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 BOOK

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

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ABSTRACT BOOK

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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

PROGRAMME

at a glance

19-23 June 2011

Sunday 19 June 2011

13.00 – 14.00	Registration and welcome
14.00 – 17.00	Pre conference workshops
17.00 – 19.00	Social activity: Get-together happening
19.00 – 22.00	Social activity: Pub Crawl

Monday 20 June 2011

08.00 – 08.30	Welcome with coffee and tea
08.30 – 09.00	Opening ceremony
09.00 – 10.45	Plenary session 1
10.45 – 11.15	Break, info market and poster sessions sponsored by Eli Lilly Nederland
11.15 – 12.45	Plenary session 2
12.45 – 14.00	Lunch, info market and poster sessions
14.00 – 15.30	Plenary session 3
15.30 – 16.00	Break, info market and poster sessions sponsored by Chainfood
16.00 – 17.30	Plenary session 4
17.30 – 17.40	Walk to Townhouse of Maastricht
17.40 – 18.30	Social activity: Welcome reception
18.30 – 21.00	Social activity: Dine Around (facultative)
21.00 – 23.00	Social activity: Nightcaps

Tuesday 21 June 2011

08.00 – 08.30	Welcome with coffee and tea
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10.30 – 11.00	Break, info market and poster sessions sponsored by IDEXX Livestock and Poultry
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17.15 – 18.30	Time to freshen up
18.30 – 19.00	Organised transportation to gala dinner venue
19.00 – 22.30	Social activity: Gala diner at GaiaPark Zoo
22.30 – 23.00	Organised transportation *There are 3 meeting points; busses will leave from Hotel Bastion, Hotel Townhouse and Maasboulevard (close to the Conference Venue).*

Wednesday 22 June 2011

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10.30 – 11.00	Break, info market and poster sessions sponsored by IDT Biologika GmbH
11.00 – 12.15	Plenary session 10
12.15 – 12.30	Best poster & best oral award ceremony
12.30 – 12.45	Presentation next meeting location
12.45 – 13.00	Closing SafePork 2011
13.00 – 14.00	Lunch, info market and poster sessions

Thursday 23 June 2011

09.00 – 17.00	Post conference tours *There are 3 meeting points; busses will leave from Hotel Bastion, Hotel Townhouse and Maasboulevard (close to the Conference Venue).*

Scientific
PROGRAMME

20-22 June 2011

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ORALS

in order of programme

Modernization of meat inspection

S. Buncic

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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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Alban, L., Stenberg, B.

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When slaughtering pig-carcasses at some of the Danish abattoirs meat-percentage and weight is estimated before the meat inspection is conducted. In the search for new and risk-based ways of conducting meat inspection, a pilot-study with the aim of investigating whether carcass-weight and/or meat-percentage, could be indicators for total-rejection of finisher pig carcasses was conducted. Potential clustering on producer-level was also checked.

From 2009 to 2010; 5,663 pig carcasses with meat-inspection-remarks that lead to total-rejection was collected from a Danish abattoir. Another 16,478 pig carcasses, some with no remark and others with remarks but not leading to totally rejection, were used as controls. Observations that had the remark code for cajectia, unknown gender and weight above 109.9 kg (maximum weight for slaughter-pigs) were excluded.

The statistical analyses were conducted in SAS (logistic regression in Glimmix).
The remarks regarding the acute diseases; pleuritis, lung inflammation, peritonitis and endocarditis were recorded in 15.8%, 6%, 3.4% and 2.8% of the cases respectively. Infected tail-biting wounds, osteomyelitis, chronical pleuritis, pyemia and feet abscesses were recorded in 19.3%, 14.1%, 7.4, 5.2% and 4.8% of the cases respectively. The most frequent recorded remark in the controls were chronic pleuritis; 15.8%. There was some correlation between; infected tail-biting wounds and osteomyelitis and the remarks; acute- pleuritis and acute lung-inflammation.
In the cases, the mean of meat-percentage and carcass-weight were 60.4%, confidence interval (CI): 60.3%-60.5% and 77.8 kg (CI: 77.5kg-78.2kg) respectively. In the controls, mean meat% and carcass-weight were 60.1% (CI: 60.1%-60.2%) and 82.0 kg (CI: 81.9kg-82.2kg).
The logistic regression indicated that total rejection could be predicted by carcass-weight and meat-percentage but the effect was low in order to be practically suitable.
The effect of producer was, however, very significant. The remark-history of deliveries for each producer will therefore be further explored as a possible indicator for total-rejection.

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State of Art of meat inspection of pigs in the EU

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The current meat inspection regulation in the EU is based on principles that are around 100 years old. However, the hazards and 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 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.

First, the detailed aims of meat inspection were evaluated on a workshop with different stakeholders: is the purpose to ensure food safety, animal health, notifiable diseases, trade, or meat quality? Next, FVO reports were studied to obtain information of level of implementation of the current regulations. Next, a questionnaire was sent out to EU member states to obtain detailed information about the way that meat inspection is conducted in pigs.

The results show that currently only few countries have introduced modified meat inspection in pigs (including among others omission of routine incision into the mandibular lymph node and the heart of finisher pigs). The limitation is caused by the requirement that the pig herds should be declared as originating from integrated production systems. In countries where the pig population is considered free from Bovine Tuberculosis and Trichinella, only few elements of traditional meat inspection in pigs are related to food safety. Moreover, other food safety hazards like Salmonella are not dealt with. 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.

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

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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.

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Experiences with a risk based meat inspection standard in pigs

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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.

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

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Salmonellosis is one of the most important bacterial zoonotic diseases in humans and Salmonella infections are often linked with the consumption of contaminated pork. The serovar most frequently isolated from pigs is Salmonella enterica subspecies enterica serovar Typhimurium (Salmonella Typhimurium), which is also the most prevalent serovar in humans. In order to reduce Salmonella Typhimurium infections in humans, minimization of the Salmonella intake into the food chain is important. A combined approach using hygienic measures, the use of feed additives and 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: ΔrfbA, ΔrfaL, ΔrfaJ, ΔrfaI, ΔrfaG and ΔrfaF. Inoculation of BALB/c mice with the parent strain, ΔrfaL, ΔrfbA or ΔrfaJ strains but not the ΔrfaG, ΔrfaF or ΔrfaI strains protected significantly against subsequent infection with the virulent Salmonella Typhimurium strain NCTC12023. Immunization of piglets with the ΔrfaJ or ΔrfaL 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 ΔrfaJ or ΔrfaL strains and sera from pigs infected with their isogenic wild type strain. 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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In this study, the effects of three intervention strategies on the transmission of Salmonella Typhimurium in 3 to 14 week old pigs were evaluated: (1) feed supplemented with 0,3 % coated calcium-butyrate salt (Green-Cab-70®, Sanluc International), (2) acidified drinking water (pH 3.5-3.8) (Agrocid Super®, AgroLogic), and (3) oral vaccination (Salmoporc®, IDT). A positive (4) (infected/untreated) and negative (5) (uninfected/untreated) control group were also included. At 8 weeks old, two pigs of each group (n=8) were challenged orally with 108 cfu of Salmonella Typhimurium strain 112910a. Then, for all pigs, blood samples were analyzed once/week for the presence of Salmonella-specific antibodies by an indirect ELISA (Idexx Laboratories) and individual feces were examined twice/week for the presence of Salmonella according to ISO 6579 annex D. At necropsy (38 Days Post Infection) ileum contents, caecum, caecum contents, ileocaecal lymph nodes and tonsils were tested for the presence of Salmonella spp., and diaphragm fluid was analyzed for Salmonella-specific antibodies.

Seroconversion was observed in all infected groups, with significantly higher antibody titers in groups 2 and 3. Vaccinated animals seroconverted upon vaccination. The overall level of Salmonella-specific antibodies in meat juice was significantly lower compared to serum. Significantly less contact animals were observed during the transmission experiment in groups 1 and 3. Calculated reproduction ratio's were significantly lower in group 1 (1.76 [1.02; 9.01]) and group 3 (2.6 [1.21; 9.45]) compared to groups 2 and 4 (+∞ with lower limit 1.88).

These 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 is a limitation if serology is used as a means to identify Salmonella positive herds. Salmonella-specific antibody levels in meat juice differ from those in serum, which might have consequences in surveillance programmes based on serology.

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

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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, 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 farm model includes extensive modelling of individual pigs and the pig pen environment. The inclusion of the environment, and a novel dose-response model for Salmonella in pigs, allowed the modelling of detailed interventions based on the cleanliness of the environment, and the susceptibility/immunity of the pig. Three potential sources of infection are considered: infected piglets, contaminated feed and environmental sources (e.g. rodents).

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.

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

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In Denmark, actions to mitigate the risk related to antimicrobial resistance have been put in place continuously. Actions have for instance been: monitoring of antimicrobial use at herd-level, herd health agreements, restricted use of fluroquinolones and supervision of all veterinarian prescriptions. Despite of this, the consumption of antimicrobials in the Danish pig production steadily increased to 3.8 g per pig in 2009. This is 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. Hence, the current risk related to antimicrobial resistance in Denmark seems low. However, among politicians and stakeholders there was a growing concern due to the increased use and the occurrence of MRSA and ESBL. Therefore, further actions were required. These were: a voluntary ban on use of cephalosporin in Danish swine herds for 2 years and a so-called "Yellow card" initiative from the Danish Veterinary and Food Administration (DVFA). The yellow card is given to farmers who use more than twice the average amount of antimicrobials. The consumption in pigs is evaluated as animal daily doses (ADD) per 100 animals seen over the last 9 months (by age group). Current 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 consumption close to these limits were warned by the DVFA, that unless actions were taken to reduce antimicrobial use, they could 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 monthly consumption to pigs of approximately 10%.

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Association between Salmonella sp. and Yersinia enterocolitica infection in swine

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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.

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

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Clostridium difficile ribotype 078 is the dominant ribotype in pigs and is the third most common found ribotype in humans with C. difficile infection. Pigs may be a reservoir for human infection with this ribotype, however zoonotic transmission routes have not been established.
A study was conducted at a farrow-to-finish farm, known to have a high prevalence of C. difficile 078, to determine whether airborne dissemination of spores inside and outside the farm could play a role in transmission of C. difficile.
Air samples were taken using a MB1 Microbio Air Sampler. A continuous airflow of 100L/min air was directed upon C. difficile agar plates (BioMérieux) for 5 minutes. Samples were taken in the wards, ventilation system and outside the farm. All plates were anaerobically incubated for 48 hours at 37°C.
Preliminary results reveal that highest numbers of spores can be found at the farrowing ward with piglets of 1-2 weeks old, while the numbers of spores decrease until weaning age of the piglets is reached. No spores were detected at the weaned pig wards, however at the boar ward and sow ward spores were present.
Movement of weaned pigs from the farrowing ward to the weaned pigs ward led to a 15-fold increase in the number of spores compared to the sample taken directly before at the same location. Spores were present in the air directly outside the ventilation exits and at 20 meters distant of these exits.
These preliminary results indicate that C. difficile spores are disseminated outside the pig farm by ventilation. However, it is unclear how these spores are diffused once in the outside air and what role this dissemination plays in the possible zoonotic transmission. Further research is needed to determine the significance of the aerial dissemination of C. difficile spores in the outside environment.

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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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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, such as transport to the slaughter house, recrudescence of Salmonella may occur. The mechanism of stress related re-excretion of Salmonella by pigs is poorly understood. Therefore, the aim of the presented study was to determine the role of the stress hormone cortisol on Salmonella re-excretion by pigs.
First, an in vivo infection model was optimized to reproduce stress induced re-excretion of Salmonella Typhimurium 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, a synthetic member of the glucocorticoids, at a concentration (2 mg/kg) that does not cause immunosuppression of the pig (Flaming et al., 1994). Furthermore we demonstrated that cortisol promotes intracellular proliferation of Salmonella Typhimurium in porcine alveolar macrophages, but not in intestinal epithelial cells (IPEC-J2), at a concentration (1 µM) that did not exert a notable effect on porcine cell viability. Whether this increased intracellular proliferation is triggered by cortisol-induced changes in the host cell or by direct interaction of the bacterium with cortisol, was investigated via microarray analysis. This pointed out that cortisol (1 µM) does not cause any significant changes in the gene expression of Salmonella Typhimurium, which implies that the enhanced survival of the bacteria is probably caused by an indirect effect through the cell. These 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.

