Trends and sources in human salmonellosis

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Contents

Introduction

Overall, the number of human infections with *Campylobacter* (3,782 cases) and *Salmonella* (1,122 cases) remains at the same level in 2014 as in the last two years. Compared to 2013, fewer foodborne outbreaks were registered in 2014 (60 outbreaks compared with 73), with a total of 2,209 cases registered of which 295 were confirmed in the laboratory.

Although the number of human *Salmonella* cases was similar to 2013, some changes occurred. The number of infections with *S.* Enteritidis was reduced by 22.5% compared to 2013, where a large travel-related outbreak with this serotype occurred, despite three outbreaks with *S.* Enteritidis in 2014, including the first outbreak from domestic eggs since 2009. Overall, human *S.* Typhimurium cases (incl. monophasic *S.* 1,4,[5],12:i:-) increased by 26.7% in 2014 compared to the low level in 2013, primarily due to an 81.1% increase in monophasic *S.* 1,4,[5],12:i:- infections mainly related to five outbreaks. In four of these outbreaks, the source was domestic pork or beef. Thus, in 2014 monophasic *S.* 1,4,[5],12:i:- became the second most common human *Salmonella* serotype and for the first time exceeded the number of genuine *S.* Typhimurium infections.

In animals *S.* 1,4,[5],12:i:-has been more common than genuine *S.* Typhimurium in domestic pigs and pork since 2012, and in 2014, this serotype was also the most common *Salmonella* serotype in the domestic broiler production and in imported pork.

An outbreak of *S.* Agona running in 2013 and 2014 had an uncommon age and gender distribution. Extensive investigations concluded that whey powder was a possible outbreak source, but the final identification of the source was not possible.

In the 2014 *Salmonella* source account, Multi-Locus Variable number tandem repeat Analysis (MLVA) was introduced for subtyping of *S.* Dublin similar to the existing MLVA-typing of *S.* Typhimurium and *S.* Enteritidis. Therefore, in this years' report, top-10 MLVA types for these serotypes are reported. The *Salmonella* source account 2014 attributed more cases to domestic pork, eggs and imported broilers and fewer cases to imported pork and beef than in 2013. For the first time since 2011, *Salmonella* was detected in the surveillance of domestic broiler meat, and human cases were attributed to this source.

Several isolations of *S.* Typhimurium DT40 and DT41 in the broiler and egg production from the end of 2013 and the beginning of 2014 were investigated. A common source of introduction was not identified, and the impact on human disease appeared to be limited.

Another characteristic of the year 2014 was a 84.0% increase in L*isteria monocytogenes* cases including four outbreaks, which are described in Chapter 3. In particular one serious outbreak of listeriosis was in focus in 2014 with a total of 41 cases reported and a mortality of 41.4%. Overall, the number of cases with VTEC and *Yersinia enterocolitica* increased by 33.3% and 25.2%, respectively. Increased awareness and improved diagnostic methods play an important role in the increasing number of VTEC cases. For both pathogens, outbreaks contributed to the increase, but only for one small VTEC outbreak the source of infection was identified (beef of Danish origin).

The number of norovirus outbreaks in 2014 was similar to the number in 2013, while the number of patients in these outbreaks more than doubled. An increasing number of cases was caused by transmission of norovirus from healthy carriers, ill persons among kitchen staff or kitchen staff attending ill persons at home prior to handling food.

New initiatives on *Listeria*

In 2014, Whole Genome Sequencing (WGS) was introduced as the routine typing of *Listeria* isolates in Denmark, and parallel on-time testing of human and food isolates with WGS and Multi-Locus sequence Typing (MLST) was introduced. Aided by WGS, the outbreak source of the large and serious outbreak of *Listeria monocytogenes* in the summer 2014 was identified to be "rullepølse", a Danish cold cut ready-to-eat speciality, from a specific producer.

Following the outbreak, the Danish Ministry of Food, Agriculture and Fisheries initiated a critical review in order to evaluate and improve the existing Danish *Listeria* efforts and initiatives, and new initiatives will be instigated from 2015-2018 targeted for groups at risk and health care

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 2014. 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 year's report. The report is also available at www.food.dtu.dk.

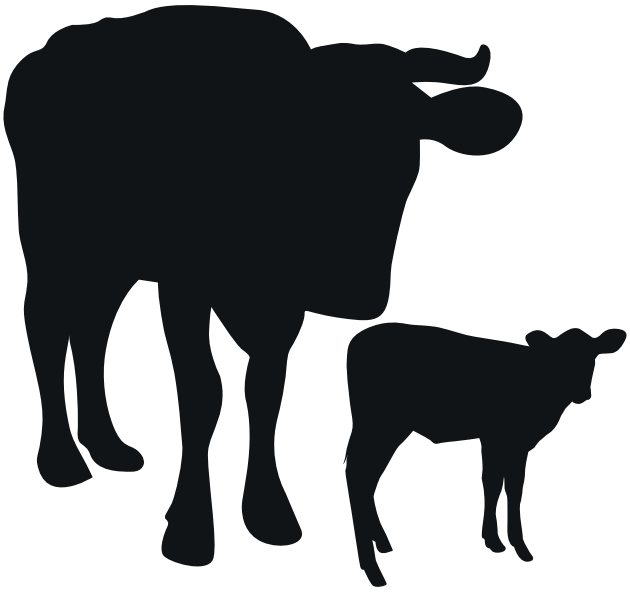
professionals, kitchens, food businesses and the official control. Other initiatives support *Listeria* typing, tracing and sampling. Additionally, several research initiatives were proposed, including development of predictive tools, studies of the genetic diversity and use of indicators for *Listeria* and studies of factors related to the high incidence of *Listeria* in Denmark.

Risk ranking of human Verotoxin-producing *E. coli*

Investigation of associations between the virulence factors of human Verotoxin-producing *E. coli* (VTEC) and occurrence of haemolytic uraemic syndrome (HUS) in a Danish cohort of 2001 VTEC cases from 1983-2012 indicates, that primarily the verotoxin subtype *vtx2a* is associated with HUS. The well-documented association between *vtx2* and the gene for either attaching and effacing properties (the *eae* gene) or the ability to colonize and persist in the gut (regulated by the *aggR* gene) has resulted in a basic and primary definition of HUS associated *E. coli* from humans for first line public health action: *vtx2* in a background of an eae or aggR positive *E. coli*. Such isolates are *vtx* subtyped and further characterized. Other VTEC are generally considered low risk and are treated as other enteropathogens, although careful evaluation of each patient is important in order to consider other risk factors for progression to severe disease. This approach is generally accepted and practical and easy to use in an operational environment.

Campylobacter and *Salmonella* seroincidence

As an alternative to defining the occurrence of human infections with *Salmonella* and *Campylobacter* from the incidence of registered culture confirmed cases which is biased from the passive laboratory surveillance, Statens Serum Institut, has calculated the seroincidence in the general population from the levels of antibody classes against *Salmonella* and *Campylobacter* based on the kinetics of the antibody decay. Measuring the seroincidences for a selection of European countries and comparing these to e.g. the national incidence of these infections, and the occurrence of *Salmonella* and *Campylobacter* in food animals has provided new information on the exposure from these sources and their relative importance.



Surveillance of vectors and vector-borne zoonoses

The National Veterinary Institute, Tecnical University of Denmark, monitors vectors and vector borne diseases in Denmark. Surveillance of mosquitoes and biting midges is running and constitutes an early warning system for vector borne diseases, which are currently not present in Denmark. These diseases have been detected elsewhere in Europe, even as close as in Germany. The surveillance systems have identified new vectors, and DNA screening of vectors using a new DNA screening chip has revealed new pathogens carried by the vectors, and e.g. a widespread occurrence of *Neoerlichia Mikurensis*, which has been associated with severe human disease in Sweden. Weekly surveillance data are available at the internet.

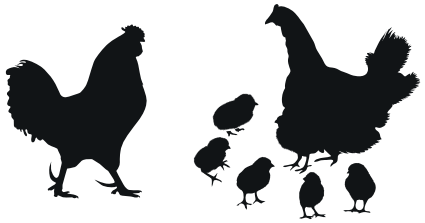
National and EU targets on zoonoses

In this years' report, chapter 7 gives an overview of the targets for the national and EU action plans and programs on *Salmonella* and *Campylobacter* and assess the achievement of the targets in 2014.

Whereas most targets were met in 2014, the occurrence of specific targeted *Salmonella* serotypes in breeding flocks of *Gallus gallus* exceeded the EU-target of a maximum of 1% positive flocks in 2014. From 4 out of 5 positive flocks *S.* Typhimurium or *S.* 1,4,12:i:- were detected.

Assessment of Ebola risk from imported bush meat

The outbreak of Ebola virus in West Africa in 2014, led the European Food Safety Agency (EFSA) to assess the risk from handling and consuming bush meat. Ebola is primarily transmitted between humans from blood or bodily fluids, but has a reservoir in certain wild animals, and a large illegal import of bush meat to Europe occurs. EFSA concluded, that the risk for introduction and transmission of Ebola virus via bush meat to Europe is currently low, although the size of the risk could not be estimated due to lack of data and knowledge. The Danish border control is aware of the risk.



