

Relative human risk of *Salmonella* Enteritidis in table eggs

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Annual Report on Zoonoses in Denmark 2013



Annual Report on Zoonoses in Denmark 2013

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Introduction

In 2013, the total number of human *Salmonella* cases remained at a level similar to the two previous years with 1,136 reported cases. The number of *S. Enteritidis* cases increased considerably compared to previous years, primarily due to a large travel-related outbreak of *S. Enteritidis*, where 88 cases were known to have visited Turkey during or just prior to disease onset. The number of cases infected with *S. Typhimurium* or monophasic *Typhimurium*-like strains and other serotypes decreased and were at the lowest observed for decades.

Ten *Salmonella* outbreaks were reported in 2013 of which seven were domestic; two of these outbreaks were caused by *S. Typhimurium* in pork. 2013 was a year with more travel-related outbreaks than usual; three *Salmonella* outbreaks and two outbreaks with other causative agents taking place at tourist destinations were reported, compared to none in 2012 and two in 2011. The most severe outbreak in 2013 was a large Nordic outbreak of hepatitis A virus with 117 cases, including 72 Danish cases. The source of the outbreak was narrowed down to frozen strawberries most probably originating from Morocco or Egypt.

No human cases were attributed to domestic broiler meat in 2013 according to the *Salmonella* source account. This was the third year in a row. In total, 52% of all human cases was estimated to be travel related; 40% was sporadic cases and 12% was known to be part of travel-related outbreaks. This was similar to previous years. For the cases not related to travel, Danish pork (11%) was estimated to be the most important source and increased compared to 2012 (8%), although this was mainly due to the two outbreaks related to this source, as the proportion of sporadic cases attributed to this source was at a similar level in these two years. The cases attributed to beef decreased again after a considerable increase last year.

The number of human *Campylobacter* cases in 2013 (3,766 cases) remained at a level similar to 2012 (3,728 cases) and thereby continues to be the most commonly reported zoonotic pathogen in Denmark, contributing more than 50 % of all cases.

Whole genome sequencing

In recent years, new DNA-sequencing techniques have been developed and made available for easy and fairly cheap sequencing of bacterial genomes. In the near future, this will enable laboratories to implement whole genome sequencing (WGS) as a replacement or complement to existing typing methods. WGS has already proved very useful in the surveillance of human listeriosis, where Statens Serum Institute implemented the method in 2013. WGS has also been applied with success as a tool in the analysis of isolates from two human *Salmonella* outbreaks. During an *S. Bareilly* outbreak in 2013, WGS of human and broiler isolates of this relatively rare serovar made it possible to conclude that the two groups of isolates must have had a close common ancestor, but the infected broiler flock did not seem to be the direct source of the outbreak. Based on these first and promising uses of WGS it is concluded that WGS will be of great value in future cluster detection and outbreak investigation, and initiatives will be taken to make sure that sharing of WGS data between the laboratories at Statens Serum Institute and the National Food Institute also remains plausible in the future.

Salmonella in table eggs

The last two decades of intensive *Salmonella* control and eradication programmes in the Danish table egg production have been highly successful, and in the last ten years only few flocks have been found *Salmonella* positive.

In order to evaluate the effect of an abolishment of the warning against the use of raw eggs in dishes eaten without heat treatment, the Danish Veterinary and Food Administration asked The National Food Institute to estimate the relative risk of salmonellosis resulting from increased use of raw eggs by consumers. A model was developed and used for estimating the risks for a number of different scenarios, where different factors such as interval between routine testing, prevalence in countries from which eggs are imported and proportion of Danish laying hen flocks infected with *S. Enteritidis* varied. Based on all

The Annual Report on Zoonoses presents a summary of the trends and sources of zoonotic infections in humans and animals, as well as the occurrence of zoonotic agents in food and feeding stuffs in Denmark in 2013. Greenland and the Faroe Islands are not represented. The report is based on data collected according to the Zoonoses Directive 2003/99/EC, supplemented by data obtained from national surveillance and control programmes as well as data from relevant research projects. Corrections to the data may occur after publication resulting in minor changes in the presentation of historical data in the following years report. The report is also available at www.food.dtu.dk.

the known factors and results, the Danish Veterinary and Food Administration abolished the recommendation to consumers on using pasteurised eggs in dishes that are not heat treated. However as eggs are biological products it is not possible to give a 100% guarantee that all eggs are free from *Salmonella*. The recommendation to use pasteurised eggs, if the dishes are meant for vulnerable consumers like elderly, immunosuppressed or children was maintained.

Salmonella Dublin in cattle

In 2002, a national surveillance programme for *Salmonella* Dublin in cattle was initiated in Denmark. The main purposes were to monitor the national and regional prevalences in the cattle population over time and to provide farmers with a tool to protect their herds against introduction of *S. Dublin*. All Danish cattle herds are classified into three levels of infection based on repeated antibody measurements in bulk tank milk, individual blood sampling, bacteriological cultivation of herds with clinical outbreaks of salmonellosis and cattle trade patterns. The programme rapidly had an effect on trade behaviour and the trend in the proportion of herds not assumed to be free from *S. Dublin* decreased. After a period of stagnation, a national eradication plan was initiated in 2007. The third and last step of this plan was set in place in 2013. This step include a regionalisation of Denmark into high prevalence and low prevalence regions, with movement of animals from high to low prevalence regions generally prohibited, and compulsory eradication of *S. Dublin* in infected herds.

Salmonella Choleraesuis and Salmonella Typhimurium DT41

In late 2012 *Salmonella* Choleraesuis, a serovar linked to the pig reservoir with the potential to cause severe clinical disease in pigs and humans, was observed in a Danish pig herd for the first time since 1999, followed by two additional findings in 2013. However, it has not been possible to identify the primary introduction of this pathogen.

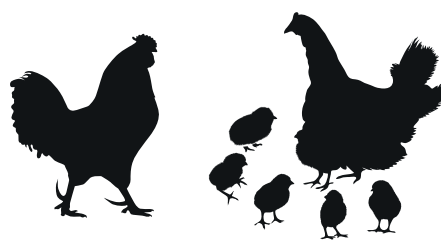
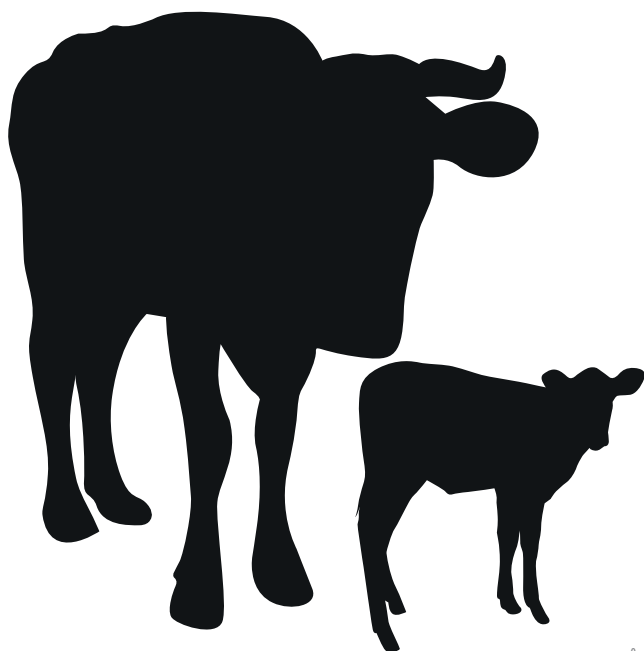
After a very low incidence of *Salmonella* in the Danish broiler and table egg production in the beginning of 2013, an unusually high number of flocks with *S. Typhimurium* DT41 was observed in the last quarter of 2013. This particular type was isolated from both hatcheries, production flocks, at slaughterhouse and from feed. After additional subtyping of the isolates coupled with epidemiological investigations, the source of the sudden increase of *S. Typhimurium* DT41 in the poultry production remained unclear, although it could be concluded that a combination of spread within the production pyramid and new introductions had played a role. No human cases infected with *S. Choleraesuis* or the relevant subtypes of *S. Typhimurium* DT41 were reported in 2013.

Risk assessment and predictive microbiology tools available online

Two user-friendly tools for risk assessment and predictive microbiology developed at the National Food Institute are freely available on the internet; TriMiCri and Food Spoilage and Safety Predictor (FSSP) software.

TriMiCri is a tool, which aims at facilitating the establishment and application of risk based microbiological criteria on *Campylobacter* in broiler meat. TriMiCri can e.g be used to apply the Danish case-by-case risk assessment approach for the users own situation or to evaluate the expected effect of setting specific microbiological criteria in terms of effect on consumer health risk and the percentage of (potentially costly) non-complying food lots.

The Food Spoilage and Safety Predictor (FSSP) software contains various models to predict the effect of product characteristics and storage conditions on shelf-life and safety of food. The software can among many other things be used to document if *Listeria monocytogenes* is able or unable to grow in a product.



1. Trends and sources in human salmonellosis

By Leonardo de Knecht (ledkn@food.dtu.dk) and Tine Hald

In 2013, *Salmonella* was the second most common cause of bacterial foodborne infections in Denmark with 1,136 cases reported. During the last three decades, broilers, pigs and laying hens have been ascribed the role of main reservoirs for this pathogen in different time periods, thus demanding different control strategies in the food chain [1]. In order to identify the main food-animal sources of human salmonellosis, Denmark has since 1995 relied on the routine application of a source attribution model. The source attribution model compares the number of human cases caused by different *Salmonella* subtypes with the distribution of the same subtypes isolated from the various food-animal sources.

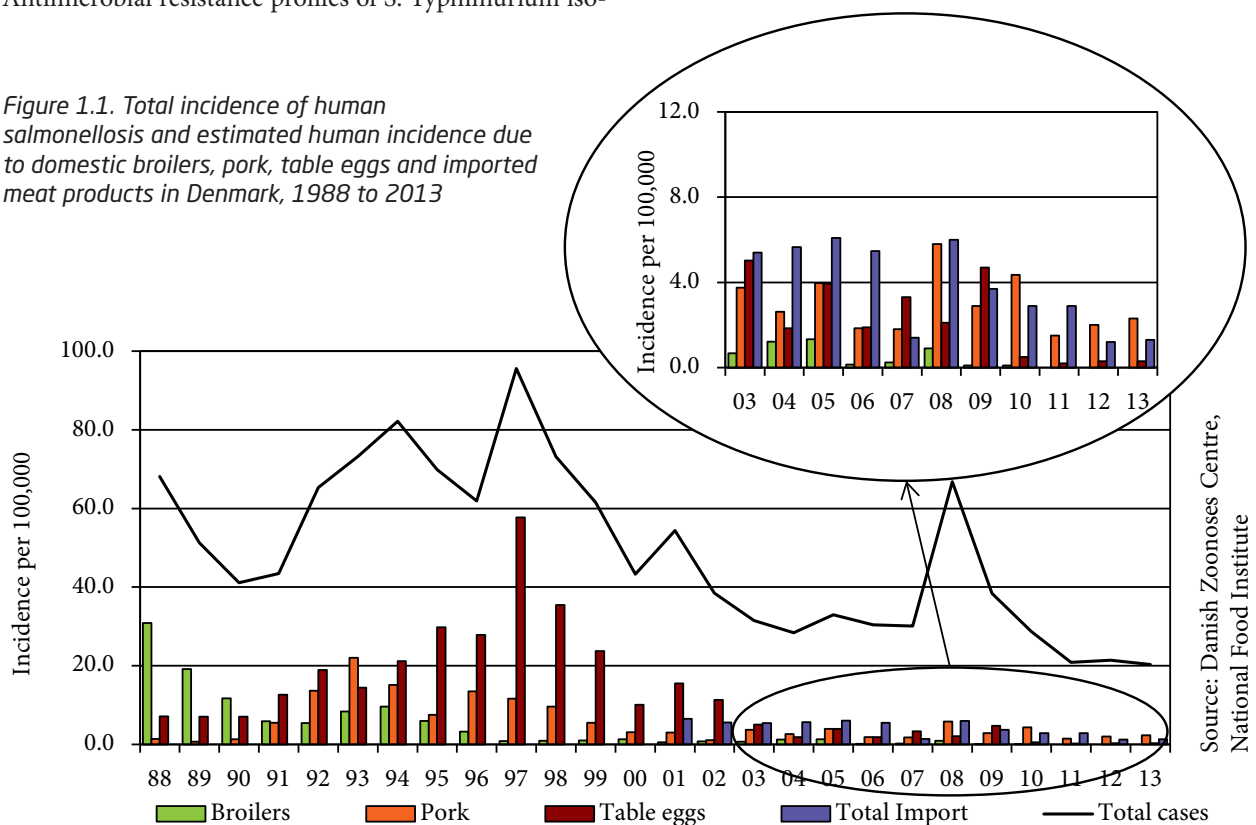
New MLVA-based *Salmonella* source account

Until 2012, the subtyping methods used to characterize human cases and animal samples were serotyping, followed by phage typing of *S. Enteritidis* and *S. Typhimurium*. Antimicrobial resistance profiles of *S. Typhimurium* iso-

lates were also included to further distinguish between similar phage types. Based on an opinion published in 2010 by the European Food Safety Authority [2], strains with just one phase of the H-antigen (e.g. S.1,4,[5],12:i:-) are considered “Monophasic *S. Typhimurium*” in the source account.

Phage typing of *Salmonella* has been applied for surveillance, source attribution and outbreak investigations in Denmark since the mid-nineties. It is a phenotypical method that depends very much on the experience of the laboratory doing the typing and on the maintenance of a reference phage collection [3]. Frequently, phage typing has been supplemented with the use of molecular methods, such as pulsed-field gel electrophoresis (PFGE) or multiple locus variable number tandem repeat analysis (MLVA), to improve the discrimination of *Salmonella*

Figure 1.1. Total incidence of human salmonellosis and estimated human incidence due to domestic broilers, pork, table eggs and imported meat products in Denmark, 1988 to 2013



Note: In 2013, imported sources may be underestimated, as imported ducks were not included for lack of data, and imported beef corresponds to an estimation based only on *S. Dublin* from previous years (2009-2012).
Source: Danish Zoonosis Centre, National Food Institute

subtyping for detection of outbreaks and identification of outbreak sources [3-6], and efforts are currently being made to establish MLVA as a tool for *Salmonella* surveillance in several countries [5, 7]. Starting with the 2013 data, MLVA substituted phage typing as the subtyping method for isolates of *S. Enteritidis* and *S. Typhimurium* from animal or food samples originating from surveillance or monitoring programs, as well as from clinical human cases in Denmark. Serotyping, as well antimicrobial resistance profiling for *S. Typhimurium*, are still performed.

The MLVA profiles for *Salmonella* are defined by the number of repetitions observed in five independent loci, namely SE9, SE5, SE2, SE1 and SE3 for *S. Enteritidis*, and STTR3, STTR6, STTR10, STTR5 and STTR9 for *S. Typhimurium*. Compared to phenotypical methods, MLVA provides a more detailed profiling of isolates. However, the source attribution model used in Denmark depends on a perfect match between the *Salmonella* subtypes from human cases and animal reservoirs, and therefore a full MLVA profile may be too discriminatory for attribution purposes. Therefore simplified MLVA profiles for *Salmonella* isolates from 2009 and 2010 were tested in different model adaptations. The models with the best fit for each profile were chosen, and the results compared with the results from the phage type-based model from the same years. The selected profile for *S. Typhimurium* is composed by loci STTR3, STTR10 and STTR9, while for *S. Enteritidis* a profile with all five loci was selected.

Since 2008, data from multiple years have been used in the model to improve the robustness and accuracy of the results, but a single year's results are based on that year's data only. As MLVA is a newly-introduced method in the

Danish surveillance system, data from previous years were not available, and therefore the 2013 model included only one year of data.

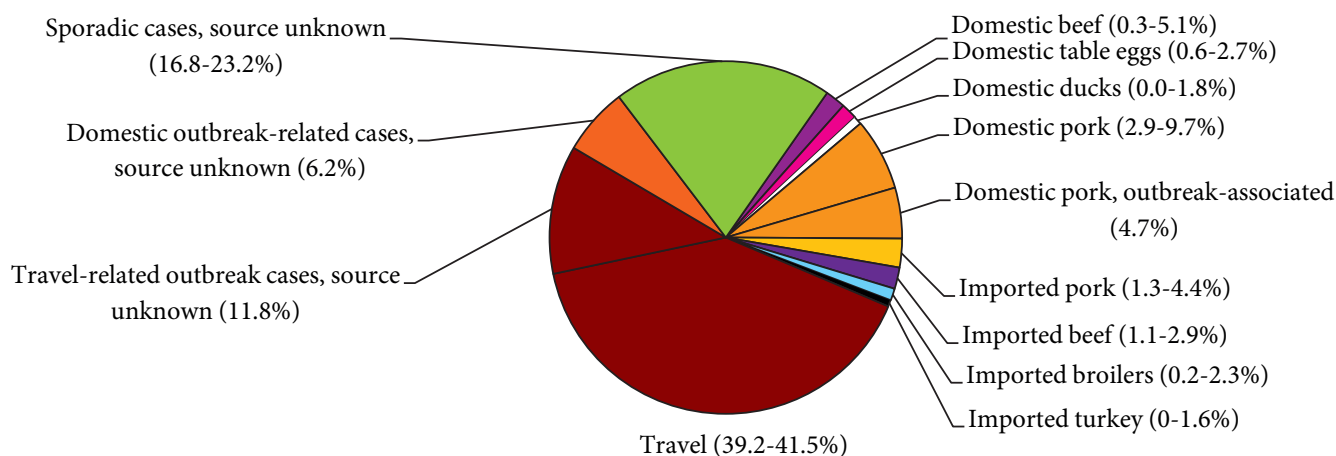
Secondly, the change in typing method and differences in profile matching means that some shifts in the attributed shares are to be expected, when compared to results from previous years. Thirdly, the amount of data available for feeding the model changed from 2012 to 2013. In 2013 no data were available from imported beef or imported ducks. The number of cases attributed to imported beef was calculated using the proportion of cases of *S. Dublin* from imported beef in 2009 to 2012 to estimate the 2013 *S. Dublin* cases from this source. This means that the overall contribution of cases from imported food is likely to be underestimated, given the absence of data.

Salmonella source account 2013 - results

The incidence of human salmonellosis in 2013 was 20.3 cases per 100,000 inhabitants, including an increase of the incidence of *S. Enteritidis* (6.2), when compared to an incidence of 4.3 reported in 2012. The incidence of *S. Typhimurium*, on the other hand, decreased from 7.3 to 6.0, with a decrease in both typical and monophasic strains (3.7 and 2.3 from 4.0 and 3.4 respectively) (Appendix Table A2). The overall trend in human salmonellosis cases attributable to the major food-animal sources is presented in Figure 1.1.

The most important food source of salmonellosis in Denmark in 2013 was estimated to be domestic pork (Figure 1.2 and Appendix Table A1), to which 11.3% of all *Salmonella* cases were attributed. This represents an increase when compared with the previous year, where 8.0% of the cases were attributed to domestic pork. Ex-

Figure 1.2. Estimated sources of 1,136 cases of human salmonellosis in Denmark, 2013 (See also Appendix Table A1)



Note: Sporadic and outbreak-related cases with unknown source include all sources not in the model, e.g. vegetables and fruit. 121 cases of the travel-related outbreaks were from a travel-related outbreak due to eggs / egg products
Source: Danish Zoonosis Centre, National Food Institute

cluding the outbreak related cases, around 7.0% of the cases both years were sporadic cases related to domestic pork. In 2012, a large outbreak involving domestic beef was responsible for around 3.0% of human cases, with an extra 4.1% of sporadic cases. No outbreaks from domestic beef were recorded in 2013, and the estimated percentage of sporadic cases attributed to this source decreased to 1.8%, while the proportion of cases attributed to imported beef increased from 0.9% in 2012 to 1.9%. Domestic table eggs were estimated to be responsible for 1.5% of human cases in 2013, not changing from 2012. A larger share of cases was attributed to imported pork, with an increase from 0.2% to 2.6%, due to the contribution of two main factors: 1) there was an increase in the observed prevalence of attributable *Salmonella* subtypes in this source, when compared to last year; 2) imported beef, imported pork and imported duck historically share some common subtypes, mainly *S. Anatum* and *S. Saintpaul*. However, because data on imported beef and imported duck was missing in 2013, there were fewer sources “sharing” cases with imported pork to compare among, which may also explain part of the increase in the contribution from imported pork. Imported chicken and imported turkeys were estimated to be responsible for 1.1% and 0.6% of cases, respectively, which is a decrease compared to the 1.8% and 1.1% observed in 2012. This decrease may be explained by few subtypes observed in those sources, and few domestic human cases caused by those subtypes. Since 2007, approximately 45-50% of the human cases have been acquired abroad. In 2013, 592 (52%) of all human cases was travel related;

40% was estimated to be sporadic cases and 12% was part of travel-related out-breaks.

Around 20% of reported sporadic *Salmonella* cases could not be associated with any of the included food sources. These cases may be caused by imported or domestically produced fruits and vegetables (survey data presented in Appendix Table A21), food-animals not included in the national surveillance or by non-food sources of infection, such as direct contact with pet animals. The fraction observed in 2013 was similar to the proportion of cases not attributable to sources included in the model from the last five years (around 15-20%).

Of the 346 reported *S. Enteritidis* cases, 207 (59.8%) reported a history of international travel and 84 (24.3%) were considered possible travellers. Of those, 134 (38.7% of all *S. Enteritidis* cases) were reported as part of outbreaks happening abroad. Since 2010, there have been no *S. Enteritidis* outbreaks known to be related to Danish products.

A total of 337 *S. Typhimurium* cases were reported in 2013 (including the 127 cases of monophasic *S.1,4,[5],12:i:-*). Of the *S. Typhimurium* cases, 26.5% were estimated to be related to international travel and 24.9% were associated with five outbreaks. Among the *S. Typhimurium* outbreaks, one was a national outbreak with 43 cases (MLVA pattern 0550) and one a local outbreak with 21 cases involving a retail outlet (MLVA pattern 0007); both had domestic pork as their source. The remaining three outbreaks did not have a specific animal reservoir identified as the source (Appendix Table A4).

Where do we acquire *Salmonella* infections?

By Luise Müller (lum@ssi.dk)

In 2013, as in the previous years, Statens Serum Institut attempted to interview all registered *Salmonella* cases where no travel information was reported by the general practitioner. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease onset. This information was complemented with information from general practitioners' reports. Travel information was obtained from a total of 67.8% of the *Salmonella* cases in 2013. Among the cases with known travel history, 52.1% were infected abroad (Table 1.1). However, the proportion of travel-related cases varied greatly between the different serotypes hence 79.0% of the *S. Enteritidis* cases, 17.5% of the *S. Typhimurium* cases, 32.5% of the monophasic *S. 1,4,[5],12:i:-* cases and 52% of cases with other serotypes were infected abroad. The distribution pattern of travel-related and domestically acquired *Salmonella* infections (not including outbreak related cases) was comparable to previous years (Figure 1.4). In 2013, Turkey was the country where most of the travel-related cases were infected (31%) and this was mainly due to a large outbreak of *S. Enteritidis* on the south-east coast (see chapter 2). Apart from that most travel-related cases were infected in Thailand (13%), Egypt (8%), and Spain (6%).