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Genetic characterization of Yersinia enterocolitica collected from tonsils of slaughtered pigs

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From January to March 2009, detection of Yersinia enterocolitica was done from 900 tonsils swabs collected from 45 pig batches in one slaughterhouse. 316 isolates of Yersinia enterocolitica were collected and then considered for their virulence genes and their PFGE profiles.

Real Time PCR was used to evaluate the presence of genes ail, myfA, ystA and ystB on the genome and the gene yadA on the pYV plasmid. PFGE analysis using XbaI enzyme was also realised. Those characterizations were also realised on human strains of biotype 3 and 4 purchased from Pasteur institute and also on one strain collected from pig tonsils at the same slaughterhouse in 2006.

Most of the isolates belonged to biotype 4 with 269 to 316 (85.12 %) and 47 to 316 (14.88%) were biotype 3. All the 316 isolates carried the ail and myfA genes, none of them carried the ystB gene. All the isolates of biotype 4 harbour the ystA except one. Moreover, only 41 of the 269 (15.2%) isolates of biotype 4 harbour the pYV plasmid. All the isolates of biotype 3 carried the pYV plasmid.

The PFGE revealed 7 Xba1 genetic profiles. More than 50% of isolates highlighted the same major PFGE patterns. No clear correlation with the biotypes or the plasmid carriage was evidenced. On the 31 positive batches, one or two different PFGE patterns could be found on one swab and from one to three different genotypes could be found in one batch. Nor Biotype 2 or 3 human strains could match with the different isolates found in these porks, but the biotype 4 CIP 81.41 and the isolate from 2006 belonged to the major PFGE pattern.

The study on this collection of isolates showed that Yersinia enterocolitica is very clonal with a major PFGE patterns. Moreover all the isolates have virulence genes and could be pathogenic.

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The infection biology of pig-associated Salmonella

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The infection of UK pigs with Salmonella is a continuing public and animal health problem that has high zoonotic potential, particularly S. Typhimurium and its highly clonal monophasic variants (that lack phase two flagellar (H2) antigen). It has been estimated that within the EU 10-20% of all human cases of salmonellosis are related to the consumption of pork. Improved control is essential in the face of EU legislation that now demands a reduction in the number of slaughter pigs testing positive for Salmonella as an effort to limit entry into the food chain. Currently there are no reliable, cost-effective control measures or an effective vaccine. This is partly due to there being limited knowledge available regarding the infection biology of Salmonella at the level of the intestinal epithelium of pigs. Similarly, since many different Salmonella types are found in pig rearing environments, a Salmonella vaccine will only be successful if it can offer protection against challenge by several different Salmonella serovars and a range of S. Typhimurium phage types (PTs). Previous work has already shown that even within the same PT there can be marked differences in virulence. Therefore, through the use of two established lines of porcine intestinal epithelial cells IPEC-1 and IPEC-J2, this in vitro work examines the invasion and attachment characteristics of different Salmonella serovars and phage types; the innate immune response of pig intestinal epithelial cells in response to infection; and the fitness of multi-resistant strains. The latter was assessed by studying the growth of the strains in vitro and their survival in the environment.

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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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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 week 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) targeted at invA was performed in a subset of the culture negative samples (549) and in all the culture positive samples. At the time of submission, Salmonella was cultured from 397 (9%) 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 17% (10 week old) to 4% (24-26 week old). At the present, 99.8% of the 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 quantifiable range (>10^3). Of those samples within the quantifiable range, the bacterial concentration ranged from 1.05x10^3 to 1.73x10^6 invA gene copies/g feces. The results suggest that point estimates of Salmonella prevalence may not accurately describe the Salmonella status of the finishing pigs. The majority of the pigs shed Salmonella at low concentrations. These preliminary data can contribute to quantitative risk assessments of the association between concentration of Salmonella shed by pigs during the finishing phase and contributions to carcass contamination at slaughter.

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Salmonella Typhimurium interference with the humoral immune response of pigs

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Foodborne salmonellosis is one of the most important bacterial zoonotic diseases worldwide. Salmonella Typhimurium is the serovar most frequently isolated from persistently infected pigs in Europe. Circumvention of the host's immune system by Salmonella might attribute to persistent infection of pigs. We found that Salmonella Typhimurium strain 112910a phage type 120 was able to persist in pigs and did not induce seroconversion up to 4 weeks post inoculation (pi), which was associated with downregulation of major histocompatibility class II (MHC II) expression on porcine alveolar macrophages (PAM). The MHC II downregulation capacity was abolished when bacteria were opsonized with Salmonella-specific antibodies. Furthermore, intracellular proliferation of Salmonella Typhimurium opsonized with serum containing anti-Salmonella Typhimurium antibodies was significantly impaired compared to that of bacteria opsonized with negative pig serum. We also found that the MHC II downregulation capacity was absent in strain MB2216 and thus clearly differed among Salmonella Typhimurium strains. From these in vitro data it is proposed that Salmonella Typhimurium might interfere with the humoral immune response to promote intracellular survival in PAM, contributing to Salmonella persistence in pigs. In a subsequent in vivo experiment, 2 groups of pigs (n = 9 and n = 19 respectively) were orally inoculated with either Salmonella Typhimurium strain 112910a or strain MB2216. Over a 40 days period, MB2216 was isolated less frequently from the faeces, although this strain induced earlier seroconversion, as assessed by a commercial LPS-based and an in-house whole-cell Elisa. Furthermore, the MB2216 strain was isolated in smaller numbers from the tonsils and lymph nodes of pigs than strain 112910a (P = 0.094) at 40 days pi. Together, these data show that a high serum antibody titre correlates with reduced Salmonella Typhimurium shedding by and persistence in pigs and vice versa. These findings might have major implications for Salmonella-monitoring programs primarily based on serology.

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

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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 immunofluorescence 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 50.1% and 44.3% of samples, respectively. 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, 92.1% of the Giardia isolates were Assemblage B and 7.9% were Assemblage E. The most prevalent Cryptosporidium genotypes were C. parvum (55.4%) and pig genotype II (37.5%). Only one (1.8%) 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.

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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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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.

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

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Giving decision makers support in case of crisis as in normal mode becomes increasingly important. By presenting decision guidance for determining restriction zones as well as optimizing surveillance systems this work focuses on either of these issues.
With the help of network analysis of animal movement databases (HI-Tier) it was shown that the German pig trade is work is modular and can be divided into communities [Lentz et al, 2011; Lentz et al, 2009]. Such structure can be of crucial importance in case of a crisis like an epidemic. Simulations have shown that 80% of all primary infections are trapped within their initial community. Considering closed trade networks this knowledge can be highly relevant in terms of disease control.
The database on pig trade movements can also be used to develop risk-based surveillance schemes, where at risk means disease, contamination or any dysfunctional production. Risk-based surveillance focuses on critical control points, which can be identified by simple network parameters as trade volume, trade frequency or number of trade partners. Furthermore data on bio-security and health status can be included as well. Here simulations have shown that surveillance on such points can detect a contamination or disease up to two months earlier than surveillance on randomly chosen control points. In terms of prevalence this can make the detection up to one hundred times faster.
With the help of these methods useful tools for crisis intervention as well as prevention and surveillance can be developed.

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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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In this work, we tested a new selective chromogenic plate, YECA, for its specificity, sensitivity and capacity to detect pathogenic Y.enterocolitica from tonsil pigs. Specificity of YECA was tested with a panel of 26 strains and compared with CIN and YeCM media. Sensitivity of YECA was tested from a 10-fold dilution of pure cultures of pathogenic biotypes (2, 3, and 4) and the non pathogenic-biotype (1A) and compared with PCA, CIN and YeCM media. Capacity of YECA to detect pathogenic Y.enterocolitica from tonsil pigs was carried out on 50 pig tonsils collected in one slaughterhouse. From each pig, 10 g of tonsil were put into a bag containing 90 ml of PSB broth and 1 ml was transferred in 9 ml of ITC broth. Streaking on YECA and CIN was done in direct after stomaching tonsils, after a 24h-incubation of ITC and after a 48h incubation of PSB and ITC. All the plates were incubated at 30°C during 24H. Presence of typical colonies on CIN and on YECA was checked. Confirmation was done by biochemical assays as described in ISO 10273:2003 method.
The three 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. 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 which high inhibition was observed. 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 during 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. In addition, the ITC- YECA way generates a time-saver by giving a positive test in 72H.

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

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INTRODUCTION:DNA isolation is a key issue for its impact on the effectiveness of molecular analyses for detection of foodborne pathogens. In this study four nucleic acid extraction methods have been evaluated for their ability to recover Salmonella DNA from pig faeces and pork samples.
MATERIAL AND METHODS: forty-four samples of pig faeces and 44 samples of pork were spiked with 3 different concentrations of S. Typhimurium (from 2 to 10 ufc/g), and were subsequently pre-enriched. Salmonella DNA was extracted from each sample using different extraction methods.
The methods selected to extract DNA from pig faeces were: 1) InviMag Stool DNA kit (Invitek), 2) QIAamp DNA Stool kit (Qiagen), 3) Lysis reagent (iCheck-Bio-Rad) and 4) Extraction DNA mix (AES Chemunex, Warnex), while for pork samples: 1) Charge switch gDNA mini Bacteria Kit (Invitrogen), 2) Lysis reagent (iCheck-Bio-Rad), 3) Extraction DNA mix (AES Chemunex) and 4) boiling method were used. Each method was tested both on pre-enriched samples and on the same ones treated with Dynabeads Anti-Salmonella (Dynal, Invitrogen). Finally, a Salmonella real-time PCR assay (AES Chemunex, Warnex) was used to compare the four extraction methods for the recovery of bacterial DNA from the two matrices.
The comparison of extraction methods was performed taking into account their extraction efficiency (amount and purity of DNA extracted) and the Ct-values obtained by real-time PCR reactions.
CONCLUSIONS: The QIAmp and Warnex Kits were the most suitable methods to detect Salmonella in pig faeces. For pork samples the best performances were obtained using the Invitrogen kit; however, the Warnex kit and the boiling method also gave satisfactory results. The treatment with Dynabeads, following pre-enrichment step, did not improve the recovery of Salmonella DNA.

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

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Human cases of Toxoplasmosis are caused by contact to Toxoplasma shedding cats, resp. to their excretions, transmission from pregnant women to theirfoetuses or by eating Toxoplasma burdenedraw 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). To add data on subclinical infections of pig herds to the 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 fordeciding on the appropriate inspection method and selecting carcasses for e.g. the production of minced meat. The paper gives an overview onways to integrate the Toxoplasma seroprevalence of pig herds into programmes for implementing the risk-based meat inspection.
Two studies were coupled: 1) 291 paired samples from pigs out of six herds (serum and meat juice from the same pigs), and 2) 3000 meat juice samples from 50 herds were tested for Toxoplasma gondii antibodies with the ANIMALTYPE® Toxoplasma AbELISA test (Labor-Diagnostik Leipzig, Leipzig, Germany).
It could be shown, that there are more Toxoplasma seropositive confinement herds than expected, and that including Toxoplasma serology into existing multi-serological herd profiles is meaningful in terms of improving the safety for pork.

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

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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.

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

DikMevius

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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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%). Of these, 16 (59%) were tested positive in all six swabs obtained before and after absence from work; five famers (19%) were tested positive before and negative in all three swabs obtained after the holidays; five (19%)were tested negative in the swab obtained on the first day after return only. 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.