1. Trends and sources in human salmonellosis

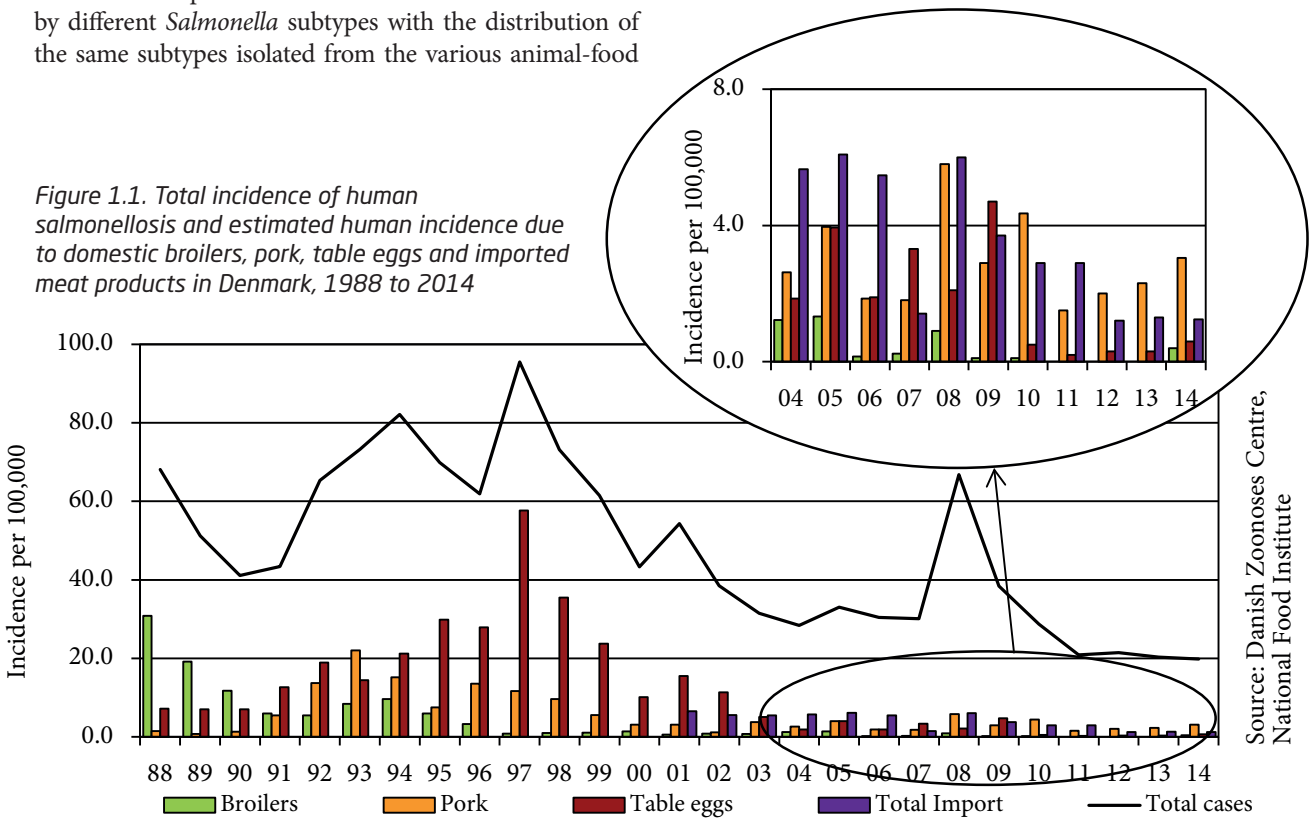
By Leonardo de Knegt (ledkn@food.dtu.dk) and Tine Hald

Salmonella enterica is an important cause of foodborne disease in humans throughout the world and is a significant cause of morbidity, mortality, and economic loss [1]. In 2014, a total of 1,122 human cases of salmonellosis were reported in Denmark, and the incidence was 19.9 cases per 100,000 inhabitants, which is similar to 2013. The incidence of *S.* Typhimurium was 7.6/100.000 (Appendix Table A2), and for the first time with a predominance of the monophasic *S.* Typhimurium strains 1,4,[5],12:i:- (4.1/100.000) [2]. The observed incidence for *S.* Enteritidis was 4.8/100,000, which is a decrease when compared to 2013 (6.2/100,000), where a large travel related *S.* Enteritidis outbreak took place.

Since the mid-nineties, *Salmonella* control strategies have been guided by the results of a *Salmonella* source attribution model (SSA). The routine application of such model has helped the identification of the main animal-food sources of human salmonellosis, as well as the monitoring of shifts in importance among those sources throughout time. This has identified broilers, pigs or laying hens as the main reservoirs for this pathogen at different time points in the last 30 years, thus allowing Danish risk managers to define different control strategies [3].

The method currently used for source attribution in Denmark compares the number of human cases caused by different *Salmonella* subtypes with the distribution of the same subtypes isolated from the various animal-food sources. Additionally to serotyping of all *Salmonella*, isolates of *S.* Typhimurium and *S.* Enteritidis have, since 2013, been subtyped using multiple-locus variable-number tandem repeat analysis (MLVA). MLVA-typing of *S.* Dublin was first presented by Kjeldsen et al. in 2014 [4], and is being used for the first time in the *Salmonella* source account in 2014. MLVA profiles for *Salmonella* are defined by the number of repetitions observed in five independent loci, namely SE1, SE5, SE2, SE9 and SE3 for *S.* Enteritidis, STTR9, STTR5, STTR6, STTR10 and STTR3 for *S.* Typhimurium, and SE1, SE2, SE5 and SD1 for *S.* Dublin. To better fit source attribution purposes, the level of discrimination of the original schemes was adjusted by simplifying the MLVA profile for *S.* Typhimurium to STTR9|STTR10|STTR3, and for *S.* Dublin to SE5|SE2|SE1, according to observations on loci stability and the epidemiological value of MLVA discrimination [5,6]. The complete scheme SE1|SE5|SE2|SE9|SE3 was used for *S.* Enteritidis. Finally, antimicrobial resistance profiles of *S.* Typhimurium and *S.* Dublin isolates was included, to further distinguish between similar MLVA types. In the source account model, strains of *S.* 1,4,[5],12:i:- were separated from genuine *S.* Typhimurium.

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 2014



Source: Danish Zoonosis Centre, National Food Institute.

Salmonella source account 2014

The overall trend in human salmonellosis cases attributable to the major food-animal sources is presented in Figure 1.1.

As in 2013, domestic pork was estimated to be the most important food source of salmonellosis in Denmark in 2014 (Figure 1.2 and Appendix Table A1), with 15.4% of laboratory-confirmed cases. Three outbreaks related to domestic pork contributed 4.6%, while sporadic cases accounted for the remaining 10.7%. More than 60% of the sporadic cases attributed to pork belong to the three subtypes involved in the outbreaks. The fraction of cases attributed to domestic beef in 2014 (2.2%) was similar to 2013, but as opposed to 2013, more than 75% of the cases from domestic beef in 2014 were related to an outbreak. Domestic table eggs were estimated to be responsible for 3.0% of human cases in 2014. Approximately half of the cases were part of an outbreak and 1.4% were sporadic cases, which is similar to 2012 and 2013. For the first time since 2010, *Salmonella*-positive Danish broiler carcasses were detected by the surveillance system, and as a result, 2.0% of cases was attributed to this reservoir. A total of 2.8% of cases were estimated as attributable to imported chicken, with 0.7% belonging to an *S.* Infantis outbreak and 0.4% to an *S.* Enteritidis outbreak (Appendix Table A4).

Imported duck was estimated as the reservoir of 2.0% of cases, which cannot be compared to last year, as samples from this source were not available in 2013. This number is, however, in accordance with 2.4% estimated in 2011 and 1.9% in 2012, suggesting that no major changes have occurred. Imported pork was estimated as the source of 1.1% of cases. No positive samples were observed from imported turkey meat in 2014, therefore no cases were attributed to this source. No samples were available from domestic ducks or turkey meat, and consequently no attribution estimates are presented for these sources. A total of 48% of patients were estimated to have travelled abroad within seven days prior to onset of symptoms. This number varied from 40.3% to 46.9% in the last seven years, except from 2008 and 2009, where extraordinary large, domestically acquired outbreaks took place.

A total of 19.2% of reported *Salmonella* cases were sporadic cases which could not be associated with any of the included food sources. This fraction has been relatively stable since 2008 and may be caused, for example, by imported or domestically produced fruits and vegetables (Appendix Table A22), food-animals not included in the national surveillance or by non-food sources of infection, such as direct contact with pet animals.

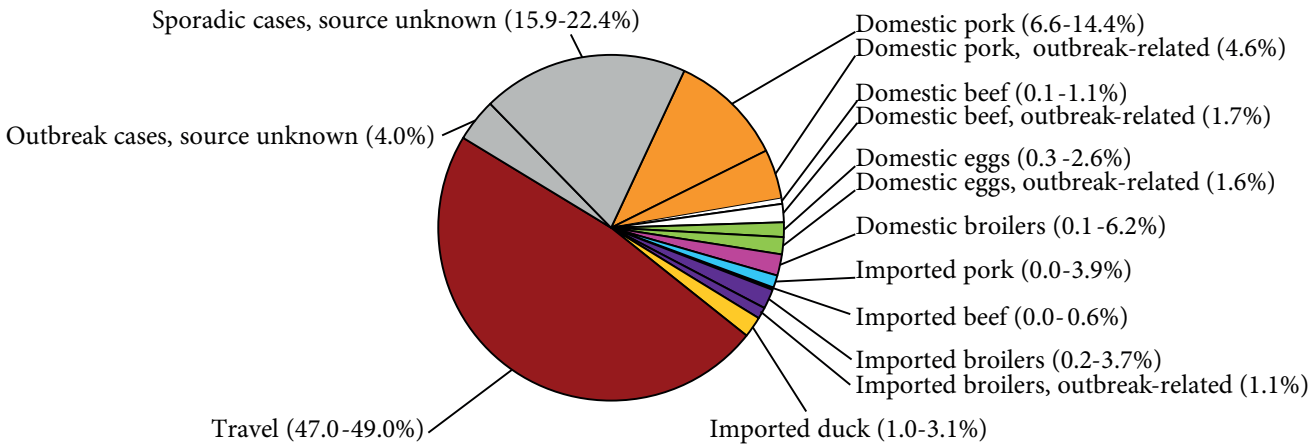
Of the 268 reported *S.* Enteritidis cases, the SSA estimated 77.9% to be related to international travel and 10.1% were part of three outbreaks. One of those outbreaks (outbreak number: FUD1379), corresponding to 6.7% of *S.* Enteritidis cases, had Danish eggs identified as the source. This is the first *S.* Enteritidis outbreak related to Danish eggs since 2009. Of the remaining two *S.* Enteritidis outbreaks, one was associated with imported chicken, whereas the source of the third outbreak could not be identified (see Chapter 2 for more information).