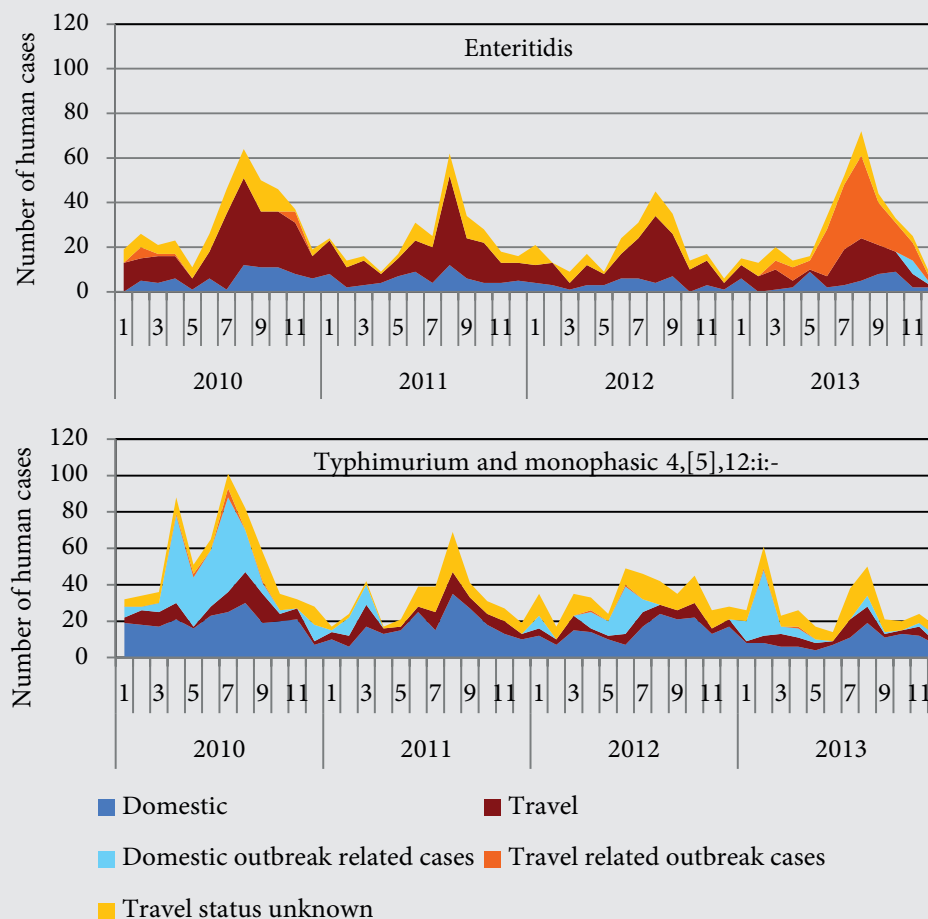
Table 1.1. Top 10 *Salmonella* serotypes in humans and place of infection, 2012-2013

2013	Number of patients (%)	% patients infected ^a		2012	Number of patients (%)	% patients infected ^a	
		Abroad	Domestically			Abroad	Domestically
Enteritidis	346 (30.5)	79.0	21.0	Enteritidis	241 (20.1)	77.0	23.0
Typhimurium	210 (18.5)	17.5	82.5	Typhimurium	223 (18.6)	20.1	79.9
Typhimurium (monophasic) ^b	127 (11.2)	32.5	67.5	Typhimurium (monophasic) ^b	192 (16.0)	21.7	78.3
Dublin	27 (2.4)	0	100	Dublin	50 (4.2)	0	100
Newport	27 (2.4)	62.5	37.5	Poona	28 (2.3)	26.7	73.3
Stanley	23 (2.0)	84.6	15.4	Stanley	28 (2.3)	68.4	31.6
Agona	22 (1.9)	25.0	75.0	Infantis	25 (2.1)	36.4	63.6
Infantis	22 (1.9)	42.9	57.1	Newport	24 (2.0)	62.5	37.5
Virchow	17 (1.5)	75.0	25.0	Virchow	21 (1.8)	81.3	18.8
Corvallis	13 (1.1)	87.5	12.5	Saintpaul	17 (1.4)	25.0	75.0
Other serotypes	302 (26.6)	51.1	48.9	Other serotypes	349 (29.1)	54.3	45.7
Total	1,136 (100)	52.1	47.9	Total	1,198 (100)	45.2	54.8

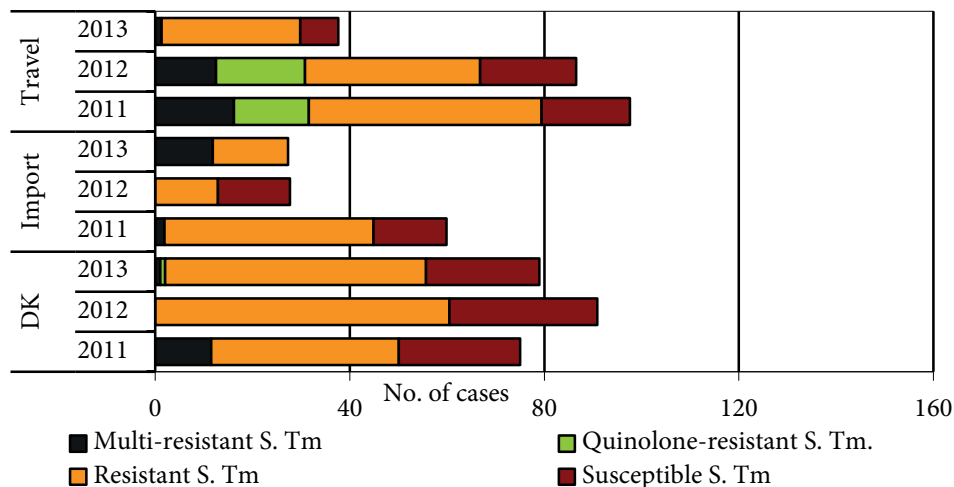
a) Patients with unknown travel information (32.2% of all patients in 2013 and 32.1% of all patients in 2012) were excluded from the percent calculations.

b) Typhimurium (monophasic) includes the *Salmonella* strains 1,4,[5],12:i:-.

Source: Statens Serum Institut

Figure 1.3. Monthly distribution of *S. Enteritidis*, *S. Typhimurium*, and monophasic *S. 1,4,[5],12:i:-* cases, 2010-2013

Source: Statens Serum Institut

Figure 1.4. Estimated sources of antimicrobial resistant^a *S. Typhimurium* infections in humans, 2011-2013

a) Resistant: Resistant towards one to three antimicrobial agents; Multi-resistant: Resistant towards four or more antimicrobial agents. Source: Danish Zoonosis Centre, National Food Institute

From the 78 *S. Typhimurium* cases with information on antimicrobial resistance, which were attributed to domestic products in 2013, 30.9% were caused by strains susceptible to all tested antimicrobial agents, 66.6% by strains resistant to one to three antimicrobial agents, 1.3% by strains resistant to four or more antimicrobial agents (multi-resistant) and 1.2% by strains resistant to quinolones (Figure 1.3). This is the first time since 2010 that quinolone-resistant strains are observed in human cases attributed to domestic food-animal reservoirs, and reflects the return of multi-resistant strains in domestic pork, where no multi-resistant strains were observed in 2012. Multi-resistant strains were isolated from 44.4% of *S. Typhimurium* cases with information on antimicrobial resistance which have been attributed to imported products. This is a change from last year, when this profile was not observed. Cases attributed to imported products with resistant *Salmonella* strains increased from 46.6% to 55.6%, and no fully susceptible strains were observed in 2013 in those sources. From the 38 *S. Typhimurium* cases acquired abroad for which resistance information was available, 79.3% were caused by resistant types, 17.2% by types susceptible to all tested antimicrobial agents and 3.5% by multi-resistant types.

References

1. Wegener HC (2010). Danish initiatives to improve the safety of meat products. *Meat Science* 84:276-283.
2. Panel on Biological Hazards (BIOHAZ) (2010). Scientific Opinion on monitoring and assessment of the public health risk of “*Salmonella* Typhimurium-like” strains. *EFSA Journal* 8(10):1826.

3. Baggesen DL, Sørensen G, Nielsen EM, Wegener HC (2010). Phage typing of *Salmonella* Typhimurium – is it still a useful tool for surveillance and outbreak investigation? *Eurosurveillance* 15(4).

4. Torpdahl M, Sørensen G, Lindstedt BA, Nielsen EM (2007). Tandem repeat analysis for surveillance of human *Salmonella* Typhimurium infections. *Emerg. Infect Dis* 13: 388–395.

5. Petersen RF, Litrup E, Larsson JT, Torpdahl M, Sørensen G, Müller L, Nielsen EM (2013). Molecular characterization of *Salmonella* Typhimurium highly successful outbreak strains. *Foodborne Pathogens and Disease* 2013; 8(6):655-661.

6. Wuyts V, Mattheus W, De Laminne de Bex G, Wilde-mauwe C, Roosens NHC, et al. (2013). MLVA as a tool for public health surveillance of human *Salmonella* Typhimurium: prospective study in Belgium and evaluation of MLVA loci stability. *PLoS ONE* 8(12): e84055. doi:10.1371/journal.pone.0084055

7. Lindstedt BA, Torpdahl M, Vergnaud G, Le Hello S, Weill FX, Tietze E, Malorny B, Prendergast DM, Ní Ghallchóir E, Lista RF, Schouls LM, Söderlund R, Börjesson S, Åkerström S (2013). Use of multilocus variable-number tandem repeat analysis (MLVA) in eight European countries, 2012. *Euro Surveillance* 18(4).

Molecular serotyping of *Salmonella* isolates

By **Jeppe Boel** (jboe@food.dtu.dk) and **Charlotta Löfström**

Serotyping is the standard method for subtyping *Salmonella* and information about serotypes is pivotal for understanding the epidemiology of *Salmonella*. Serotyping is done by slide agglutination using specific antisera that detects differences on cell surface structures: Somatic (O) lipopolysaccharides and flagellar antigens (H). Some *Salmonella* strains, particularly from non-human sources, autoagglutinate (exhibit a rough phenotype) when typed by traditional slide agglutination. This feature is associated with the loss of the outer membrane, which changes the hydrophobicity of the bacteria. Therefore serotyping by agglutination cannot be performed on such isolates and this causes data gaps, when subtyping data is needed, e.g. for *Salmonella* source attribution. To overcome this obstacle, serotypes can instead be determined on DNA level, i.e. by determining the presence of genes encoding the O and H antigens using molecular serotyping.

During 2012 a molecular DNA based serotyping method was implemented at the National Food Institute, Technical University of Denmark, and during 2013 this method was used as a supplement to the traditional serotyping based on agglutination. The DNA based molecular method detects genes mediating the corresponding O and H antigens and thus it is based on the White-Kauffmann-Le Minor scheme. The assay is a multiplex bead-based suspension array based on the Luminex xTAG technology and detects the major O groups and H antigens [1, 2]. It can directly identify the most important serovars of *Salmonella*, but in some cases there is a need to supplement the DNA based data with additional slide agglutination tests. DNA based molecular serotyping has proved to be a valuable tool in the routine typing of *Salmonella*, and further the method enables serotyping of rough isolates.

The possibility to serotype rough isolates was utilized in a study of rough *Salmonella* isolates from pig carcasses obtained in the national surveillance program for fresh meat. A total of 211 rough strains isolated during the period 2005-2012 were analyzed using molecular serotyping. The typing enabled serovar identification of 168 of the strains (80%). The identified serotypes were Typhimurium (n=92; 44% of the 211 strains), Derby (n=40; 19%), 4,[5],12:i:- (n=22; 10%), Infantis (n=8; 4%) and others (n=6; 3%). Serotyping results for strains isolated during the same years from pig carcasses, where traditional serotyping was possible (smooth strains) (n=1,233) showed a similar serovar pattern, where Typhimurium (n=547; 44%), Derby (n=399; 32%) and 4,[5],12:i:- (n=72; 6%) also were the most commonly identified serotypes. Studies are in progress to serotype the remaining 43 rough strains that could not be typed in the first approach, using other DNA based techniques to further assess the difference in results between molecular and traditional serotyping. In conclusion, our preliminary data suggests that molecular serotyping of rough *Salmonella* strains mirror the serotypes obtained using traditional serotyping for smooth strains.

References

1. Fitzgerald, C, Collins, M, van Duynne, S, Mikoleit, M, Brown, T & Fields, P (2007). Multiplex, bead-based suspension array for molecular determination of common *Salmonella* serogroups. *Journal of Clinical Microbiology* 45 (10): 3323-3334
2. McQuiston, JR, Waters, RJ, Dinsmore, BA, Mikoleit, ML & Fields, PI (2011). Molecular determination of H antigens of *Salmonella* by use of a microsphere-based liquid array. *Journal of Clinical Microbiology* 49 (2): 565-573.



2. Food- and waterborne outbreaks

By the Central outbreak management group

Food- and waterborne outbreaks in Denmark are reported in the Food- and waterborne Outbreak Database (FUD). Outbreaks that occurred in 2013 are presented in Appendix Table A4. Figure 2.1 shows the relative distribution of these outbreaks by the different causative agents. Household outbreaks and clusters that could not be verified as common source outbreaks are not included. The outbreak investigation procedures in Denmark are described in further detail in Chapter 10.

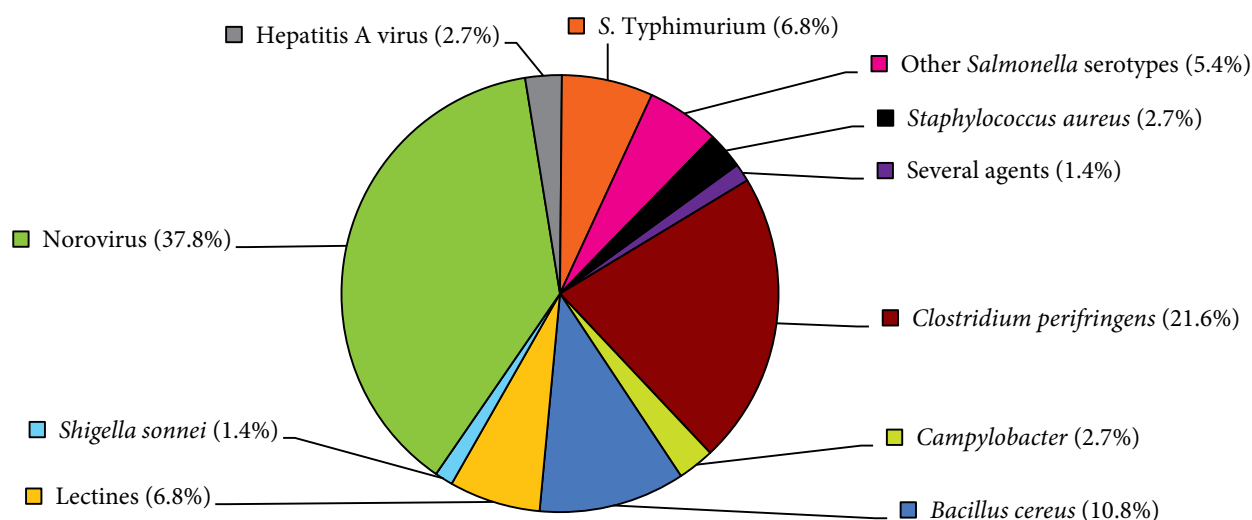
In total, 74 foodborne outbreaks were reported to FUD in 2013 (Appendix Table A4) which is a decrease from 2012, where 82 food- and waterborne outbreaks were reported. There were no notifications of waterborne outbreaks in 2013. The outbreaks were mainly regional outbreaks (93.2%) and only five outbreaks were national outbreaks with patients distributed over several regions. The largest of these outbreaks was caused by hepatitis A virus. Compared to previous years an increase in travel related outbreaks was observed (five reported in 2013, none in 2012 and two outbreaks in 2011).

In total, the number of persons affected by foodborne outbreaks was 2,066 persons with a median of 12 persons

per outbreak (range 2 – 425). The largest outbreak involving 425 persons was an outbreak caused by *Clostridium perfringens*. All affected persons had dined at the same event, and the outbreak investigation conducted by the Food Control Office¹ in Northern Jutland, pointed out patty shells with sauce containing hen meat and asparagus as the source of infection (FUD1325). As in previous years norovirus (NoV) was the most frequent cause of foodborne outbreaks (28 outbreaks) and in total, 655 persons were affected by NoV outbreaks. The transmissions routes of NoV causing foodborne outbreaks are multiple. In Table 2.1 a breakdown of the number of outbreaks and the persons affected per route of infection for 2013 is shown. From the data it is evident that the most common ways of infection was contamination from either an ill dining guest attending a buffet and thereby contaminating the buffet (cutlery or food items) or a member of the kitchen staff contaminating the food. Less often the infection originated from contaminated ready-to-eat products like oysters and frozen berries.

In 2013 *Clostridium perfringens* was more frequently associated with foodborne outbreaks than in previous years. In total, 16 outbreaks of *C. perfringens* affecting a

Figure 2.1. Aetiology of the 74 foodborne disease outbreaks reported with a causative agent in the Food- and waterborne Outbreak Database (FUD), 2013 . Percentage of total outbreaks indicated in brackets



Source: Food- and waterborne Outbreak Database (FUD)

1. The Danish Veterinary and Food Administration (DVFA) is one authority but operates from more locations throughout the country. To be able to distinguish the locations the terms DVFA is used synonymous with the location in Glostrup and Food Control Office (FCO) synonymous with the location in question

total of 753 persons were reported in 2013, compared to eight and seven outbreaks caused by this agent in 2012 and 2011, respectively.

When dividing the outbreaks into reported settings, "Restaurants, cafés and bars" was the most frequent setting (45.9%) with 34 reported outbreaks affecting 984 persons (mean: 29 persons per outbreak). Outbreaks taking place in workplace canteens (10 outbreaks) also affected a high number of persons (327 persons) and on average had slightly more persons per outbreak than "Restaurant, café and bar outbreaks" (mean: 33 persons per outbreak). Composite meals (25) and buffet meals (25) were the most frequently reported source of outbreaks in 2013 and most often these outbreaks were associated with NoV and *C. perfringens* (Appendix Table A4).

Outbreaks with *Salmonella*

In 2013, ten *Salmonella* outbreaks were reported in FUD, where three of these outbreaks were related to persons travelling abroad. Five domestically acquired outbreaks of *S. Typhimurium*, one outbreak of *S. Enteritidis* and one outbreak of *S. Mikawasima* were reported. Two of the national outbreaks of *S. Typhimurium* were suspected to have been caused by Danish produced pork. The first one was an outbreak of *S. Typhimurium* MLVA type 0007 with a resistance pattern rarely seen for this MLVA type; Ampicillin, Streptomycin and Sulfamethoxazole (ASSu) (FUD1245) and was a continuation of an outbreak with 12 patients already reported in 2012. Further investigation was initiated and the patients were interviewed. In total, 22 patients were identified with date of onset of illness from November 2012 to April 2013. The patients were 11 females and 11 males aged 1-82 years (median 62 years). The majority of patients lived in the eastern part of Denmark, primarily on the island of Falster. This outbreak was severe as several patients were hospitalised and two patients – aged 71 and 79 years - died within three weeks after the infection. However, it was not possible to establish whether the *Salmonella* infection was the cause of death. Results of interviews with the patients indicated that the source of the outbreak was pork meat

from a specific butcher shop. This was supported by the trace-back investigation of the meat. No food items were available for testing, but several of the possibly implicated swine herds were reported to have had *Salmonella* seropositive responses at the time of the outbreak, although none were known to be positive with the epistrain; MLVA type 0007. Furthermore, MLVA type 0007 was not identified by MLVA-typing of selected *S. Typhimurium* ASSu isolates from pig slaughterhouses obtained in the same period.

The second outbreak was caused by *S. Typhimurium* U312 MLVA type 0550 fully sensitive to antibiotics (FUD1254). A total of 43 patients were reported from January to May 2013. The patients were 24 females and 19 males aged 0-94 years (median age 51 years). The patients were from all over Denmark. Interviews pointed at pork meat as the possible source. Fully sensitive *S. Typhimurium* isolates from the *Salmonella* surveillance and control of food and animals in Denmark were selected for MLVA typing. Thirty-two isolates mainly from October 2012 to January 2013 and mainly from pig slaughterhouses or pork samples were typed by MLVA. The outbreak MLVA type was detected in two isolates from pork isolated during January 2013. The isolates were from a medium-sized pig slaughterhouse and from a cutting plant receiving meat among others from the above-mentioned slaughterhouse, however it was not possible to explain all patients from this source and no final conclusions could be made of the origin of the meat, although a strong indication towards the source being a pork product was obtained.

Nordic hepatitis A outbreak

In February 2013, Statens Serum Institut saw an increase in the number of domestically acquired hepatitis A cases (FUD1259) [1-4]. The cases were geographically distributed all over the country and did not belong to any of the known risk groups (drug users, men having sex with men, homeless people and travellers to endemic countries). Subtyping of the hepatitis A virus (HAV) showed that the majority of cases were genotype 1B with two closely related sequences indicating a common foodborne source. After extensive

Table 2.1. Norovirus outbreaks in 2013 per route of transmission based on number of cases or number of outbreaks

Transmission route/source	No. of outbreaks	No. of persons ill
Ill kitchen staff or healthy carrier of virus among kitchen staff	9	226
Kitchen staff tending to ill persons at home before entering the kitchen	5	129
Ill person/guest attending a buffet	10	261
Seafood (oysters)	3	27
Frozen raspberries	1	12
Norovirus in total	28	655

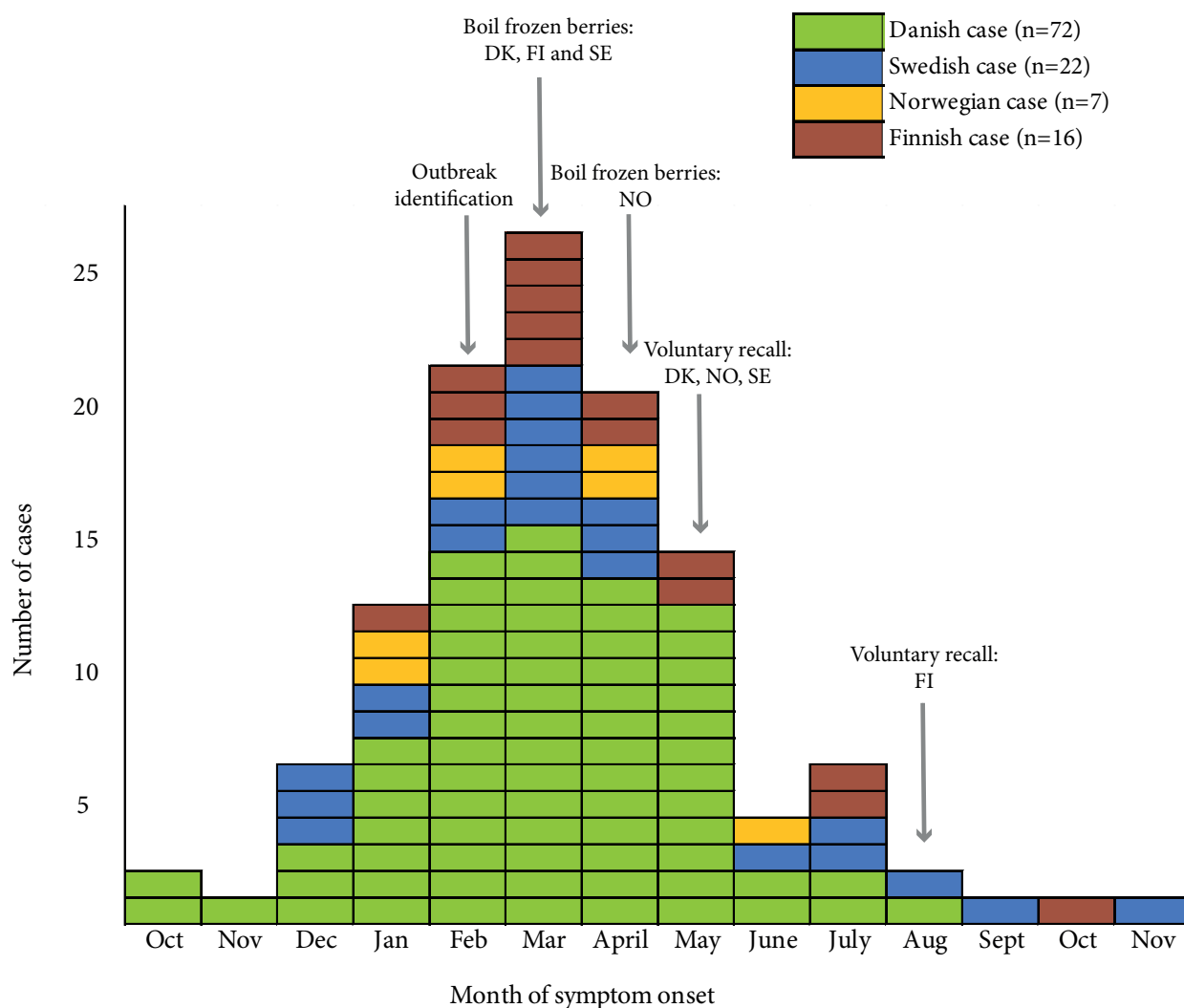
Source: Food- and waterborne Outbreak Database (FUD)

interviews with the initial cases, a case-control study was performed pointing out frozen berries and strawberries in particular – used without heat treatment in e.g. desserts and smoothies - as the most likely source of the outbreak. The Danish Veterinary and Food Administration then issued recommendations to heat treat all frozen berries before consumption. An international inquiry revealed that cases had also been reported in Norway, Sweden and Finland. Case-control studies were performed in these countries and a joint analysis of the results pointed out frozen strawberries to be the source of infection. In total 117 cases were identified with date of onset from October to November 2013 (Figure 2.2). The majority of cases was from Denmark where 72 cases were registered from October 2012 to August 2013. Of these 43 (60%) were females

and 29 (40%) were males. Cases were 2-81 years old with the median age of 22 years. Seven cases were considered to be secondary cases. Norway reported 7 cases, Sweden 22 cases and Finland 16 cases.

An extensive food source trace-back investigation involving all four countries was performed. The investigation was performed using purchase data collected from patients (receipts) and where possible, information on purchase of berries was obtained by using the supermarkets data on exact purchase by patient credit cards. Information from the packages of berries and fruit sampled for virological testing was also included in the investigation. This information pointed at frozen strawberries provided by a specific packaging establishment in Belgium. Presented with this information, the supermarket chain retailing the product in

Figure 2.2. Hepatitis A cases by month of symptom onset^a and country, n=117



a. When date of symptom onset is unknown, date of diagnosis has been used or thirdly date of testing or fourthly date of hospitalisation or date of notification
Source: Statens Serum Institut

Denmark made a voluntary recall of this product. Further trace-back investigation performed by the Danish Veterinary and Food Administration in cooperation with the supermarket chain and the Belgian establishment narrowed the possible vehicles down to strawberries most probably originating from either Morocco or Egypt, and from the harvest year 2012. In all, 57 samples of frozen berries and frozen mango fruit were tested (20 from Denmark, 14 from Finland, 11 from Sweden, 12 from Norway). The samples were collected in the patients' homes and from retail shops identified during the investigation. The National Food Institute, Technical University of Denmark investigated the Danish and Finnish samples. Unfortunately, it was not possible to detect hepatitis A virus in any of the tested samples. There might be several explanations for that, including uneven distribution of virus in berries, limitations in method sensitivity, or simply because the bags tested did not contain the virus. In conclusion, this outbreak was a very large and long-lasting challenge involving both interdisciplinary collaboration between public health institutes, laboratories and food authorities, and also a good example of international collaboration and collaboration with the establishments involved in the trade of frozen berries.

Outbreaks at tourist destinations

In 2013, several outbreaks at tourist destinations involving Danish travellers were reported. In the period from June to October 2013 an increased number of *S. Enteritidis* cases with six specific subtypes were seen among Danish travellers (FUD1290) [4]. In total, 121 cases were registered and 88 of these were known to have visited Turkey during onset or just prior to disease onset. In comparison, during the previous years' summer peaks (June to September) 29-37 travel-related *S. Enteritidis* cases from Turkey were reported annually in 2010-12. Interviews with the cases showed that they had stayed in different hotels and different towns – primarily at the south-east coast of Turkey, so there was no common place of exposure. The interviews showed that cases mainly had booked all-inclusive stays (73%). The interviews also showed that half of the cases had eaten eggs during their stay and since consumption of eggs is considered to be the main risk factor for *S. Enteritidis* infection, this was the main hypothesis of the source – however, it was not possible to confirm this.

