is a relevant, or the most relevant, zoonoses in pig meat production. The recent EU baseline study showed significant differences in Salmonella prevalence at herd and abattoir level across Europe.

A significant part of Salmonella contamination of pig carcasses and pork can be prevented by proper abattoir hygiene. Accordingly, prevention of faecal contamination and good slaughter hygiene contribute for a large part to the number of points that can be acquired in the questionnaire. Legal requirements urge the operators to take action when Salmonella is found on carcasses. Salmonella control should happen along the entire supply chain. For that reason it is good that pig farmers, whose herd is categorised in the highest Salmonella category, are under the obligation to reduce the Salmonella prevalence in their herd. Some abattoirs process pig from high risk herds separately.

The present tool, that is still under development and needs more validation, can be a valuable tool to identify weak spots in the management of either farmers or food production companies. Finally, this tool can never be the holy grade with the final solutions. It will help operators and their advisors to structurally check and think about the risky processes. The weighing of the answers is based on experts' opinion. Experts base their insight on experience from the past and evidence from literature. Final scientific validation of all points will however not take place. The weight factors will stay guesstimates. The tool is now applied in the field to tests its applicability as support tool for advisory work in the pig supply chain, by veterinarians testing it at farm level and food safety advisors applying it in medium size abattoirs and cutting plant.

Another very promising area of application for this tool is education. The attendees can fill in the questionnaire, discuss the outcomes, as well as the importance that is given by the weighing of the answer. These types of exercises are a pleasant way to educate the participants in control of food borne zoonoses. Besides bachelor students in food science, veterinary medicine and agricultural science, this type of courses can be typically useful for auditors and quality managers within the pork production chain.

Conclusions

A modern internet based diagnostic tool was developed to advice responsible persons and their advisers for the control of Salmonella in primary and meat production.

Acknowledgement

A practical framework for tracing sources of Salmonella in a pig slaughter plant

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Introduction

Salmonella causes around 30 000 cases of human illness per year in The Netherlands, of which an estimated 25% is caused by pork. Salmonella carrying pigs and resident flora on slaughter equipment are relevant sources of carcass contamination. Although recognized, these sources from which and the routes through which Salmonella is transmitted to the pig carcasses during slaughter are not well understood in a quantitative way.

Here, we present the application of a sampling scheme at predefined potential sources and at downstream sampled carcasses to get insight in the change in Salmonella numbers throughout a slaughter plant. The resulting data are implemented in a biotracing system for Salmonella in the pork chain. This results in a framework that:

- Gives insight in the most important source of Salmonella upon a contamination event.
- Becomes more powerful in source tracing each time new data is added.
- Can be used as a monitoring system on a day-to-day basis.
- Points to targeted intervention

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