A total of 427 *S.* Typhimurium cases were reported in 2014, including 197 cases of classical *S.* Typhimurium and 230 cases of monophasic *S.* 1,4,[5],12:i:- strains. In total, 98 cases were part of six outbreaks with *S.* Typhimurium, of which 86 cases (87.8%) were monophasic. Sixty-five cases of *S.* 1,4,5,12:i:- were implicated in four outbreaks, of which two were connected to pork, one to beef and one to an unknown source. Monophasic *S.* 1,4,12:i:- was isolated in one pork-related outbreak with 22 registered cases, and a classical *S.* Typhimurium strain was responsible for five cases in a third pork-related outbreak (see Chapter 2 and Appendix Table A4 for more information). Among cases infected with *S.* Typhimurium, 97 cases (22.7 %) were estimated to have travelled abroad prior to disease onset, with a predominance of classical strains over monophasic (approximately 2:1).

Antibiotic resistance in *S.* Typhimurium

Of the 134 *S.* Typhimurium cases (including monophasic strains) attributed to domestic food products, with resistance information available, 22.8% were caused by strains susceptible to all tested antimicrobial agents, 74.4% by strains resistant to one to three antimicrobial agents and 2.8% by strains resistant to four or more antimicrobial agents (multi-resistant) (Figure 1.3). No quinolone-resistant strains were observed in cases attributed to domestic food animal sources

Figure 1.2. Estimated sources of 1,122 cases of human salmonellosis in Denmark, 2014. (See also Appendix Table A1)



Source: Danish Zoonosis Centre, National Food Institute.

in 2014, returning to a situation observed between 2010 and 2012, and interrupted in 2013, when 1.2% of *S.* Typhimurium cases, attributed to domestic sources, were resistant to this antibiotic group.

Large annual changes in the relative distribution of resistance patterns are observed among *S.* Typhimurium cases attributed to imported food sources 2012-2014 (Figure 1.3). However, these changes must be interpreted with caution, as the number of *S.* Typimurium cases attributed to imported food sources these years were limited, and available data from these sources changed, with no data from imported beef or imported duck available in 2013 (Appendix Table A1).

From the 94 *S.* Typhimurium cases estimated to be acquired abroad, for which resistance information was available, 33.4% were caused by resistant types, 37.4% by types susceptible to all tested antimicrobial agents, 15.7% by types resistant to quinolones and 13.5% by multi-resistant types.

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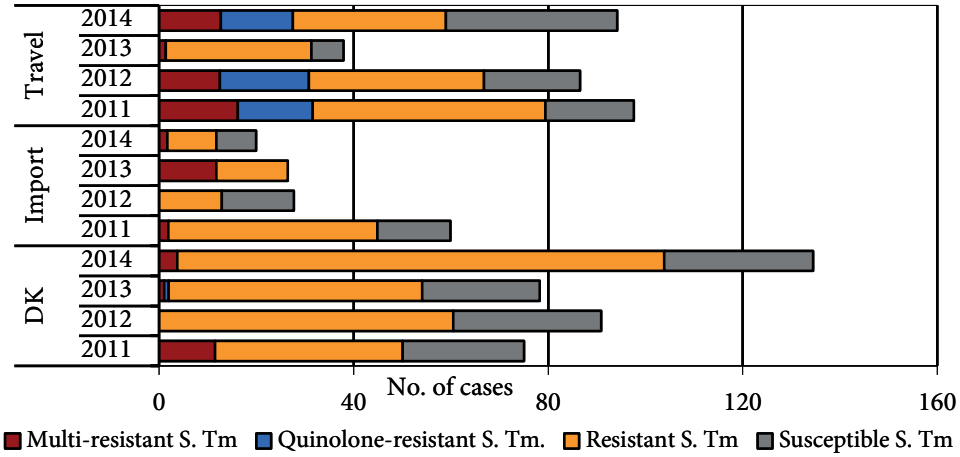
Figure 1.3. Distribution of antimicrobial resistance[a] in S. Typhimurium, including S. 1,4,[5],12:i:-, from human infections attributed to domestic or imported food sources, or travel in the Salmonella *source account, 2011-2014*



a) Resistant: Resistant towards one to three antimicrobial agents; Multi-resistant: Resistant towards four or more antimicrobial agents. Antimicrobials in the resistance profile for the *Salmonella* source account were: ampicillin, ceftiofur or cefotaxime/ceftazidine, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, sulphamethoxazole, tetracycline and trimethoprim.
Source: Danish Zoonosis Centre, National Food Institute.

Where do we acquire *Salmonella* infections?

By Luise Müller (lum@ssi.dk)

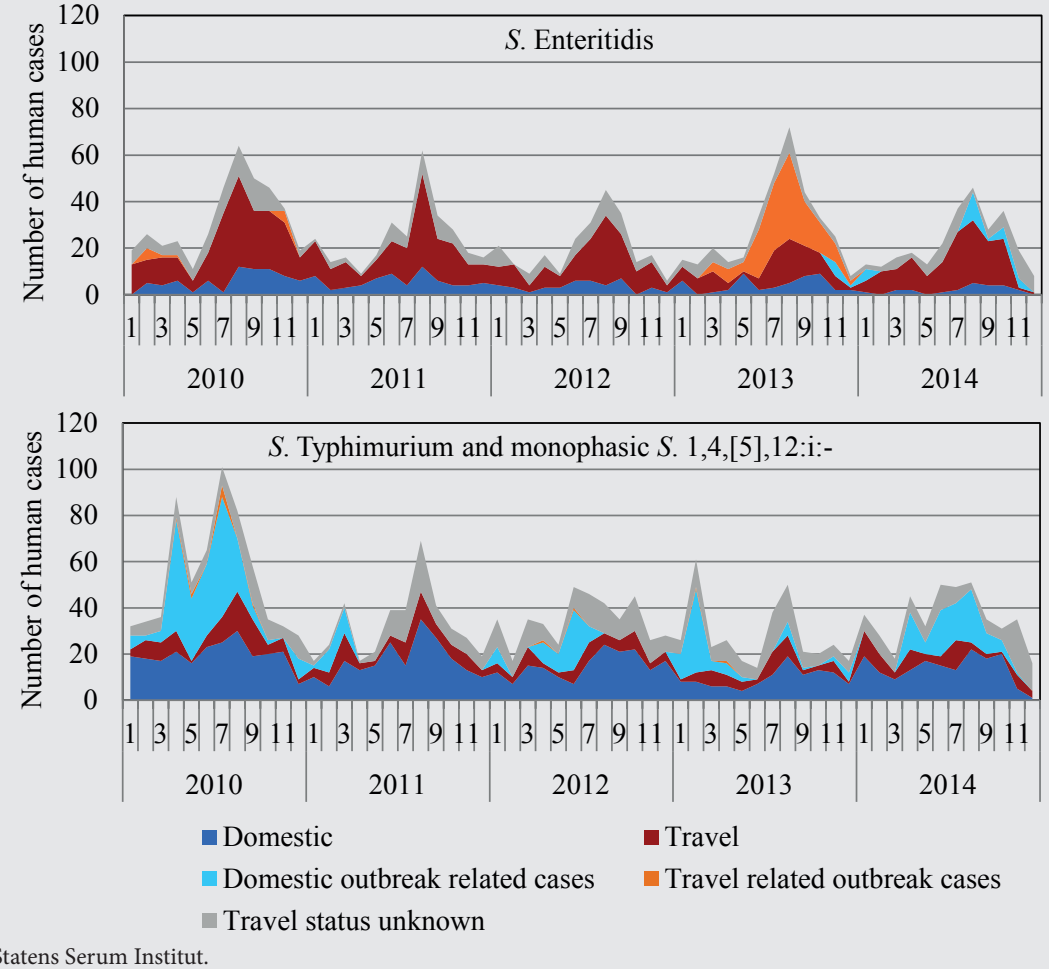
In 2014, as in the previous years, Statens Serum Institut attempted to interview all registered *Salmonella* cases where no travel information was reported by the general practitioner. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease onset. This information was complemented with information from general practitioners' reports. Travel information was obtained from a total of 71.2 % of the *Salmonella* cases in 2014. Among the cases with known travel history, 46.4 % were infected abroad (Table 1.1). However, the proportion of travel-related cases varied greatly between the different serotypes, hence 77.2 % of the *S.* Enteritidis cases, 31.2 % of the *S.* Typhimurium cases, 12.7 % of the monophasic *S.* 1,4,[5],12:i:- cases and 54.1% of cases with other serotypes were infected abroad. In 2014, the majority of travel-related cases travelled to Thailand (17.5%), Turkey (15.4%), and Spain (6.4%). In contrast to 2013, no travel-related outbreaks due to *S.* Enteritidis were registered (Figure 1.4).

Table 1.1. Top 10 Salmonella *serotypes in humans and information about travel aboad, 2013-2014*

2014	Number of patients (%)	% of patients[a] infected Abroad[b]	Domestically	**2013**	Number of patients (%)	% of patients[a] infected Abroad[b]	Domestically
Enteritidis	268 (23.9)	77.2	22.8	Enteritidis	346 (30.5)	79.0	21.0
1,4,[5],12:i:-	230 (20.5)	12.7	87.3	Typhimurium	210 (18.5)	17.5	82.5
Typhimurium	197 (17.6)	31.2	68.8	1,4,[5],12:i:-	127 (11.2)	32.5	67.5
Infantis	38 (3.4)	26.1	73.9	Dublin	27 (2.4)	0	100
Dublin	21 (1.9)	0	100	Newport	27 (2.4)	62.5	37.5
Stanley	21 (1.9)	81.3	18.8	Stanley	23 (2.0)	84.6	15.4
Newport	19 (1.7)	50.0	50.0	Agona	22 (1.9)	25.0	75.0
Virchow	18 (1.6)	77.8	22.2	Infantis	22 (1.9)	42.9	57.1
Agona	16 (1.4)	28.6	71.4	Virchow	17 (1.5)	75.0	25.0
Kentucky	16 (1.4)	58.3	41.7	Corvallis	13 (1.1)	87.5	12.5
Other serotypes	278 (24.8)	59.0	41.0	Other serotypes	302 (26.6)	51.1	48.9
Total	1,122 (100)	46.4	53.6	Total	1,136 (100)	52.1	47.9

a) Patients with unknown travel information (28.8 of all patients in 2014 and 32.2% of all patients in 2013) were excluded from the percent calculations.
b) Infected abroad is defined as travel abroad in a seven-day period prior to disease onset.
Source: Statens Serum Institut.