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

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MRSA ST398 has not been detected in pigs in Ireland. However, other strains of MRSA, including t002, have been isolated from animals and humans.
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 such strains.
Six pigs were inoculated intra-nasally with 2 x 10^9cfu 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 at regular intervals 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. Upper respiratory tract samples were taken at post-mortem for MRSA culture.
All inoculated pigs were MRSA-positive for 5 days post-inoculation with counts up to 7.5 x 10^4 cfu/swab. Thereafter the number of positive pigs decreased with no inoculated pig positive after 17 days PI. However, MRSA was isolated from three inoculated pigs following post-mortem 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 intermittently 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.

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

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Despite its global occurrence in pigs, the kinetics of colonization of MRSA ST398 and other hospital and community acquired MRSA in pigs are largely unknown. We investigated the kinetics of colonization and transmissibility of MRSA after nasal inoculation of three different MRSA strains in weaned piglets.
In the study 58 piglets from one farm were randomly divided into four test groups and one control group. Three trial groups were inoculated with MRSA ST8, MRSA ST9 and MRSA ST398, respectively. The fourth group consisted of MRSA ST398 infected and non inoculated "sentinel" animals. The infectious dose of 5.0×10^8 cfu/animal had been determined in preliminary animal studies.
Clinical symptoms, the nasal, conjunctival and skin colonization of MRSA, faecal excretion and organ distribution of MRSA, as well as different environmental samples were examined for three (?) weeks after treatment.
After nasal inoculation of 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 sentinel animals. Likewise, faecal excretion and internal colonization of head and gut associated lymph nodes with MRSA ST8, ST9 and ST398 could be shown regularly in each group. However faecal excretion and the colonisation of the nasal mucosa with MRSA ST9 was significantly lower in the first days after inoculation compared to the test groups inoculated with ST8 and ST398.
This is the first study to demonstrate colonization of lymphnodes (e.g. the ileocolic lymphnode) after nasal inoculation of pigs with MRSA. The results of this study suggest a variability of colonization mechanisms of the different MRSA types in pigs.

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

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Purpose: 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.

Methods: 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).

Results: MRSA was detected in 40% (4/10) of the herds included in the study. The prevalence of MRSA in pigs was higher at lairage and ranged from 0 to54.2% per farm compared to that same batch of pigs on-farm (0 to 12.5%). The proportion of MRSA positive isolates was relatively higher in nasal compared to peri-anal samples. We detected MRSA in 1.6% (4/240) of the carcass swab and 3.7% (5/135%) of the retail pork samples. MRSA isolates showing phenotypic and genotypic similarity were recovered from batch of pigs before slaughter at the lairage, carcass swabs and retail meat.

Summary: MRSA was prevalent in market age pigs on-farm and at the lairage, on carcass and subsequently on retail meat. Persistence of MRSA in the food chain implies potential food safety significance. Further detailed study of representative swine population in the US to elucidate the extent of food safety risk is essential.

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

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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.

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

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Since 2005 there has been a dramatic rise in reports of tetraresistant monophasic Salmonella Typhimurium (mST) DT193 or DT120 in many European countries. This new 'epidemic' has been focussed on porcine and human hosts, but infection has also been found in cattle, horses and companion animals. These closely-related S.4,5,12:i:- and S.4,12:i:- strains lack fljB, that codes for the phase 2 flagellar antigens, but are still fully motile. In pigs, mST has been reported from clinical cases of enteritis and septicaemia in weaned pigs but the distribution and persistence on pig farms is unknown. A study was therefore carried out which was aimed at investigating and quantifying the occurrence of mST in different categories of pigs, wildlife on pig farms and the farm environment. Visits were carried out to three outdoor breeder/finisher farms, three indoor breeder/finisher farms, as well as one outdoor and one indoor rearer/finisher where mSt had been reported previously. Samples of individual faeces, pooled faeces, large gauze environmental swabs and faeces of wildlife pests were taken from all parts of the farms. Representative individual samples were also tested using a semi-quantitative dilution-enrichment method. mST was commonly found in both adult and postweaning sectors of the herds and in some age groups the within-group prevalence approached 100%. On most farms a wide variety of other serovars was also found in pigs and often predominated, but mST was more common in wild birds in such situations. mST was also more commonly found in newly introduced gilts and weaners than in the adult breeding herd. On one farm five different serovars, including S.4,5,12:i:-, were found in one batch of new gilts on arrival at the farm. In most positive faeces samples the numbers of Salmonella organisms was low, but some individual samples contained up to 105 cfu/g.

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

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Salmonella is one of the major causes of food-borne disease in the European Union (EU). The EU Regulation No 2160/2003 imposes a reduction of the prevalence of this agent in pigs. The Member States prevalence of the different serotypes varies, and some risk factors could be more associated with different serotypes. The data used in this 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 172 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. At the time of the collection of the samples a questionnaire about the herd management and potential risk factors was applied. A multinomial multilevel analysis of the dataset was done 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. In the GLMM the factors associated with the pen fecal samples were considered the first level of the explanatory variables and the factors associated with the herds were the second level. Comparing to "no Salmonella" as reference the significant associations ($p<0.05$) found for "serotypes Typhimurium or serotype 4,5,12:i:-" were: mixture of gilts and sows in the pen, herds with more than 203 breeding pigs, pen samples with more than 10 animals/sample. For the "other serotypes": control of rodents, region of the country, semen from others 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.

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

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The aim of this study was to evaluate the prevalence of Salmonella enterica in different points of the pork production chain. In order to achieve this objective, holding pens, slaughter lines, carcasses and cutting plants from two slaughterhouses were evaluated three times distributed at the beginning, half and end of the working week. The prevalence of Salmonella contamination in holding pens was 39.4% before the entry of the pigs, 83.3% at half working day, 69% at the end of the day and 63.3% after cleaning procedures. Regarding the slaughter line, Salmonella was detected in 156 out of 425 samples (36.8%) and at least one positive sample was found in 86% of the 26 points sampled. The prevalence within the slaughter line varied from 20% to 70% depending on the slaughterhouse and visit. In total, 445 hot carcasses were evaluated and the prevalence estimated at this point was 40.2%. Moreover, 323 carcasses could be sampled once more after chilling and cooling procedures and a significant decrease in Salmonella contamination was demonstrated (10.8%). A lower level of contamination was found in the cutting plant; Salmonella was detected in 67 out of 598 samples (11.2%) collected at this level, although 89% of the 27 checked points presented contamination in at least one sampling day. All the isolates were serotyped and part of them typed by molecular methods using Multi Locus Variable Analysis (MLVA). The results of this analysis provided relevant information about the variation of serotypes between sampling days and the relationships between isolates and molecular types found in different points.

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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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In order to reduce the incidence of human salmonellosis, Community 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). Information on the potential risk factors for Salmonella was gathered in this survey and analysed to assess associations with pen positivity.

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 all 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 4,[5],12:i:- were also found in several MSs.

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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Introduction.
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.
Methods.
In total, 150 pig carcasses were sampled in 10 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.
Results.
Pathogenic Y. enterocolitica were isolated from the tonsils of 91 pigs (60.7%) and rectal contents of 36 pigs (24.0%). Twenty-eight pigs were positive for pathogenic Y. enterocolitica in both tonsils and rectal content, while 62 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 respectively rectal content from one pig were positive for Y. pseudotuberculosis. Regarding carcass samples, 71 (47.3%) pig carcasses were contaminated with enteropathogenic Yersinia spp. Pathogenic Y. enterocolitica were mostly recovered from masseter muscles (55/150), followed by the sternal region (30/150), ductus pelvis (16/150), and medial site just before the sacrum (15/150).
Conclusions.
During slaughter, a high proportion of pigs carried pathogenic Yersinia spp. in their tonsils or intestines. Moreover, nearly 50% of the carcasses was positive for pathogenic Y. enterocolitica on one or more of the sampled carcass sites. 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.

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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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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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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.

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

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Introduction

Antibiotic consumption in Danish pig production has close attention of the public. Recently, a threshold level for an acceptable number of daily doses (ADD) per pig was set by the authorities, and herds with ADD/pig above the threshold are forced to imply action plans to reduce their antibiotic consumption.

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: Identification of a focus area for reduction, development of an action plan and follow-up on results.

Methods

In part 1, Farm Facts calculate ADD/pig for all herds in a veterinary practice. Each pig herd gets two histograms: One comparing their ADD/pig to other herds, and another showing diagnoses and treatments for their herd. The vet and the farmer can then identify diagnoses and treatments responsible for the main part of the consumption.

With the main consumption identified, vet and farmer proceeds to part 2: Creation of an action plan for reduction. For each disease group, Farm Facts has built-in suggestions for alternative ways to reduce disease, including management changes, feeding strategies and vaccination programmes. The action plan is specific for one herd and includes success criteria based on selected parameters.

As the farmer implements new preventive measures to reduce antibiotic consumption, part 3 in Farm Facts gives a graphical presentation of the development of selected parameters. The graph illustrates performance before and after the action plan and shows, whether success criteria are met.

Results and conclusion

Until now, Farm Facts has mainly been used for introduction of vaccination programmes. Here, it has proved helpful to implement vaccines as a preventive measure, and in some herds, ADD/pig has been reduced by more than 50%.

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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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ResPig is a digital diagnostic and monitoring program for veterinarians involving 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 the investigated diseases. The program helps the veterinarian to take the necessary steps towards a structured approach to respiratory problems with restrictive use of antibiotics.
Data were used from 300 farms involving 936 cross-sectional serology results in 2008 and 2009. Farms provided performance and slaughterhouse data and a vaccination history. Odds ratios were calculated between the serological results and the technical and slaughterhouse data provided in the farm anamneses. Two multiplying farms measured their antibiotic used in daily doses (DDD) 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.
Significant relationships were demonstrated between ResPig serology results for App, PRRS, M hyo and PCV2 and the technical performance and slaughterhouse data. 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. Positive M hyo serology was a risk factor for high mortality in finishers. 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 and the identification of risk factors and subsequent interventions undertaken are helpful in reducing antibiotic usage. This may contribute to even better technical performance, but is certainly beneficial for human food safety. An antibiotic use score will also be implemented in the ResPig program.

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

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

Therefore ninety-five Belgian closed or semi-closed pig herds were randomly selected. 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). Additional data concerning production characteristics (daily weight gain and mortality of fattening pigs) and the use of antimicrobial drugs, quantified by the treatment incidence (TIUDDpig) were collected. This means the number of pigs treated with one UDDpig (used daily dose in pigs)/1000 pigs at risk/day.

The external biosecurity score was on average 65 (min 45; max 89), whereas the internal biosecurity score was on average 52 (min 18; max 87). The daily weight gain of fattening pigs was positively correlated with both external ($r=0.36$, $p<0.01$) and internal ($r=0.29$, $p<0.01$) biosecurity. This indicates that in herds with higher biosecurity the production is also better. The correlation between mortality of fattening pigs and the external biosecurity was slightly negative ($r=-0.11$, $p=0.31$) giving an indication of the link between biosecurity and an improved health status of the pigs.

The correlations between the TIUDDpig and the external ($r=-0.17$, $p=0.10$) and internal ($r=-0.22$, $p=0.03$) biosecurity were both negative. Although not all relations are statistically significant, largely because of the huge variation in treatment incidence, they do suggest a trend of decreased antimicrobial use with increasing biosecurity. This indicates that the use of antimicrobial drugs on a herd might be decreased by increasing the biosecurity level.

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Garlic reduces effect of Actinobacillus pleuropneumoniae infection in pigs

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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 Actinobacillus pleuropneumoniae (APP).