In the end of July 2013 an outbreak of gastroenteritis among children from four Nordic countries staying at the same hotel in Turkey was reported (FUD 1302) [5]. A total of 61 laboratory confirmed cases were reported from Norway (23), Sweden (27), Finland (3) and Denmark (8). The laboratory results showed a mix of *S. Enteritidis*, *Shigella sonnei* and Verotoxin producing *Escherichia coli* (VTEC). A cohort study in Norway pointed out slush-ice as the most likely source of infection.

An international outbreak was seen from November 2012 – April 2013 with hepatitis A virus (HAV) in travellers returning from Egypt (FUD 1277) [6]. In total, 15 confirmed cases of HAV infections with genotype 1B and identical RNA sequence of which 9 were identified in Denmark, and 89 probable cases, were reported in 14 other EU/EFTA countries. All 104 cases had been travelling to Egypt. An international case-control study was conducted. It was not possible to identify the source of the outbreak; however, the results indicated that consumption of strawberries or mango fruit could have been involved [7].

In October 2013, a Danish travel agency informed about an outbreak from a specific hotel in Tunisia (FUD 1307). Further investigation identified five cases in the Danish surveillance system with *Shigella sonnei* infection and travel to Tunisia. In comparison 0-1 case have been reported each year from 2009-2012. Interviews with four of the five cases showed that they had stayed at the same hotel during the first two weeks in August 2013. Three patients had not been eating outside the hotel. An international inquiry did not reveal cases from other countries. Outbreaks implicating Danish tourists in other countries are difficult to investigate and it was not possible to confirm the sources of any of the above-mentioned outbreaks. However, for several of the outbreaks the Danish tourists have stayed at resorts and have only eaten at the hotels and it is likely that these places are not perceived as risk areas. Efforts should be made to make tourists aware of the risks and preventive measures such as taking general hygiene precautions even when staying at closed resorts with all inclusive, and getting vaccinated against HAV when travelling to endemic areas.

References

1. Statens Serum Institut (2013). EPI-NEWS, week 22.
2. Statens Serum Institut (2013). EPI-NEWS, week 48.
3. Lassen SG, et al. (2013). Ongoing multi-strain food-borne hepatitis A outbreak with frozen berries as suspected vehicle: four Nordic countries affected, October 2012 to April 2013. *Euro Surveillance* 2013 18(17).
4. Nordic outbreak investigation team (2013). Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected. *Euro Surveillance* 18(27) .
5. Statens Serum Institut (2013). EPI-NEWS, week 38
6. MacDonald E, et al. (2013). Increase in hepatitis A in tourists from Denmark, England, Germany, the Netherlands, Norway and Sweden returning from Egypt, November 2012 to March 2013 *Euro Surveillance* 18(17).
7. Sane J, Et al. (2014). Multistate foodborne hepatitis A outbreak among tourists returning from Egypt – need for reinforced vaccination recommendations. Submitted for *Euro Surveillance*, February 2014.

3. Whole genome sequencing

By Mia Torpdahl (mtd@ssi.dk), Charlotta Löfström and Eva Møller Nielsen

In recent years, new DNA-sequencing techniques have been developed and made available for easy and fairly cheap sequencing of bacterial genomes. These post-‘Sanger’ sequencing techniques are commonly referred to as ‘next-generation-sequencing’ (NGS). NGS includes sequencing technologies that have high sequence capacity, but produce short sequences (e.g. as developed by Illumina and IonTorrent), which is compensated for by high sequence coverage (Figure 3.1). This technological development has dramatically reduced the cost of determining the complete, or nearly complete, genetic information for bacterial isolates. The technology and price will in the near future allow reference laboratories to implement whole genome sequencing (WGS) for typing of bacterial isolates as a replacement or complement to the conventional phenotypic and molecular typing methods.

The use of WGS for epidemiological purposes, such as routine surveillance and outbreak investigations, rely on the analysis of the large amount of data produced. Presently, there are two main approaches for analysing data, the mapping of sequence reads to a reference and “de novo” assembly (without a reference). These approaches can be used independently or in combination. The sequences from different bacterial isolates can be compared to each other directly e.g. by identifying single-nucleotide polymorphisms (SNPs) compared to a reference or gene-by-gene comparisons across assembled genomes. Conventional typing information can also be extracted. Currently, the analytical approaches are still under development and no international standards have been decided on for comparing bacterial isolates based on WGS data.

In the last two years, the use of WGS of foodborne bacteria has been under development at Statens Serum Institut and the National Food Institute, Technical University of Denmark, for epidemiological purposes. At both institutes, WGS has been used for several outbreak investigations in 2012-2013 and WGS was tested for the routine surveillance of VTEC infections for a 3-months period in 2012 [1]. Furthermore, Statens Serum Institut implemented WGS for the continuous surveillance of listeriosis in 2013. The analytical approaches used for the routine surveillance might be markedly different from the approach used in outbreak situations where a limited number of closely related isolates are compared, e.g. by SNP-based analysis.

Surveillance of listeriosis

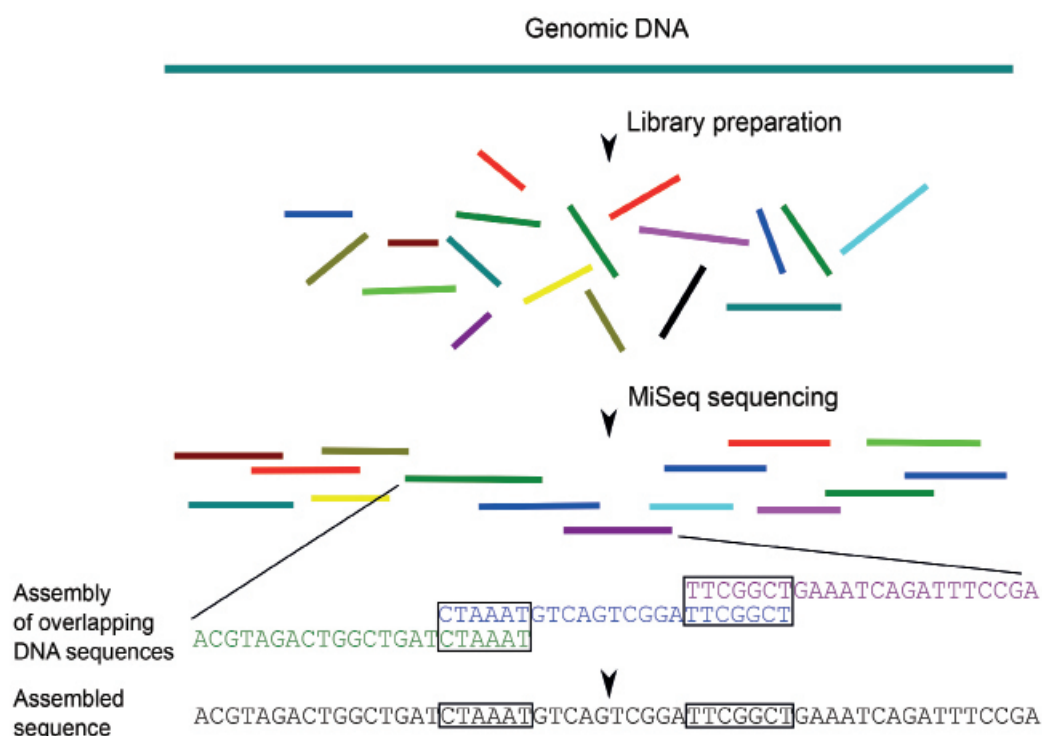
The surveillance of listeriosis cases in Denmark is based on the ‘real-time’ typing of isolates from all human infec-

tions. The regional clinical laboratories submit the isolates to Statens Serum Institut where typing is performed and used for early detection of outbreaks and the surveillance of trends over time. For the past ten years, isolates have been typed by pulsed-field gel electrophoresis (PFGE) (Annual report on Zoonoses 2011). Although PFGE with two restriction enzymes is internationally standardized and gives a good discrimination for typing of *Listeria*, PFGE is relatively labour-intensive to perform on a few isolates at a time and PFGE-profiles can generally be difficult to interpret and compare. As replacement of PFGE and other characterisation methods used for *Listeria* isolates, Statens Serum Institut implemented WGS for surveillance of listeriosis in 2013. The incoming isolates are sequenced on a weekly basis and the aim of the developed pipeline and the analytical approach is to obtain a clear indication of any related isolates and thereby potential outbreaks by an easy and fast analysis. The new isolates should be compared to older isolates in the database without re-analysis of these. Therefore, the analysis is based on extracting the genes from the multiple-locus sequence typing (MLST) method, which has an international standard nomenclature [2]. Only in the situations where isolates with the same MLST type are identified within a few months, a SNP-based analysis is performed to directly compare the relevant isolates. This algorithm for analysis was developed and validated based on the knowledge obtained from analysing representative isolates from the previous ten years’ surveillance, including isolates with known epidemiological links.

In 2013, 50 cases of listeriosis were registered. In concordance with the PFGE-based surveillance, WGS showed that the majority of isolates were from sporadic cases. However, five clusters of two to four isolates each were identified. Within these clusters, isolates had the same MLST profile and had less than 10 SNP differences. One cluster seemed to be linked to a specific hospital, but otherwise it was not possible to find a common source of infection or any other link between the patients. Epidemiological investigations of *Listeria* cases are often quite difficult due to the long incubation period, the high mortality rate and the fact that *Listeria* cases are often very old and suffer from underlying diseases.

WGS is a cost-efficient method for typing if used as the only laboratory method, i.e. if the hitherto used methods (serotyping and PFGE) are discontinued. As always when shifting laboratory methods, this can give problems with comparability between laboratories and countries in a transient period. Statens Serum Institut has implemented WGS

Figure 3.1. Principles of next generation short read sequencing



Source: Statens Serum Institut

for typing of *Listeria* isolates from human cases of infection before national or international systems for exchange of WGS-typing data is available. However, the principle of extracting the seven MLST loci with an established nomenclature from the NGS raw data (possible extended with more loci in the future) gives optimal conditions for easy exchange of typing results, as the results are independent of the bioinformatics tools used in each laboratory, e.g. for assembly and SNP-calling.

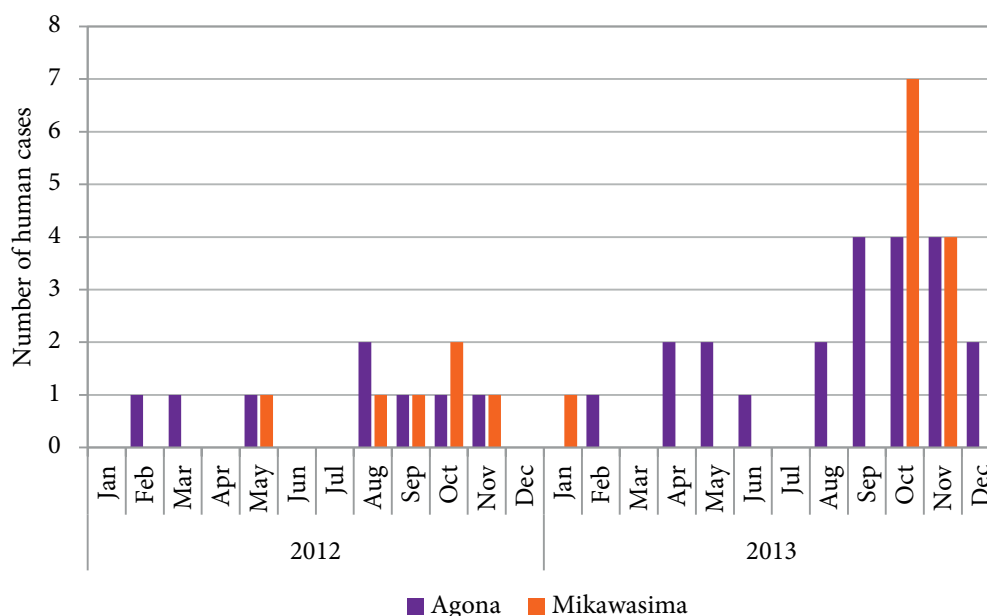
However, PFGE is the standard method for *Listeria* typing in Europe – for typing of human isolates as well as food isolates – a PFGE profile will still be produced for outbreak isolates for national and international comparison, including upload to ECDC’s Molecular Surveillance System.

WGS for *Salmonella* cluster investigations

The national surveillance of human *Salmonella* cases is based on the ‘real-time’ typing of isolates from human infections by serotyping of all isolates, multiple-locus variable number of tandem repeats analysis (MLVA) of the two most frequent serotypes (Enteritidis and Typhimurium, including the monophasic variant) and PFGE of other serotypes. Results are used for cluster analysis, outbreak investigations, comparison with food and animal isolates as well as making international inquiries. There are some

interpretative implications that have to be considered when using PFGE in outbreak investigations, e.g. the significance of minor band differences when defining clusters within different *Salmonella* serotypes. In 2013, Statens Serum Institut investigated the use of WGS for cluster detection and analysis of *Salmonella*. The incoming isolates were screened for clusters based on serotype and when potential outbreaks emerged, isolates were selected for sequencing. Two clusters appearing in the autumn were investigated using WGS, one being the rare serotype Mikawasima and the other the more prevalent serotype Agona (Figure 3.2).

Mikawasima isolates from patients in 2012 (6 isolates) and 2013 (12 isolates) were analysed by WGS and PFGE. The 11 isolates from October and November 2013 clustered together by both methods. Analysis of WGS data showed that the isolates in this cluster had no SNP differences, whereas all other isolates clustered into several groups more than 70 SNPs apart (Figure 3.3). The cluster isolates displayed the same unique PFGE profile although the profiles found in Mikawasima isolates generally showed high similarity (Figure 3.3). The data indicate that the increase in Mikawasima seen in October and November 2013 was an outbreak. It also showed another cluster from 2012 consisting of 3 cases. The SNP tree and the PFGE data showed a high degree of concordance, although the sequence data had a higher resolution in comparison to

Figure 3.2. Prevalence of *Salmonella* serotypes Agona and Mikawasima in 2012 and 2013

Source: Statens Serum Institut

the few band differences seen with PFGE.

The 16 *S. Agona* isolates received from February to November 2013 were analysed by PFGE and WGS. Both PFGE and WGS data identified the same 8 isolates received during August to November as being part of an outbreak. However, WGS data had a higher resolution and separated isolates with indistinguishable PFGE profile in correlation with age group and date of isolations, indicating a unique epidemiological relationship among these isolates.

WGS has also been evaluated as a tool to compare *Salmonella* isolated from humans and foods in outbreak investigations and to investigate the plausibility of sharing data and results between the laboratories at Statens Serum Institut and the National Food Institute, Technical University of Denmark. One example is an outbreak in 2012 where seven patients were infected with *S. Bareilly* with an identical PFGE profile. Patient interviews traced the source to unidentified food served at a specific restaurant [3]. At the same time four broiler flocks were tested positive for *S. Bareilly*. PFGE profiles from the veterinary and human isolates were found to differ by two bands. WGS results showed that the broiler and human isolates belonged to the same MLST type and that they were divided into two closely related groups based on SNPs. It was concluded that there was a close common ancestor of the two isolate groups, but that the broiler flock did not seem to have been the direct source of the human outbreak [4]. Although the

same conclusion was drawn based on PFGE and WGS data, a more precise determination of the relationship between isolates was obtained using WGS thereby contributing to the conclusions of the outbreak investigation.

From these first and very promising uses of WGS analysis for cluster investigations on human *Salmonella* infections and outbreak investigations of food and human isolates, we therefore conclude that WGS is a highly sensitive method that will contribute tremendously in cluster detection and outbreak investigations. Over the next few years it is the goal at Statens Serum Institut to replace the current flow of different subtyping schemes in *Salmonella* with one method and therefore implement WGS for *Salmonella* for the national human surveillance.

At the National Food Institute, WGS is planned to be implemented within a few years' time and used as a complement or to replace existing typing methods. In line with this, a PhD project was initiated in 2013 aiming at evaluating different approaches suitable for application of WGS in a routine laboratory setting. In this project cases related to outbreak investigations will first be investigated and thereafter, using the experiences gained, strategies for applying WGS for surveillance of e.g. *Salmonella* will be investigated. During this process it will be important to harmonize the protocols used in order to assure that data can easily be exchanged between SSI and DTU, e.g. in joint outbreak investigations.

WGS for the future surveillance of foodborne infections/zoonoses in DK

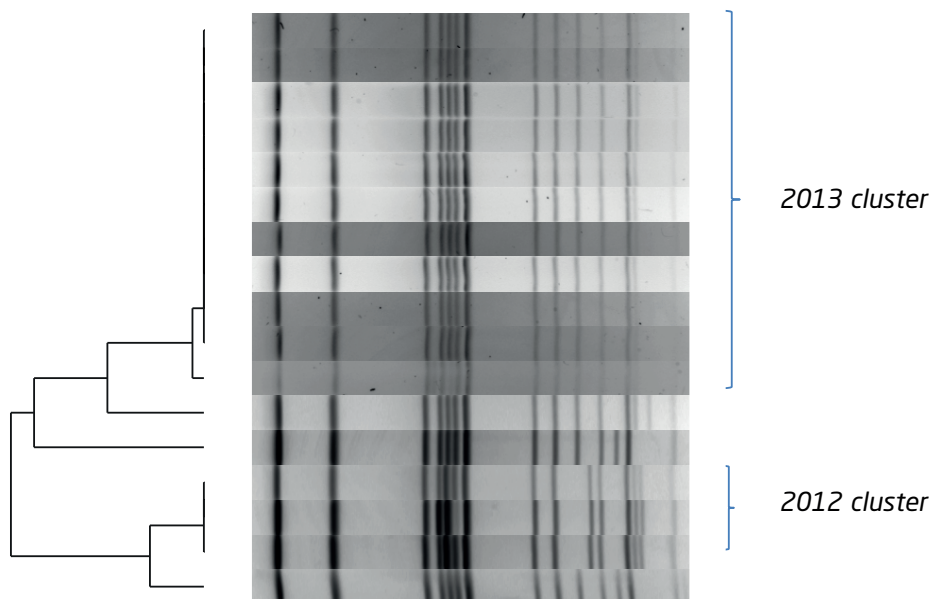
The vision for implementing WGS in reference laboratories is that analysis of WGS-data can replace the multitude of different methodologies presently used for characterisation of bacterial isolates, e.g. serotyping, PCR/Sanger sequencing for determining virulence factors and antimicrobial resistance genes, antimicrobial resistance profiling, and molecular typing methods for high discriminatory typing. If the relevant information can be extracted from WGS data, it will be cost-effective to replace the conventional methods by routine WGS in the surveillance of foodborne infections. Statens Serum Institut and the National Food Institute, Technical University of Denmark are working on further developing and implementing WGS for characterisation of foodborne pathogens such as verotoxin-producing *E. coli*, *Salmonella*, *Listeria* and *Campylobacter*. The great benefits of WGS will be achieved when tools are developed for extracting key conventional typing information, primarily the serotype of *Salmonella* and *E. coli*. Conventional serotyping has formed the primary categorization of these organisms for decades and it can be anticipated that serotype information will still be important for a period of several years to come. This will allow the discontinuation of conventional serotyping in laboratories performing WGS as they will still be able to compare to

laboratories only performing the conventional serotyping. In contrast, it is less likely that backwards comparability is feasible and important for typing methods like PFGE. To enable sharing of data between different laboratories, it is important to assure that harmonized protocols are applied.

References

1. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM (2014). Evaluation of Real-Time WGS for Routine Typing, Surveillance and Outbreak Detection of Verotoxigenic *Escherichia coli*. JCM PMID:24574290 (Epub ahead of press).
2. Ragon M, Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, Brisse S (2008). A new perspective on *Listeria monocytogenes* evolution. Plos Pathog Plos Pathog doi: 10.1371/journal.ppat.1000146.
3. Anonymous (2013). Annual Report on Zoonoses in Denmark 2012, National Food Institute, Technical University of Denmark.
4. Torpdahl, M, Kiil, K, Litrup, E, Sørensen, G, Aarestrup, FM (2013). A Danish *Salmonella* Bareilly outbreak investigated by the use of whole genome sequencing. In 10th International Meeting on Microbial Epidemiological Markers (IMMEM-10) - Abstract Book. (pp. 215). Paris, France.

Figure 3.3. Dendrogram based on SNPs retrieved from the core genomes of the *S. Mikawasima* strains. PFGE profiles from the same strains are also shown



Source: Statens Serum Institut

4. Relative human risk of *Salmonella* Enteritidis in table eggs in Denmark

By Helle Korsgaard (hkor@food.dtu.dk), Tina Struve, Tine Hald and Håkan Vigre

Since 1980, the use of raw (unpasteurised) eggs in dishes that are not thoroughly heat treated has not been permitted in restaurants, canteens and other public food handling businesses in Denmark. Since the late-1990s consumers have also been advised to avoid using raw eggs in dishes that are not heat treated, such as certain desserts and cakes.

In Denmark, laying hen flocks are tested intensively for *Salmonella* and when a flock is found positive, eggs from this flock are subjected to heat treatment. However, eggs from infected flocks can reach the retail market as table eggs during the period between the time of infection and when the routine testing reveals the *Salmonella* infection.

The last two decades of intensive *Salmonella* control and eradication programs in the Danish table egg production have been highly successful, and in the last ten years only few flocks have been found *Salmonella* positive (Appendix Table A6).

In order to evaluate the effect of the possible abolishment of the warning against the use of raw eggs in dishes eaten without heat treatment, the Danish Veterinary and Food Administration asked The National Food Institute, Technical University of Denmark, to estimate the relative risk of salmonellosis resulting from increased use of raw eggs by consumers. The relative human risk was to be estimated for the current testing intensity (every 9th week) and for a more intensive program with testing every 2nd week.

In Denmark, most of the table eggs available at retail are domestically produced (97% in 2008-2010). The rest is primarily imported from Sweden and Finland where the occurrence of *Salmonella* in poultry is very low. A second question from the Danish Veterinary and Food Administration was to estimate the changes in relative human risk, if imported eggs available for consumption were to originate from other EU Member states with higher levels of *Salmonella* among laying hen flocks.

Risk modelling

To answer the questions, a probabilistic simulation model was set up to describe the infection dynamics of *Salmonella* during table egg production. In the model, the Danish consumer's risk of purchasing contaminated table eggs depends not only on the risk of introduction of *Salmonella* into a laying hen flock, but also on how fast the infection spreads within the flock, the total number of eggs produced and the frequency and sensitivity of the routine testing.

The Danish *Salmonella* source account (chapter 1) shows that domestically acquired human infections attributed to table eggs are primarily caused by *S. Enteritidis*, even though other serotypes such as *S. Typhimurium* are also detected in Danish flocks of laying hens. Therefore, it was decided that the model only should include introduction and spread of *S. Enteritidis*.

Proportion of eggs produced by infected birds

On average 0.8% of the laying hen flocks tested in 2008-2010 were found positive for *S. Enteritidis* by the Danish control program (testing every 9th week), and the model predicts that 0.03% of the Danish table eggs reaching retail were produced by infected birds. In comparison, the model predicts that sampling every 15th week would have increased the proportion of Danish eggs produced by infected birds reaching the market by a factor of almost two, whereas a more intensive routine testing (every 2nd week) would have reduced the proportion to a fifth. Please note that the model predict number of eggs produced by infected birds, not the number of contaminated eggs.

In 2013, *S. Enteritidis* was detected in one of 373 laying hen flocks (0.3%). In October 2013, the routine testing of Danish laying hen flocks changed from every 9th week to every 2nd week. Assuming the same risk of infection per flock every day during 2013, the model estimates that during the first nine months (testing every 9th week) an

estimated 0.014% of the Danish eggs were produced by infected birds compared to an estimated 0.003% during the last three months (testing every 2nd week).

Estimating the relative human risk

To simplify the model, we assume there is a direct correlation between the number of human *S. Enteritidis* cases caused by consuming raw table eggs in non-heat treated dishes and the proportion of table eggs on the retail market produced by infected birds, given that:

- The proportion of contaminated eggs produced by the infected birds is the same in all scenarios.
- One table egg equals one portion consumed by one person.
- Infection of one or more persons due to cross contamination is not considered.

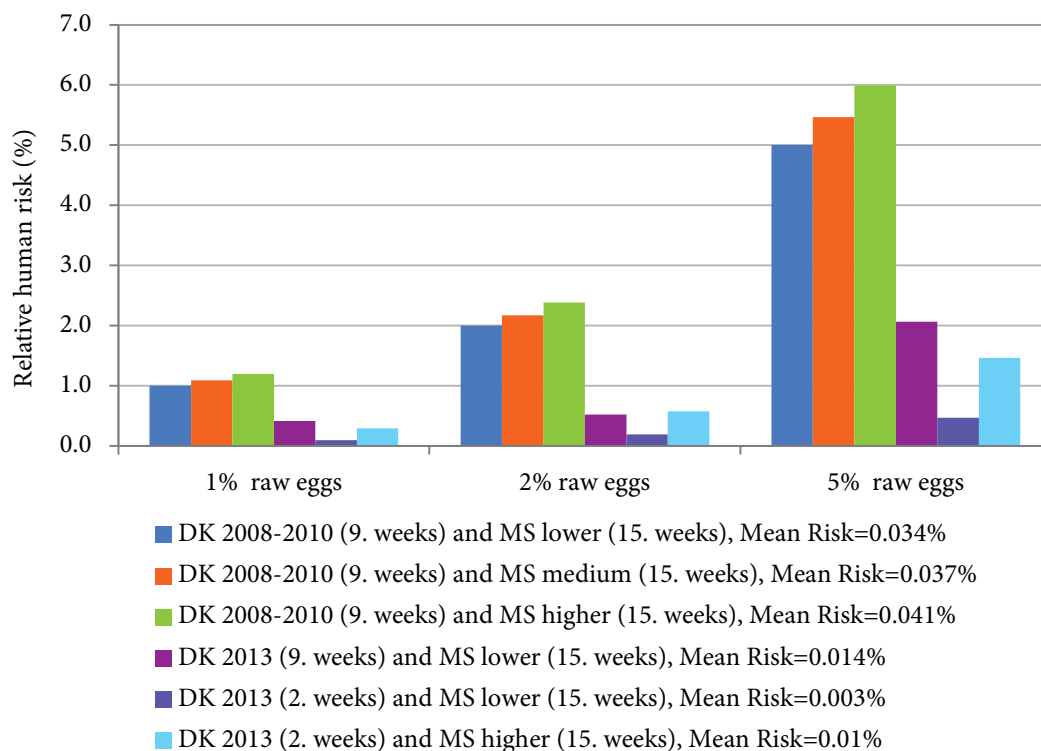
The relative human risk is calculated as:

$$RR_{\text{scenario}} = \text{Risk}_{\text{scenario}} / \text{Risk}_{\text{baseline}}$$

where the 'Risk' is the estimated mean proportion of table eggs available at retail produced by infected birds. By assuming the same proportion of *S. Enteritidis* contaminated table eggs in all scenarios, the estimated relative human risks are independent of the actual proportion of contaminated table eggs produced by the infected birds as well as the actual numbers of human cases caused by *S. Enteritidis* contaminated eggs.