Figure 1.4. Monthly distribution of S. *Enteritidis,* S. *Typhimurium,* and monophasic S. 1,4,[5],12:i:- *cases, 2010-2014*



Source: Statens Serum Institut.

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 2014 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 details in Chapter 8.

In total, 60 foodborne outbreaks were reported to FUD in 2014 (Appendix Table A4) which is a decrease from 2013, where 74 foodborne outbreaks were reported. There were no notifications of waterborne outbreaks in 2014. The outbreaks were mainly regional or local outbreaks (80%) and only 12 outbreaks were considered national outbreaks. The largest national outbreak was caused by *Listeria monocytogenes*, MLST224 (outbreak number in FUD: FUD1373).

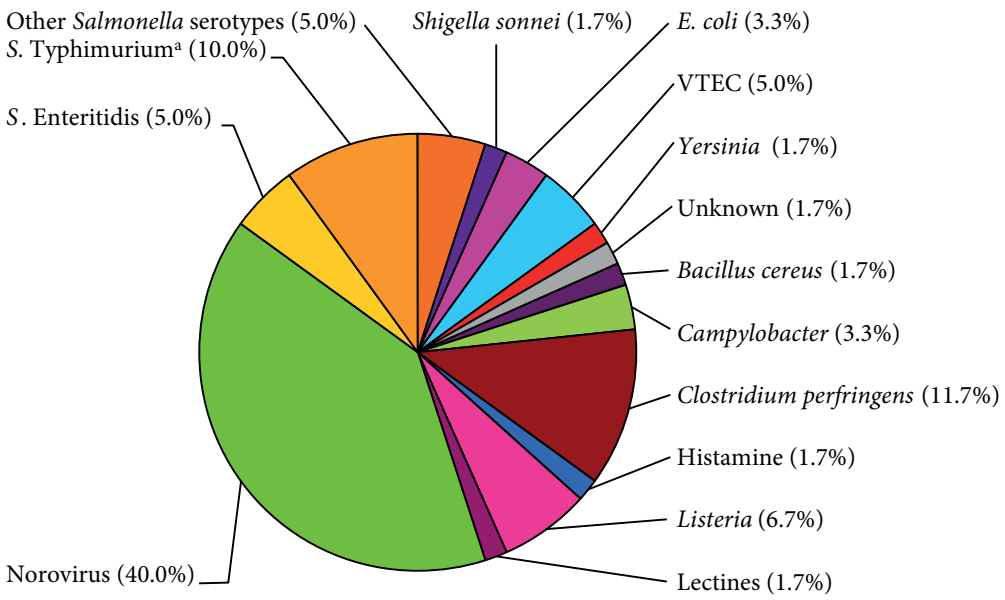
In total, the number of persons affected by foodborne outbreaks was 2,209, with a median of 11 persons per outbreak (range 2 – 430). The largest outbreak involving 430 persons was an outbreak caused by Norovirus (NoV) in a canteen serving a buffet meal. The source of the illness appeared to be one of the kitchen staff who had attended a sick child at home with the same symptoms. Analyses of stool samples from both the child and some of the guests that became ill showed the same type of NoV.

As in previous years NoV was the most frequent cause of foodborne outbreaks (24 outbreaks), and in total, 1,339 persons were affected by NoV outbreaks. The transmission routes for NoV causing foodborne outbreaks were multiple. In Table 2.1 a breakdown of the number of outbreaks and the number of people affected per route of transmission for 2013 and 2014 are shown. The most common ways of infection with NoV in 2014 were contamination from ill kitchen staff or kitchen staff attending ill family members before working in the kitchen. Less often, the infection originated from contaminated ready-to-eat products like oysters and frozen berries.

In 2014, *Clostridium perfringens* was less frequently associated with foodborne outbreaks than in 2013. In total, seven outbreaks of *C. perfringens* affecting a total of 528 people were reported in 2014, compared to 16, 8 and 7 outbreaks caused by this agent in 2013, 2012 and 2011 respectively.

When dividing the outbreaks into reported settings, the most frequent setting was restaurants (40%) with 24 outbreaks affecting 363 people (mean: 15 people per outbreak). Outbreaks taking place in workplace canteens and catering

Figure 2.1. Aetiology of the 60 foodborne disease outbreaks reported with a causative agent in the Food- and waterborne Outbreak Database (FUD), 2014 . Percentage of total outbreaks indicated in brackets



a) *S.* Typhimurium includes the *Salmonella* strains 1,4,[5],12:i:-.
Source: Food- and waterborne Outbreak Database (FUD).

(9 outbreaks) also affected a high number of people (1,262 people) and affected on average 140 persons per outbreak. Composite meals (20 outbreaks) and buffet meals (nine outbreaks) were the most frequently reported sources of outbreaks in 2014 and most often these outbreaks were associated with NoV or *C. perfringens* (Appendix Table A4).

2.1 Outbreaks with *Listeria*

The most severe foodborne outbreak in 2014 was caused by *Listeria monocytogenes* (FUD1373). with a total of 41 cases reported. Seventeen cases died within 30 days from the laboratory sample date. The outbreak vehicle was identified as "rullepølse" – a Danish cold cut ready-to-eat speciality made from pork. Apart from this outbreak, listeriosis has been in focus in 2014, where the implementation of new laboratory methods with whole-genome sequencing (WGS) have improved identification and comparison of clusters over long periods of time. Read more about *Listeria* in Chapter 3.

2.2 Outbreaks with *Salmonella*

In 2014, eight outbreaks of *Salmonella* with patients in two or more regions were reported in FUD. The first outbreak - caused by *S.* Agona - started in August 2013 and lasted 14 months (FUD1317). In total, 21 cases were registered from August 2013 to October 2014 with *S.* Agona PFGE0022. Isolates were analysed by WGS which supported the identification of a cluster already identified by PFGE and in addition, sub-clustered isolates from patients being less than one year old. The age distribution was characterized by more than half of the cases being under five years of age, a group of men aged 20-24 years and a few elderly people above 70 years old (Figure 2.2). Seven cases were infants 4-9 months old and infant formula was therefore suspected as the source. Hypothesis generating interviews showed that young male cases were working out to build muscles and they consumed protein shakes made from protein powder. Furthermore, two of the elderly cases had consumed protein drinks during hospital admissions while recovering from illness. These observations led to the hypothesis of whey powder as the source of the outbreak, as whey powder is used in infant formula, protein powder and in products for special nutritional purposes e.g. protein drinks used by ill and elderly people with a low calorie intake. This hypothesis was tested in a case-control study, which showed that the young adult cases were more likely than age matched controls to consume protein products. In addition, infant cases were more likely to consume infant powder and/or pre-made baby food than the infant controls – however, the study had its limitations due to a small number of cases in the different age groups. Samples of protein powder from two patients' homes were tested, but *Salmonella* was not

The role of asymptomatic food handlers in calicivirus outbreaks

New research has shown that one of five calicivirus outbreaks is caused by contamination from asymptomatic food handlers. Data from FUD on 191 calicivirus (189 norovirus and 2 sapovirus) outbreaks that occurred from 2005-2011 was reviewed and categorized in order to clarify the routes of contamination. For 51 (27%) outbreaks, the contamination had occurred during production, and the most common food items implicated were frozen berries, lettuce and oysters. Another 55 (29%) outbreaks had supposedly occurred after an ill guest had attended a self-serve buffet. Contamination from food handlers took place during the preparation or serving of the food in 64 (34%) of the outbreaks of which 41 (64%) were caused by asymptomatic food handlers – either food handlers who had contact to ill household members (but remained asymptomatic), or retrospectively were found to be in the incubation- or recovery period at the time of handling the food. [1]

Table 2.1. Norovirus outbreaks in 2014 per route of transmission based on number of cases or number of outbreaks

Transmission route/source	2014		2013	
	No. of outbreaks	No. of persons ill	No. of outbreaks	No. of persons ill
Ill kitchen staff or healthy carrier of virus among kitchen staff	11	507	9	226
Kitchen staff tending to ill persons at home before entering the kitchen	6	729	5	129
Ill person/guest attending a buffet	2	43	10	261
Seafood (oysters)	3	40	3	27
Frozen raspberries	2	20	1	12
Norovirus in total	24	1,339	28	655

Source: Food- and waterborne Outbreak Database (FUD).

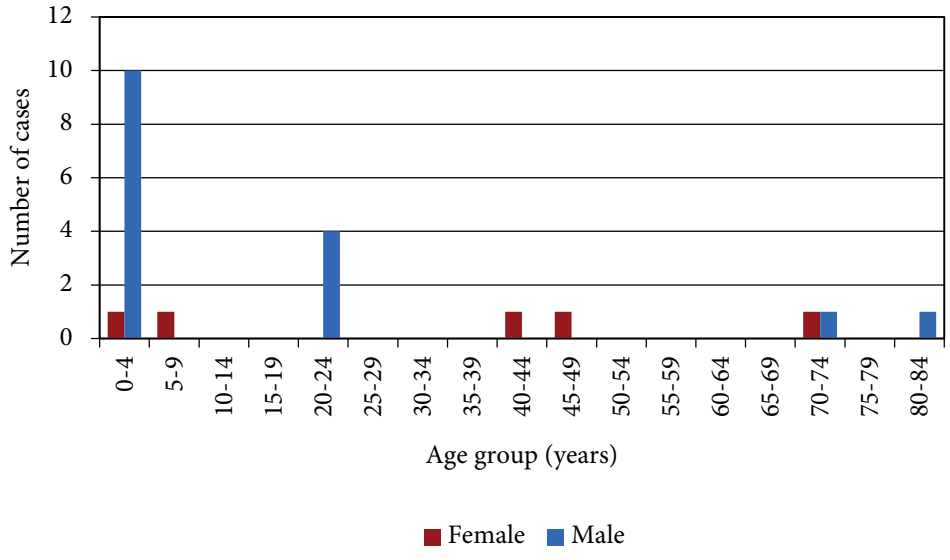
detected. No tested animal, food or feed isolates matched the PFGE type. Trace back investigation of whey powder used for infant formula, baby food, protein powder and the products for special nutritional purposes mentioned by the patients was conducted. The result of the tracing was very complex and showed that the whey powder used originated from the major producers on the market with an even more complex supplier network to these establishments. No specific product or supplier of whey powder or raw material for the production of whey powder could be pointed out to be the outbreak source. The long lasting outbreak indicates that the vehicle was a low-contaminated product with a long shelf life or a contaminated factory with occasionally contaminated products or a combination of both. In conclusion, the investigation pointed at whey powder as a possible source for several reasons: The specific age distribution, the fact that *S.* Agona is previously known to have caused outbreaks in infant formula [2, 3], the long shelf life of the product and the case-control study supporting the hypothesis although the epidemiological evidence from the case-control study was not strong.