Thirty male pigs of about seven weeks of age were aerosol challenged with APP serotype 2. Fifteen pigs received from two days prior to infection until four days after infection 2.5%(w/w) garlic added to their diet (Garlic group). The others received a standard diet (Control group). The pigs were monitored for temperature, appetite, and behavior. White blood cells were counted before the exposure and at day one and three after exposure. Four days after exposure the pigs were euthanized. Gross pathology findings were recorded, tissue samples were taken and lymph nodes were histological and bacteriological examined.

There was no difference in the number of pigs with symptoms of lung problems. 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 50% of the animals showed typical signs of an App infection. Of the Garlic group 20% of the pig showed these signs. 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 ($p=0.15$). It seems that feeding high concentrations of freeze dried garlic has no preventive effects to an APP infection, however it reduces the effects on the course and severity of an APP infection.

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Risk management with regard to dioxin residues in pork meat

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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.

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

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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.

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HEV inactivation assessment using viable virus

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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.

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

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EU regulations require disinfection of slaughter-equipment with water of at least 82°C. However alternative system having an equivalent effect may be used when equivalence can be shown. Inspexx© 200 (Inspexx) a solution containing peracetic acid and peroctanoic as active substances, a product of Ecolab, is a product of which was shown that it is an equivalent alternative. It is applied in cold water and does not have to be rinsed after application.
An additional study was done to study whether Inspexx is effective in the disinfection of knives used at slaughter. The standard method of disinfection with water of 82°C was used as reference. Enterobacteriaceae and mesophilic aerobic counts were the outcome variables. Knives were dipped in hot water or Inspexx for 0, 1, 10, 30 and 60 seconds.
Results show that Inspexx reduces the bacterial contamination of the knives significantly faster, within 1 second, than hot water, which needs at least 10 seconds. It is concluded that Inspexx 200 could serve as a more effective aid for disinfection of slaughter knives.
The outcome of this study confirms a previous efficacy study on slaughter equipment. That study in combination with safety and toxicity studies were the basis for approval of official authorities from several European countries to apply Inspexx as an alternative method for disinfection of slaughter equipment during slaughter. We discuss that Inspexx is therewith an in practice applicable and more robust alternative for disinfection of slaughter equipment. Moreover, it can also save water and energy as the water has not to be heated. Accordingly, Inspexx does not only improve meat safety, but will also save environmental resources.

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

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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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More than 20,000 samples are analysed each year for presence of antibacterial residues in Danish slaughter pigs. Surveillance data indicate that the true antibacterial residue prevalence in Danish slaughter pigs is negligible (~0.01%). 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. Danish surveillance data from 2005-2009 and prior knowledge about true prevalence and test sensitivity and specificity were included in the model.

The probability of detecting at least one confirmed sample presenting residues above 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 a 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 antibacterial use and post-mortem meat inspection data. A similar approach is considered for surveillance for antibacterial residues in Danish sows.

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Modelling of Salmonella dynamics in the pig slaughterhouse

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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.

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

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Targets will shortly be set by the European Union (EU) for each individual Member State (MS) to reduce the prevalence of salmonella in pigs at slaughter. A Quantitative Microbiological Risk Assessment (QMRA) was funded by the European Food Safety Authority (EFSA) to support the scientific opinion required by the EC, and which was subsequently adopted by the BIOHAZ panel.

The QMRA characterizes the variability both within and between EU MSs. This was achieved by developing a generic model with a clearly defined set of MS-dependent parameters. The QMRA estimates the number of human salmonella cases for three product types: pork cuts, minced meat and fermented ready-to-eat sausages. The QMRA was primarily designed to investigate current and future interventions, resulting in a highly mechanistic model. The generic model was parameterised for four case study MSs. 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. The model was validated by comparing the QMRA estimated prevalence to the observed prevalence at the point of lairage and retail; the QMRA outputs were deemed to be plausible at these two points. The total number of cases attributable to the three product types was also estimated. Similar to other farm-to-consumption QMRAs the model overestimates the number of cases, which can be attributed to a variety of factors including a lack of data regarding both human immunity and dose response. However, the QMRA still allows for the prediction of the relative impacts of different interventions during production on the Farm, Transport and Lairage of pigs and at the Slaughterhouse, which was the main purpose of the QMRA. The QMRA will be, where possible, used by EU MSs as part of an evidence base that will inform the design of their National Control Plans for Salmonella in pigs.

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

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Healthy food, environmentally friendly production and animal welfare are regarded by the public as being better taken care of in organic and free-range production systems. The close contact between production animals in these systems and the environment could, however, lead to transmission of pathogens from wild to domestic animals and subsequently to humans. Food safety could then be compromised.

The objectives of this semi-quantitative 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 would be suggested.

Inputs were taken from nationally and internationally published (and non-published) scientific literature. 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 and high). A similar scale was used for uncertainty in the outcomes. The final relative ranked risk estimates were obtained by multiplying outcomes from the three steps. These risks are only relevant in the context of comparing one to another.

No differences were identified between indoor and outdoor pig production in regard to food safety related risks of Salmonella, Campylobacter, Y. enterocolitica and T. spiralis (if carcasses are tested).

There is a risk for humans to acquire T. gondii from sub-optimal 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. To ensure adequate food safety, freezing or heat treatment to the extent of killing all T. gondii cysts present in the meat destined for such productions could be conducted.

The largest uncertainty was related to the likelihood of survival of T. gondii in the above mention products. Further research is therefore required.

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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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It is well recognised in meat inspection circles that older animals have a higher prevalence of pathological lesions, but there is limited baseline microbial hazard data on sow meats or comminuted products. These surveys were conducted between 2008 and 2010 to address that data gap.

Processors: 101 sow meat samples were collected on a proportional basis from four Australian abattoirs, and associated processors (representing around 80% of total sows slaughtered). Samples comprised mince or small pieces of meat destined for comminuted products. The mean Total Viable Count (TVC) concentration was 4.1 log10 cfu/g, E .coli prevalence was 42.6% (95% CI: 32.8-52.8%) and the mean concentration for E. coli positive samples was 1.28 log10 cfu/g. The overall prevalence of Salmonella was 8.9% (95% CI: 4.2-16.2%) with all isolates originating from one abattoir. Isolates included S. Anatum (×3), S. Rissen (×3), S. Typhimurium pt 35 (×2), S. Infantis (×1). Verotoxin producing E. coli were not detected in any of the samples (<2.9%), but Listeria prevalence was 14.9% (95% CI: 8.6-23.3%). Isolates included L. monocytogenes (×6 – all from mince), L. welshimeri (×5) and L. ivanovii (×4).

Retail: 116 fresh pork sausages and 148 fresh pork mince samples were purchased from supermarkets (n=87, n=107) and butcher shops (n=29, n=43), respectively. For sausages, concentrations of TVC averaged 4.3 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 average TVC were high (6.2 log10 cfu/g) and a large variation in TVC was observed from product with the same number of days until the use-by-date. In addition, commercial retail criteria (maximum TVC of 6 log10 cfu/g) were not met by 54.1% of samples. However, levels of E. coli (6%), coagulase positive Staphylococcus (1.3%), Campylobacter spp. (2.7%) and Salmonella spp. (1.5%), were low. No E. coli O157:H7, MRSA or Yersinia enterocolitica were detected (i.e. prevalence <3.5%).

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Modelling the use of different enforcement strategies to improve food safety

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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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Data from the serological salmonella-surveillance in breeding herds were combined with data from the Danish movement data-base, and the Danish Salmonella-surveillance, based on meat-juice.
646 integrated herds with both sows and finisher pigs received gilts or boars from 158 different breeding herds.
Serological results were obtained from the year 2008 from the breeding herds. A seroprevalence based on the cut-off 20 OD% was calculated for each breeding herds.
All sow herds receiving pigs from the breeding herds from July 1. 2008 to July 1. 2009 were identified in the movement data-base. For each sow herd with finisher production serological data from the meat-juice surveillance was obtained from the period from October 1. 2008 to October 1 2009. Due to Danish risk-based surveillance, only the first sample obtained in each month was used for the analysis to avoid bias from more samples being taken in seropositive herds.
Standard logistic regressions showed a clear association between the serological status of the breeding herd and the sow herd with finisher production. Increasing seroprevalence in breeding herds was significantly ($p<0.0001$) associated with higher average seroprevalence in finisher herds.
To further explore this result, zero-inflated models were used to explore the data. The zero-inflated models showed that the effect of seroprevalence in the breeding herds had a high impact on the risk of the sow herd with finisher production to be seropositive whereas the effect on the prevalence in positive sow herds with finisher production was low.
Based on these results it seems that buying in gilts or boars from high prevalence herds in-creases the risk of becoming serologically positive, but it has little direct effect on the quantita-tive level in the herd.

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Relation between antimicrobial use and resistance in Belgian pig herds

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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 tested against 15 different antimicrobial agents. 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. Also quantitative 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 of each antimicrobial drug gives an idea of the correctness of dosing.

The TI_{ADDpig} for group level use was 235.7 per 1000 pigs at risk per day, whereas the TI_{UDDpig} equaled 200.7. This means that in reality, less pigs are being treated with the same amount of antimicrobials than theoretically possible. Generalized linear regression analysis showed a significant link between the TI_{ADDpig} and the ARI ($p < 0.01$), whereas no significant link could be found for the TI_{UDDpig} ($p > 0.05$). This is an indication that the frequency of treatment may play a role in the selection of resistance. Incorrect dosing was not a significant factor in the selection of resistance.

Results from this study indicate that the frequency of drug administration may play a role in the spread of antimicrobial resistance in commensal E. coli. The hypothesis that incorrect dosing is a risk factor for antimicrobial resistance selection cannot be supported whereas it seems that the frequency of drug administration may play a role in the selection of resistance.

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Salmonella in pigs and pork and their antimicrobial resistance - 10 years of surveillance in Germany

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Salmonella isolates from pigs and pork have been submitted to the National Reference Laboratory for Salmonella. In this study data on Salmonella isolates and their antimicrobial resistance generated between 2000 and 2009 were analysed retrospectively. A total of 1839 isolates from pork and 4163 isolates from pigs submitted to the NRL were serotyped and tested for their antimicrobial resistance using the broth microdilution method. Minimum inhibitory concentrations (MIC) were evaluated using epidemiological cut-offs as provided by EUCAST.

The majority of isolates from pigs and pork belonged to three serovars: S. Typhimurium (66 and 52 %), S. 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 S. 1,4,[5],12:i:- 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 S. 1,4,[5],12:i:- 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 S. 1,4,[5],12:i:-. 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 this resistance. Resistance to fluoroquinolones was constantly on a low level in pigs and pork (3 to 4 %). Similarity of trends in serovar and resistance patterns supports the assumption of a vertical transmission along the food chain.

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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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Purpose: 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 co-selection with antimicrobial resistance. **Methods:** 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 two fold 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. A non-parametric Wilcoxon Rank Sum test for trends across ordered groups (Stata 10, College Station, TX) was used to determine association. **Results:** 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 value for association between copper tolerance and MDR AmStTeKm was 4.6 (Chi-square=17.9; $P<0.05$). 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. 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. **Summary:** 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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Basic principle of traditional meat inspection is the observation for pathological lesions which may indicate a disease or the presence of an agent.
Traditionally, the aim of cutting lymph nodes (LN) was to detect tuberculosis in both, cattle and swine. These days for swine, observation of mandibular LN for abscesses possibly caused by MAIC agents is still mandatory. However, visual inspection is not reliable enough, and such lesions are notoriously underreported. So, improvement of inspection (and prevention) procedures is required.

In this ongoing project, PCR techniques were modified for the analysis of lymph node tissues. 44 LN samples (mandibular and gut) with visible and suspicious lesions from finisher pigs were taken at the position of meat inspection and underwent a PCR- based analysis. Samples were taken from visually unaffected tissue and in parallel from the abscess directly. 28 out of 44 LN were positive for MAIC, positive results were obtained from tissue without any visible lesion, too.
Results indicate, that a PCR based procedure works despite the presence of NL tissue.