It is unknown how frequently raw eggs are used in non-heat treated dishes in Denmark, both with and without the authorities warning against such use. Therefore, the relative human risk for Danish consumers is estimated assuming 1%, 2% and 5% of the table eggs is consumed without heat treatment.

Figure 4.1. Relative human risk^a of *S. Enteritidis* infection for selected scenarios^b, assuming 1%, 2% or 5% of the table eggs are consumed in non-heat treated dishes



a) Relative human risk for scenario_x = Risk_x / Risk_{baseline}, where the risk is the 'mean proportion of table eggs available at retail produced by infected birds', when 97% of the table eggs originate from Danish laying hen flocks and 3% is imported from other EU Member States (MS)

b) Scenarios where the model varies: the proportion of Danish laying hen flocks infected with *S. Enteritidis* as observed in 2008-2010 (0.7 %) and in 2013 (0.3 %), the proportion of *S. Enteritidis* infected laying hen flocks in other EU Member States (lower = 0.07 %, medium = 1.4 % or higher = 2.8 % infected flocks), and interval of routine testing of every 2, 9 or 15 weeks.

Source: National Food Institute

A baseline scenario, set to the value of 1, is estimated assuming:

- 1% of the table eggs are consumed in non-heat treated dishes.
- 97% of the eggs originate from Danish laying hen flocks tested every 9th week during 2008-2010.
- The remaining 3% originate from EU Member States with low levels of *S. Enteritidis* among laying hen flocks (0.07% positive flocks) tested every 15th week.

Results

Figure 4.1 presents the estimated relative human risks for the baseline scenario (set to 1) and the alternative scenarios where the imported table eggs originate from flocks in EU Member States with higher levels of *Salmonella* among the laying hen flocks (1.4% and 2.8% positive flocks). Even though only 3% of the table eggs are assumed to be imported, the relative human risk increases by a factor of 1.1, if the imported eggs originate in EU Member States where *S. Enteritidis* in layer flocks is detected twice as often as the average in Denmark during 2008-2010. If the consumption of raw eggs in non-heat treated dishes also doubles, the relative risk increases by a factor of 2.4.

The proportion of *S. Enteritidis* infected laying hen flocks in Denmark was lower in 2013 (0.3%) compared to 2008-2010 (0.7%), and flocks were tested every 9th week until October 2013 and every 2nd week thereafter. Compared with the baseline situation in 2008-2010, the relative human risk was reduced to 0.4 during the first months in 2013 and to 0.1 during the last months of 2013, assuming 3% of the table eggs available at retail were import from EU Member states with low *S. Enteritidis* occurrence (Figure 4.1).

When the proportion of eggs produced by *S. Enteritidis* infected birds in Denmark is as low as estimated for the last months of 2013, the contribution of *S. Enteritidis* from imported eggs becomes increasingly important. If 3% of the eggs were imported from an EU Member State with higher occurrence of *S. Enteritidis* (2.8% infected flocks), the relative human risk would more than double (Figure 4.1).

Conclusion

In response to the questions raised by the Danish Veterinary and Food Administration, it must be concluded that as long as there are *Salmonella* positive table egg laying hen flocks in Denmark and in the countries from where we import table eggs for direct consumption, there is a risk of salmonellosis when consuming raw eggs in dishes that are not heat-treated. Therefore it must be expected that not warning consumers against the use of raw eggs in non-heat treated dishes may lead to an increased consumption of raw eggs and therefore an increased human risk.

Contaminated eggs will be produced in the period between infection of the flock and until detection of the contaminated flock. Increasing the frequency of routine testing of the Danish laying hen flocks in 2013 from every 9th to every 2nd week decreased the relative human risk by approximately a factor 5.

In order to be able to evaluate effects of changes in consumers' use of raw table eggs as well as changes in import, the Danish Veterinary and Food Administration was provided with a an Excel tool, giving outputs on the form similar to Figure 4.1. The proportion of eggs imported from different EU Member States can be entered, and then the tool will calculate the weighted mean proportion of eggs produced by infected birds available for the Danish consumers as well as the relative human risk.



New initiatives on *Salmonella* in table eggs

By Annette Perge (ape@fvst.dk)

In 1997, the number of people suffering from salmonellosis due to the consumption of table eggs was an estimated 3.000, the highest number ever in Denmark. As a consequence the Danish Veterinary and Food Administration introduced a recommendation to consumers about using pasteurised egg products in dishes consumed without prior heat treatment. A requirement for food businesses to heat treat dishes containing raw eggs, alternatively to use pasteurised egg products was already in place in the legislation. The following years Denmark made a huge and successful effort to reduce the occurrence of *Salmonella* in the table egg production. As a result of the low prevalence of *Salmonella* achieved, in 2007 Denmark applied to the EU for special guarantees for *Salmonella* in table eggs [1], a position attained by Sweden and Finland at the time of their accession into the EU in 1995. In July 2012, special guarantees for *Salmonella* in table eggs were granted to Denmark [2]. This was an acceptance of Danish eggs being as safe as eggs produced in Sweden and Finland. The Danish Veterinary and Food Administration therefore found it necessary to reconsider the current legal requirements for heat treatment of eggs in food businesses and the recommendations to consumers. In order to make the right decision the Danish Veterinary and Food Administration asked the National Food Institute, Technical University of Denmark, to provide advice on the consequences in relation to human health, if the legal requirement and consumer recommendation were repealed.

In October 2013, the Danish Veterinary and Food Administration abolished the recommendation to consumers on using pasteurised eggs in dishes that are not heat treated. As eggs are biological products it is not possible to give a 100% guarantee that all eggs are free from *Salmonella*. In order to be on the safe side, the Danish Veterinary and Food Administration still recommend the use of pasteurised eggs, if the dishes are meant for vulnerable consumers like the elderly, the immunosuppressed or children.

The requirement for food businesses to heat treat dishes containing raw eggs, alternatively to use pasteurised eggs was maintained. The rationale was that the use of imported eggs is common in the catering sector and these eggs do not necessarily have the same level of safety as eggs produced in Denmark. Also, if a restaurant or a cafeteria serves dishes with raw eggs, the consumer is not necessarily aware of this choice. Consequently the consumer cannot himself make the choice of whether to eat dishes containing raw eggs or not. Finally, hospitals and homes for elderly serve meals for vulnerable groups where the use of a contaminated egg could have fatal consequences. The Danish Veterinary and Food Administration will follow the situation closely, including the number of human cases of salmonellosis from table eggs, and after a period the legal requirement for heat treatment of raw eggs in food businesses will be reconsidered.

References

1. Regulation (EC) No 853/2004 stating that any MS or region of MS that has a control programme recognized as equivalent to that approved for Sweden and Finland in respect to food of animal origin concerned can obtain the same special guarantees as Sweden and Finland.
2. Commission implementing regulation (EU) no. 427/2012 of 22nd May 2012 on the special guarantees concerning *Salmonella* laid down in Regulation (EC) No 853/2004 of the European Parliament and of the council to eggs intended for Denmark.



5. *Salmonella* Dublin in cattle

By Erik Rattenborg (era@vfl.dk) and Gudrun Sandø

Salmonella enterica subspecies *enterica* serovar Dublin (*S. Dublin*) is a foodborne zoonotic bacteria that can cause severe invasive manifestations in humans [1] and in cattle. *S. Dublin* is to a high degree host-adapted to cattle, and it can cause both welfare and economic losses in infected herds.

In 2002, a national surveillance programme for *S. Dublin* was initiated in Denmark. The main purposes were to monitor the national and regional prevalences in the cattle population over time and to provide farmers with a tool to protect their herds against introduction of *S. Dublin*. Subsequently all Danish cattle herds are classified into one of three levels of infection based on repeated antibody measurements in bulk tank milk, individual blood sampling, bacteriological cultivation of herds with clinical outbreaks of salmonellosis and cattle trade patterns (Table 5.1).

The programme is administered by the Knowledge Centre for Agriculture, Cattle and controlled by the Danish Veterinary and Food Administration.

The programme rapidly had an effect on trade behaviour among cattle farmers. Within the first six months after initiation of the programme, the trading pattern stabilized, i.e. animals purchased into Level 1 herds decreased from random to only 2-3% of animals purchased from Level 2 herds. This was followed by an economical effect, i.e. the market price of live animals from a herd in Level 1 became considerably higher than animals from Level 2.

Development in infection levels

Since the beginning of the programme, the trend in the proportion of Level 2 herds has been decreasing. Initially the main driver probably was the change in trading behaviour mentioned above [2]. The decreasing trend has not been equal in different types of production (Figure 5.1 and 5.2) and different geographical areas.

In Figure 5.1 the development of the proportion of Level 2 herds is shown for dairy herds and non-dairy herds (all other production types). In Figure 5.2 the trends of the non-dairy herds are specified according to production type.

The motivation for control of the infection within the herds has differed depending on the production type and geographical area. For dairy herds the motivation has been high from the beginning because of the difference in market value of live animals. Among slaughter calf producers the motivation initially was lower because they buy the calves from several dairy herds and continuously received infected calves (Figure 5.2). As these producers do not sell live animals, no economic consequence was imposed on them. Later, however, many of them discovered the positive influence of *Salmonella* control on calf health and these now exclusively buy calves from Level 1 dairy herds.

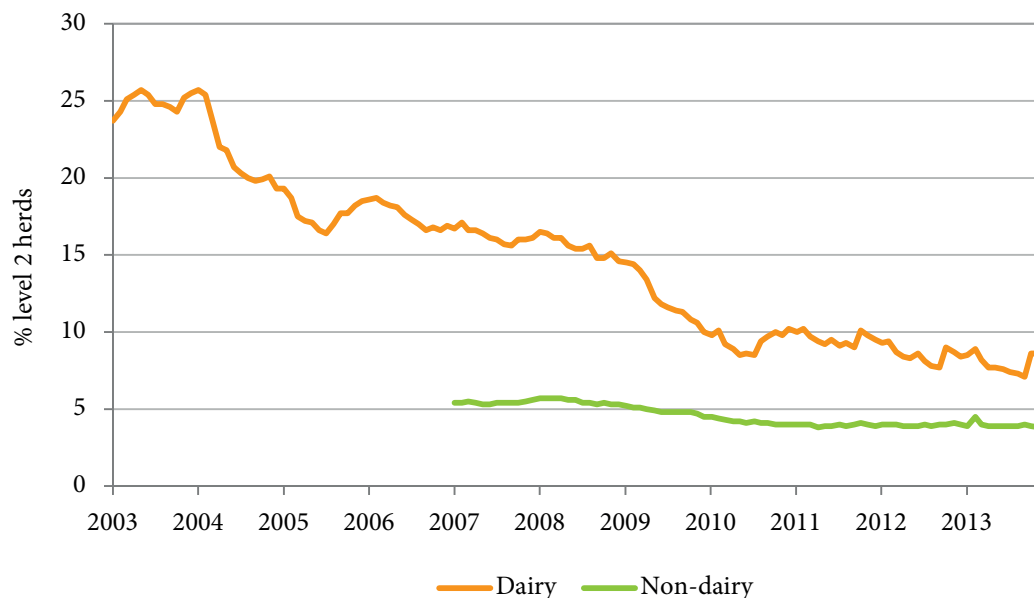
The difference in prevalence by geographical region is pronounced (Figure 5.3). Regions with low prevalence also reflect areas with few herds (e.g. Zealand). Furthermore the decreasing trend has been proportionally more

Table 5.1. Definition of the three *S. Dublin* herd levels

	Milk producing herds: Bulk tank milk	Non-dairy herds: Individual blood samples (slaughterhouse or live animals)
Level 1	Antibody negative based on 4 consecutive samples	Antibody negative based on up to 8 consecutive samples
Level 2	Antibody positive based on 4 consecutive samples or cattle purchased from a level 2 or 3 herd	Antibody positive based on up to 8 consecutive samples or cattle purchased from level 2 or 3 herd
Level 3	Clinical signs of salmonellosis and culture positive samples	Clinical signs of salmonellosis and culture positive samples

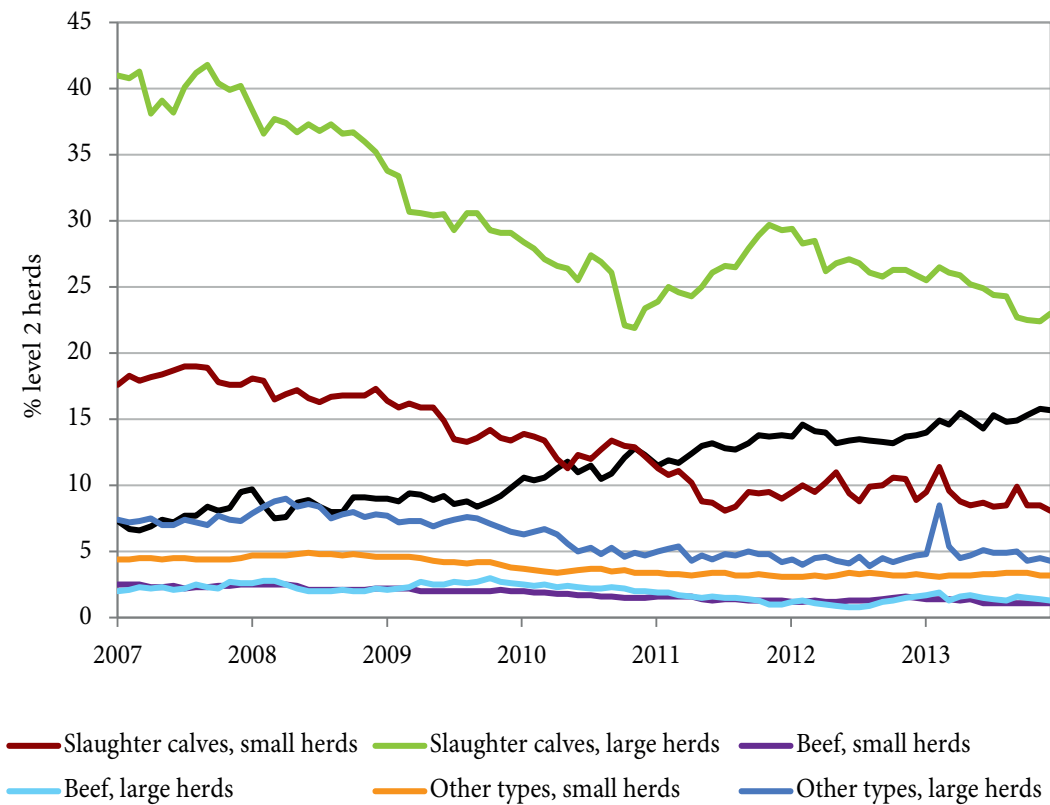
Source: Knowledge Centre for Agriculture, Cattle

Figure 5.1. Trend in proportion of Level 2 herds shown in percent for dairy and non-dairy herds



Source: Knowledge Centre for Agriculture, Cattle

Figure 5.2. Trend in proportion of Level 2 non-dairy herds shown in percent specified by production type



Source: Knowledge Centre for Agriculture, Cattle

pronounced in regions with an initial low prevalence. This is suggested to be attributed to neighbour effects among other reasons [2].

Further national initiatives

The decrease in the proportion of dairy herds in Level 2 declined in 2005 and again in 2010. In 2005, the initial effect of the programme had 'burned off'. Therefore new initiatives were developed and agreed upon between the authorities and the industry.

In 2007 a voluntary eradication campaign was decided by the industry with the aim of eradicating *Salmonella* Dublin from the Danish cattle population. Subsequently in 2008 a national control plan was prepared in a collaboration between the Knowledge Centre for Agriculture, Cattle, Department of Large Animal Sciences at the University of Copenhagen, The National Food Institute at Technical University of Denmark and the Danish Veterinary and Food Administration. The control plan sets means and targets for the reduction in different production types and at carcass level. The overall aim was to eradicate *Salmonella* Dublin from the cattle production by 2014.

The control plan has involved three steps (see text box). In 2010 restrictions were imposed upon Level 2 herds with high ELISA values as live trade was prohibited. New initiatives were outlined in 2012 when also the target of eradication by 2014 was postponed to 2016 because of in-

sufficient progress. The revision came into force July 2013 and includes regionalization of Denmark into high prevalence and low prevalence regions (Figure 5.3). Movement of animals from high to low prevalence regions is generally prohibited, and positive herds in the low prevalence region are placed under official restrictions.

Furthermore all positive herds are obliged to outline an action plan, including herd specific control measures, in collaboration with a veterinarian. The effect of the control measures must be documented and official restrictions are imposed on the herds if there is no progress. The provisions are laid down in order no. 954 of 13th of July 2013 on *Salmonella* in cattle a.o. species.

References

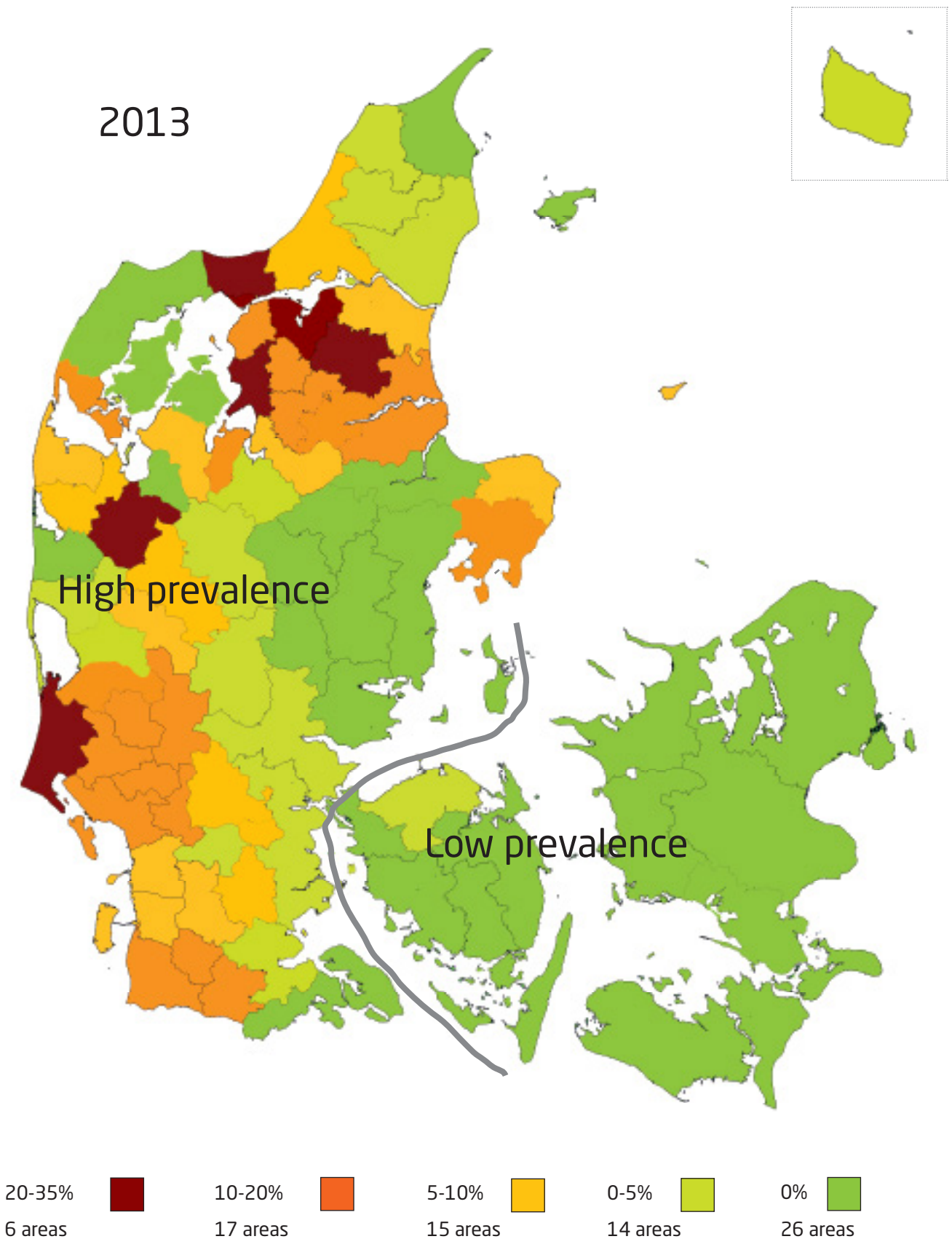
1. Helms M, Vastrup P, Gerner-Smidt P, and Mølbak K (2003). Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. *British Medical Journal*, (326) 357-361.
2. Nielsen LR, Rattenborg E, Nielsen J (2006). Development of the national surveillance program for *Salmonella* Dublin in Danish cattle. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia, Aug 2006.

The *S. Dublin* eradication programme

The eradication has been organized in three phases. The aim of the national strategy is to eradicate *S. Dublin* by 2016. By eradication is meant that the prevalence has to be close to 0% and appearing cases have to be controlled in order not to spread.

- Phase 1: Voluntary intervention in infected cattle herds from 2007 to end of 2009. All Level 2 and 3 herds were offered to be part of a network group that meets several times together with a consultant or veterinarian acting as facilitator to inspire each other to keep up the intervention work.
- Phase 2: Level 2 herds with high ELISA values were placed under restrictions and live trade was prohibited.
- Phase 3: In 2013 regionalisation of the country in low and high prevalent regions was implemented. The partition in low and high prevalent regions by legislation is shown in Fig. 5.3. Furthermore herd specific eradication programmes in all Level 2 and 3 herds is required.

Figure 5.3. Map of Denmark showing high and low prevalent regions and area prevalences



Source: Knowledge Centre for Agriculture, Cattle

6. Outbreak of *Salmonella* Choleraesuis in Danish pig herds

By Charlotta Löfström (chalo@food.dtu.dk), Gitte Sørensen and Dorte Lau Baggesen

In the end of 2012 one Danish pig herd was found positive for *Salmonella* Choleraesuis. This event was followed by the isolation of *S. Choleraesuis* from three herds during 2013 in January, August and December, respectively. In all herds there were signs of clinical disease. Finding *S. Choleraesuis* was unexpected since it is rarely found in pigs in Denmark and the last isolation was during an outbreak in 1999 [1]. Infections with *S. Choleraesuis* in humans are also very rare in Denmark. The last human case of *S. Choleraesuis* was in June 2012 and since 2000 only six cases has been reported.

At the National Food Institute, Technical University of Denmark, studies were initiated to investigate the possible connection between the infections. PFGE typing showed that the isolates from the three occasions (1999, 2012 and 2013) had different PFGE profiles, suggesting that several introductions of *S. Choleraesuis* to Denmark had occurred. Epidemiological investigations showed that pigs from the herd found positive in 2012 have been delivered to the herds found positive in January 2013, and that isolates from these two herds had the same PFGE profile. Isolates from the two herds infected in the second half of 2013 shared a different PFGE profile. It has not been possible to identify the primary introduction of the infection to the Danish pig herds. This was also the case for the outbreak in 1999/2000

[1]. Apart from the delivery of pigs from one farm to another taking place in December 2012 and January 2013, no new pigs had to our knowledge been brought into the infected herds, ruling out horizontal spread between herds as a source. Other plausible causes include cross contamination from contaminated vehicles during transport, contaminated feed, contact with infected humans and cross contamination from wildlife, but presently there is no clear evidence pointing at any of these sources.

The lack of source identification may have been caused by limitations in the epidemiological information available, but also by an insufficient resolution of isolates by the epidemiological typing methods applied (mainly PFGE and antimicrobial resistance profiles). Future use of methods with higher resolution, such as whole genome sequencing (see chapter 3 for more information), might help in revealing the source in this and other outbreaks.

References

1. Baggesen, DL, Christensen, J, Jensen, TK, Skov, MN, Sørensen, G, Sørensen, V (2000). Udbrud af *Salmonella enterica* subsp. *enterica* serovar Choleraesuis var. Kunzendorf (*S. Choleraesuis*) i en dansk svinebesætning. Dansk Veterinærtidsskrift 83, 6-12.



7. *Salmonella* Typhimurium DT41 in poultry

By Charlotta Löfström (chalo@food.dtu.dk), Ann-Sofie Hintzmann, Gitte Sørensen and Dorte Lau Baggesen

Salmonella Typhimurium is one of the serovars most frequently involved in human salmonellosis in Europe [1], including Denmark (Appendix Table A2). Worldwide, poultry and poultry related products have been reported as an important reservoir of *S. Typhimurium*. Due to the successful implementation of an efficient *Salmonella* control program the prevalence of *Salmonella* in poultry in Denmark has been very low for a number of years (Appendix Table A6 and A8).