Four outbreaks with patients in two or more regions were caused by *S.* Typhimurium or monophasic *Salmonella* 1,4,[5],12:i:-. Three of the outbreaks were found to have been caused by Danish meat. For the fourth outbreak, no source could be identified.

From March to May 2014 an outbreak occurred with 22 cases of monophasic *Salmonella* 1,4,12:i:- MLVA0007 fully sensitive to antibiotics (FUD1368). Patients were 1-81 years old, 13 were male and the majority of cases were from Jutland. Hypotheses generating interviews were performed with 20/22 cases. Results indicated that the source most likely was fresh pork sold either from the retail part of a specific cutting plant or in a small supermarket chain at the same geographical locations as the cases. Trace back investigation showed that the supermarket chain was supplied with fresh meat and minced meat from the cutting plant. Furthermore, it was possible to support the hypothesis of pork from the supermarket chain with information from one patient's shopping receipts showing that the patient had bought pork from the supermarket chain. However, it was not possible to point out a specific time period or a specific batch of meat that could have caused the outbreak and thus no meat could be withdrawn from the market.

Another outbreak caused by monophasic *Salmonella* 1,4,5,12:i:- MLVA0201 with the resistance pattern: Ampicillin, Streptomycin, Sulfamethoxazole, and Tetracycline (ASSuT) occurred between May and September 2014 (FUD1372). This outbreak was discovered, when an unusually high number of food isolates from one specific slaughterhouse and one cutting plant in late May tested positive for the same monophasic *Salmonella* type. The slaughterhouse had received pigs from a farm, where the herd was categorized as *Salmonella* positive. The pigs from this farm were slaughtered as the last herd on May 22, 2014 and sampled as scheduled in the legislation. The carcasses were all retained until the analysis results were known. The results were positive and the carcasses consequently sent for heat treatment. However, insufficient cleaning and disinfection of the plant and equipment led to contamination with the same *Salmonella* type of carcasses from pigs slaughtered the following day. The carcasses were cut in a cutting plant, and pork sampled as part of the normal procedures prior to export was found to contain the same strain of *Salmonella*. Consequently, both establishments were closed down for extra cleaning and disinfection. Environmental

Figure 2.2. Salmonella Agona PFGE0022 by age and sex, (FUD1317, n=21)



Source: Statens Serum Institut

samples taken after cleaning showed satisfactory results. No further positive samples were seen. The meat from these pigs was not withdrawn from the market, as there with few exceptions is no legal requirement demanding absence of *Salmonella* in fresh meat. In the beginning of July, the first eight patients infected with the same *Salmonella* type were identified. In total, 25 cases were reported from May to September scattered throughout Denmark. Interviews with cases showed that 18/20 had consumed fresh pork in the week prior to disease onset – eleven of these had eaten minced meat. Two cases had tasted raw minced meat. Eight patients had bought the minced pork in a local butcher shop – four in the same butcher chain. No other food item was identified as eaten by the majority of cases. In conclusion, this outbreak was very likely caused by fresh meat from this specific slaughterhouse.

Another outbreak, in which the agent had already been detected in foodstuff at the time a patient cluster was identified, was an outbreak of monophasic *Salmonella* 1,4,5,12:i:- MLVA1277 not previously seen in Denmark (FUD1374). A total of 19 cases were identified in the outbreak with disease onset from June to August 2014. The foodstuff isolate originated from minced beef from a specific meat production establishment, which had been tested as part of the company's own check program. According to Reg. (EC) No 2073/2005 concerning microbiological criteria for certain food items the isolation of *Salmonella* in minced meat should have resulted in an immediate recall, but unfortunately the contaminated batch was not recalled from the market and consumers were not informed until much later. The food consumption pattern found through interviews with 10 cases and the trace forward investigation of the batch of minced meat supported, that cases could have been exposed to meat from this specific establishment.

Three outbreaks of *S.* Enteritidis were reported in 2014. The first outbreak was caused by *S.* Enteritidis MLVA0206 and a total of 12 cases were reported from November 2013 to January 2014 (FUD1327). Patient interviews with a focus on egg consumption did not reveal the source, but it seemed unlikely that eggs should be the source, since some cases did not eat eggs in the week prior to disease onset, and those who did, reported consumtion of eggs from different producers and of different types.

The second outbreak with *S.* Enteritidis outbreak investigation started in August 2014, when a large egg producing unit in Denmark experienced severe clinical illness among their laying hens and the herd was found positive for *S.* Enteritidis phage type 21 (FUD1379). The eggs were immediately recalled from the consumers. In August and September, 13 patients without travel history outside Denmark in the week prior to disease onset, were identified with the same phage type, identical by MLVA typing (MLVA0019) to the poultry isolate. Interviews showed that all patients - but one - had consumed or handled eggs from the recalled batch - after the time of the recall. The outbreak was considered to be over and successfully and timely controlled and thereby presumably several cases of salmonellosis had been prevented. However, a few weeks later another five cases with the same type were registered. This second peak of cases indicated a new introduction of the same *Salmonella* type, but interviews showed that cases had not eaten eggs from the same producer and 3/5 cases had eaten eggs from local farms in the area near the large producer.

The third outbreak with *S.* Enteritidis MLVA0017 (FUD1391) took place in September to October 2014. WGS proved useful to specify the case-definition, as different resistance profiles were observed for cases. In total, four patients were related to this outbreak. Relation between human isolates and an isolate from a batch of fresh chicken breast filet, originating from Poland, and tested positive in the case-by-case control program, was detected by SNP analysis of WGS results (Table A17). When the batch of chicken was found to be contaminated, it was recalled from the consumers. Fresh chicken breast filet seemed to be a plausible source of this outbreak since three interviewed cases reported that they had consumed chicken prior to disease onset. However, this could not be finally proved since the places of purchase reported by the cases differed from the places where the incriminated batch had been sold.

The *Salmonella* outbreaks in 2014 emphasize the importance of close collaboration between the surveillance of human *Salmonella* and the surveillance of *Salmonella* in food, especially when the food item (e.g. pork products) is consumed by many inhabitants on a daily or weekly basis which limits the potential of epidemiological investigation. Therefore, knowledge of a possible match between food isolates and human isolates is essential in the outbreak investigation in order to prevent new cases of illness.

2.3 References

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3. *Listeria*

In 2014, an increase in the number of human listeriosis cases was observed mainly due to several foodborne outbreaks caused by *Listeria monocytogenes*. Thorough investigations were carried out to find the sources to the outbreaks, and a critical review was initiated to come up with recommendations to improve the general control and management of *Listeria* in the food production.

3.1 Whole-genome sequencing as part of the *Listeria* surveillance

By Eva Møller Nielsen (emn@ssi.dk) and Charlotta Löfström

During 2014, several foodborne outbreaks of *Listeria* were detected using the whole-genome sequencing (WGS). WGS was introduced in routine typing for surveillance of listeriosis in Denmark in September 2013 and has increased the discrimination of isolates. During 2014, procedures with parallel, on-time, analysis of clinical and food isolates have been introduced. The new technique and procedures have increased the ability to find the link between human cases and food sources

During 2014, a 1-year pilot project was initiated in a collaborative effort between Statens Serum Institut, National Food Institute at the Technical University of Denmark, and the Danish Veterinary and Food Administration, where *L. monocytogenes* food isolates obtained from the official control programs were sequenced and analysed in parallel with the human clinical isolates and results were compared. WGS was performed on a weekly basis on both human and food isolates. The WGS data analysis was done by initial determination of the classical multi-locus sequence type (MLST) according to the established international nomenclature [1]. Isolates with the same MLST were then compared in a single nucleotide polymorphism (SNP)-based analysis using a reference genome within the group (i.e., same MLST). For more information see chapter 3 in Annual Report 2013.

During the course of the project, 28 isolates from the routine food control were sequenced along with the 92 human isolates from 2014. Furthermore, as part of outbreak investigations, 68 isolates were obtained by sampling in several food producing companies, e.g. as follow-up on the large outbreak of MLST sequence type 224 (ST224) (FUD 1373). These isolates were also subjected to WGS. The use of WGS and the procedure with parallel, on-time analysis of clinical and food isolates, allowed identification of the source of several foodborne outbreaks of *Listeria* during 2014.

3.2 Listeria outbreaks in 2014

By Luise Müller (lum@ssi.dk) on behalf of the Central Outbreak Management Group

3.2.1 Extensive *Listeria* outbreak caused by Danish cold cut ("rullepølse")

On the 26th of June 2014 a cluster of three recent *Listeria monocytogenes* ST224 patients similar to four known patients from 2013 was detected by WGS. An outbreak investigation was initiated to reveal the source in order to stop the outbreak (FUD1373). As part of a project initiated in 2013, all listeriosis patients were routinely followed up with extensive contact to the hospitals for further clinical information and, if possible, patients or relatives were interviewed with a trawling questionnaire on symptoms, food intake in the 30 days prior to disease onset, place of food purchase and food handling habits at home.

In total, 41 patients were reported (four from 2013 and 37 from 2014 (Figure 3.1)). The patients were 43-90 years old with a median age of 72 years, and 23 (56%) were women. Seventeen patients (41%) died within 30 days from the sample date. Clinical information was obtained for all patients, and interviews were possible for 25 patients. The place of exposure could be established for 35 patients and showed, that five patients had been exposed in hospital/elderly home, 14 in private homes and 16 in either one of these. All patients had underlying diseases, in particular cancers and haematological diseases, and many were in treatment with immunosuppressive drugs rendering them more susceptible to listeriosis.