Simultaneously, post mortem data (2005 to 2009) from animals of the respective farms of origin were arranged according to the frequency of recorded NL lesions. For each year, farms with high numbers of suspicious NL lesions were identified and the 4 farms for each year were compared. Of the total of 20 farms (mandibular lesion) and another 20 farms (gut lymph nodes), five farms appeared twice during these 5 years interval.

For the implementation of reliable inspection systems, agent-specific tracing back systems should be developed. For MAIC, a step-by-step approach was established:
- visual observation of LN post mortem
- ranking of farms with a high frequency of suspicious lesions,
- confirmation by PCR- based analysis of lesions
- search for ports of entry at the site.

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Case studies: Tuberculination in pig herds suspected of infection with Mycobacterium avium

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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 flock 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.

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A retrospective analysis of Salmonella isolation trends from UK pigs since 1994, with special reference to monophasic S. Typhimurium and antimicrobial resistance trends

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The number of Salmonella reports from pigs in the UK have come down considerably since the mid- 1990s, when up to 766 positive epidemiological group reports (incidents) per year were recorded, and numbers have been relatively stable since 2003 with less than 300 incidents reported per year. S. Typhimurium has been the most common serovar throughout the study period (between 60 and 70% of incidents). S. Derby, the second most common serovar, has shown a downward trend since 2007, accounting for 8% 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 7.1% of incidents in 2009, whereas S. 4,12:i:-, after showing a small peak in 1997, has also increased since 2007 and accounted for 2.1% of incidents in 2009. The most commonly seen phage type among S. Typhimurium isolates throughout the study period has been DT193, although U302 showed a peak between 1998 and 2003. Nearly all S. 4,5,12:i:- isolates were phagetype DT193.
The percentage of S. Typhimurium isolates from pigs showing resistance to six or more antimicrobials has increased from 0% in1994 to 43.7% in 2009, with peaks of 57.9% in 2003 and 65.1% in 2007 respectively. Resistance of S. Typhimurium to tetracycline, sulphonamides, ampicillin, trimethoprim/sulphamethoxazole and chloramphenicol increased considerably since 2003, with more than 90% of isolates being resistant to tetracycline, sulphonamides and ampicillin at some point. The most common resistance pattern observed in S. 4,5,12:i:- (ampicillin, streptomycin, compound sulphonamides, tetracycline) was seen in 64.7% 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.

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Clostridium difficile in pork and retail meat in Texas

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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).

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Salmonella sp. in edible offal (liver and tongue) from pigs slaughtered for consumption

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In recent years, the importance of pork as a source of human Salmonellosis has been highlighted by the increasing number of research studies in this area. However, the information on 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. 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 along 8 sampling days during 5 months. Salmonella sp. was isolated using the conventional microbiological methods and isolate strains were analyzed using serotyping, antimicrobial testing and macrorestriction profiling by 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 the liver swab (5/40; 12.5%) and the 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 (R1 and R4) were observed in tongue and swab liver samples collected in different days, suggesting common contamination sources and the persistence of Salmonella sp. clones along the slaughter process. Additionally, the presence of the same MRP (R1, R4 and T2) in liver parenchyma samples seems to indicate that the pig is one of the possible vehicles of Salmonella sp. to those edible products. The results 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 slaughter process and along liver and tongue processing and selling chain.

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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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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.

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Herd-level risk factors influencing serological Yersinia prevalence in fattening pig herds

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Recent epidemiological evidence has demonstrated that pork is an important source of yersiniosis in humans. If a pig herd has a history of infection with Yersinia (Y.). enterocolitica, antibodies are widely distributed among the pigs. The objective was to find those herd factors associated with finishing pig herds testing seropositive for pathogenic Yersinia. Serological prevalence and data on farm management and production of 80 fattening herds from Lower Saxony, Germany, were included in the analysis. Detecting antibodies against Yersinia was performed using the Pigtype® Yopscreen ELISA. Risk-factor analysis was performed by using the Wald chi-square test.
The serological within-herd prevalence varied from 0% to 100 %. 16.3% of the herds had no serological reactors, whereas 52.5% had a seroprevalence above 90%.
Although more than 70 farm characteristics were included in the risk assessment, only four herd characteristics were associated with higher seropositivity for Yersinia spp.: the floor design, the source of the drinking water, health problems and the daily weight gain.
Y. enterocolitica is spread by faecal-oral transmission. The analysed risk factor "no housing on fully slatted floor" is directly associated with frequent contact to faeces, while this connection is not automatically given by the factors "recurring health problems in the herd" and "comparatively low daily weight gain". A low daily weight gain might be the consequence of recurring health problems, because illness causes inadequate feed intake and loss in weight e.g. diarrhoea. Diarrhoea might lead to an increased shedding of the agent. Unfortunately, the information about the cause of health problems was invalid for analytical purposes. The factor "no use of supply water" was not further proven by bacteriological analysis of the drinking water. But strains described in literature, which were isolated from untreated water, belong mainly to non-pathogenic species of Y. enterocolitica.

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Clostridium difficile in a farrowing pen

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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.

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Variety of Clostridium difficile PCR ribotypes in pigs arriving at the slaughterhouse

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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.

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Carriage of Campylobacter by sows and spread to fattening pigs in farrow-to-finish farms

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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.

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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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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.

Isolation of Salmonella spp. in pigs during transport, lairage, slaughterline and quartering

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The aim of this study was to determine the prevalence of Salmonella in the different points of the chain (transport, lairage, slaughterline and quartering) in order to determine Hazard Analysis and Critical Control Points (HACCP) in the pig slaughter process. We carried out eight systematic sampling at six different stages: (i) sampling of the transport at its arrival to the slaughterhouse and after cleaning and disinfection (C+D), (ii) lairage before entry of the pigs, and after departure to slaughter, (iii) pig carcasses of ten pigs in different stages of slaughter-dressing, (iv) tonsils, lymph nodes and feces, (v) environmental samples, (vi) quartering. All samples were analyzed according to ISO 6579:2002 and colonies were serotyped for confirmation of Salmonella spp. with specific antisera (Biorad). Preliminary results of this study showed a total of 134 Salmonella isolates from 1180 different samples (11.3%). The highest percentage of isolates were detected at the point of pre-scalding (30/80, 37.5%), feces (18/80, 22.5%), transport (13/60, 21.6%), tonsils (15/80, 18.7%), 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 samples (5/80, 6.25%). Moreover, the higher number of isolates was produced coinciding with the higher workload of the slaughterhouse, when more than 400 pigs were slaughter. These data show the isolation of Salmonella spp. from samples of different source, which constitute a great risk for pigs both before and after slaughter. Thus, the HACCP must focus on 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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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. Therefore, 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.

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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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Salmonella is one of the major causes of food-borne disease in the European Union (EU) and the EU approved legislation (EU Regulation No 2160/2003) 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 baseline survey on the prevalence of Salmonella in breeding pigs in Portugal. A total of 1670 pen fecal samples from 167 herds were tested. Of these samples 170 were positive to Salmonella. Along with the samples' collection a questionnaire was applied to collect information about the herd management and potential risk factors. A multilevel analysis was applied to the dataset using generalized linear mixed models (GLMM). The outcome variable was presence/absence of Salmonella in the pen sample. In the GLMM the first level for the explanatory variables was assigned to the pen samples and the second level was assigned to herds. The results show significant risk associations ($p<0.05$) at herd level: North Region versus Alentejo Region (OR=3.86), control rodents (OR=0.23), more than 90% of boars from an external source versus more than 90% of boars home-raised or no boars (OR=1.85), 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 first 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). Further studies should be done to evaluate these risk associations giving a positive contribute to improve Salmonella Control Plans in swine, in Portugal.

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Post harvest reduction of Salmonella by use of vaccination in growing pigs

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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.

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Prevalence and characterization of Salmonella and Listeria monocytogenes in french raw pork meat at the distribution level

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A French study was undertaken in 2010 to estimate the occurrence of Salmonella and Listeria monocytogenes in raw pork meat (Minced pork meat, pork chop, fillet and roast, and other various pieces). A total of 320 samples have been collected at the distribution level in various geographical areas distributed on all France. Detection and enumeration were conducted for the two pathogens as described in ISO methods. All isolates were serotyped and genotyped by standardized PFGE method.
Salmonella was detected in 2.5% of the samples (8 on 320) but not numerable. Prevalence of L. monocytogenes was higher with 41 positive samples (13.1%) and significantly more important in minced pork meat (25%). Eight samples could be numerated and number of ufc/g varied from 10 to 730. Five samples were contaminated by both, Samonella and L. monocytogenes.
The 27 Salmonella isolates were of serotypes Derby (4), Typhimurium (2), 4,5,12:i:- (monophasic variant of S. Typhimurium) (1), Anatum (1) and Infantis (1). One isolate for each serotype for each positive sample were subtyped by PFGE using XbaI enzyme (n=9). The method highlighted a distinct PFGE type for each serotype except for serotype Derby which exhibited 2 PFGE patterns.
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 serogroup and 5 PFGE patterns.
This study provided recent valuable information on the occurrence of Salmonella (2.5%) and L. monocytogenes (13.3%) in raw pork meat at the distribution level. Minced pork meats were particularly contaminated by both indicated that transformation of meat increases the risk to contaminate pork meat.

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A study of the distribution of Salmonella serovars in an integrated pig company

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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, 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. Disinfectants that were used between batches of pigs were often used at insufficient concentration.

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The influence of good farming practice on the occurrence of Salmonella on pig farms

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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 questionnaire, 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.

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Growth and survival of exponential and stationary phase Salmonella during sausage fermentation

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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.

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Evaluation of cleaning and disinfection procedures against Samonella enterica at swine farms, transport and lairage facilities

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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.

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Yersinia enterocolitica prevalence and diversity in a pig slaughterhouse

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Yersinia enterocolitica is involved in human infections from food. It was isolated mainly in pork. The aim of this study was to contribute to the assessment of the prevalence in France with an optimization of the research methods based on ISO 10273-2003. 516 samples from 344 pigs (24 batches) were analyzed over 23 consecutive months in a single pig slaughterhouse. Enumeration and isolation were achieved by using CIN agar and YeCM chromogenic medium (modified from the Weagant medium, 2008). Irgasan, Ticarcillin and potassium Chlorate broth (ITC) was used as enrichment media. The identification was performed on lactose negative, oxidase negative, urea positive, TDA negative strains using the API 20E gallery. Biotypes were identified by biochemical typing. The bioserotypes most frequently encountered in Europe (i.e. 1A, 4 O:3, 2 O:9 and 3 O:5.27) were determined by m-PCR, targeting the ail virulence gene, the plasmid (virF), the RfbC and 16S RNA genes. The total prevalence of Yersinia enterocolitica in the slaughterhouse, 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 by PFGE typing. Only a few different genotypes were obtained: 8 distinct profiles with ApaI, 4 with NotI and 9 combined profiles. More diversity was observed with ApaI than with NotI. The 4 strains of bioserotype 1A present 2 distinct profiles which were clearly different from the 96 strains of bioserotype 4 O:3. This study contributes to a better understanding of the food infections risks caused by Yersinia enterocolitica. That's why it is important to develop more efficient protocols using classical microbiology and especially molecular biology.

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Serological characterization of Salmonella spp. infection in finishing pigs from NE Spain

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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.