Despite the low prevalence of *Salmonella* in the Danish poultry production, reoccurring isolations of different *Salmonella* types, particularly *S. Typhimurium* phage type DT41, has been made in broiler breeder flocks over the past decades without identifying a clear source [2]. During the last quarter of 2013 an unusually high number of flocks were found positive for *S. Typhimurium* phage types DT40, DT41 and unspecific phage types (RDNC). Isolates were found in the whole poultry production chain, including hatcheries, breeding flocks for the broiler production line, broiler flocks and at the slaughter house. One DT41 isolate was also found in animal feed, but to our knowledge this feed was not intended for the poultry sector. These DT41 isolates were of particular interest due to the previous history with repeated findings in poultry breeding flocks and the isolates were therefore characterized in more detail using the DNA based typing method, multi locus variable number of tandem repeat analysis (MLVA).

MLVA-results showed that the three isolates, where the phage type could not be determined (RDNC), were related to the DT41 isolates. Based on the MLVA typing the DT41 and the RDNC isolates (41 isolates in total) were split into eight groups. When a maximum divergence at one locus was permitted these could be gathered into four groups. Using this criterion, combined with epidemiological information, a spread of the infection between some of the flocks within a short time could be documented. Furthermore, one subtype of DT41 was found in the whole production pyramid, from broiler breeding flocks to broilers and the poultry slaughter house. The feed isolate was found to be different from the rest of the isolates.

In conclusion, the source of the sudden increase of *S. Typhimurium* DT41 in the poultry production remains

unclear, although it could be concluded that a combination of spread within the production pyramid and new introductions had played a role. However, further studies using more discriminatory typing methods to investigate this in more detail are in progress.

References

1. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012 (2014). The EFSA Journal 2014;12(2):3547.
2. Litrup, E, Christensen, H, Nordentoft, S, Nielsen, EM, Davies, RH, Helmuth, R, Bisgaard, M (2010). Use of multiple-locus variable-number tandem-repeats analysis (MLVA) typing to characterize *Salmonella* Typhimurium DT41 broiler breeder infections. J Appl. Microbiol. 109, 2032-2038.



8. Online tools - risk assessment models and predictive microbiology

8.1 TRiMiCri: Easy access to risk based microbiological criteria

By Maarten Nauta (maana@food.dtu.dk)

Microbiological criteria (MC) are used to assess the acceptability of food based on the microorganisms found in samples taken from food lots. Traditionally, MCs are defined on the basis of expert knowledge and a general agreement on feasibility. However, with the rise of quantitative microbiological risk assessment, there is an increased interest in making MCs risk based [1]. This means that the effect of implementing specific MCs is evaluated in terms of their effect in terms of decreased consumer health risks. A new type of risk based MC has been proposed in Denmark, when the case-by-case risk assessment was introduced. Instead of a traditional microbiological limit (ML) on the number of samples that is allowed to carry more than a threshold concentration, it applies a limit for the relative consumer risk (RRL) associated with concentrations found in the samples.

The establishment and evaluation of risk based MCs is often considered to be a difficult task, that requires more than average knowledge of risk assessment. In a project financed by the Nordic Council of Ministries (NMDD) and supported by partners from other Nordic countries, National Food Institute, Technical University of Denmark therefore developed a user friendly software “TRiMiCri”. This “Tool for Risk based Microbiological Criteria” aims to facilitate establishment and application of risk based microbiological criteria, with a focus on *Campylobacter* in broiler meat.

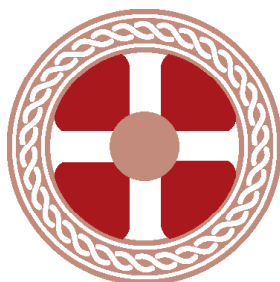
TRiMiCri has three main functionalities:

- Quantitative concentration data found in samples taken from a food lot can be evaluated for compliance against user-defined MCs.
- Semi-quantitative concentration data found in representative samples can be entered to define a baseline risk, that is needed for the evaluation of RRL MCs. This allows the user to apply the Danish case-by-case risk assessment approach for its own situation.
- The expected effect of setting specific microbiological criteria can be evaluated in terms of effect on consumer health risk and the percentage of (potentially costly) non-complying food lots. This can help food safety managers in defining the most suitable microbiological criterion, based on associated health risks.

TRiMiCri, and its tutorial, are freely available via the website <http://tools.food.dtu.dk/trimicri>. The developers are happy to receive feedback on the tool.

References

1. Codex Alimentarius Commission (2013). Principles and guidelines for the establishment and application of microbiological criteria related to foods CAC/GL 21 – 1997, Rev. 2013.



8.2 Food Spoilage and Safety Predictor (FSSP) Software

By Paw Dalgaard (pada@food.dtu.dk)

Mathematical models for growth, survival or inactivation of microorganisms can be valuable tools to evaluate safety and shelf-life of food. However, such predictive microbiology models can be difficult to use in practice unless they are included in user-friendly application software such as Food Spoilage and Safety Predictor (FSSP) [1]. This software contains various models to predict the effect of product characteristics and storage conditions on shelf-life and safety of food. The first version of the software was launched as far back as in January 1999 and it is now widely used in 118 countries.

In 2014 a new and expanded version of the FSSP software is launched. FSSP contains new predictive models and new facilities in addition to all the features already available as part of the former version called Seafood Spoilage and Safety Predictor (SSSP), e.g. models to predict the effect of temperature storage conditions on product shelf-life, models for growth of specific spoilage micro-organisms to predict shelf-life of fresh fish and models to predict food safety including histamine formation in marine fin-fish. New predictive models in FSSP include:

- Growth and growth boundary model for lactic acid bacteria in meat and seafood products. This new model has been extensively validated and it can be used for a wide range of products [2].
- Expanded model to predict the simultaneous growth of lactic acid bacteria and *Listeria monocytogenes* in various meat and seafood products including some mayonnaise based seafood salads.
- Product specific models for the simultaneous growth of lactic acid bacteria and *Listeria monocytogenes* in chilled cottage cheese.
- A generic growth and growth boundary model for any microorganism/food combination where cardinal growth parameter values like the minimum temperature and pH for growth have been determined. This generic model can take into account the effect of various product characteristics and storage conditions. Predictions can be obtained for constant or for dynamic temperatures, pH and lactic acid conditions.

FSSP can for example be used to document if *Listeria monocytogenes* is able or unable to grow in a product [3]. This specific use of the software is described on the Danish Veterinary and Food Administration website (www.fvst.dk, in Danish). In addition, FSSP can be used to facilitate development or reformulation of especially lightly preserved foods.

FSSP is an important tool for the public sector consultancy, teaching and industry advice given by the Predictive Microbiology research group at the National Food Institute, Technical University of Denmark.

FSSP is available for free at <http://fssp.food.dtu.dk>. To help interested FSSP users benefit from this tool, one-day workshops are organized as indicated on the FSSP homepage or on request to the author.

References

1. Dalgaard, P (2009). Modelling of microbial growth. Bulletin of International Dairy Federation, 433, 45-60.
2. Mejlholm, O, Dalgaard, P (2013). Development and validation of an extensive growth and growth boundary model for lactic acid bacteria in processed and ready-to-eat seafood and meat products. International Journal of Food Microbiology 167, 244-260.
3. Mejlholm, O, Gunvig, A, Borggaard, C, Blom-Hansen, J, Mellefont, L, Ross, T, Leroi, F, Else, T, Visser, D, Dalgaard, P (2010). Predicting growth and growth boundary of *Listeria monocytogenes* – an international validation study with focus on processed and ready-to-eat meat and seafood. International Journal of Food Microbiology 141, 137-150.



9. International topics

By Gudrun Sandø (gus@fvst.dk)

9.1 Control of zoonoses in animal populations

9.1.1 EU coordinated monitoring studies

Based on Zoonosis Directive 2003/99/EC and Regulation (EC) No 2160/2003, the Commission can initiate harmonised studies in order to generate comparable prevalence data from all Member States with the purpose of setting common EU targets for the reduction of the pathogens in question. So far, eight such baseline studies have been carried out concerning *Salmonella*, *Campylobacter*, *Listeria* and MRSA. The EU results have been published on the EFSA website (www.efsa.eu), except from the results of the baseline study on *Listeria* for smoked fish. The Danish results from these studies have been presented in Annual Reports on Zoonoses 2005-2009.

9.1.2 EU harmonised *Salmonella* surveillance programmes

Based on the results of baseline studies in flocks of poultry, harmonised regulation on targets and surveillance in the poultry production has been laid down by the Commission.

In 2010, the EFSA published a scientific opinion [1] on monophasic *S. Typhimurium*-like strains in which it was concluded that the monophasic *Salmonella* strains with the antigenic formula *S. 1,4,[5],12:i:-* should be treated equal to *S. Typhimurium*. Subsequently EU regulations on target end criteria set on *Salmonella* have included these types as well.

According to Regulation (EC) No 584/2008, the EU target at 1% for breeding and fattening turkey flocks positive for *S. Typhimurium* or *S. Enteritidis* had to be reached at the end of 2012. This regulation has now been replaced by Regulation (EC) No 1190/2012 laying down a permanent target of maximum 1% positive breeding and fattening turkey flocks. The regulation enters into force in 2013. In Denmark, no turkey flocks were positive with *S. Typhimurium* or *S. Enteritidis* in 2013 (Appendix Table A9).

In breeding flocks of *Gallus gallus*, the target of 1% positive adult flocks had to be reached by the end of 2009 according to Regulation (EC) No 1003/2005. The target was set for *S. Typhimurium*, *S. Enteritidis*, *S. Hadar*, *S. Infantis* and *S. Virchow*. This regulation has been replaced by Regulation (EC) No 200/2010 laying down a permanent target of maximum 1% adult flocks positive for *S. Typhimurium* including the monophasic *S. 1,4,[5],12:i:-* strains, *S. Enteritidis*, *S. Hadar*, *S. Infantis* and *S. Virchow*. In the legislation no distinction is made between breeding flocks

from the table egg and broiler production lines.

In Denmark, one rearing flock from the broiler production was positive with *S. Typhimurium* DT41 in 2013 (Appendix Table A6 and A8).

The EU baseline study on table egg laying flocks carried out in 2004 showed large differences in the prevalence between Member States. Therefore, Member States specific targets were set either as an annual 10-40% reduction of positive adult flocks dependent on the prevalence of positive adult flocks in the Member State the previous year or a maximum of 2% adult flocks positive (Regulation (EC) No 1168/2006). The target was set for *S. Typhimurium* and *S. Enteritidis* and had to be reached at the end of 2010. This regulation has been replaced by Regulation (EC) No 517/2011 laying down permanent targets for the reduction of *Salmonella* in laying flocks. The new regulation maintains the previous targets. For Denmark, the target is a maximum of 2% adult flocks positive for *S. Typhimurium* (including the monophasic *S. 1,4,[5],12:i:-* strains) and *S. Enteritidis*. The prevalence in Denmark has been lower than 2% since 2004. In 2013, four flocks were positive for the target serotypes (One flock with *S. Enteritidis* and three flocks with *S. Typhimurium*) (Appendix table A6).

In broiler flocks of *Gallus gallus*, the target of maximum 1% flocks positive for *S. Typhimurium* and *S. Enteritidis* had to be reached at the end of 2011 according to Regulation (EC) No 646/2007. This regulation has been replaced by Regulation (EC) No 200/2012, which maintains the previous target at 1% including the monophasic *S. 1,4,[5],12:i:-* strains. Denmark has had intensive *Salmonella* control programmes since the 1990's and the target of 1% was reached in 2000. In 2012, 0.5% of broiler flocks was positive with *S. Typhimurium* including the monophasic *S. 1,4,[5],12:i:-* strains (Appendix Table A8).

9.2 Antimicrobial resistance

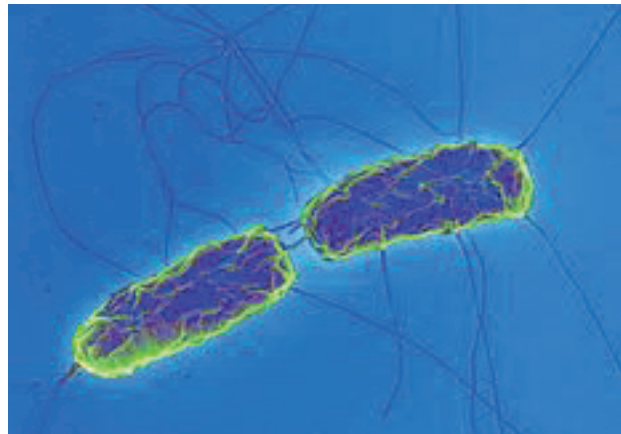
The Commission has implemented a harmonised monitoring of antimicrobial resistance within the EU from 2014. The decision is based on a recommendation from the European Food Safety Authority (EFSA). The harmonized monitoring of antimicrobial resistance is implemented for poultry, pigs and calves (under 1 year) and meat of broilers, pigs and cattle. The monitoring includes antimicrobial resistance in *Salmonella*, *Campylobacter jejuni*, *E. coli* and



ESBL in *Salmonella* and *E. coli*. It is optional for Member States to monitor antimicrobial resistance in enterococci and *Campylobacter coli*. The samples will be collected during a two-year rotation period. In 2014 poultry will be sampled and in 2015 pigs and calves will be sampled. The sampling strategy is harmonised to ensure comparable results between Member States. The idea is that the knowledge gained from the monitoring will raise awareness of the potential antimicrobial resistance problems in the individual Member States and thus lead to increased problem solving.

9.3 Microbiological criterion for verotoxin-producing *E. coli* in sprouts

In July 2013, four legal acts and a guidance document on different aspects of production of sprouts came into force. These new provisions included among other things a new microbiological criterion for verotoxin-producing *E. coli* in sprouts. See more in Annual Report on Zoonoses 2012.



10. Surveillance and control programmes

The collaboration between national and regional authorities, the industry and non-governmental organizations is presented in Figure 10.1. According to the Danish legislation, 41 infectious diseases are notifiable in Denmark. An overview of the notifiable and non-notifiable human and animal diseases presented in this report is provided in Appendix Table A29 and Table A30, respectively, including reference to the relevant legislation.

10.1 Surveillance of human disease

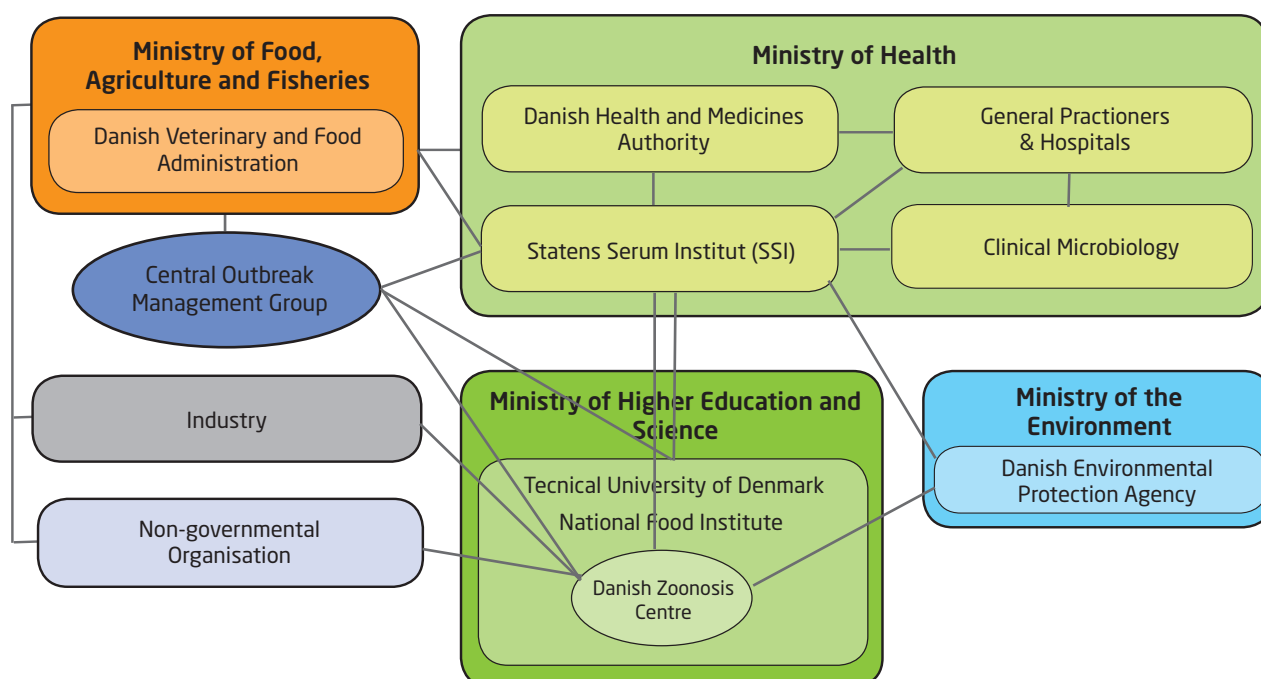
Information on human cases due to zoonotic pathogens presented in this report is reported to Statens Serum Institut through different channels depending on the disease:

- Notifiable through the laboratory surveillance system: *Salmonella*, *Campylobacter*, *Yersinia*, Verotoxin-producing *E. coli* (VTEC) and *Listeria*.
- Individually notifiable zoonotic pathogens: *Chlamydia psittacci* (ornithosis), *Leptospira*, *Mycobacterium*, Bovine Spongiform Encephalopathy (BSE) prions (var. Creutzfeldt-Jakob Disease), Verotoxin-producing *E. coli* (VTEC) and Lyssavirus (rabies).
- Non-notifiable zoonotic pathogens: *Brucella*, *Cryptosporidium*, *Echinococcus*, *Toxoplasma* and *Trichinella*.

In Denmark, the physicians report individually notifiable zoonotic diseases to the Danish Health and Medicines Authority and the Department of Infectious Disease Epidemiology at Statens Serum Institut. Physicians send specimens from suspected cases to one of the clinical microbiology laboratories depending on the geographical region. Positive cases diagnosed by a clinical microbiological laboratory are reported through the laboratory surveillance system to the Unit of Gastrointestinal Infections at Statens Serum Institut. The laboratories must report positive results to Statens Serum Institut within one week. Furthermore, all *Salmonella* and VTEC isolates are sent to the reference laboratory at Statens Serum Institut for further sero- and genotyping. The results are recorded in the Register of Enteric Pathogens maintained by Statens Serum Institut. Positive cases are reported as episodes, i.e. each patient-infectious agent combination is only recorded once in any six-month period. Overviews of results from the Register of Enteric Pathogens are presented as follows:

- All laboratory confirmed human cases are presented in Appendix Table A2.
- VTEC O-group distribution in humans is presented in Appendix Table A3.
- The *Salmonella* serovar distribution is presented in Appendix Table A5.

Figure 10.1. Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feedstuffs in Denmark, 2013



Source: Danish Zoonosis Centre, National Food Institute

10.2 Outbreaks of zoonotic gastrointestinal infections

In Denmark, local and regional foodborne outbreaks are typically investigated by the Food Control Offices¹ in collaboration with the medical officers at the Danish Health and Medicines Authority, and the regional clinical microbiology laboratories. Larger regional and national outbreaks are investigated by Statens Serum Institut, the National Food Institute, Technical University of Denmark and the Danish Veterinary and Food Administration in collaboration. These institutions may also aid in the investigation of local outbreaks. Representatives from these institutions meet regularly in the Central Outbreak Management Group to discuss surveillance results, compare the reported occurrence of zoonotic agents in animals, food and feedstuffs with that in humans, and coordinate the investigation of outbreaks. The formal responsibility of investigating food- or waterborne outbreaks is currently divided between three ministries based on the outbreak source: the Ministry for Interior and Health for infectious diseases; the Ministry of Food, Agriculture and Fisheries for foodborne and animal related diseases; and the Ministry of the Environment (along with the municipalities) for waterborne diseases.

Outbreaks may be detected in various ways. Individuals who experience illness related to food intake in settings such as restaurants or work place cafeterias may report these incidents directly to the Food Control Office. General practitioners and hospitals are obliged to report all suspected

water- and foodborne infections to the Danish Health and Medicines Authority and to Statens Serum Institut. Clusters of cases may also be noted in the laboratory or identified at Statens Serum Institut through the laboratory surveillance system of gastrointestinal bacterial infections or through subtyping of bacterial isolates from patients.

A list of verified outbreaks (not including household outbreaks) reported to the Food- and waterborne Outbreak Database (FUD) are presented in Appendix Table A4 and some of the outbreaks from 2013 are outlined in Chapter 2.

10.3 Surveillance and control of animals and animal products

Salmonella surveillance and control programmes for poultry, pigs and cattle are presented in Appendix Tables A31-A36. Sample analysis is performed at authorised private laboratories, the Danish Food and Veterinary Administrations laboratory, the National Food Institute and the National Veterinary Institute at Technical University of Denmark. *Salmonella* isolates are forwarded to the National Food Institute for serotyping, some isolates are also phage- and genotyped as well as tested for antimicrobial resistance. An overview of the methods used for subtyping is presented in Appendix Table A37.

Overviews of results from surveillance and control of *Salmonella* are presented as follows:

- Results from the Table egg production are presented in Appendix Tables A5-A7.

1. The Danish Veterinary and Food Administration (DVFA) is one authority but operates from more locations throughout the country. To be able to distinguish the locations the terms DVFA is used synonymous with the location in Glostrup and Food Control Office followed by the location synonymous with the location in question

The new human microbiology database - MiBa

By Luise Müller (lum@ssi.dk)

In 2008, Statens Serum Institut took the initiative to establish a nationwide database with the aim to enable real-time surveillance of communicable diseases and microorganisms as well as provide nationwide access for healthcare personnel to microbiology reports [1]. The database was entitled the Danish Microbiology Database (MiBa) and the establishment and management has been a collaborative process between departments of clinical microbiology at the hospitals (DCM) and Statens Serum Institut. MiBa is mainly financed by a grant from the Danish Ministry of Health from 2009. MiBa is a national database that receives copies of reports from all DCM. The key principle is the simultaneous submission to MiBa of an electronic copy every time a new or updated report is sent from a DCM to the general practitioner or hospital department that requested the test. Since January 2010, copies of all reports submitted by Danish DCMs have been transferred to MiBa. By December 2013, approximately 11 million reports had been uploaded and there were an increasing number of accesses, where health-care employees look up reports in the database, reaching more than 30,000 accesses per month. So far the nationwide sharing of data in MiBa has been a success and has already proved very useful e.g. in the surveillance of influenza and other respiratory infections [2]. The full benefits of a timely and complete surveillance system are still to be experienced. Finally, it is expected that MiBa will provide numerous opportunities for national surveillance of zoonoses in humans also for those that are not currently notifiable [1].

References

1. Voldstedlund M et al. (2014). The Danish Microbiology Database (MiBa) 2010 to 2013. Euro surveillance 19(1)
2. Bragstad K et al. (2013). Low vaccine effectiveness against influenza A(H3N2) virus among elderly people in Denmark in 2012/13 – a rapid epidemiological and virological assessment Euro surveillance 18(6).

- Results from the broiler production are presented in Appendix Tables A5 and A8.
- Results from the duck and turkey productions are presented in Appendix Table A5 and A9.
- Results from the pig production are presented in Appendix Tables A5, A13 and Figures A1-A3.
- Results from the cattle production are presented in Appendix Tables A5, A14-A15 and Figure A4.
- Results from the feeding stuff production are presented in Appendix Tables A18-A19.
- Results from the rendering plants are presented in Appendix Table A20.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Table A22-A23.

Overviews of results from monitoring of *Campylobacter* are presented as follows:

- Results from the broiler production are presented in Appendix Tables A10-A11.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Table A22-A23.
- Results on the relative distribution of *Campylobacter* species in broilers, pigs and cattle are presented in Appendix Tables A12 and A16.

Pig and cattle carcasses are screened for *Mycobacterium* and *Echinococcus* during meat inspection at the slaughterhouse. Although Denmark is assigned as a region where the risk of *Trichinella* in domestic swine is negligible, all slaughter pigs are still examined for *Trichinella* at slaughter as well as wild boars, and horses slaughtered for human consumption. In addition, boars and bulls are tested for *Brucella* and bulls are tested for *Mycobacterium* at semen collection centres. All positive results for notifiable infectious diseases are reported to the Danish Veterinary and Food Administration. Results are presented in Appendix Table A13-A14.

Results from the surveillance for Bovine Spongiform Encephalopathy (BSE) in cattle, Transmissible Spongiform Encephalopathy (TSE) in sheep/goat are presented in Appendix Tables A24-A26.

Results from the monitoring of *Coxiella burnetii* (Q fever) in cattle are presented in Appendix Table A14.

Results based on suspicion of diseases with *Chlamydia psittacci*, *Cryptosporidium*, *Trichinella*, classical rabies and European Bat *Lyssavirus* in zoo animals, pets and wild life are presented in Appendix Table A22-A23.

10.4 Official testing of zoonotic pathogens in foodstuffs

In Denmark, control of zoonotic microorganisms in foodstuffs is mainly carried out as projects which are coordinated at the central level of the Danish Veterinary and Food Administration. Sampling and testing are carried out with the following purposes:

- To verify that food business operators comply with microbiological criteria laid down in the legislation
- To verify the microbiological safety of food for which no microbiological criteria are laid down at EU Community level.
- To monitor the effect of established risk management procedures in order to evaluate if these provide the desired results or need to be reconsidered.
- To generate data for the preparation of risk profiles and risk assessments to support microbial risk management
- To discover emerging problems with microbiological contaminants.

Appendix Table A27 provides information on the centrally coordinated studies conducted in 2013. An overview of these results presented in this report is found in table A27.

For further information consult the website of the Danish Veterinary and Food Administration, www.fvst.dk.

New action plans

New action plan on *Campylobacter* in broilers

By Gudrun Sandø (gus@fvst.dk)

The *Campylobacter* action plan 2013 - 2016 was adopted in spring 2013. This is the second *Campylobacter* plan, and it was developed during 2012 by the Danish Agriculture & Food Council, the Danish Broiler Association, the National Food Institute and the National Veterinary Institute at Technical University of Denmark, and the Danish Veterinary and Food Administration.