The 16th of July, a match was found by WGS between the human isolates and *Listeria* isolated from "rullepølse"– a Danish cold cut speciality - made from pork from a Danish manufacturer (Company A). In the interviews 18/23 patients confirmed that they had consumed "rullepølse" in the 30 days prior to disease onset. A production and distribution ban for Company A was induced and an extensive withdrawal including downstream businesses was initiated. In total, approximately 6000 companies in 2nd–5th distribution layer were affected, and all these were contacted by telephone. Furthermore, 600 companies were inspected physically.

The outbreak investigation included extensive sampling with more than 800 samples from Company A and downstream businesses. In total, 32/43 *Listeria* positive samples were detected positive with ST224, and these included samples from Company A and from the production environment of a large cutting plant receiving products from

Company A. These findings led to further product recall.

In conclusion, this outbreak was successfully controlled and supposedly, further illnesses/deaths were prevented, despite the fact that outbreaks of listeriosis are often very difficult to investigate because of the long incubation period and patients often being severely ill. The outbreak demonstrates that surveillance of *Listeria* in food as well as typing of isolates from food and patients are crucial to identify the source.

As a consequence of this large outbreak, the Danish Ministry of Food, Agriculture and Fisheries initiated a critical review of the Danish efforts against *L. monocytogenes* which resulted in several recommendations for initiatives to improve the control of *Listeria* in Denmark. These included increased knowledge on *Listeria* risk in food handlers preparing food to vulnerable groups, e.g. in hospitals, and increased information on *Listeria* to risk groups, increased knowledge on *Listeria* risk in food companies and in the food control, and optimized procedures for sampling and source tracing. See 3.3 [2].

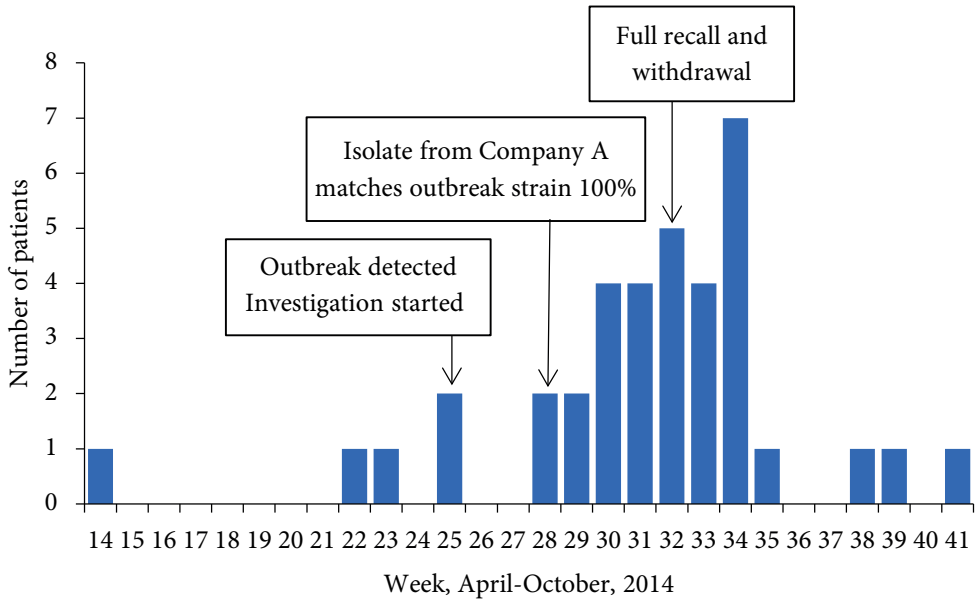
3.2.2 Other *Listeria* outbreaks

In 2014, three additional outbreaks/clusters of *Listeria monocytogenes* detected by WGS were investigated. In April, a cluster of four cases with *L. monocytogenes* ST391 was reported with three cases from 2013, the first one in June 2013 (FUD1376). No known food isolates matched this type, and the next months a new case occurred every second month. The latest ST391 case was detected in November 2014 and in total, eight cases were identified. It is uncertain whether more cases will occur. One case was a child aged 12 years and the other seven were aged 47-89 years. Four were women and three cases died within 30 days from the sample date. Interviews were possible for only three cases, which was not enough to identify a common exposure.

Another *Listeria* cluster dating back to 2013 was detected in September 2014. This was an outbreak of *L. monocytogenes* ST399 (FUD1384). The possibility of hospital-acquired infection was discussed already in July 2013 and the occurrence of new cases supported this hypothesis. Furthermore, the same type was found in an own control sample of asparagus soup from the hospital kitchen and, later on, in a sample of frozen meatballs taken before being added in the soup. Improper heating of the soup after addition of the frozen meatballs was identified as a factor contributing to the possibility for *Listeria* to survive in the soup. From March 2013 to September 2014, six cases could be related to this outbreak – four of these could be identified as possibly infected in the hospital. Because of this outbreak, the hospital kitchen now changed the procedures for re-heating pre-cooked soup.

In September, a cluster of *L. monocytogenes* ST6 was detected (FUD1385). Again, cases had occurred over a long period with date of onset for the first case in May 2013 and the latest in November 2014. Six cases were identified;

Figure 3.1 Week of sampling for Listeria *ST224 patients, April to October 2014[a], n=37*



a) Four cases from 2013 are not included in the figure.
Source: Statens Serum Institut.

five women and one man, aged 43-89 years old. Two cases died within 30 days from the sample date. In September 2014, food isolates of *L. monocytogenes* from a Danish producer were found to be closely related based on WGS. The isolates were from smoked trout and halibut and these products were withdrawn from the market. Furthermore, the producer temporarily stopped the production of smoked halibut. For the five patients, who became ill in 2014, a link was established with eating smoked fish products purchased before the withdrawal in supermarkets, which were selling products from the producer.

3.3 New initiatives on *Listeria*/Critical review

By Stine Thielke (stith@fvst.dk)

Following the large outbreak of *Listeria monocytogenes* during the summer 2014 the Danish Ministry of Food, Agriculture and Fisheries initiated a critical review of the Danish *L. monocytogenes* effort. The critical review was initiated in order to evaluate and improve current efforts and initiatives as well as to identify future focus areas relevant to *L. monocytogenes*.

National and international experts took part in the evaluation and critical assessment of the Danish effort including representatives from Danish research institutes and universities, the food industry, the Danish consumer council, the authorities and two international experts from Belgium and Austria. The critical review was based on an initial workshop followed by comprehensive assessment of identified focus areas. The result was a report presenting six overall themes and associated initiatives based on the assessments (Table 3.1).

The initiatives within each theme are presented below. The initiatives in theme 1-4 will be instigated during the next four years as part of a political agreement. The industry has committed itself to instigate the initiatives within theme 5, and an effort is put into commencing the initiatives within theme 6 through research funds. Overall, the initiatives are intended to improve the general control and management of *Listeria* in the food production as well as to enhance the knowledge and skill base of the food control, the food businesses and food handlers, and groups at risk in relation to *Listeria* with the general aim to secure safe food and reduce the number of cases of listeriosis.

Theme 1 – Knowledge of *Listeria* and information for groups at risk

It is imperative to increase the knowledge and awareness of *Listeria* for groups at risk to minimize the risk of infections with *L. monocytogenes*. The initiatives will be a revision of the current recommendations to groups at risk and definitions of groups at risk. Concurrently, information on the risk of *L. monocytogenes* must be communicated to groups at risk and healthcare professionals in contact with these groups. The intention is among others to get a better distinction of the groups at risk and thereby to a greater extent target the relevant information, recommendations and precautions.

Theme 2 - Knowledge of *Listeria* in kitchens preparing food primarily to groups at risk

Kitchens and food handlers preparing food or delivering food to groups at risk need to understand and manage the risk of *Listeria* since errors during production can be fatal. Hence one of the initiatives is to develop tools and guidance material specifically for this food sector. Moreover the Danish Veterinary and Food Administration will conduct a control campaign directed at kitchens preparing food to groups at risk, and the control frequency in food businesses preparing food to groups at risk will be evaluated.

Theme 3 – Knowledge of *Listeria* in the food control and in the food businesses

It is necessary to continuously increase the knowledge, both within to the food control and the food businesses, on how to manage and control *Listeria* in the food production. Consequently the existing guidance material on *Listeria* will be adjusted and extended, i. e. with practical tools, to better match the needs of the food businesses and the food control. The elaboration of online guidance material will be based on the results of a communication project, which will be conducted to obtain insight and information on how to ensure efficient distribution, communication and design of written material. The Danish Veterinary and Food Administration will also convene specialization courses for the food control inspectors and establish a group of specialists within the food control.

Theme 4 – Source tracing and sampling

Typing of food isolates have proved to be valuable for source tracing and outbreak investigation and can also provide important information to food businesses in relation to the management of *L. monocytogenes* in the food production. Therefore, a collaboration between the authorities and research institutes will be initiated to determine, which isolates from official samples that should be typed using whole genome sequencing. Parallel to this initiative a collaboration between the authorities, the research institutes and the food industry will take place in order to investigate the possibilities to use own control samples and whole genome sequencing in the official control. Sampling is an important part of the control and management of *L. monocytogenes* in the food production

if it is performed correctly and based on a risk assessment and as an integrated part of a HACCP plan, also referred to as intelligent sampling. Hence another initiative will be the development of guidance material and description of principles of intelligent sampling to the food businesses. The Danish Veterinary and Food Administration will also elaborate guidelines for official sampling in withdrawal and recall cases to better ensure efficient corrective measures by the food businesses and a close follow up.

Theme 5 - Initiatives by the food industry

A number of initiatives will be conducted by the food industry, including cross-disciplinary development of tools and practical guides for the food businesses, evaluation and if necessary adjustment of the labour market training courses to ensure, that the education of the food handlers sufficiently covers the subject of *L. monocytogenes,* more focus on *L. monocytogenes* in the collaboration with the production equipment industry, and more focus on *L. monocytogenes* at the annual seminars between the food industry and the Danish Veterinary and Food Administration.

Theme 6 - Areas of research

L. monocytogenes comprise a complex problem regarding food production and food safety, and more research is needed in several areas. Research in these areas can contribute to increase the overall level of knowledge and to continuously improve the effort towards *L. monocytogenes*. Among the identified research areas considered beneficial are further development of existing predictive models, determination of the genetic diversity of *Listeria* isolates from food and humans, indicators for *Listeria* and identification of factors influencing the high incidence of listeriosis in Denmark matched to other comparable countries.