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Salmonellosis in wild birds and its relationship with the infection in finishing pigs

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Despite the prevalence of Salmonella infection in wild birds seems to be quite low there are evidences of the relationship between salmonellosis in farm animals and wild birds. In this ongoing study we investigate the potential relationships between Salmonella infection in birds living around pig farms and pig salmonellosis. So far, 12 finishing pig farms have been sampled. Bird nets were set up close to pig farms, in areas with vegetation where they could be unnoticed. Birds were captured alive and kept in dark cages by species and in groups of up to 5 animals until they defecate. Feces were collected through sterile swabs and birds released after tagged. Pools of feces were collected from a minimum of 7 boxes from the barns. Samples were cultured by triplicate and following the ISO 6579:2002 standard. In 8 farms (66.6%) Salmonella was isolated from pig feces and in 4 (50%) from at least one bird sample. Out of these 4 farms, in 3 (75%) the serotype observed in pig feces and in bird feces was the same, i.e. Typhimurium. Out of the 87 bird samples analyzed, 9 (10.3%) were Salmonella positive (3 from sparrows, 3 from starlings, 1 from swallows, 1 from a warbler, and 1 from a nightingale). Only one bird sample, from the starlings, presented multiple resistance (to ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline and nalidixic acid). Interestingly this pattern was also observed in the Salmonella strain isolated from the corresponding pig farm. From these preliminary results it can be speculated that birds can be either victims or responsible of the infection in the pig farm. In any case, isolation of pigs from birds is encouraged to prevent the maintenance of the infection cycle. Ongoing molecular characterization of the isolated strains will help to better understand these relationships.

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The addition of galacto-oligosaccharides on the feed for the control of salmonellosis in fattening pigs

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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.

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Meat Juice serology underestimates prevalence of Salmonella in pig herds

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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 quantify the relationship between salmonella ELISA test results from meat juice and from blood serum.
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 the observed differences into account.

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Practical experiences with the reduction of prevalence of Salmonella infections in pig herds

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PIC Deutschland started in 2001 with concrete actions to reduce Salmonella prevalence in their breeding herds. The problem farms were identified by high serological titres in the slaughter animals and the results of the PIC Health Database.
Regional vets assisted the high positive farms using strict protocols and advises to reduce the Salmonella prevalence. It involved in total 98 farms (nursery, pretest and grow out units).
The basis of the reduction program is covered by 3 main aspects:
1. Reduction of the introduction in the herd.
2. Reduction of the internal spread in the herd.
3. Improving the level of immunity and intestinal health of the animals.
Four cases will be used to illustrate our actions and their success:
1. Sudden increase of OD-values in 2001. Problems: door ventilation, high pressure cleaning of central corridor with animals present in compartments, no strict AI-AO and >50% wheat and triticale. Direct ELISA-monitor improvement after changes.
2. Extreme increase in OD values. Actions: rodent control, more barley in the feed, meal feeding, organic acids in feed. A few months later there were less than 10% positive blood samples.
3. Category III, outdoor farm. Identified problems: piglets were often put back in nursery, hard manure on solid floors, and many pest animals. Four months after implementation of our advices the average OD was 4%.
4. Category II in spite of good hygiene and feed. Values decline only after stopping sorting the animals.
Concluding we were able to reduce the Salmonella prevalence on all farms, especially with biosecurity measures (quarantine, working routes, AI-AO, age segregation, pest control, stress reduction), hygienic measures (cleaning and disinfection, correct high pressure cleaning, trough hygiene, general health situation) and feed adjustment (meal/pellets, mycotoxines, feed composition, fermentation, soya, barley, feed/water additives). All PIC herds now have Salmonella-status I.

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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. 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 supported the program in assisting and improving farms with higher serological 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%). One year later strict control measures on salmonella reduction decreased the percentage of indexes over 20% from 12 to 7%. A checklist was used to control nursery, pretest and grow out units.

Main actions were focussed on:

- reduce introduction
- reduce spreading on farm
- stabilize health of the gastrointestinal tract

2004 the German QS program (Quality and Safety) started. The PIC salmonella monitoring program became part of the salmonella program of QS. According to the QS regulations 10 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 Salmonella monitoring and reduction program of 2001 till present resulted in a lot of information, protocols and success stories. This whole period 55 PIC farms are categorized. 100% is at the moment in category I.

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Effect of different treatments on swine carcasses surface contamination with Salmonella Typhimurium

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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.

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Identification of Salmonella clonal groups and enterobacteria quantification in different risk areas of manufacturing process in four Brazilian feed mills

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Manufacturing and delivering of high quality feed is essential to the success of pig production. It has also long been recognized that Salmonella may be effectively spreaded via contaminated feed. The flow of ingredients and feeds through each feed manufacturing facility contributes to the level of contamination. Thus, the identification of critical points for contamination and Salmonella clonal groups spreading may contribute to develop contamination control plans. The objective of this study was to determine the frequency of Salmonella isolation and to identify Salmonella clonal groups in risk areas in the feed mill. Sampling was conducted in feed manufacturing facilities belonging to four swine producing companies. Each facility was visited six times after the flow chart of each facility was studied and sample spots were defined. Samples were taken from ingredients and dust found in the storage bins, bucket elevators, mills, mixers, scales and pelleting chamber and cooler. A total of 1,341 samples were analyzed for Salmonella and enterobacteria. A total of 65 (4.8%) samples were Salmonella-positive, and the highest number of positive samples was found in the transportation area (bucket elevator and conveyor belt). In three facilities, Salmonella was isolated from samples of the end product. Serovars Montevideo, Infantis, Newport, Orion, Senftenberg, Agona, Worthington and Tennessee were found in more than one step of the manufacturing process, and were submitted to molecular typing by pulsed-field gel electrophoresis. Clonal groups were identified among the isolated strains. Storage bins showed highest enterobacteria counts in all studied feed mills, but no correlation could be found between enterobacteria enumeration and Salmonella isolation. Problems to keep feed mill machinery in clean condition and high production flows were identified in all mills and these may have contributed to spread Salmonella clonal groups.

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In-vitro experiment of Listeria reduction in ready-to-eat dry cured sausages

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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 methods to reduce Listeria such as bacteriophage (ListexTM), bacteriocin (nisin and pediocin (Fargo 37)), organic acids (sorbate and lactate), lyzozyme and culture of bacteriocin-producing lactic acid bacteria (HoldbagTM Listeria 10IP (Lactobacillus plantarum), HoldbagTM 261 (Staphylococcus xylosus, Lactobacillus lactis, and Lactobacilus plantarum), and Fermitrat N (Micrococcus varians and Staphylococcus carnosus). The in vitro experiments, performed in 96 well plate, using Brain Heart Infusion media with pH adjusted to pH 6 (which similar to that of sausages), offer additional benefits, such as save time and cost. 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 (ListexTM, nisin, and pediocin), and bacteriostatic effect (organic acids, lyzozyme and lactic acid bacteria). Using the optimum concentration, the effective combinations among treatments were studied at two different temperature, 30ºC and 10ºC (which mimic the curing process). The optimum treatment against Listeria is Pediocin (Fargo 37, 2.8 mg/ml) which could be applied alone or in combination with bacteriophage (ListexTM) or bacteriocin-producing lactic acid bacteria (Fermitrat N). The obtained results will be further proceed, to direct application in different types dry cured sausages.

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Attachment of Salmonella spp. to pork meat

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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 production optimization and thereby minimizing contamination. Attachment is an important prerequisite for adhesion and persistent of Salmonella in the food production chain. Using the IFR Gel Cassette System it is possible to mimic growth on biological surfaces so growth in food (as immobilized cells) and planktonic growth (cells in solution) can be compared under controlled conditions. Together with a developed meat model it is possible to study whether immobilized Salmonella reacts differently according to attachment ability, detachment probability and expression of attachment genes when transferred to pork surfaces compared to planktonic cells. In the meat model, a bacterial suspension (either immobilized or planktonic cells) is spread onto pork surfaces and at different time points, the surfaces are treated to remove loosely and strongly attached cells. Samples for RNA purification are withdrawn for the real-time PCR gene expression analysis. The attachment potential of the strains is measured by cell counts of loosely and strongly attached cells. From experiments with a wildtype S. Typhimurium, four knockout strains were constructed and investigated to see if they behaved differently compared to the wildtype. Preliminary results with one knockout strain (deletion of the prg operon) indicate that when applying planktonic cells to a pork surface, the detachment probability increases to 12% from 5% compared to the wildtype. These results and the further work that is in progress, may lead to an indication of what kind of food production environments that facilitate expression of adhesion genes and thereby food contamination.

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Salmonella in pork – Lessons to be learned from salmonella control in poultry

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Based on Reg. (EC) No. 2160/2006, programs to control Salmonella in primary production in poultry have been decided and implemented in Europe. The regulations are based on flexible reduction targets to combine public health needs (reduction in human salmonellosis cases) with highly variable baseline prevalences in the poultry population in the Member States. The control programs focus on serovars that have continuously been associated with high incidence rates in the human population: 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. This talk will highlight the potential lessons to be learned from the remarkable success that the implementation of the control programs in poultry has had with respect to reducing human salmonellosis.

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Pig fecal and tonsil contamination of Yersinia enterocolita in one French slaughterhouse

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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 cm^2; 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.

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Identification of plasmids in a Salmonella Typhimurium septicemic isolate without the classical 95 kb virulence plasmid

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Salmonella Typhimurium is an important pathogen in swine and also a zoonotic agent. 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, most of affected pigs will become asymptomatic carriers and can be the source of meat contamination when slaughtered. It is thus important to better characterize these isolates in order to understand pathogenesis of infection and develop appropriate control measures. In this study, some plasmids of a S. Typhimurium strain isolated from a septicemic pig were characterized. Based on preliminary results, this isolate did not possess the classical 95 kb plasmid associated to virulence, but contained many low molecular weight plasmids. This isolate was one of the most invasive in intestinal epithelial cell line (58.34% ± 7.32) and showed no acquire resistance to tested antimicrobial agents. We therefore sequenced these plasmids using conjugation. 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 function and some hypothetical proteins from various bacterial species. Finally, a third plasmid is currently being characterized. The size of this plasmid is higher than the two sequenced plasmids. These plasmids contained several genes of unknown functions that will need to be further studied for their putative role in virulence.

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Evaluation of ozonated water as a microbiological risk mitigation option in pork production

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Ozone is an oxidative molecule that has a bactericidal effect. This molecule can be solubilized in water and was proposed to be used in food production plants. In fact it possesses, as a disinfecting solution, many industrially relevant characteristics such as the absence of residues following the application, usability at meat industries room temperature and applicability during activities. The objective of this study was to evaluate the bactericidal effect of an ozonated water rinse on the wrapping of meat logs at the entrance of the 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. Total aerobic counts were measured, Salmonella and Listeria monocytogenes detection were also individually conducted on each units and enumeration of E.coli and coliforms completed the bacteriological analysis. 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/meat log 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 effective tool to reduce aerobic flora contamination before meat slicing processing. It also indicates that ozonoted water could represent an alternative option to the chlorinated water treatments and represent effective ways to control product wrapping contamination before entrance at the slicing plant.

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Salmonella in Irish pig farms; prevalence, antibiotic resistance and molecular epidemiology

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The objective was to examine the prevalence of Salmonella in manure from 30 Irish pig farms and to characterise any recovered isolates in order to assess potential risks and epidemiological relationships. In 2009-2010, manure was sampled from the finisher houses of 30 commercial pig farms, chosen based on their Salmonella category; 10 each from categories 1 (<10% seroprevalence), 2 (11-49% seroprevalence) and 3 (>50% seroprevalence). The presence/absence of Salmonella was determined in 25 g manure samples using standard procedures (ISO 6579:2002 Annex D, 2005). Salmonella isolates were serotyped, phage typed, tested for antibiotic resistance and typed by pulsed field gel electrophoresis (PFGE). Overall, Salmonella was detected in manure from 50% (15/30) of herds; 30% (3/10) of Category 1 herds and 60% (6/10) of Category 2 and 3 herds. In total, 29 isolates, comprising seven serotypes were recovered. S. Typhimurium predominated; 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; DT104b within three herds, DT104 in two and U288, DT193, U311 and DT17 each in one herd. In addition, S. Manhattan, Goldcoast, Bredeney, Brandenburg, Livingstone and Derby were each isolated from one herd. 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-resistant (resistant to ³6 antibiotics). Molecular analysis revealed 15 PFGE types and facilitated tracking of isolates across farms. For example, one S. Typhimurium DT104 isolate was common to three herds. Overall, Salmonella prevalence in our study 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.