The action plan covers initiatives in the broiler production at farm level as well as at the slaughterhouse, information to consumers, and as a new element it also covers sources and routes of transmission other than from the broiler production. Targets have been set in the broiler production: The prevalence of positive flocks shall be reduced by 20% by 2016 compared to the level in 2012. The target for fresh broiler meat at the slaughterhouses is formulated as a reduction of the relative risk compared to the level in 2012 and depends on the prevalence as well as the concentration of *Campylobacter* in fresh broiler meat. By the end of 2014 and 2016 the relative risk should be reduced by 25% and 50%, respectively.

The content of the plan and the background for it is described in more detail in Annual Report 2012. Development and target achievement will be evaluated on a yearly basis. The report is available at the Danish Veterinary and Food Administration's website www.fvst.dk (in Danish).

New action plan on *Salmonella* Dublin in cattle

By Gudrun Sandø (gus@fvst.dk) and Erik Rattenborg

The action plan against *Salmonella* Dublin in cattle was adopted in 2008. The overall aim was to eliminate *Salmonella* Dublin from the cattle production by the end of 2014. As the reduction in the occurrence of seropositive herds did not progress as expected, new initiatives were agreed on in 2013. The initiatives were outlined by the Knowledge Center for Agriculture, Cattle, Department of Large Animal Sciences at the University of Copenhagen, The National Food Institute and the Danish Veterinary and Food Administration.

The new initiatives include classification of regions in Denmark into high prevalence and low prevalence regions. There are restrictions on relocations of animals between regions with different status. Furthermore all positive herds are obliged to initiate herd specific control measures. The provisions are laid down in order no. 954 of 13th of July 2013 on *Salmonella* in cattle. The new aim is to eliminate *Salmonella* Dublin from the cattle production by the end of 2016. The action plan and the new initiatives are described more detailed in chapter 5.

New action plan on *Salmonella* in pigs

By Gudrun Sandø (gus@fvst.dk)

The first action plan against *Salmonella* in pigs and pork was drawn up in 1995. The fourth action plan expired by the end of 2013 and the fifth plan for the period 2014-2017 was adopted in December 2013. The plan was prepared by the Danish Agriculture & Food Council, the Danish Butcher Association, the Danish Meat and Research Institute, the National Food Institute, and the Danish Veterinary and Food Administration.

A technical working group and a steering committee are continuously following up on the action plan, and in 2012 the technical working group evaluated the ongoing fourth action plan and the development in the occurrence of *Salmonella* in herds, slaughter pigs, on carcasses and in humans. The evaluation showed, among other things, that the prevalence of infected slaughter pigs has increased markedly since 1998. Especially the prevalence of pigs infected with *S. Derby* but also the prevalence of pigs infected with *S. Typhimurium* including the monophasic types had increased. The target for the fourth action plan was to reduce the carcass prevalence to 1.0% *Salmonella* by the end of 2013. This target was not reached as the prevalence for 2013 was 1.3%.

The target for the new plan is to reach a prevalence of 1.0 % *Salmonella* at carcass level in 2014, and to maintain this low prevalence throughout the period (2014-2017).

The following issues have been examined and described in the report on the fifth action plan:

- The background for the increase of *Salmonella* in herds
- The significance of the increase in herds on the prevalence at carcass level
- Hygiene monitoring as a tool at the slaughter houses
- Transmission of infection from pigs, manure and offal

A substantial part of the increase in the proportion of positive herds is caused by live trade of infected pigs. Since 2010 all herds are categorized as *Salmonella* positive or negative, and purchasers are obliged to inform buyers on the status of the herd. However, *Salmonella* is not the only factor of interest when purchasing pigs, and farmers might choose to buy pigs from known *Salmonella*-infected herds because of other characteristics important for the pig producer. The typical herd size and structure has changed in the period since the first action plan was adopted as herds are becoming larger and more specialized. This leads to more purchases and a higher risk of introducing *Salmonella* into the herd. The use of finely ground feed has increased, and this can also lead to increased *Salmonella* prevalence, as it affects gut health.

An increased level of *Salmonella* in the herds leads to a higher risk of contaminated carcasses, but in the period 2001-2012 the slaughterhouses have been able to take the necessary hygiene precautions to manage the increased herd prevalence without increases in carcass prevalence.

To further strengthen and support the hygiene at the slaughterhouse, tools will be developed based on analysis of the registration of contamination at the slaughter line and results of the *E. coli* monitoring. The tools shall contribute to prevent high prevalence of *Salmonella* in the meat. Furthermore, the criterion for intensified *Salmonella* intervention at slaughterhouse level will be included in the yearly evaluation of the plan. The intensified *Salmonella* intervention is imposed on a slaughterhouse, if the 12-months prevalence of *Salmonella* in carcasses is 2 % or above in 4 of 6 consecutive months.

The plan contains recommendations on good practice that can reduce the risk of spread of infection from pigs to other livestock and crops, directed at farmers and growers. In 2014 the Danish Veterinary and Food Administration will also examine the prevalence of *Salmonella* in organically grown vegetables, as the use of manure as fertilizer, poses a risk for transmitting *Salmonella* to crops.

The development and target achievement will be evaluated on a yearly basis. The report on the fifth action plan is available at the Danish Veterinary and Food Administration's website www.fvst.dk (in Danish).

Trends and sources in human salmonellosis

Table A1. Estimated no. of reported human cases and percentage of cases per major food source, travel or outbreaks, 2011-2013

Source	2013		2012		2011	
	Estimated no. of reported cases (95 % credibility interval ^a)	Percentage of reported cases	Estimated no. of reported cases (95 % credibility interval ^a)	Percentage of reported cases	Estimated no. of reported cases (95 % credibility interval ^a)	Percentage of reported cases
Domestic pork	128 (86- 163)	11.3	110 (84-139)	8.0	86 (44-131)	7.4
Domestic beef	21 (4-58)	1.9	85 (72-100)	7.1	6 (0-28)	0.5
Domestic table eggs	17 (7-31)	1.5	15 (1-35)	1.3	11 (2-22)	1.0
Domestic broilers	0	0	0	0	0	0
Domestic ducks	9 (0-21)	0.8	10 (1-23)	0.8	19 (4-37)	1.7
Imported pork	30 (15-50)	2.6	3 (0-10)	0.2	65 (28-104)	5.6
Imported beef	22 (13-33)	2.0	11 (4-20)	0.9	33 (10-48)	2.8
Imported broilers	12 (2-26)	1.1	21 (3-47)	1.8	24 (2-51)	2.0
Imported turkey	7 (0-18)	0.6	13 (1-28)	1.1	13 (1-38)	1.1
Imported duck	No data	-	22 (13-34)	1.6	28 (8-54)	2.4
Travels	458 (445-471)	40.3	539 (527-550)	45.0	538 (531-546)	46.2
Unknown source	228 (191 - 264)	20.1	332 (293-369)	27.7	288 (252-330)	24.7
Outbreaks, unknown source ^b	204	18.0	37	4.3	55 ^b	4.3
Total	1,136		1,198		1,166	

a) The model is based on a Bayesian framework which gives 95 % credibility intervals.

b) Includes 134 *S. Enteritidis* cases, which were part of outbreaks happening abroad. 121 of these cases were from one travel-related outbreak due to eggs / egg products

Source: Danish Zoonosis Centre, National Food Institute

Human disease and outbreak data

Table A2. Zoonoses in humans, number of laboratory-confirmed cases, 2008-2013

Zoonotic pathogen	Incidence per 100,000 inhabitants		Reported no. of cases				
	2013	2013	2012	2011	2010	2009	2008
Bacteria							
<i>Brucella abortus/melitensis</i> ^{a,d}	-	4	2	7	6	7	8
<i>Campylobacter coli/jejuni</i> ^b	67.1	3,766	3,728	4,068	4,035	3,352	3,454
<i>Chlamydia psittaci</i> ^b	0.2	12	12	7	9	14	6
<i>Leptospira</i> spp. ^b	0.1	3	7	11	10	12	13
<i>Listeria monocytogenes</i> ^b	0.9	50	50	49	62	97	51
<i>Mycobacterium bovis</i> ^b	0	0	0	1	2	0	1
<i>Salmonella</i> total ^b	20.3	1,136	1,198	1,166	1,598	2,129	3,656
<i>S. Enteritidis</i> ^b	6.2	346	242	293	388	600	638
<i>S. Typhimurium</i> ^{b,c}	6.0	337	415	386	521	767	2,002
Other serotypes ^b	8.1	453	541	487	689	762	1,016
VTEC total ^b	3.3	186	190	224	184	165	159
O157	0.4	23	36	27	25	24	14
other or non-typeable	2.9	163	154	197	159	141	145
<i>Yersinia enterocolitica</i> ^b	6.2	345	291	224	192	238	330
Parasites							
<i>Cryptosporidium</i> spp. ^{a,d}	-	6	8	31	25	35	92
<i>Echinococcus multilocularis</i> ^{a,e}	-	3	7	4	1	0	0
<i>Echinococcus granulosus</i> ^{a,e}	-	9	20	31	10	11	5
<i>Trichinella</i> spp. ^{a,e}	-	0	0	0	0	0	0
Viruses							
<i>Lyssavirus</i> ^b	-	0	0	0	0	0	0

a) Not notifiable hence the incidence cannot be calculated.

b) Notifiable.

c) *S. Typhimurium* and monophasic *S. 1,4,[5],12:i:-* strains.

d) Data presented are from one laboratory (Statens Serum Institut) only, representing a proportion of the Danish population. The proportion of the population represented varies from year to year, thus results from different years are not comparable. Testing for these pathogens is carried out only if specifically requested on the submission form.

e) The cases were imported.

Source: Statens Serum Institut

Table A3. VTEC O-group distribution in humans^a, 2013

O-group	Number of episodes	O-group	Number of episodes
O157	23	O128	6
O103	22	O63	5
O146	21	O-rough	13
O26	18	Notification ^b	18
O145	8	Other O-groups or non-typeable	64
O117	6	Not confirmed	10
Continued in the next column		Total	214

a) All O-groups that resulted in five or more episodes are listed.

b) The cases are reported through the notification system, isolates not available for analysis.

Source: Statens Serum Institut

Table A4. Food- and waterborne disease outbreaks^a reported in the Food- and Waterborne Outbreak Database (FUD) (n=73), 2013

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
<i>Bacillus cereus</i>	28	-	Sports event	Buffet meal	1260
<i>Bacillus cereus</i>	7	-	Hotel	Buffet meal	1264
<i>Bacillus cereus</i>	11	-	Restaurant	Buffet meal	1292
<i>Bacillus cereus</i>	4	-	Restaurant	Composite meal	1286
<i>Bacillus cereus</i>	22	-	Restaurant	Composite meal	1281
<i>Bacillus cereus</i>	2	-	Restaurant	Composite meal	1295
<i>Bacillus cereus</i>	10	-	Restaurant	Composite meal	1310
<i>Bacillus cereus</i>	3	-	Restaurant	Composite meal	1340
<i>Campylobacter</i>	3	2	Private party	Chicken	1294
<i>Campylobacter</i>	4	4	Regional	Unknown	1313
<i>Clostridium perfringens</i>	2	-	Restaurant	Buffet meal	1267
<i>Clostridium perfringens</i>	8	-	Restaurant	Buffet meal	1299
<i>Clostridium perfringens</i>	40	1	Catering	Buffet meal	1300
<i>Clostridium perfringens</i>	425	-	Restaurant	Buffet meal	1325
<i>Clostridium perfringens</i>	22	-	Restaurant	Buffet meal	1335
<i>Clostridium perfringens</i>	28	-	Restaurant	Buffet meal	1338
<i>Clostridium perfringens</i>	20	-	Canteen	Composite meal	1256
<i>Clostridium perfringens</i>	33	-	Canteen	Composite meal	1270
<i>Clostridium perfringens</i>	19	-	Catering	Composite meal	1271
<i>Clostridium perfringens</i>	9	-	Restaurant	Composite meal	1279
<i>Clostridium perfringens</i>	14	-	Restaurant	Composite meal	1280
<i>Clostridium perfringens</i>	50	-	Restaurant	Composite meal	1304
<i>Clostridium perfringens</i>	4	-	Restaurant	Composite meal	1320
<i>Clostridium perfringens</i>	55	-	School	Composite meal	1321
<i>Clostridium perfringens</i>	9	-	Restaurant	Pork	1298
<i>Clostridium perfringens</i>	15	-	Production	Pork product	1287
Hepatitis A virus	72	40	National	Fruit (imp)	1259
Lectines	100	-	Shop	Beans, fresh (imp)	1240
Lectines	7	-	Canteen	Dried beans (imp)	1278
Lectines	8	-	Canteen	Dried beans (imp)	1312
Lectines	23	-	Canteen	Dried beans (imp)	1324
Lectines	2	-	Restaurant	Dried beans (imp)	1343
Norovirus	20	3	Private party	Buffet meal	1272
Norovirus	6	1	Restaurant	Buffet meal	1293
Norovirus	24	-	Hotel	Buffet meal	1315
Norovirus	7	-	Restaurant	Buffet meal	1319
Norovirus	15	-	Private party	Buffet meal	1329
Norovirus	25	-	Canteen	Buffet meal	1330
Norovirus	5	-	Canteen	Buffet meal	1331
Norovirus	58	4	Canteen	Buffet meal	1332
Norovirus	138	-	Canteen	Buffet meal	1333
Norovirus	10	2	Private party	Buffet meal	1339
Norovirus	47	-	Restaurant	Buffet meal	1341
Norovirus	14	-	Private party	Buffet meal	1342
Norovirus	23	-	Shop	Buffet meal	1351
Norovirus	53	8	Restaurant	Composite meal	1257
Norovirus	6	5	Shop	Composite meal	1268

Continued on the next page

Table A4. Food- and waterborne disease outbreaks^a reported in the Food- and Waterborne Outbreak Database (FUD), 2013 (Continued from previous page)

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
Norovirus	27	-	Restaurant	Composite meal	1282
Norovirus	20	-	Restaurant	Composite meal	1283
Norovirus	37		Restaurant	Composite meal	1284
Norovirus	16		Restaurant	Composite meal	1285
Norovirus	8		Restaurant	Composite meal	1288
Norovirus	11		Restaurant	Composite meal	1303
Norovirus	32		Catering	Composite meal	1311
Norovirus	10		Canteen	Composite meal	1326
Norovirus	4		Restaurant	Composite meal	1337
Norovirus	12		Producer	Fruit (imp)	1349
Norovirus	9		Restaurant	Oysters (imp)	1241
Norovirus	8	2	Restaurant	Oysters (imp)	1255
Norovirus	10	-	Restaurant	Oysters (imp)	1263
<i>S. Enteritidis</i>	12	12	National	Unknown	1327
<i>S. Mikawasima</i>	11	11	National	Unknown	1314
<i>S. Typhimurium</i> DT120, MLVA0007 ^b	22	22	Shop	Pork	1245
<i>S. Typhimurium</i> U312, MLVA0550	43	43	National	Pork	1254
<i>S. Typhimurium</i> , MLVA0008	7	7	Regional	Unknown	1305
<i>S. Typhimurium</i> , MLVA0642	6	6	Restaurant	Composite meal	1348
<i>S. Typhimurium</i> , MLVA0817	7	7	National	Unknown	1258
<i>Staphylococcus aureus</i>	80	-	Restaurant	Buffet meal	1275
<i>Staphylococcus aureus</i>	10	-	Restaurant	Buffet meal	1308
Outbreaks related to travel					
Hepatitis A virus	9	9	Travel (Egypt)	Unknown	1277
<i>S. Enteritidis</i> , MLVA0004, -0005, -0020	121	121	Travel (Turkey)	Eggs/egg products	1290
<i>S. Enteritidis</i> , MLVA0066	5	5	Travel (Egypt)	Unknown	1323
<i>S. Enteritidis</i> + <i>Shigella sonnei</i> + VTEC	16	8	Travel (Hotel, Turkey)	Buffet meal	1302
<i>Shigella sonnei</i>	5	5	Travel (Hotel, Tunisia)	Unknown	1307
Total	2,055	321			

Note: (imp)= imported product

a) In addition to the above mentioned outbreaks, one household outbreak (FUD 1318) with 2 persons involved was registered in 2013. The outbreak was suspected to have been caused by *C. perfringens* in a beef product containing herbs and spices cooked in the household not paying sufficient attention to the duration of the cooling process after preparation.

b) 12 of the 22 cases in FUD1245 had disease onset in 2012.

Source: Food- and Waterborne Outbreak Database (FUD)

Monitoring and surveillance data

Table A5. Top 15 (humans) serotype distribution (%) of *Salmonella* from humans, animals, carcasses and imported meat, 2013

Serotype	Human	Pig ^a	Pork ^b	Beef ^b	Layer ^c	Broiler ^c	Duck ^{c,d}	Turkey ^c	Imported meat (batch)		
	N=1,136	herds N=216	batches N=158	batches N=20	flocks N=4	flocks N=34	flocks N=17	flocks N=2	Pork ^e N=27	Broiler ^e N=11	Turkey ^e N=9
Enteritidis	30.5	0.5	0	0	25.0	0	0	0	0	0	0
Typhimurium	18.5	11.6	20.9	0	75.0	38.2	5.9	0	44.4	0	0
Typhimurium (Monophasic) ^f	11.2	18.1	22.8	5.0	0	8.8	0	0	25.9	0	0
Dublin	2.4	0	0	45.0	0	0	0	0	0	0	0
Newport	2.4	0	0	0	0	8.8	29.4	0	0	0	22.2
Stanley	2.0	0	0	0	0	0	0	0	0	0	0
Agona	1.9	0.5	0	0	0	2.9	0	0	0	0	0
Infantis	1.9	1.4	5.1	0	0	2.9	0	0	3.7	27.3	0
Virchow	1.5	0	0	0	0	0	0	0	0	0	0
Braenderup	1.1	0	0	0	0	0	0	0	0	0	0
Corvallis	1.1	0	0	0	0	0	0	0	0	0	0
Java	1.1	0	0	0	0	0	0	0	0	27.3	0
Kottbus	1.1	0	0	0	0	0	0	0	0	0	0
Mikawasima	1.1	0	0	0	0	0	0	0	0	0	0
4,5,12;b:-	1.1	0	0	0	0	0	0	0	0	0	0
Others	21.1	67.6	46.2	5.0	0	35.3	64.7	100	25.9	45.5	77.8
Unknown	0.3	0.5	5.1	45.0	0	2.9	0	0	0	0	0
Total	100	100	100	100	100	100	100	100	100	100	100

a) Isolates collected from coecum samples taken randomly at slaughter.

b) Sampling of beef and pork carcasses at slaughterhouses according to surveillance programmes (Tables A35 and A36).

c) Sampling in production flocks prior to slaughter according to surveillance programmes (Tables A32-A34).

d) Since 20/09/2013 these samples were no more taken

e) Case-by-case control of imported meat. For further information regarding case-by-case control programme, see Annual Report on Zoonoses in Denmark 2007.

f) Typhimurium (monophasic) includes the *Salmonella* strains 1,4,[5],12:i:-.

Source: Danish Veterinary and Food Administration, Statens Serum Institut, and National Food Institute

Table A6. Occurrence of Salmonella in the table egg production^a, 2004-2013

	Rearing period (parent flocks)		Adult period (parent flocks)		Pullet-rearing flocks		Table egg layer flocks	
	N	Positive	N	Positive	N	Positive	N	Positive
2004	9	2	9	0	368	1	641	5
2005	16	0	9	0	355	6	655	7
2006	17	0	11	0	289	2	565	2
2007	11	0	12	0	326	0	510	5
2008	10	0	6	0	258	1	508	4
2009	13	0	6	0	253	0	454	8
2010	15	0	9	0	225	0	455	8
2011	8	0	9	0	195	0	410	2
2012	9	0	8	0	197	1	359	3
2013	10	0	7	0	173	0	373	4 ^b

a) See Tables A31 and A33 for description of the surveillance programmes.

b) One flock positive with *S. Enteritidis* and three flocks positive with *S. Typhimurium*

Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration

Table A7. Occurrence of Salmonella in the table egg layer flocks sorted by type of production, 2004-2013

	Deep litter		Free range		Organic		Battery	
	N	Positive	N	Positive	N	Positive	N	Positive
2004	214	0	72	2	175	1	177	2
2005	217	3	70	0	178	0	175	4
2006	185	0	62	0	164	2	148	0
2007	155	2	56	0	146	2	146	1
2008	151	0	61	2	145	1	135	1
2009	133	1	78	0	130	4	110	3
2010	117	0	45	2	136	1	157	5
2011	109	0	40	0	130	1	131	1
2012	101	0	37	1	136	1	131	1
2013	108	0	37	1 ^a	137	3 ^b	94	0

a) One flock positive with *S. Enteritidis*

b) Two flocks positive with *S. Typhimurium* DT40 and one flock with double infection with *S. Typhimurium* DT40 and DT41

Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration

Table A8. Occurrence of *Salmonella* in the broiler production^a, 2004-2013

	Rearing period (parent flocks)		Adult period (parent flocks)		Broiler flocks		Slaughterhouse (flocks/batches)	
	N	Positive	N	Positive	N	Positive	N	Positive
2004	275	1	155 ^b	6	4,246	64	1,472	24
2005	214	0	185 ^b	0	4,034	87	1,174	27
2006	190	0	282	5	3,621	71	875 ^c	17
2007	152	0	258	3	3,703	60	884	10
2008	146	0	293	2	3,845	43	518 ^d	3
2009	140	0	225	4	3,767	35	375	3
2010	126	0	200	5	3,773	43	346	1
2011	114	0	213	0	3,795	47	306	0
2012	123	0	183	0	3,342	27	368	0
2013	128	0	152	1 ^e	3,498 ^f	34 ^g	288	0

a) See Tables A31 and A32 for description of the surveillance programmes.

b) In 2003-2005, only one flock per house was registered per year although there may have been more than one flock in the house, however all flocks were sampled according to the surveillance programme.

c) From 2006, data cover only samples taken following the *Salmonella* programme. Verification samples taken once a week by producers of poultry meat approved to market *Salmonella*-free poultry meat are not included. Collection of verification samples started in the middle of 2005.

d) From 2008, all AM positive flocks are heat treated at slaughter. Sampling is now carried out as verification of the AM results of the negative flocks.

e) *S. Typhimurium* DT41.

f) Data include 91 organic flocks.

g) *S. 4,5,12:i:-* (3), *S. Agona* (1), *S. Bareilly* (1), *S. Berta* (1), *S. Derby* (1), *S. Give* (5), *S. Infantis* (1), *S. Manhattan* (1), *S. Mbandaka* (1), *S. Newport* (3), *S. Senftenberg* (1), *S. Typhimurium* (13), *Salmonella spp* (1) and one double infection with *S. Anatum* and *S. Regent*.

Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration

Table A9. Occurrence of *Salmonella* in turkey and duck flocks^a, 2006-2013

	Duck flocks		Turkey flocks	
	N	% pos	N	% pos
2006	266	80.5	11	0
2007	-	-	13	0
2008	68	64.7	10	10.0
2009	85	63.5	15	0
2010	108	56.5	24	4.2
2011	95	58.1	38	2.6
2012	96	49.0	23	0
2013	64 ^c	20.3	56	3.6 ^b

a) See Table A34 for description of the surveillance programmes. The two major turkey and duck slaughterhouses in Denmark closed down in 2004 and 2007, respectively. Therefore, most commercially reared duck and turkey flocks are transported abroad for slaughter.

b) Two flocks were positive with *S. Indiana*.

c) Since 20/09/2013 these samples were no more taken.

Source: Danish Agriculture and Food Council

Table A10. Occurrence of *Campylobacter* in broiler flocks, 2004-2013

	Cloacal swabs at slaughter		Sock samples at farm	
	N	% pos	N	% pos
2004	5,157	27.0	-	-
2005	4,952	30.4	-	-
2006	4,522	30.8	-	-
2007	4,527	26.8	-	-
2008	4,950	26.3	-	-
2009	4,591	29.4	-	-
2010 ^a	-	-	3,132	16.5
2011	-	-	3,379	14.4
2012	-	-	3,376	11.6
2013	-	-	3,508	13.1

a) From 2010, results from broiler flocks are not comparable to results from previous years, as the sampling method changed from cloacal swabs at slaughter to boot swabs collected in the stable 7-10 days before slaughter according to Regulation No. 1469 of 15/12/2010 as amended.

Source: Danish Agriculture and Food Council, Danish Veterinary and Food Administration, and National Veterinary Institute

Table A11. Occurrence of *Campylobacter* in non-heat treated broiler meat at slaughter and retail^a, 2012-2013

		Chilled broiler meat (samples)					
		At slaughter		At retail			
		Denmark		Denmark		Import	
		N	% pos	N	% pos ^b	N	% pos ^b
2012	Conventional	1,044 ^d	21.5	-	-	-	-
	Organic/free-range	-	-	-	-	-	-
	In total	-	-	521	9.7	154	28.2
2013	Conventional	870 ^c	28.2	849	12.1	170	12.8
	Organic-free-range	93 ^c	90.3	35	42.9	38	71.1
	In total	-	-	884	17.8	208	31.9

a) Centrally coordinated studies (see section 10.4 for description). Limit of quantification: 10 cfu/g.

b) The prevalence is calculated as a mean of quarterly prevalences, except organic/free-range results.

c) Leg-skin samples only.

d) Included are 238 leg-skin samples, prevalence = 24,4%.