3.4 References

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Table 3.1 Overview of the 6 Listeria monocytogenes themes and initiatives to be instigated from 2015-2018

1. Knowledge of *Listeria* and information for groups at risk	• A better definition of groups at risk • Targeted information and recommendations to groups at risk and healthcare professionals
2. Knowledge of *Listeria* for kitchens preparing food primarily to groups at risk	• Improved guidance on risks related to *Listeria* • Intensified control • Control campaign on riscs of food to groups at risk
3. Increased knowledge to the food businesses and the official control	• Online *Listeria* guideline • Increase of *Listeria* specialists within the official control • Increased knowledge on how to efficiently communicate guidance material
4. Source tracing and sampling	• Typing by whole genome sequencing • Guideline to intelligent sampling • Official samples in relation to outbreaks and withdrawal/recall
5. Initiatives by the food industry	• Practical tools and guides to control *Listeria* • Evaluation of labour market training courses • Focus on *Listeria* in relation to production equipment • Annual seminars between the food industry and the Danish Veterinary and Food Administration
6. Areas of research	• Predictive models • Genetic diversity of *Listeria* isolates • Indicators • Factors related to high incidence in Denmark

Source: The Danish Veterinary and Food Administration.

4. Verotoxin-producing *E. coli* (VTEC)

By Flemming Scheutz (fsc@ssi.dk) and Luise Müller

In 2014, a total of 280 episodes of verocytotoxin- producing *Escherichia coli* (VTEC) were notified. VTEC isolates were obtained from 229 episodes, of which 37 (16%) were caused by O157 (Appendix Table A3). A total of 12 patients developed haemolytic uraemic syndrome (HUS). Nineteen patients were tested positive for VTEC by PCR techniques but isolates were not obtained or submitted for further characterization. Laboratory confirmed VTEC infection thus had an incidence of 4.4 per 100,000 (Appendix Table A2 and A3). Further 32 VTEC cases (including 4 HUS cases) were notified but without information on the method and validity of the diagnose. Overall, the annual number of episodes has been increasing during the last decade. Improved diagnostic methodologies and increased awareness play an important role in this increase.

The incidence of VTEC infections in children less than five years old is approximately 10 fold higher than the incidence in adults (Table 4.1). Nine of the 12 HUS cases were in children less than 10 years. This is the highest number of HUS cases yearly since HUS became notifiable in 2000.

4.1 Outbreaks with Verocytoxin-producing *E.coli*

In 2014, three outbreaks – an unusual high number, were registered with verocytotoxin-producing *E. coli* (VTEC). On average, less than one outbreak per year has been registered in Denmark the last five years, and the last outbreak of VTEC was in October 2012. The first outbreak in 2014 was detected in August, when PFGE typing revealed a cluster of VTEC O103 cases (FUD1377). Hypothesis generating interviews showed, that two cases were from the same household, but no further connection with the third case could be established. Four weeks later, two new cases with the same PFGE pattern were identified, and new interviews were conducted. The five cases were 0-71 years old and lived in different parts of Denmark. No common events or environmental sources were evident, and the outbreak was closed as presumably foodborne, where the source could not be identified.

In October-November, an outbreak of VTEC O157:H-, was identified in a day-care centre in Jutland (FUD1392). Three children in the day-care were diagnosed with VTEC, of which a two-year old child developed HUS. A fourth case – a child living more than 100 km away from the day-care centre had no connection to the other cases. The medical officer and the Food Control Office in Northern Jutland put intensive investigation and control measures at the day care facilities in place. The outbreak source was not identified, but either a common food, environmental exposure or person-to-person transmission in the day-care centre could have played a role.

In November-December an outbreak of VTEC O157:H7 occurred (FUD1409). In total, seven cases were identified, of which four were laboratory-confirmed. The age distribution was unusual for this agent, as cases were young people aged 14-27 years. The cases were from the areas of Copenhagen and Aarhus and they had all been eating kebab (beef) from kebab restaurants. A Swedish kebab restaurant in Malmö had also had a small outbreak of VTEC O157 and comparison of the human isolates by whole genome sequencing (WGS) showed related types. The Danish Veterinary and Food Administration undertook trace-back investigation, but no common batch of

Table 4.1. Number of Verocytoxin-producing E. coli *(VTEC) cases and HUS-cases (haemolytic uraemic syndrome) by age group and incidence per 100,000 population, 2014*

Age group	VTEC cases No.	VTEC cases Incidence per 100,000	HUS cases No.	HUS cases Incidence per 100,000
<1 year	18	31.5	0	-
1-4 years	79	32.7	7	2.9
5-9 years	18	5.4	2	0.6
10-19 years	28	4.1	1	0.1
20-29 years	27	3.7	0	-
30-39 years	16	2.4	0	-
40-49 years	20	2.5	0	-
50-59 years	23	3.1	1	0.1
60-69 years	13	1.9	0	-
>69 years	38	5.4	1	0.1
Total	280	4.9	12	0.2

Source: Statens Serum Institut.

meat or producer could be found. As a control measure, The Danish Veterinary and Food Administration visited all restaurants serving kebab in Denmark in January and February 2015, to ensure proper heating of kebab meat and proper hygiene procedures in the kitchens. Approximately 1,200 establishments were visited during this campaign. No further outbreak cases were reported after this period. An overview of the outbreaks can be seen in appendix Table A4.

4.2 Virulence factors and HUS

VTEC infection and HUS were made notifiable in Denmark in 2000, but the dataset from the Danish Cohort presented below includes patients from 1983 to 2012. The virulence profile for each strain has been related to the clinical data, in particular during the period 1997-2006, where detailed information was obtained on all cases to investigate the cause of disease and complications.

For the period 1983 to 2012, VTEC infection was complicated with HUS in three per cent (70/2,001) of the patients registered in the Danish Cohort (Table 4.2); 6% (44/698) in children less than five years old, 5% (12/229) in children aged 5-15 years and one per cent (14/1,074) in adults. However, great variation was found among different virulence types. The *vtx2* gene together with the *eae* gene is particularly often associated with HUS in children less than 14 years old. Furthermore, preliminary results (data not presented) indicate, that it is primarily the VT subtype *vtx2a*, which is associated with HUS. Only 0.7% (4/552) of the patients developed HUS following infection with a strain of genotype *vtx1* and *eae*, and they all had several predisposing or underlying risk factors, e.g. antibiotic treatment during the acute phase, nephrotic syndrome. For each case, it is therefore important that unusual associations between HUS and specific virulence profiles are carefully examined for underlying disease, epidemiologic relation and antibiotic treatment during the acute phase of illness.

The observed and well documented association between *vtx2* and either presence of either the *eae* gene (attaching and effaing properties) or the ability to colonize and persist in the gut, e.g. enteroaggregative *E. coli* (EAEC), has resulted in a basic and primary definition of HUS associated *E. coli (*HUSEC) for first line public health action: *vtx2* in a background of an *eae* or *aggR* (master regulator gene in EAEC). For such human strains *vtx* is subtyped and further characterized. VTEC with the virulence profile *vtx1*, *vtx1* and *eae*, *vtx2*, *vtx1* and *vtx2* regardless of serotype are considered "low-risk" VTEC and are treated as other enteropathogens like e.g. *Salmonella* or *Campylobacter*.

This simplified approach has been generally accepted by the primary diagnostic clinical microbiology laboratories and public health officers in Denmark. Though not complete in the characterization of each VTEC isolate, this approach is practical and easy to use in an operational environment, which is expected to quickly:

- evaluate the risk of progression of the disease in individuals,
- minimize transmission of HUSEC,
- rehabilitate individuals in order to prevent the worsening of an individual's health, and
- reduce the socio-economic impact on affected families.

However, such a simple approach should be applied with prudence because each individual case is unique and each patient should be carefully evaluated with regard to predisposing factors, general clinical condition, contact with other VTEC infected individuals, and possible link to an outbreak. The identification of "low-risk" VTEC does not per se exclude the risk of progression to severe disease, dehydration or HUS, and patients with bloody stools and/or affected kidney function must be carefully monitored.

Table 4.2. The Danish Cohort: HUS-cases (haemolytic uraemic syndrome) in the period 1983-2012 among Danish cases with Verocytoxin-producing E. coli *(VTEC) stratified according to virulence types and age*

	< 5 years		5-14 years		> 14 years	
Virulence type	VTEC cases No.	HUS cases No. (%)	VTEC cases No.	HUS cases No. (%)	VTEC cases No.	HUS cases No. (%)
eae + vtx2	180	34 (18.9)	49	7 (14.3)	120	4 (3.3)
eae + vtx1 + vtx2	95	6 (6.3)	38	3 (7.8)	110	2 (1.8)
eae + vtx1	332	3 (0.9)[a]	51	1 (2.0)[a]	169	0
vtx2	36	0	25	1 (4.0)[a]	233	8 (3.4)[b]
vtx1 + vtx2	22	0	31	0	187	0
vtx1	33	1 (3.0)[a]	35	0	255	0
Total	698	44 (6.3)	229	12 (5.2)	1,074	14 (1.3)

a) These patients were treated with antibiotics during the acute phase of illness, had several predisposing or underlying risk factors, or (one case) had a double VTEC infection (also infected with O157:H7 (*eae + vtx1 + vtx2*). One patient was positive for VTEC O13, O73:K1:H18 (*vtx2d*).
b) Eight of 25 (32%) patients with culture confirmed EAEC-VTEC O104:H4 (*vtx2a*) developed HUS and were part of the German outbreak in 2011.
Source: Statens Serum Institut.

5. New insights in the European epidemiology of *Salmonella* and *Campylobacter*

By Kåre Mølbak (krm@ssi.dk), Hanne-Dorthe Emborg and Steen Ethelberg

Measuring human incidence of foodborne infections including *Salmonella* and *Campylobacter* infections is affected by biases inherent in passive laboratory surveillance as reflected in the surveillance pyramid. The pyramid describes the chain of events that have to occur so that a case of *Salmonella* or *Campylobacter* in the population will become a reported case, including factors like health-seeking behavior, clinical and laboratory practices regarding microbiological diagnostics and finally reporting rules and compliance. The attributes of the surveillance pyramid are different in different countries and therefore it is often misleading to compare reported figures of culture confirmed cases between countries. To overcome this problem, we hypothesized that seroepidemiologic methods could provide a stringent approach to measure the force of infection, i.e. the rate at which humans are exposed to *Salmonella* or *Campylobacter* to a degree that this exposure is recognized by the immune system as an antibody response.