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The Salmonella monitoring programme in The Netherlands

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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.

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Effect of transportation and mixing with unfamiliar pig on Salmonella susceptibility in market weight pigs

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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.

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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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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 reference 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 ºC 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.

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Enrichment or maceration influence post harvest isolation of Salmonella from mesenteric lymph nodes

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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.

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Rapid detection of Salmonella in meat: Comparative and collaborative validation of a non-complex and cost effective pre-PCR protocol

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Cost-effective and rapid monitoring of Salmonella in the meat production chain can contribute to food safety. The objective was, for the first time, to validate an easy-to-use pre-PCR sample preparation method based on a simple boiling protocol for screening of Salmonella in meat and carcass swab samples using a real-time PCR method. The protocol included incubation in buffered peptone water, centrifugation of an aliquot and a boiling procedure. The validation study included comparative and collaborative trials recommended by the Nordic Organization for Validation of Alternative Methods (NordVal). The comparative trial was performed against a culture based reference method (NMKL187, 2007) and a previously NordVal approved PCR method with a semi-automated magnetic bead-based DNA extraction step using 122 artificially contaminated samples. The limit of detection (LOD50) was found to be 3.0, 3.2 and 3.4 CFU/sample for the boiling, magnetic bead-based and NMKL187 methods, respectively. When comparing the boiling method with the magnetic beads, the relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) were found to be 98%, 102% and 98%, respectively (Cohen's kappa index 0.95). When comparing results obtained by the boiling to the culture based method, the AC, SE and SP were found to be 98%, 102% and 98%, respectively (kappa index 0.93). In the collaborative trial valid results from 11 laboratories were included and apart from two false positive samples, no deviating results were obtained (SP, SE and AC were 100%, 95% and 97%, respectively). This test is under implementation by the Danish meat industry, and can be useful for screening of large number of samples in the meat production, especially for fast release of minced meat with short shelf-life.

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Progress in salmonella isolation and their serovar composition in pigs

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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 (2009)

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Regulation EC 2075/2005 ensures official inspection of food of animal origin with specific rules on official controls for Trichinella in meat. For surveillance programs this regulation allows serological methods.
During the 1st national ring trial, 21 laboratories from 11 states of Germany tested serum samples (including dilution series) from 22 experimentally infected pigs, as well as pig serum and meat juice samples from their routine submission using the PIGTYPE© Trichinella ELISA. 2009 this assay was officially approved in Germany for testing antibodies against Trichinella in serum, serum pools and meat juice samples of domestic pigs and wild boars. The number of correct, false positive and false negative results per laboratory was compared to the sample status obtained by the German Trichinella reference laboratory. Furthermore the repeatability and the OD values obtained by the ring trial participants were analysed. The result variation of the labs was compared to the mean variation by Mandel´s k.
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.
The participating laboratories tested a total of 234 serum samples, 33 plasma samples and 147 meat juice samples from pigs and 26 serum samples from wild boars from their routine submissions. All 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. 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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Introduction

Sera from slaughter pigs can be tested for antibodies against several pathogens with ELISA. For the detection of antibiotic residues, a microbial inhibition test inrenal pelvis fluid (preurine) is used. As a faster alternative, these (small) residues can also be detected in sera with an immunoassay, i.e. an inhibition test. The purpose of the research described here is to combine these different assay principles in one protocol for a bead-based Luminex assay.

Materials & methods

A previously developed Trichinella Luminex assay was chosen as a representative serological assay, while the detection of sulfamethoxazol was chosen as a representative inhibition assay. A panel of serologically well-described swine serum samples were spiked with antibiotic residues and were used for setting up the protocols.

Results

To combine the serological test with the inhibition assay, the effects of various conditions were investigated. Differences between the two assays are the number of incubation steps and the required serum dilutions. A 'split-and-pool' sample preparation protocol was developed which resulted in a sample suitable for simultaneous detection of both antibodies and antibiotic residues.

Discussion & conclusions

The results demonstrate that it is possible to combine a serological assay and an inhibition assay in one Luminex protocol. To further enhance the sensitivity, optimization of variables like bead production, buffer composition and labelling need to be performed.

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Comparison of different enrichment media for the isolation of Salmonella from naturally infected slaughter pigs

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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.

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Title Performance of four different diagnostic tests for C. difficile infection in piglets.

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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.

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Risk assessment of MRSA isolates from swine using a diagnostic DNA microarray

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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 (Fetsch et al. 2008). 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. Additionally, the phenotypic resistance of some isolates was investigated by broth microdilution to compare the genotypic and phenotypic resistance profiles.

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. After a precipitating reaction an image of the array was recorded and analysed using a designated reader and software. In addition the clonal complex of the isolate is defined based on all hybridisation reactions.

More than 100 isolates were included in this genotype characterisation. The tests showed a good correlation between genotype and phenotype resistance.

The array provides information about genes for PVL. toxic shock syndrome, and exfoliativ toxins, and enterotoxins and in addition resistance genes for example pleuromutiline and streptothricin.

The characterisation via DNA-Chip has is superior to phenotypic methods since the application is quick and simple for personal trained in molecular biology.

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.

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A longitudinal study on the persistence of Livestock Associated-MRSA in swine herds

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In recent years a new type of MRSA, now called livestock associated MRSA (LA-MRSA), belonging to the CC398 clone, has globally emerged in swine world wide. Aim of this study was to monitor the LA-MRSA-status of swine herds over a longer period. To investigate this, we selected sixteen LA-MRSA-positive herds from a previous study. Starting in September 2009, five dust samples were collected every two months, on the first occasion by employees of the AHS, and subsequently by the herd owner or employees. Samples were analysed by a two-step selective enrichment and growth on chromogenic agar. MRSA was confirmed by PCR. Typing of the S. aureus protein A gene (spa) was done at the Institute of Hygiene.
Of the 16 herds, two were excluded from the study due to inconsistent sampling. Two herds were positive on all sampling occasions and were consistently contaminated with spa-type t011. In the remaining herds, occasionally no positive dust samples were found at some sampling moments. The predominanting 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 in a swine herd 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 the, occasionally found, negative samplings in overall positive herds. However, a true change of positive LA-MRSA-status to a negative status, followed by re-introduction cannot be ruled out in our study design.

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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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This study aimed to assess the antimicrobial resistance pattern of Salmonella enterica subsp. enterica serovar (S.) Derby and Typhimurium isolated from healthy pigs slaughtered in southern Brazil. Thirty-two S. Derby and seventeen S. Typhimurium strains isolated from carcasses (n=29), intestinal contents (n=16), and lairage environment (n=4) were tested. The antimicrobial resistance was determined by the agar disk diffusion test according to the document M31-A2 of the CLSI using the following disks: trimethoprim (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). The isolates were resistant to tetracycline (100%), sulfonamides (79.5%), streptomycin (77.5%), ampicillin (38.8%), gentamicin (32.6%), kanamycin (30.6%), nalidixic acid (24.5%), chloramphenicol (18.4%), and ceftazidime (2%). All isolates were susceptible to cefotaxime and ciprofloxacin. Five resistance patterns were found in S. Derby isolates, and TE-S-S3 was detected in 25 strains (78.1%). The pattern TE-S-S3-NA was found in four strains. Two isolates showed resistance to six antimicrobials K-TE-C-CN-S3-AMP and K-TE-S-C-S3-AMP. One S. Derby, isolated from pig carcass, presented resistance to eight antimicrobials, with the pattern TE-CAZ-S-C-CN-S3-NA-AMP. For S. Typhimurium, eight patterns were found and the most prevalent were K-TE-CN-AMP and K-TE-CN-NA-AMP, detected in four isolates each. Two strains showed resistance to seven antimicrobials K-TE-S-C-CN-S3-AMP; five were resistant to six antimicrobials TE-S-C-CN-S3-AMP, K-TE-S-CN-S3-AMP, and K-TE-S-CN-NA-AMP; and one showed resistance to four antimicrobials TE-S3-NA-AMP. One isolate from carcass presented resistance only against tetracycline. The large number of resistant isolates underlined the potential hazard that S. Derby and S. Typhimurium can pose to human health when they enter the food chain.

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Methicillin resistant S. aureus (MRSA) in the pork food chain in Germany

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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 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 against fluoroquinolones 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.

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Reduction of antibiotics after implementing PCV2 vaccination on 460 sow Dutch pigfarm

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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).

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Implementing PCV2 vaccination resulting in reduction of antibiotic use on Dutch farrow-to-finish farm

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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 November 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 11 months in which only vaccinated pigs were present on the farm (5438 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 48 % (4,03 vs 2,10%), 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 81 % (40,61 vs 7,59 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.

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Significantly reduced use of antimicrobials with PCV2 and ileitis vaccination in a Danish herd

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Introduction and objectives

Both in Denmark and in other countries, pig producers are under severe political pressure to reduce the consumption of antimicrobials. Therefore, the present study was designed to examine whether vaccination against both ileitis and PCV2 could give a significant reduction in the use of antibiotics and still be economically beneficial for the farmer.

Materials and methods

The study herd received 1000 SPF weaners every 7th week, which were moved to a finishing unit 7 weeks later. Antibiotics were used only when indicated by the clinical status of the pigs.

Pigs were vaccinated with Ingelvac CircoFLEX® (Boehringer Ingelheim) i.m. at 2 weeks of age and with Enterisol® Ileitis Vet. (Boehringer Ingelheim) in drinking water at 4 weeks of age.

Efficacy reports was collected every 7th week and data antibiotic prescriptions were obtained from the Vetstat database. Data were collected 1 year before and 1 year after start of vaccination. Comparison of before and after was done with ANOVA, using $p \leq 0.05$ as significance level.

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. The reduction was mainly seen in oral medication.

In the finishing unit, the economical benefit was calculated as follows:

Significant increase of ADWG with 44 g/day ($p=0.0133$)
Significant reduction of FCR with 0.21 Feeding units/kg gain ($p=0.0005$)
Significant reduction of mortality with 1.79% ($p<0.0001$)
Significant reduction of antibiotic expenses with 1.15 €/pig ($p=0.0156$)
ROI 1:2.5 (one € spent on vaccine was paid back 2.5 times)

Conclusion

With a reduced use of antibiotics, vaccinated pigs grew faster and had a better FCR than the non-vaccinated pigs getting more antibiotics, thus demonstrating, that prevention (vaccination) is better than cure (antibiotics), also from an economical point of view.

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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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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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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.

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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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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.

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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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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 oxytetracyclin 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.

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Improvement in pig growth and feed conversion due to knowledge transfer about disease prevention and improving immune response

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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.

We tested the effect of the pig farmers' awareness of the importance of these management factors to improvement in average daily gain in weight, feed conversion, the mean percentage of lymphocytes and I-FABP values.

For one year 17 pig farmers attended a trail 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 introduction of diseases. Eighteen pig farmers, the control group, did not attend these meetings. The meetings consisted of knowledge transfer, farmers discussions to get insight in their possibilities for optimizing their farms, group discussions with farmers, researchers and veterinarians and setting up a plan of action with set deadlines. Farmers carried out this plan for at least half a year. The three meetings were held every four till six weeks.

After one year, the farmers of the Study group 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 meeting. The number of actions of the farmers in the trial group was significantly higher. No difference was found in the mean percentages of lymphocytes and I-FABP values. The average daily gain in weight and the feed conversion were significantly better for the trial group compared to the test group, but only during the first three months after the meeting.

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Antibacterial and antioxidant activity of oregano essential oil

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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 pneumoniae, 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 (48,200). 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.

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Emerging Hepatitis E viruses from swine in Europe

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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.