Source: National Food Institute

Table A12. Relative distribution of *Campylobacter* species (%) in broilers before slaughter^{a,b}, 2003-2013

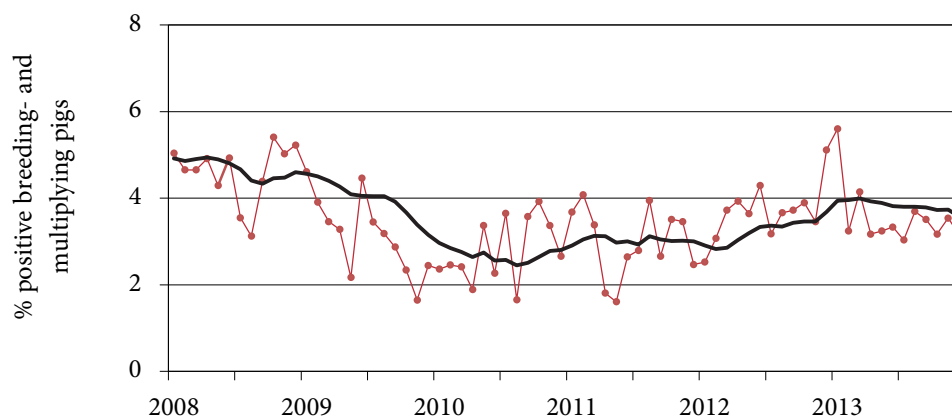
	N	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. upsaliensis</i>	NT/other
2004	101	94.1	5.9	0	0
2005	109	90.8	0	2.8	6.4
2006	113	92.0	7.1	0.9	0
2007	111	91.9	0.9	5.4	1.8
2008	100	90.5	0	2.8	6.6
2009	105	89.0	11.0	0	0
2010	-	-	-	-	-
2011	46	95.7	4.3	-	-
2012	44	93.2	6.8	-	-
2013	60	91.7	8.3	-	-

a) Samples were collected as part of the DANMAP programme and isolates were examined using conventional microbiological methods.

b) Since 2010, samples were only tested for *C. coli* and *C. jejuni*.

Source: National Food Institute

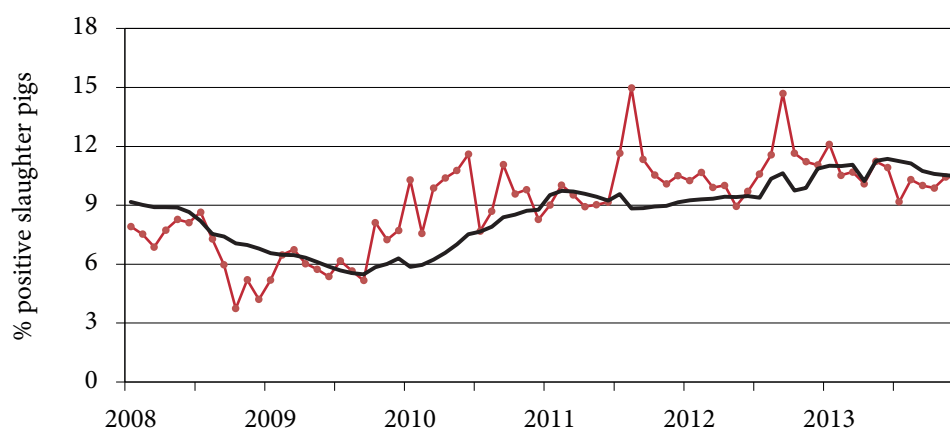
Figure A1. Serological surveillance of *Salmonella* in breeding and multiplying pigs^a based on monthly testing of blood samples, 2008-2013



a) For more information about the surveillance programme, see Table A37.

Source: Danish Agriculture and Food Council

Figure A2. Serological surveillance of *Salmonella* in slaughter pigs^a, 2008-2013. Percentage of seropositive meat juice samples (first sample per herd per month)^b



a) For more information about the surveillance programme, see Table A37.

b) The peak in late summer 2007, the very low level during 2008 and the peak in June 2012 were due to technical problems in the laboratory. Peaks in January 2010 and August 2011 were due to data transfer problems.

Source: Danish Agriculture and Food Council

Table A13. Occurrence of zoonotic pathogens in pigs and pork in Denmark, 2013

Zoonotic pathogen	Herds		Animals/Samples		
	N	Pos	N	Pos	% pos
At farm					
<i>Brucella abortus</i> ^a	-	-	36,453	0	-
<i>Leptospira</i> ^b	106	1	230	1	0.4
At slaughterhouse (slaughter pigs)					
<i>Salmonella</i> spp. ^{c,d}	6,914	343 ^e	-	-	-
<i>Salmonella</i> spp. ^{c,f} (slaughtering >50 pigs/month)	-	-	18,015	-	1.3 ^g
<i>Salmonella</i> spp. ^{c,f} (slaughtering 50 or less pigs/month)	-	-	593	-	0.8
<i>Salmonella</i> spp. ^{c,h}	-	-	894	216	24.2
<i>Trichinella</i> spp. ⁱ	-	-	19,586,503	0	-
<i>Mycobacterium bovis</i> ^j	-	-	18,549,103	0	-
<i>Echinococcus granulosus/multilocularis</i> ^j	-	-	18,549,103	0	-

a) Including samples from boars (examined at pre-entry, every 18 month, and prior to release from semen collection centres) (15,918 samples), samples collected in connection with export (20,404 samples), import (16 samples) or diagnostic samples (115 samples). 5-8 ml blood samples were analysed using either the SAT, RBT, CFT or ELISA methods.

b) Sampling is based on suspicion of leptospirosis due to increased abortions or other reproductive problems in a herd. Samples are investigated using immunofluorescence techniques.

c) See Table A37 for description of the *Salmonella* surveillance programme.

d) Data are from December 2013. Slaughter pig herds monitored using serological testing of meatjuice samples collected at slaughter.

e) Includes herds belonging to level 2 and 3 only.

f) Swab samples from four designated areas of the half-carcass were collected at the slaughterhouse after min. 12 h chilling. Sample size is 4x100 cm². Samples from five animals were pooled, except at slaughterhouses where 50 pigs or less were slaughtered per month, in which case samples were analysed individually.

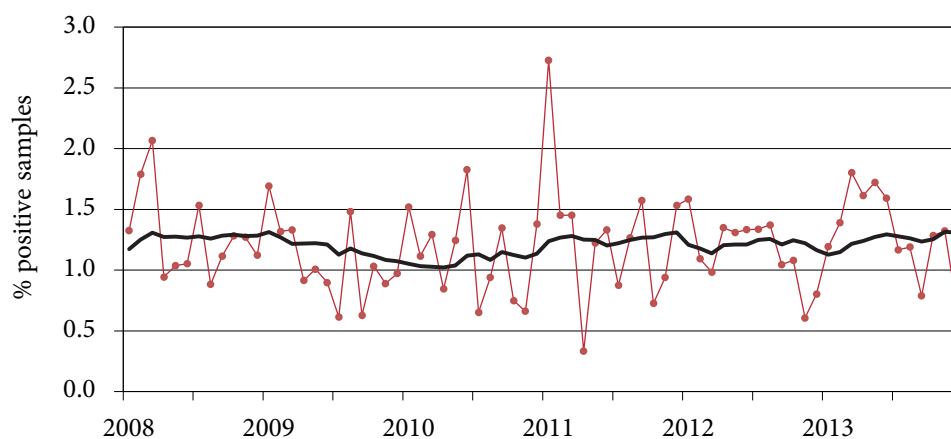
g) When estimating the prevalence of *Salmonella*, both the loss of sensitivity and the probability of more than one sample being positive in each pool are taken into consideration. A conversion factor has been determined on the basis of comparative studies, as described in Annual Report 2001.

h) Coecum samples are randomly collected from slaughter pigs at slaughter.

i) Samples collected from slaughter pigs at slaughter were examined using the method described in Directive 2075/2005/EEC. In 2007, Denmark achieved official status as region with negligible risk of *Trichinella*, according to EU Regulation (EC) No 2075/2005.

j) Slaughter pigs were examined by meat inspectors at slaughter.

Source: Danish Veterinary and Food Administration, National Veterinary Institute, and National Food Institute

Figure A3. *Salmonella* in pork, monitored at slaughterhouses^a, 2008-2013

a) For more information about the surveillance programme, see Table A36.

Source: Danish Veterinary and Food Administration

Table A14. Occurrence of zoonotic pathogens in cattle and beef in Denmark, 2013

Zoonotic pathogen	Herds		Animals/Samples		
	N	Pos	N	Pos	% pos
At farm					
<i>Brucella abortus</i> ^a	-	-	1,716	0	-
<i>Mycobacterium bovis</i> ^{b, c}	-	-	1,250	0	-
<i>Coxiella burnetii</i>	40 ^d	26	262 ^e	19	7.3
At slaughterhouse					
<i>Salmonella</i> spp. ^f (slaughtering >50 cattle/month)	-	-	5,620	-	0.3 ^g
<i>Salmonella</i> spp. ^f (slaughtering 50 or less cattle/month)	-	-	602	-	0.8
<i>Mycobacterium bovis</i> ^{b, h}	-	-	491,790	-	-
VTEC O157 ⁱ	135	17	-	-	-
<i>Echinococcus granulosus/multilocularis</i> ^h	-	-	491,790	0	-

a) Denmark has been declared officially brucellosis free since 1979. The last outbreak was recorded in 1962. Including samples from bulls (examined at pre-entry, every year, and prior to release from semen collection centres), samples collected in connection with export, import or diagnostic samples. 5-8 ml blood samples were analysed using either the SAT, RBT, CFT or ELISA methods.

b) Denmark has been declared officially tuberculosis free since 1980. The last case of TB in cattle was diagnosed in 1988.

c) Analysis using the tuberculin test. Including samples from bulls (examined at pre-entry, every year, and prior to release from semen collection centres) and samples collected in connection with export.

d) Bulk tank milk samples taken for diagnostic testing and analysed using an ELISA method.

e) Serum samples taken for diagnostic testing (76 samples, 18 pos), export (175 samples, 1pos) and breeding (11 samples, no pos) and analysed using an ELISA method. An additional 8 samples from placenta was analysed using the FISH method, none were positive.

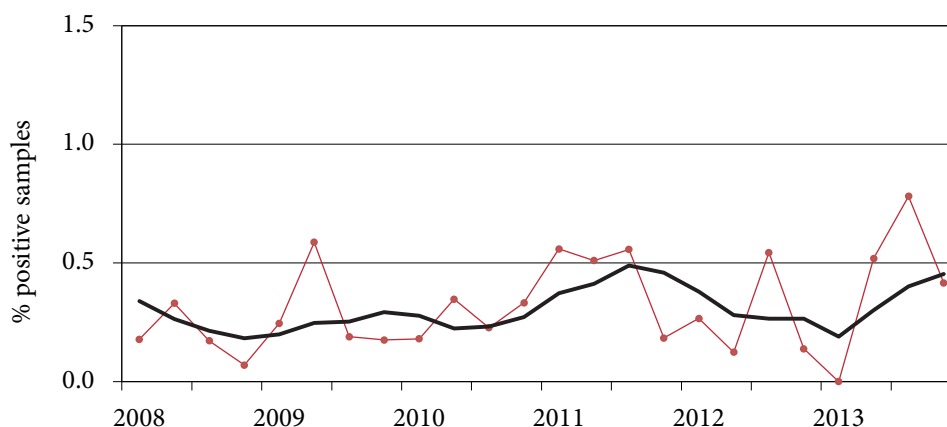
f) See Table A35 for description of the surveillance programme. Swab samples from four designated areas of the half-carcass were collected at the slaughterhouse after min. 12 h chilling. Sample size is 4x100 cm². Samples from five animals were pooled, except at slaughterhouses where 50 cattle or less were slaughtered per month, in which case samples were analysed individually.

g) When estimating the prevalence of *Salmonella*, both the loss of sensitivity and the probability of more than one sample being positive in each pool are taken into consideration. A conversion factor has been determined on the basis of comparative studies, as described in Annual Report 2001.

h) Slaughtered cattle were examined by the meat inspectors at slaughter.

i) Caecal content are tested from one animal per herd, collected at slaughter (DANMAP programme). A 25 g faecal sample from one slaughter calf per herd is examined using overnight enrichment, immunomagnetic separation method and plating on CT-SMAC plates for O157.

Source: Danish Veterinary and Food Administration, Danish Agriculture and Food Council, National Veterinary Institute, and National Food Institute

Figure A4. Salmonella in beef, monitored at slaughterhouses^a, 2008-2013

a) For more information about the surveillance programme, see Table A36.

Source: Danish Veterinary and Food Administration

Table A15. Cattle herds in the *S. Dublin* surveillance programme^a, January 2013

<i>Salmonella</i> Dublin level		Non-milk producing herds		Milk producing herds	
		N	% pos	N	% pos
Level 1	On the basis of milk samples	-	-	3,288	92.1
	On the basis of blood samples	14,060	95.5	-	-
Total	Probably <i>Salmonella</i> Dublin free	14,060	95.5	3,288	92.1
Level 2	Titer high in blood- or milk samples	219	1.5	259	7.3
	Contact with herds in level 2 or 3	316	2.1	15	0.4
	Other causes	124	0.8	5	0.1
Level 3	Salmonellosis, official supervision	0	-	4	0.1
	Total	Non <i>Salmonella</i> Dublin free	659	4.5	283
Total number of herds sampled		14,719		3,571	

a) See Table A35 for description of the surveillance programme.
Source: Knowledge Centre for Agriculture, Cattle

Table A16. Relative distribution of *Campylobacter* (%) in pig and cattle herds^a, 2004-2013

	Pigs				Cattle			
	N	<i>C. coli</i>	<i>C. jejuni</i>	other/unknown	N	<i>C. coli</i>	<i>C. jejuni</i>	other/unknown
2004	152	98.0	1.3	0.7	43	2.3	97.7	0
2005	158	97.5	2.5	0	31	0	100	0
2006	154	97.4	2.6	0	99	15.2	84.8	0
2007	205	97.6	2.4	0	93	4.3	95.7	0
2008 ^b	198	97.5	2.5	-	103	4.9	95.1	-
2009	160	85.6	14.4	-	110	2.7	97.3	-
2010	-	-	-	-	-	-	-	-
2011	168	96.4	3.6	-	123	8.1	91.9	-
2012	160	88.1	11.9	-	113	2.7	97.3	-
2013	0	-	-	-	95	0	100	-

a) Samples were collected as part of the DANMAP programme. Caecal content was tested from one animal per herd. Data does not reflect the national prevalence of *Campylobacter* in pigs and cattle.

b) Since 2008, samples are only tested for *C. coli* and *C. jejuni*.

Source: National Food Institute

Table A17 Results from the intensified control of *Salmonella* and *Campylobacter* in fresh meat based on a case-by-case risk assessment, 2013

		Batches tested	No. of batches positive	No. of batches deemed unsafe based on a risk assessment	Batches deemed unsafe based on Microbiological criteria ^a	Mean prevalence in positive batches ^b	Mean relative human risk in positive batches ^c
<i>Campylobacter</i>							
Danish	Broiler	121	33	1	-	32.0	2.43
Imported	Broiler	149	63	6	-	34.4	4.39
<i>Salmonella</i>							
Danish	Pork	231	29	5	-	11.7	5.74
	Broiler	100	0	0	-	-	-
Imported	Pork	230	24	3	-	7.4	4.85
	Broiler	182	16	2	5	16.5	7.39
	Turkey	58	8	1	0	16.3	101.93

a) Regulation (EC) No 1086/2011.

b) The *Salmonella* prevalence in each batch is based on the proportion of positive pooled samples (12 pools per batch) and number of subsamples per pool. Only results for batches subjected to risk assessment have been included.

c) Calculated as the risk relative to a batch of the same size with a mean prevalence (weighted average in Danish and imported meat) of *Campylobacter* or of a *Salmonella* type with an average impact to cause human infection.

Source: Danish Veterinary and Food Administration, and National Food Institute

Table A18. Feed business operators own sampling of *Salmonella* in compound feeds, feed processing and feed material (batch-based data), 2011-2013

	2013		2012		2011	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control) ^a :						
Ordinary inspections - clean zone	7,132	2 ^d	7,105	11	7,105	11
Ordinary inspections - unclean zone	577	88 ^e	736	82	736	82
Compound feed, farm animals	375	0	316	0	316	0
Feed materials, farm animals ^b	1,295	11 ^f	1,369	25	1,369	25
Transport vehicles, clean zone/hygiene samples ^c	973	4 ^g	884	0	884	0
Transport vehicles, clean zone/hygiene samples ^c	255	0	259	0	259	0

a) Presence of *Salmonella* in compound feed is indirectly monitored by environmental samples collected during feed processing.

b) Predominantly soy bean meal and rapeseed cake.

c) Samples from transport vehicles (hygiene samples) prior to loading of feed compounds.

d) S. Idikan, S. 4,5,12:i:-.

e) Most of the findings were from one feed producer. The raw material from this feed producer is most frequently heat treated at other feed production plants during the production of final feed. S. Agona, S. Derby, S. Havana, S. Infantis, S. Livingstone, S. Montevideo, S. Mbandaka, S. Putten, S. Rissen, S. 4.5.12:i-, S. 13m23:1.w.

f) S. Ealing, S. Havana, S. Idikan, S. Infantis, S. Mbandaka, S. Minnesota, S. Schwarzengrund, S. Senftenberg, S. Worthington.

g) S. Infantis, S. Kottbus, S. Schwarzengrund, S. Typhimurium.

Source: Danish Veterinary and Food Administration and the feed business operators.

Table A19. Control of Salmonella in compound feeds, feed processing and feed material (batch-based data), 2010-2013

	2013		2012		2011		2010	
	N	Positive	N	Positive	N	Positive	N	Positive
Feed processing plants (process control) ^a :								
Ordinary inspections ^c	333	7 ^d	311	11	377	12	558	5
Feed materials, farm animals ^b	99	2 ^e	99	4	68	3	379	24

a) See footnote a) to Table A20. Companies are sampled one to four times per year.

b) See footnote b) to Table A20.

c) Primarily findings of *Salmonella* in the dirty zone.

d) *S. Derby* (clean), *S. Infantis* (dirty and clean), *S. Montevideo* (dirty), *S. Putten* (clean), *S. Rissen* (clean), *S. Typhimurium* DT41 (dirty)

e) *S. Putten*.

Source: Danish Veterinary and Food Administration.

Table A20 Salmonella in three categories of meat and bone meal by-products not intended for human consumption^a, 2013

Category of processing plant		Own-check samples		Product samples	
		N	Positive	N	Positive
1+2	By-products of this material cannot be used for feeding purposes	255	26	52	0
2	By-product of this material may be used for feed for fur animals	65	0	17	0
3	By-products from healthy animals slaughtered in a slaughterhouse. Products of these may be used for petfood ^b and for feed for fur animals	445	3	799	20
Total		765	29	868	20

a) Regulation No. 1774 of 03/10/2002.

b) For cats and dogs. Only by-products from pigs are used in this pet food.

Source: Danish Veterinary and Food Administration

Table A21. Pathogens in batches^a of ready-to-eat vegetables, herbs and fruits^b, 2013

Type of sample	<i>Salmonella</i>		<i>Campylobacter</i>		<i>E. coli</i> (>100 cfu/g)	
	N	Pos	N	Pos	N	Pos ^j
Vegetables						
Baby corn	17	2 ^g	17	0	17	7 ^k
Cucumber	7	0	7	0	7	0
Pepper	25	0	25	0	25	0
Salad	89	1 ^h	89	0	89	2 ^l
Sprouts	39	0	39	1 ⁱ	39	6 ^m
Sugar peas	21	0	21	0	21	0
Tomato	21	0	21	0	21	0
Other vegetables ^c	17	0	17	0	17	0
Herbs						
Chives	5	0	5	0	5	0
Parsley	10	0	10	0	10	2 ⁿ
Rosemary	2	0	2	0	2	0
Sage	1	0	1	0	1	0
Spearmint	2	0	2	0	2	0
Spring onions	12	0	12	0	12	0
Tarragon	2	0	2	0	2	0
Other herbs ^d	11	0	11	0	11	0
Fruit and berries						
Apples and pears	9	0	9	0	9	0
Grapes	11	0	11	0	11	0
Raspberries	14	0	14	0	14	0
Strawberries	16	0	16	0	16	1 ^o
Other berries ^e	23	0	23	0	23	0
Other fruits ^f	6	0	6	0	6	0
Total	360	3	360	1	360	18

a) Five samples per batch

b) Centrally coordinated study to control and investigate *Salmonella*, *Campylobacter* and *E. coli* in Danish and imported ready-to-eat vegetables, sprouts and herbs.

c) including cauliflower, cabbage, broccoli, beans, zucchini and onions.

d) including basil, thyme and dill.

e) including cranberries, blue- and blackberries.

f) including plums, cherries, persimmon and carambole.

g) *S. Weltevreden* and *S. Thompson* in two different batches from Thailand.

h) *S. Napoli* in one batch of leafy green from Italy.

i) One batch of alfalfa sprouts sprouted in Denmark.

j) Batches with >100 cfu/g in one or more samples.

k) Six batches from Thailand and one batch from Kenya.

l) Two batches from Spain and Germany, respectively.

m) Four batches of bean sprouts from Denmark and one batch of beet root sprouts and one batch of onion sprouts from the Netherlands.

n) Two batches of parsley from Italy.

o) One batch of strawberries from Egypt.

Source: Danish Veterinary and Food Administration

Table A22. Occurrence of zoonotic pathogens in pets and zoo animals in Denmark^a, 2013

Zoonotic pathogen	Pet animals						Zoo animals			
	Dogs		Cats		Others		Mammals & reptiles		Birds	
	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
<i>Salmonella</i> spp.	3	0	2	0	5 ^b	1 ^c	1 ^d	0	2 ^e	0
<i>Campylobacter</i> spp.	0	-	0	-	0	-	0	-	0	-
<i>Brucella canis/abortus</i> ^f	0	-	0	-	0	-	0	-	0	-
<i>Chlamydia psittaci</i>	0	-	0	-	7 ^g	2 ^h	0	-	0	-
<i>Cryptosporidium</i> spp.	4	1	1	0	0	0	3 ⁱ	0	0	-
<i>Lyssavirus</i> (classical)	1	0	0	-	0	-	0	-	0	-
European Bat <i>Lyssavirus</i>	1	0	0	-	0	-	0	-	0	-

a) All samples are analysed based on suspicion of disease, and does not reflect the country prevalence

b) One budgerigar, two red-footed tortoises, one leopard gecko and one corn snake.

c) One leopard gecko tested positive for typh II 47:b:enxz15.

d) One vincunja.

e) One oestrich, one red-billed quelea.

f) Results based on serological testing of blood samples.

g) Four psittacine birds, three parots.

h) Two parrots.

i) Two ring-tailed lemur, one southern pig-tailed macaque.

Source: National Veterinary Institute, and Danish Veterinary and Food Administration

Table A23. Occurrence of zoonotic pathogens in wild and farmed wildlife in Denmark^a, 2013

Zoonotic pathogen	Farmed wildlife				Wildlife			
	Wild boar		Minks & chincillas		Mammals		Birds	
	N	Pos	N	Pos	N	Pos	N	Pos
<i>Salmonella</i> spp.	0	-	31	1 ^b	18 ^c	3 ^d	0	-
<i>Campylobacter</i> spp.	0	-	3	0	0	-	0	-
<i>Chlamydia psittaci</i>	0	-	0	-	0	-	100 ^m	7
<i>Cryptosporidium</i> spp.	0	-	5	5	45 ^e	12 ^f	3 ^g	1 ^h
<i>Echinococcus multilocularis</i>	0	-	0	-	417 ⁱ	4 ^j	0	-
<i>Trichinella</i> spp. ⁿ	572	0	0	-	687 ^k	0	0	-
<i>Lyssavirus</i> (classical)	0	-	0	-	14 ^l	0	0	-
European Bat <i>Lyssavirus</i>	0	-	0	-	14 ^l	0	0	-

a) All samples are analysed based on suspicion of disease and does not reflect the country prevalence, except for animals analysed for *Echinococcus multilocularis*. These animals are collected as part of survey.

b) *S. Typhimurium*.

c) 1 roe deer, 5 hedgehogs, 12 badgers.

d) 2 Hedgehogs with *S. Enteritidis* and 1 badger with *S. Dublin*.

e) 1 hare, 5 minks, 14 racoon dogs, 8 otters, 2 wolves, 13 roe deer, 1 hedgehog, 1 squirrel.

f) 5 minks, 2 racoon dogs, 2 roe deer, 1 wolf, 1 hedgehog, 1 squirrel.

g) 2 sparrowhawks and 1 sea eagle.

h) 1 sea eagle.

i) Results from a survey. 305 foxes, 26 badgers, 85 racoon dogs, 1 racoon.

j) Foxes.

k) 1 dolphin, 50 badgers, 61 minks, 158 racoon dogs, 2 coatis, 386 foxes, 4 racoons, 3 porpoises, 1 gray seal, 21 harbour seals.

l) 3 foxes, 1 marten, 10 bats.

m) Mallards.

n) In 2007, Denmark achieved official status as region with negligible risk of *Trichinella*, according to EU regulation (EC) No 2075/2005.

Source: National Veterinary Institute, and Danish Veterinary and Food Administration

Table A24. The Bovine Spongiform Encephalopathy (BSE) surveillance programme^a for cattle, 2013

Type of surveillance	N ^b	Positive
Active surveillance		
Healthy slaughtered animals (>48 months)	3,342	0
Risk categories:		
Emergency slaughters (>48 months)	1,114	0
Slaughterhouse antemortem inspection revealed suspicion or signs of disease (>48 months)	0	-
Fallen stock (>48 months)	19,019	0
Animals from herds under restriction	0	-
Passive surveillance		
Animals suspected of having clinical BSE	2	0
Total	23,477	0

a) According to the EU Regulation (EC) 999/2001 as amended, Commission Decision 2009/719/EC as amended and Danish Order no. 878 of 01/07/2013 as amended.

b) Samples (brain stem material) are tested using a IDEXX technique or Prionics-Check PrioStrip. Confirmatory testing is carried out using Western blot (definitive diagnosis if positive case), else with histopathology or immunohistochemistry. Further confirmation on autolysed material is performed at the European Union TSE reference laboratory.

Source: National Veterinary Institute, and Danish Veterinary and Food Administration

Table A25. The Transmissible Spongiform Encephalopathy (TSE) surveillance programme^a for sheep and goats, 2013

Type of Surveillance	N ^b	Positive
Active surveillance		
Fallen stock (>18 months)	637	0
Animals from herds under restriction	0	-
Passive surveillance		
Animals suspected of having clinical TSE	0	-
Total	637	0

a) According to the EU Regulation (EC) 999/2001 as amended, Commission Decision 2009/719/EC as amended and Danish Order no. 1288 of 20/12/2011 as amended.

b) Samples (brain stem material) are tested using a IDEXX technique or Prionics-Check PrioStrip. Confirmatory testing is carried out using Western blot (definitive diagnosis if positive case), else with histopathology or immunohistochemistry. Further confirmation on autolysed material is performed at the European Union TSE reference laboratory.

Source: National Veterinary Institute, and Danish Veterinary and Food Administration

Table A26. Distribution^a (%) of prion protein genotype of sheep randomly selected, 2013

	Genotype	Sheep n=100
NSP 1	ARR/ARR	26
NSP2	ARR/AHQ, ARR/ARH, ARR/ARQ	16
NSP 3 (ARQ/ARQ)	ARQ/ARQ	36
NSP 3 (Other)	AHQ/AHQ, AHQ/ARH, AHQ/ARQ, ARH/ARQ, ARH/ARH, ARQ/ARH ARH/AHQ, ARQ/AHQ	16
NSP4	ARR/VRQ	1
NSP5	ARH/VRQ, ARQ/VRQ, VRQ/VRQ, AHQ/VRQ	5
Total		100

a) The genotypes were grouped in the NSP classification system according to their different susceptibility:

NSP 1: Genetically most resistant, NSP 2: Genetically resistant, NSP 3: Genetically little resistance,

NSP 4: Genetically susceptible, and NSP 5: Genetically highly susceptible.

Source: National Veterinary Institute, and Danish Veterinary and Food Administration

Table A27. Centrally coordinated studies conducted in 2013

Title of project	No. of samples	Pathogen surveyed	Further information
DANMAP, antimicrobial resistance in Danish and imported broiler, beef and pork	1,000	<i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Escherichia coli</i> , <i>Enterococcus faesium</i> , <i>Enterococcus faecalis</i>	Results are presented in the DANMAP Report 2013
ESC ^a in pigs at slaughter	400	<i>Escherichia coli</i>	Data are being processed
<i>Campylobacter</i> spp. in fresh, chilled Danish broiler meat	1,200	<i>Campylobacter</i> spp.	Appendix Table A11
<i>Campylobacter</i> spp. in fresh, chilled Danish and imported broiler meat	1,000	<i>Campylobacter</i> spp.	Appendix Table A11
Intensified control for <i>Salmonella</i> spp. and <i>Campylobacter</i> spp. in fresh Danish and imported meat	851 ^b	<i>Salmonella</i> spp., <i>Campylobacter</i> spp.	Appendix Table A17
<i>Salmonella</i> spp. - antibiotic resistance in slaughter pigs	960	<i>Salmonella</i> spp.	Data are being processed
<i>Salmonella</i> spp. in table eggs - trade	100	<i>Salmonella</i> spp.	Results are published on the DFVA website www.fvst.dk (in Danish)
<i>Salmonella</i> spp. and <i>Escherichia coli</i> in raw, frozen scallop from Greenland	50	<i>Salmonella</i> spp., <i>Escherichia coli</i>	Data are being processed
<i>Listeria monocytogenes</i> , <i>Salmonella</i> spp., <i>Escherichia coli</i> , staphylococci in fish goods from Greenland	100	<i>Salmonella</i> spp., <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , staphylococci	Data are being processed
<i>Listeria monocytogenes</i> in cold smoked fish products	1,000	<i>Listeria monocytogenes</i>	Appendix Table A28
Microbiological classification of mussel production areas in Denmark	100	<i>Salmonella</i> spp., <i>Escherichia coli</i>	Results are published on the DFVA website www.fvst.dk (in Danish)
Pathogens in Danish and imported ready-to-eat vegetables	1,000	<i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Escherichia coli</i>	Appendix Table A21
<i>Salmonella</i> in herbs (EU 699/2009)	100	<i>Salmonella</i> spp.	Data are being processed
<i>Salmonella</i> and <i>E. coli</i> - meat preparation - at retail	500	<i>Salmonella</i> spp., <i>Escherichia coli</i>	Data are being processed
<i>Salmonella</i> and <i>E. coli</i> in meat products for heat treatment	500	<i>Salmonella</i> spp., <i>Escherichia coli</i>	Data are being processed
Official verification of microbiological criteria (EU 2073/2005)	350	<i>Salmonella</i> spp., <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , total viable count	Data are being processed
<i>Salmonella</i> in animal feed	525	<i>Salmonella</i> spp.	Results are published on the DFVA website www.fvst.dk (in Danish)
<i>Salmonella</i> Dublin in beef	300	<i>Salmonella</i> spp., <i>Escherichia coli</i>	Data are being processed
Import control - Fish	300	<i>Salmonella</i> spp., <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , staphylococci	Results are published on the DFVA website www.fvst.dk (in Danish)
<i>Campylobacter</i> in organic poultry products	100	<i>Campylobacter</i> spp.	Data are being processed
<i>Campylobacter</i> - slaughterhygiene - poultry	1,900	<i>Campylobacter</i> spp., <i>Escherichia coli</i>	Data are being processed

a) ESC: Expanded-Spectrum Cephalosporin-Resistant Strains.

b) Batches.

Source: Danish Veterinary and Food Administration, and National Food Institute

Table A28. *Listeria monocytogenes* in Danish produced ready-to-eat foods^a, 2013

Food category	Sampling place	Samples analysed by a qualitative method ^b				Samples analysed by a quantitative method			
		Batches ^c		Single samples		Batches ^c		Single samples	
		N	Pos	N	Pos	N	Pos	N	Pos
Cheese, RTE	At processing	35	0	0	-	22	0	0	-
	At retail	0	-	0	-	0	-	1	0
Milk and dairy products, RTE	At processing	22	0	4	0	29	0	0	-
	At retail	0	-	0	-	0	-	0	-
Products made from broiler meat, RTE	At processing	1	0	0	-	1	0	0	-
	At retail	0	-	0	-	0	-	0	-
Products made from other poultry meat, RTE	At processing	0	-	0	-	0	-	0	-
	At retail	0	-	0	-	0	-	0	-
Products made from pork, RTE	At processing	21	2	11	-	3	0	0	-
	At retail	0	-	1	-	0	0	3	0
Products made from beef, RTE	At processing	5	2	6	0	2	0	0	0
	At retail	0	-	0	-	0	-	2	0
Fruit, RTE	At processing	0	-	0	-	0	-	0	-
	At retail	0	-	0	-	0	-	0	-
Vegetables, RTE	At processing	20	0	0	-	1	0	0	-
	At retail	0	-	0	-	0	-	0	-
Fish and Fishery products, RTE	At processing	4	2	16	0	16	0	0	-
	At retail	0	-	0	-	0	-	0	-
Shellfish and products thereof, RTE	At processing	1	0	2	-	8	0	0	-
	At retail	0	-	0	-	0	-	0	-
Other RTE products	At processing	14	0	2	0	26	0	0	-
	At retail	4	0	0	-	7	-		-

a) Samples are collected by the local food control offices according to EU Regulation (EC) No 2073/2005.

b) *Listeria monocytogenes* present in a 25 g sample of the product.

c) 5 samples from each batch, analysed individually.

Source: Danish Veterinary and Food Administration

Monitoring and surveillance programmes

Table A29. Overview of notifiable and non-notifiable human diseases presented in this report, 2013

Patogen	Notifiable	Notification route
Bacteria		
<i>Brucella</i> spp.	no	-
<i>Campylobacter</i> spp.	1979 ^a	Laboratory ^b
<i>Chlamydomphila psittaci</i> (Ornithosis)	1980 ^a	Physician ^c
<i>Listeria monocytogenes</i>	1993 ^a	Physician
<i>Leptospira</i> spp.	1980 ^a	Physician
<i>Mycobacterium bovis/ tuberculosis</i>	1905 ^a	Physician (and laboratory ^d)
<i>Coxiella burnetii</i>	no	-
<i>Salmonella</i> spp.	1979 ^a	Laboratory
VTEC	2000 ^a	Physician and laboratory
<i>Yersinia enterocolitica</i>	1979 ^a	Laboratory
Parasites		
<i>Cryptosporidium</i> spp.	no	-
<i>Echinococcus multilocularis</i>	no	-
<i>Echinococcus granulosus</i>	no	-
<i>Toxoplasma gondii</i>	no	-
<i>Trichinella</i> spp.	no	-
Viruses		
<i>Lyssavirus</i> (Rabies)	1964 ^a	Physician (via telephone)
Prions		
BSE/Creutzfeld Jacob	1997 ^a	Physician

a) Danish order no. 277 of 14/04/2000. Cases must be notified to Statens Serum Institut.

b) The regional microbiological laboratories report confirmed cases.

c) The physician report individually notifiable infections.

d) The laboratories voluntarily report confirmed cases.

Source: Statens Serum Institut

Table A30. Overview of notifiable and non-notifiable animal diseases presented in this report, 2013

Patogen	Notifiable	EU legislation	Danish legislation
Bacteria			
<i>Brucella</i> spp.	1920 ^a		
Cattle	OBF in 1979 ^b	Decision 2003/467/EC	Order no 305 of 3/5 2000
Sheep and goats	ObmF in 1995 ^c	Decision 2003/467/EC	Order no. 739 of 21/8 2001
Pigs	No cases since 1999	Directive 2003/99/EC	Order no. 205 of 28/3 2008
<i>Campylobacter</i> spp.	no	-	-
<i>Chlamydomphila psittaci</i>	1920	-	
Birds and poultry			Order no. 871 of 25/8 2011
<i>Listeria monocytogenes</i>	no	-	-
<i>Leptospira</i> spp. (only in production animals)	2003	-	Act no. 432 of 09/06/2004
<i>Mycobacterium bovis/tuberculosis</i>	1920 ^a		
Cattle	OTF in 1980 ^d	Decision 2003/467/EC	Order no. 1417 of 11/12 2007
<i>Coxiella burnetii</i>	2005	-	Act no. 432 of 09/06/2004
<i>Salmonella</i> spp.	1993 ^e	-	
Cattle			Order no. 954 of 10/07/2013
Swine			Order no. 404 of 08/05/2012
Poultry			Order no. 1512 of 13/12/2013
VTEC	no	-	-
<i>Yersinia enterocolitica</i>	no	-	-
Parasites			
<i>Cryptosporidium</i> spp.	no	-	-
<i>Echinococcus multilocularis</i>	2004	Council Directive 64/433/EC	Act no. 432 of 09/06/2004
<i>Echinococcus granulosus</i>	1993	Council Directive 64/433/EC	Act no. 432 of 09/06/2004
<i>Toxoplasma gondii</i>	no	-	-
<i>Trichinella</i> spp.	1920 ^a	Regulation 2075/2005/EC	Order no. 412 of 28/05/2008
Viruses			
<i>Lyssavirus</i> (Rabies)	1920	-	Order no. 330 of 14/04/2011
Prions			
TSE			
Sheep and goats	yes	Regulation 999/2001/EC (as amended)	Order no. 1288 of 20/12/2011
BSE			
Cattle	yes ^f	Regulation 999/2001/EC (as amended)	Order no. 878 of 01/07/2013 (as amended)

a) Clinical cases, observations during the meat inspection at the slaughterhouse, positive blood samples or finding of agents are notifiable.

b) Officially Brucellosis Free (OBF) according to Council Directive 64/432/EC as amended and Commission Decision 2003/467/EC. No cases in since 1962.

c) Officially *B. melitensis* Free (ObmF) according to Council Directive 91/68/EC and Commission Decision 2003/467/EC. Never detected in sheep or goat.

d) Officially Tuberculosis Free (OTF) according to Council Directive 64/432/EC as amended and Regulation (EC) No 1226/2002, and Commission Decision 2003/467/EC. No cases in since 1988 or in deer since 1994.

e) Only clinical cases notifiable.

f) Denmark was recognized as a country with negligible risk for BSE at OIE general session in May 2011.

Source: Danish Veterinary and Food Administration

Table A31. Salmonella surveillance programme for the rearing flocks and adult flocks of the grandparent and parent generation of the broiler and table egg production, 2013

Time	Samples taken	Material	Material
Rearing flocks		<i>Grandparent generation</i>	<i>Parent generation</i>
Day-old ^{a,b}	Per delivery	5 transport crates from one delivery: crate liners (>1m ² in total) or swab samples (>1m ² in total). Analysed as one pool	5 transport crates from one delivery: crate liners (>1m ² in total) or swab samples (>1m ² in total). Analysed as one pool
1st & 2nd week ^{b,c}	Per unit	-	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g
4th week ^{a,c}	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g
8th week ^{b,c}	Per unit	2 pairs of boot swabs (analysed as one pooled sample). Cage birds: 60x1g samples of fresh droppings. Analysed as one pool	2 pairs of boot swabs (analysed as one pooled sample). Cage birds: 60x1g samples of fresh droppings. Analysed as one pool
2 weeks prior to moving ^{a,d}	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g
Adult flocks		<i>Grandparent generation</i>	<i>Parent generation</i>
Every two weeks ^{a,b} (Every 16th week) ^e	Per flock	Hatcher basket liners from 5 baskets (>1m ² in total) or 10g of broken eggshells from each of 25 hatcher baskets (reduced to 25g sub-sample). Analysed as one pool	Hatcher basket liners from 5 baskets (>1m ² in total) or 10g of broken eggshells from each of 25 hatcher baskets (reduced to 25g sub-sample). Analysed as one pool
After each hatch ^b	Per hatch	Wet dust samples. Up to four hatchers of the same flock can be pooled	Wet dust samples. Up to four hatchers of the same flock can be pooled
Every week ^b	Per unit	-	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g
0-4 weeks after moving, 8-0 weeks before slaughter ^{b,d}	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g
After positive findings ^{b,d}	Per unit	5 pairs of boot swabs (analysed as two pooled samples), 2 dust samples (250 ml) and 5 birds (analysed for antimicrobial substances)	5 pairs of boot swabs (analysed as two pooled samples), 2 dust samples (250 ml) and 5 birds (analysed for antimicrobial substances)

a) Sampling requirements set out by Regulation (EC) No 2160/2003.

b) Samples collected by the food business operator.

c) Sampling requirements set out by Order no 952 of 10/07/2013.

d) Samples collected by the Danish Veterinary and Food Administration

e) When eggs from a flock exceed the capacity of one incubator, each incubator should be sampled as described.

Source: Danish Veterinary and Food Administration

Table A32. Salmonella and Campylobacter surveillance programme for the broiler flocks, 2013

Time	Samples taken	Material
<i>Salmonella</i>		
15 - 21 days before slaughter ^{a,b,c,d}	Per flock	5 pairs of boot swabs. Analysed individually
7 - 10 days before slaughter ^{b,e}	Per flock	5 pairs of boot swabs. Analysed individually
After slaughter ^{b,c}	Per batch	300x1g neck skin, analysed in pools of max. 60 grams. Sampling size depends on whether the slaughterhouse slaughters only AM-negative flocks or AM-negative as well as AM-positive flocks
<i>Campylobacter</i>		
7 - 10 days before slaughter ^{b,e}	Per flock	1 pair of boot swabs

a) Sampling requirements set out by Regulation (EC) 2160/2003.

b) Sampling requirements set out by Order no. 1512 of 13/12/2013 replacing 1105 of 18/09/2013 replacing 1462 of 16/12/2009.

c) Samples collected by the food business operator.

d) Once a year, one pair of socks is collected by the Danish Veterinary and Food Administration.

e) Samples are collected by a representative of the slaughterhouse, laboratory or the Danish Veterinary and Food Administration.

Source: Danish Veterinary and Food Administration

Table A33. Salmonella surveillance programme for the pullet-rearing, table egg layer and barnyard/hobby flocks in the table egg production, 2012

Time	Samples taken	Material
Pullet-rearing		
Day-old ^{a,d}	Per delivery	5 transport crates from one delivery: Crate liner (> 1 m ² in total) or swab samples (> 1 m ² in total) (Analysed as one pooled sample)
4 weeks old ^{b,d}	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples of 60 gram
2 weeks before moving ^{a,c}	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5x60 g faeces samples. 60 blood samples (serology)
Table egg layers (Production for certified packing stations)		
24 weeks old ^{a,c}	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 g. 250 ml (100 g) dust or a dust sample by a cloth of min. 900 cm ²
Every 2 weeks from age 20 weeks ^{a,b,d,e,f}	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 g.
After positive findings of other serotypes than <i>S. Enteritidis</i> , <i>S. Hadar</i> , <i>S. Infantis</i> , <i>S. Virchow</i> or <i>S. Typhimurium</i> including the monophasic strains <i>S. 1,4,[5],12:i:-</i> ^c	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples consisting of 60 gram each
Barnyard and hobby flocks		
Every 18 weeks ^{b,d,g}	Per flock	Egg samples

a) Sampling requirements set out by Order no 1260 of 15/12/2008, replaced by Order no. 953 of 10/07/2013 and no. 1134 of 27/09/2013.

b) Until 01/10/2013 sampling every 9th week only.

c) Samples collected by the Danish Veterinary and Food Administration.

d) Samples collected by the food business operator.

e) According to Regulation (EC) 2160/2003 sample collection must be carried out every 15 weeks as a minimum.

f) Until 01/10/2013 sampling every 9th week only.

g) For flocks with 30 birds or less: No testing if only delivered to a well-known circle of users.

Source: Danish Veterinary and Food Administration

Table A34. *Salmonella* surveillance programmes for the duck and turkey flocks, 2013

Time	Samples taken	Material
Duck production		
Max. 21 days before slaughter ^{a,b,c}	Per flock	2 pairs of boot swabs. Analysed individually
Turkey production		
Max. 21 days before slaughter ^{d,e}	Per flock	2 pairs of boot swabs. Analysed individually

a) Sampling requirements set out by Order no 1512 of 13/12/2013 replacing Order no.1105 of 18/09/2013 replacing Order no. 1260 of 15/12/2008.

b) Samples collected by the food business operator.

c) Since 20/09/2013 these samples were no more taken

c) Sampling requirements set out by Regulation (EC) 584/2008.

d) Samples collected by the food business operator or the local food control offices.

Source: Danish Veterinary and Food Administration

Table A35. *Salmonella* surveillance programme^a for the cattle production, 2013

No. of samples	Samples taken	Comment
Milk producing herds		
4 samples distributed over 18 months	Bulk tank samples	Calculation of herd level ^b
10 samples	Blood samples	If the owner wants a herd moved from level 2 to 1
Non-milk producing herds		
1 sample every 180 days at slaughter ^c	Blood samples	Calculation of herd level ^b
4-8 samples depending on herd size	Blood samples	Consecutive negative samples required for level 1 ^d
Beef carcasses at the slaughterhouse		
5 samples daily, pooled into one analysis	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering more than 200 cattle per day
5 samples per 200 slaughtered cattle, pooled into one analysis	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering more than 200 cattle per month but 200 or less cattle per day
5 samples every 3 rd month, pooled into one analysis	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering 50-200 cattle per month
1 sample every 3 rd month	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering less than 50 cattle per month

a) Order no. 954 of 10/07/2013 as amended. In 2013, the programme for eradication of *Salmonella* Dublin from the Danish cattle production was intensified. This implies regionalisation of the country according to prevalence and compulsory eradication plans in Level 2 herds.

b) Herd levels based on serological testing (blood and milk):

Level 1: Herd assumed free of infection based on bulk tank samples (milk producing herd) or blood samples (non-milk producing herd or milk producing-herd assumed free of infection).

Level 2: Herd not assumed free of infection.

Level 3: Herd infected based on culture and clinical signs.

c) No samples are taken, if the herd has been tested for *S. Dublin* within the last 180 days or 8 samples have been tested within the last 24 months.

d) Number of samples equals total number of animals in the herd minus 2 (max. 8 animals, min. 4 animals).

Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration

Table A36. *Salmonella* surveillance programme^a for the pig production, 2013

Time	Samples taken	Purpose
Breeding and multiplier herds		
Every month	10 blood samples per epidemiological unit	Calculation of <i>Salmonella</i> -index based on the mean from the last three months with most weight to the result from the more recent months (1:3:6)
Max. twice per year	Herds with <i>Salmonella</i> -index 5 or above: Pen-faecal samples ^{b, d}	Clarify distribution ^e and type of infection in the herd
Sow herds		
When purchaser of piglets is assigned to level 2 or 3, max. twice per year	Pen-faecal samples	Clarify distribution ^e and type of infection in the herd, and clarify possible transmission from sow herds to slaughter pig herds
Herds positive with <i>S. Typhimurium</i> , <i>S. Infantis</i> and <i>S. Derby</i> are considered positive for the following 5 years ^f	No samples are collected from the herd during the 5 year period when the herd is considered positive, unless the herd is proven negative	Reduce repeated sampling in positive herds infected with a persistent serotype
Slaughter pigs, herds		
At slaughter	Meat juice, 60-100 samples per herd per year. Herds in RBOV ^{d, e} : one meat juice sample per month	Calculation of slaughter pig index based on the mean from the last three months with most weight to the result from the most recent month (1:1:3). Assigning herds to level 1-3 and assigning herds to risk-based surveillance (RBOV) ^e
Slaughter pigs, animals		
At slaughter	Coecum samples, 80 samples per month, 11 month per year	Random collection of samples for monitoring of the distribution of serotypes and antimicrobial resistance.
Herds assigned to level 2 or 3, max. twice a year	Pen-faecal samples	Clarify distribution and type of infection in the herd
Pork carcasses at the slaughterhouse		
5 samples daily, pooled into one analysis	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering more than 200 pigs per day
5 samples per 200 slaughtered pig, pooled into one analysis	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering more than 200 pigs per month or 200 or less pigs per day
5 samples every 3 rd month, pooled into one analysis	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering more than 50 pigs per month or less than 200 pigs per month
1 sample every 3 rd month	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering less than 50 pigs per month

a) Sampling requirements set out by Order no. 1722 of 22/12/2010.

b) Herds with index above 10 have to pay a penalty for each pig sold.

c) Pigs from herds in Level 3 must be slaughtered under special hygienic precautions.

d) The herd owner must inform buyers of breeding animals about the infection level and type of *Salmonella*.

e) RBOV: risk-based surveillance where the sample size in herds with a SP-index of zero (no positive samples in the previous three months) are reduced to one sample per month.

f) These serotypes are primarily spread by live trade, and are known to persist in herds.

Source: Danish Veterinary and Food Administration

Table A37. Typing methods used in the surveillance of foodborne pathogens in Denmark, 2013

Methods	Human	Food	Animal
<i>Salmonella enterica</i>			
Serotype	All	All	All
Phage type	None	Few <i>S. Typhimurium</i> and <i>S. Enteritidis</i>	Few <i>S. Typhimurium</i> and <i>S. Enteritidis</i> , all isolates from poultry
Antimicrobial resistance	All <i>Salmonella</i> except <i>S. Enteritidis</i>	Almost all isolates	Almost all isolates
MLVA	<i>S. Typhimurium</i> and <i>S. Enteritidis</i>	<i>S. Typhimurium</i> and <i>S. Enteritidis</i> for the <i>Salmonella</i> source account, outbreak investigations and research	<i>S. Typhimurium</i> and <i>S. Enteritidis</i> for the <i>Salmonella</i> source account, outbreak investigations and research
PFGE	Outbreak investigations	Outbreak investigations	Outbreak investigations
<i>Campylobacter coli/jejuni</i>			
Antimicrobial resistance	Isolates from 3 districts for DANMAP surveillance	For DANMAP surveillance purposes and the case-by-case program	Only for DANMAP surveillance purposes
FlaA-SVR	Outbreak investigations	Outbreak investigations	None
MLST	Outbreaks investigations, research	None	None
VTEC			
Serotype	All	None	All (O157)
Virulence profile	All	None	All (O157)
PFGE	All	None	Outbreak investigations
<i>Listeria</i>			
Serogroup	All	None	None
PFGE	All	All	All
WGS	All	None	None
<i>Yersinia enterocolitica</i>			
O-group	All isolates send to SSI	None	None

Source: Statens Serum Institut, and Danish Zoonosis Laboratory, National Food Institute

Population and slaughter data

Table A38. Human population, 2013

Age groups (years)	Males	Females	Total
0-4	155,792	147,990	303,782
5-14	340,363	324,525	664,888
15-24	370,625	354,395	725,020
25-44	708,813	700,049	1,408,862
45-64	750,502	747,447	1,497,949
65+	466,184	560,550	1,026,734
Total	2,792,279	2,834,956	5,627,235

Source: Statistics Denmark

Table A39. Number of herds/flocks, livestock and animals slaughtered, 2013

	Herds/flocks ^a	Livestock ^a (capacity)	Number slaughtered
Slaughter pigs (>27 kg)	7,057	6,494,809	18,549,103
Cattle	19,798	1,599,254	491,790
Broilers	296	22,053,902	107,098,752
Layers (excl. barnyard)	400	4,979,268	-
Turkeys	36	378,199	4,075
Sheep & lambs	7,265	153,708	74,550
Goats	3,336	22, 22844 844	2,935
Horses	-	-	1,723

a) August 2013.

Source: The Central Husbandry Register and Danish Veterinary and Food Administration

Table A40. Number of farms in the broiler production, 2013

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (grandparent)	4	14	90,000
Adult period (grandparent)	4	7	90,000
Rearing period (parent)	15	90	130,000
Adult period (parent)	44	146	720,000
Hatcheries	4	-	-
Broilers	259	600	-

Source: Danish Veterinary and Food Administration and Danish Agriculture and Food Council

Table A41. Number of farms in the table egg production, 2013

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (parent)	6	10	20,000
Adult period (parent)	8	8	40,000
Hatcheries	5	-	-
Pullet-rearing	82	140	1,460,000
Layers (excl. Barnyard)	161	218	3,320,000

Source: Danish Veterinary and Food Administration, and Danish Agriculture and Food Council

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