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HEV in the pork food chain in United Kingdom

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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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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 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 pork is in place since 1972. A qualitative risk assessment was conducted to evaluate the human health risk of residues in Danish pork. The hazard identification identified the residues that could potentially be found. The release assessment evaluated the probability of release of antibacterial residues in Danish pork, based on antibacterial consumption data from 2008. The exposure assessment estimated the probability of human exposure, based on findings of residues 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 human health risk from residues other than antibacterials in Danish pork was considered negligible. 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. Examples of recent cases are given. They 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.

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Quantifying the effect of natural microflora on growth of salmonellae in fresh pork

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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 irradiated minced pork as well as in minced pork with a natural microflora. The inoculated meat was stored 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 S. Typhimurium DT104 as well as S. Derby 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. This interaction between Salmonella and the natural microflora should be included in risk assessment models regarding salmonellae in fresh pork. Besides Salmonella testing, 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 isolates 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.

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VITAL, Monitoring and Control for Virus Safe Pork

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VITAL is an ongoing (2008-2011) EU funded project on monitoring and control of foodborne 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.

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Improvements in processing hygiene indicator and microbial hazard levels in Australian finisher pigs from 1996 to 2010

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National baseline data on processing hygiene and hazard levels is needed to determine impacts of regulation in the meat industry and to inform national standard setting.

Finisher carcases at six major pig abattoirs in all mainland states were sampled in 2010 in proportion to their slaughter throughput, which represents approximately 60% of the national pig kill. Samples (n=294) were collected by swabbing 3×100 cm^2 per carcase using the E. coli and Salmonella Monitoring (ESAM) sponge technique. In total, 294 finisher carcases were sampled over two occasions six months apart and cultured for Total Viable Counts (TVC), E. coli and E. coli O157:H7. In addition, Salmonella ESAM data for the same abattoirs, collected around the sampling occasions, were also accessed. The results were compared to those from a 1996 baseline survey of Australian finisher pigs, which assessed hygiene indicators and microbial hazards using 3×20 cm^2 swab samples per carcase.

Despite the 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. 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 $\log_{10}$ cfu/cm^2 were observed. Similarly, average concentration of TVC reduced by 1.9 $\log_{10}$ cfu/cm^2 over the same period.

Salmonella levels remain low (0.4%; 95% CI: 0.0 – 2.1%) by international standards and no E. coli O157 was detected (<1.3%). Contrary to some international findings, these results indicate that E. coli O157:H7 contamination is not an issue with Australian pig carcases at this stage.

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.

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The cost-benefit of salmonella control in Swedish pigs

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Analysis of the costs and benefits of salmonella control pre-harvest in the pork production has been performed on EU level. 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, the cost of the control is shared by the tax payers and the producers. A thorough analysis of the cost-benefit of the program has been requested by various stakeholders. Pending this, a quick calculation based on previously published and unpublished data was made.
These data included the cost of: i) human illness due to salmonella from Swedish pigs, ii) eradication of salmonella from infected pig farms, iii) surveillance in pigs and pig products, iv) preventive measures in the feed sector.
The total yearly cost of salmonella control in Swedish pigs was estimated to 1.1-1.2 million Euro (average based on the past 6 years) and the current yearly cost of human salmonellosis due to pigs was estimated to 0.013 million Euro.
These costs were compared to expected costs without a salmonella control program under several "what if"-scenarios. For example, if the human sero-prevalence would become equal to that in Denmark and the proportion of cases due to pigs increased to 15% (EU-estimate), the cost of human salmonellosis due to pigs would increase to 2.8 million Euro. Under these assumptions, the saved costs exceed the cost of salmonella control in Swedish pigs. Other levels of sero-prevalence and estimates for the proportion of cases attributable to pigs were also investigated. Under exceptional circumstances, such as the large feed-borne outbreak in 2003, the costs may exceed the benefits.

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Identification of control strategies to manage microbiological risks in typical pork products

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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. 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.
Starting from 2009 a pilot project has been implemented by a local veterinary service of the Veneto region in collaboration with IZSVe with the aim of identifying control measures based on own-checks and official controls in order to manage microbiological risks.
Two different sampling schemes were designed and applied (A and B).The A sampling scheme was applied in all the farms both aimed at estimating the prevalence of selected foodborne 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 maturation) and at collecting information on the most influent parameters of fermentation and drying (pH, aw, environmental conditions). The more intensive sampling scheme B was applied in a selection of four farms 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.
According to the data obtained a control strategy was defined based on the identification of positive/negative batches by the competent authority and the monitoring of the weight decrease in sausages by the food business operator; a weight decrease of at least 25% has been correlated to an aw decrease equal or below 0.92, which is identified as hostile to foodborne pathogens growth, also according to the relevant 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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Pigs are commonly infected with Salmonella spp. at the slaughterhouse, and the consumption of pig meat is hypothesised to be a significant 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. In this presentation we describe how the baseline model was modified to describe 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. Here 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. Given the modelling of cross-contamination within the abattoir a non-linear relationship between MS slaughter pig prevalence and incidence of pigmeat-borne human salmonellosis was expected; however, the relationship was proportional. Anal bunging of the carcass during processing was shown to be the most effective intervention mechanism in reducing human illness; however, some intervention mechanisms were ineffective in reducing human illness, including cleaning and disinfection and logistic slaughter. Further investigation/validation of intervention results, including the proportional relationship between slaughter pig prevalence and human illness, will also be presented.

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A Transport & Lairage model for Salmonella transmission in pigs for individual EU Member States

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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.

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Modelling preparation and consumption of pork products

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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.

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A risk assessment for visual only meat inspection of both indoor and outdoor pigs within the UK

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This project has been commissioned by the UK Food Standards Agency in the context of its programme of work to modernise meat hygiene 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 visual-only inspection of pigs that have been reared under controlled housing conditions since weaning. However, the definition of 'controlled housing' excludes outdoor pig production, hence so far UK abattoirs have yet to introduce visual-only meat inspection because of the associated complications of having a large outdoor pig herd. We have therefore conducted a qualitative risk assessment to assess the 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 to public or animal health 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 than the traditional method. It was determined from the UK's national meat inspection database 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.

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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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The hygienic performance of three manure treatment systems (simple storage, biological treatment or thermal treatment) was evaluated for effluents 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.

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The Role of Proactive Risk Assessments in Ensuring Business Continuity in the Swine Industry during a FMD Outbreak

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Emerging 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. However, swine production systems, harvesting facilities and processing plants have limited capacity to hold live animals or to store pork products. Hence even a brief disruption in movement could result in devastating impacts to the industry as well as serious animal welfare concerns. Pro-actively evaluating the risks before an outbreak occurs enables timely movement permitting decisions to be made and supports continuity of business.

To prevent disruptions in business continuity, we developed a novel approach toward improving veterinary emergency-response preparedness. By partnering with industry and government stakeholders, we assess the routes of exposures and transmission pathways of infectious diseases prior to the occurrence of an outbreak. Using disease transmission models and exposure pathways analysis, 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. The process also determines areas of uncertainty (data gaps) that need to be addressed in order to more fully evaluate the risks during an outbreak. While FMD does not present a human food safety risk, this method could also be employed for specific foodborne pathogens.

Determine the room for improvement of processes within the management of crisis and their prevention – the maturity model

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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.

Impact of the thermal treatment of pig slurry on vegetative and spore forming bacteria

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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.

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Prevalence of MRSA CC398 in pig holdings

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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 Vet-Med-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.

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Trends in antimicrobial resistance of E. coli isolated from pigs at slaughter

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Based on the number of slaughtered pigs the representative abattoirs were chosen in 2009 and 2010 to sample, respectively, 181 and 189 rectal swabs. Single E. coli strain from MacConkey culture was used for biochemical confirmation followed by antimicrobial resistance testing with microbroth dilution method (Sensititre, TREK D.S.). MIC interpretation according to EUCAST epidemiological criteria showed non wild-type isolates to each of 13 antimicrobials tested. Cephalosporin resistance was sparse in both study periods whereas streptomycin and tetracycline resistance approached 40% of tested strains. In 2010, compared to previous year, the frequency of cephalosporin and phenicols resistance tend to increase (max. 2.6%) but in the case of other compounds it seemed to diminish with maximum decrease (5.8%) observed in ciprofloxacin. None of year-to-year changes were significant. The percentage of non-wild type strains decreased from 65.7% to 55.3% ($P \leq 0.05$) and 55 resistance profiles were observed in 2009 compared to 45 in the next year. The most resistant profiles comprised up to 11 antimicrobials from 7 antimicrobial classes. The shift in number of strains resistant to one up to seven 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. We conclude, that comparable and harmonized antimicrobial resistance monitoring introduced in pigs at slaughter revealed the most crucial resistances of indicator E. coli. Some variations were observed over two years, but they were not significant. The changes in the number of resistant E. coli strains and their profiles justify the need for continuous monitoring followed by the identification of genetic backgrounds in non-wild type MIC isolates.

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Detection of anti-salmonella antibodies in porcine serum using Luminex xMAP technology

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Introduction

Salmonella is a foodborne pathogen causing gastro-enteritis in humans. It is considered a major public health hazard with ~35000 cases in the Netherlands (2008), of which ~20% is pork-associated. Countries like Denmark and the Netherlands use serology for monitoring the Salmonella status of pig herds in order to reduce the number of infected herds and, eventually, human salmonellosis. For Salmonella serology the antigenic component lipopolysaccharide (LPS) is used. A multiplex approach was designed in which five LPS variants were coupled to individual bead sets, resulting in a comprehensive xMAP assay for anti-salmonella antibodies in porcine serum.

Materials & methods

Coupling of each LPS to the individual bead sets was confirmed with specific agglutination sera. The fiveplex assay was compared with a commercial ELISA by testing experimental sera and 175 field sera.

Results

The five LPS beads reacted with agglutination sera as expected. In a multiplex set-up each LPS bead reacted with experimental porcine sera in agreement with the serogroup of the strain used for immunization. With field sera, results of the xMAP fiveplex assay were in good agreement with that of the ELISA.

Discussion & conclusions

The results show that LPS can be coupled to xMAP beads and that the resulting assay performs comparable to a commercial ELISA. The flexibility of the platform allows future improvements by addition of other LPS variants or antigens of other relevant pathogens. Ultimately, this type of assay can be used for routine screening of porcine samples for the presence of multiple pathogens (e.g. Salmonella, SVDV, Aujeszky's disease virus, MAA, Toxoplasma, Trichinella) in one cost-reducing multiplex assay.

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Trichinella serology in swine on the Luminex platform

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Introduction

Trichinellosis is a parasitic zoonosis affecting at least 11 million people all over the world and is included in the EU white paper on food safety. New EU legislation concerning meat inspection for Trichinella spp. allows population monitoring of Trichinella-free herds using serological methods.
Using Luminex xMAP technology, a bead-based flow cytometric platform that allows identification of up to 100 analytes, an assay for the serological screening of porcine samples for anti-Trichinella antibodies was developed.

Materials & methods

E/S antigen, isolated from Trichinella spiralis larvae, was coupled to Luminex paramagnetic MagPlex beads using a standard EDC/NHS coupling. The performance of the Luminex assay was compared with that of two commercially available ELISA's using porcine serum samples.

Results

Luminex results with longitudinal serum samples from experimentally infected pigs were comparable with ELISA results. For a set of ~240 field sera an overall agreement of >90% was achieved.

Discussion & conclusions

The results of this preliminary validation show the capabilities of the Luminex xMAP platform for detecting antibodies against Trichinella in pig serum.
This study demonstrates the feasibility of the Luminex xMAP platform for veterinary diagnostics. In the near future, serological monitoring of multiple veterinary pathogens (e.g. Salmonella, SVDV, Aujeszky's disease virus, MAA, Toxoplasma, Trichinella) in one cost reducing multiplex assay will be possible on this platform.

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