Based on initial work in the European Med-Vet-Net network of excellence, we have developed methods and models to estimate the force of infections in defined populations. The advantage is, that this measure is independent of the sensitivity of public health surveillance. Because this measure is different from the incidence of clinical infections, we have named it seroincidence. The seroincidence is calculated from the analysis of the levels of antibody classes against non-typhoid *Salmonella* (*S.* Typhimurium and *S.* Enteritidis) or *Campylobacter jejuni* in the general population, and the results of these examinations are converted into a single measure (the seroincidence) based on the kinetics of the antibody decay [1-5].

5.1 Seroepidemiology of *Salmonella* infections

We applied the model to almost 10,000 serum samples randomly selected from individuals representing the general healthy population in 13 different European countries. The year of collection ranged from 2000 to 2012. There was a 10-fold difference in the seroincidence; lowest in Sweden (0.06 infections per person-year), Finland (0.07), and Denmark (0.08) and highest in Spain (0.61) followed by Poland (0.55). The figures did not correlate with the reported national incidence of *Salmonella* infections in humans (Figure 5.1), but correlated with prevalence data of *Salmonella* in laying hens ($p < 0.001$) (Figure 5.2), broilers ($p < 0.001$) (data not shown) and slaughter pigs ($p=0.03$) (data not shown) as published by the European Food Safety Authority [7-9]. The seroincidence also correlated with Swedish figures of country-specific risk of travel-associated *Salmonella* infections [10] ($p=0.001$) (Figure 5.3).

5.2 Seroepidemiology of *Campylobacter* infections

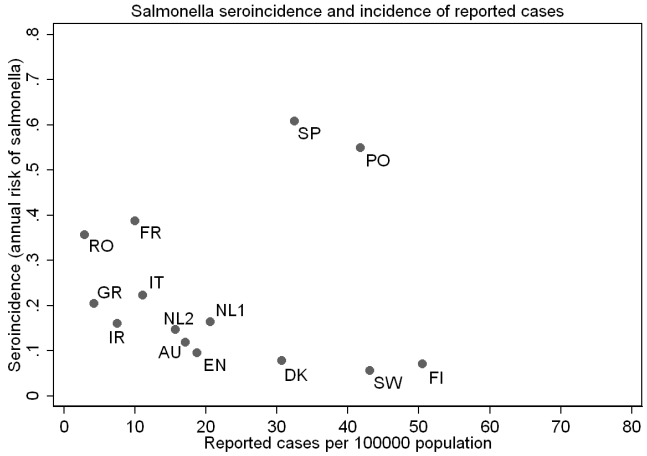
We applied the model to approximately 8,000 serum samples collected from populations in 11 different countries. Again, the samples represented the general population and were collected over the same time span as for the *Salmonella*-sera. For *Campylobacter*, only a two-fold difference was observed: Greece had the lowest seroincidence (0.55) and Poland the highest (1.11). The figures had an inverse correlation with the reported national incidence of *Campylobacter* infections in humans ($p=0.008$), and did not correlate with prevalence data of *Campylobacter* in broilers (11). Seroincidence tended to correlate with Swedish figures of country-specific risk of travel-associated *Campylobacter* infections (12) ($p=0.09$). Data on seroincidence of *Campylobacter* is not presented.

5.3 Conclusions

While *Campylobacter* and *Salmonella* remain as common bacterial foodborne zoonoses, these studies highlight some important similarities and differences. For both infections, the actual number of infections is much higher than suggested in the official surveillance figures. The multipliers are very different across Europe. Indeed, the official numbers are mainly an indicator of the overall function of public health microbiology rather than the real incidence of infections. Hence, national surveillance is useful for following trends within a country and to detect events such as outbreaks or emergence of new subtypes. But the official figures should never be used in a comparison of disease burden between countries.

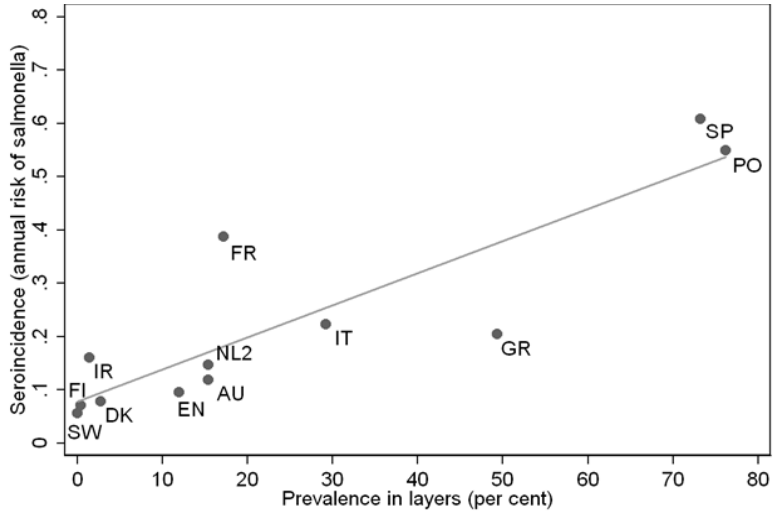
It is of importance to underline that the observed differences between reported figures and seroincidence estimates are not only due to underreporting and under

Figure 5.1. Salmonella seroincidence and the incidence of culture-confirmed cases reported to the European Union



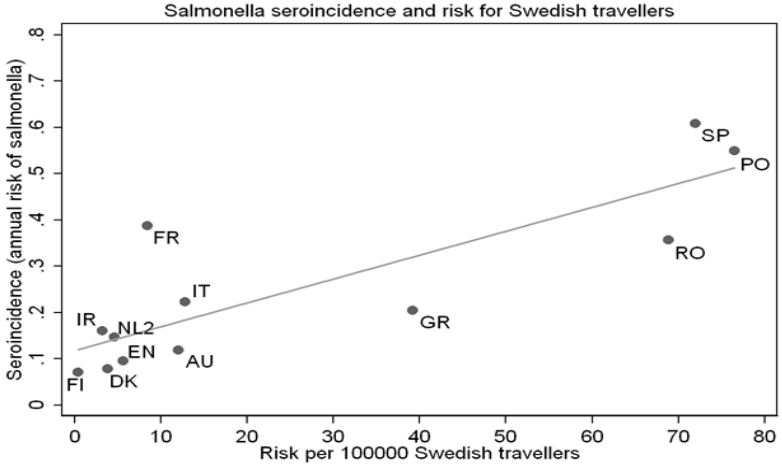
Note: Each point represents one country that took part in the study except that The Netherlands is represented by two serum collections (1998-2002 and 2006-2007). Spearmans rho = -0.367, P=0.197. Reproduced from [6].
Source: Statens Serum Institut.

Figure 5.2. Figure 2. Salmonella seroincidence and the observed prevalence of salmonella-positive holdings of laying hens in 12 European countries



Note: Each point represents one country. Spearmans rho = 0.893, p = 0.001. Reproduced from [6].
Source: Statens Serum Institut.

Figure 5.3. Salmonella seroincidence and risk of Salmonella infection in Swedish travellers



Note: Each point represents one of 12 European countries. Spearmans rho = 0.811, p = 0.001. Reproduced from [6].
Source: Statens Serum Institut.

ascertainment. Any activation of the immune system including asymptomatic infections and mild illness will also be captured by the seroincidence measure, while only a fraction of infections causing clinical illness will be included in the reported laboratory surveillance incidence estimate.

For *Salmonella* it is clear that countries such as the Nordic countries, that implemented control activities several decades ago, have a much lower rate of infections than countries in the Eastern and the Southern Europe, where many countries only started the *Salmonella* control programmes in poultry when they became mandatory in the European Union from 2008. The predominant mode of *Salmonella*-transmission is through the food chain, and there are striking correlations between the prevalence of *Salmonella* in food animals and the seroincidence. This correlation was present even though the samples were collected over a considerable time span and the EFSA baseline surveys were conducted in specific years. This may indicate, that seroincidence is a robust indicator of the level of exposure to *Salmonella*.

In contrast to *Salmonella*, there was not much difference in the seroincidence of *Campylobacter* infections across the participating countries. This shows that there across Europe is less variation in the force of *Campylobacter*-infections than the in the force of *Salmonella*-infections. It was a surprise that there was no correlation between *Campylobacter* seroincidence and prevalence of contaminated broiler carcasses in the corresponding countries. This may indicate that the prevalence of *Campylobacter* is not an appropriate measure of the risk of infection but that other measures, e.g., the degree of contamination may be suitable. Furthermore, there are other routes of transmission than poultry. This suggests that seroepidemiology is not suitable to address the impact of control activities of *Campylobacter* aimed at the poultry reservoir. These data support the notion of *Campylobacter*-transmission from a number of reservoirs, including environment and various animal sources, may be a driving factor for seroconversion and immunity whereas high-dose exposures from poultry (and during travel abroad) may still be a leading cause of clinical illness.

ECDC has developed a software tool that can be used to convert measurements from cross sectional data to a seroincidence figure. The tool has been developed in R and can be downloaded from the www.ecdc.europa.eu. The measurements should be aligned with the reference curves (decay profiles) in order to make meaningful use of the tool.

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6. Vectorborne Zoonoses

By René Bødker (rebo@vet.dtu.dk), Birgit Kristensen and Carsten Kirkeby

The National Veterinary Institute, Tecnical University of Denmark (National Veterinary Institute), monitors vectors and vector borne diseases in Denmark on behalf of the Danish Veterinary and Food Administration.

Denmark is considered free of zoonotic diseases transmitted by mosquitoes (*Culicidae*) and biting midges (*Culicoides*). But tick borne zoonotic disease transmitted by *Ixodes ricinus* are common and, due to increasing numbers of the tick vector, considered an increasing problem. The increased tick density is most likely linked to growing populations of roe deer that functions as the main host for adult egg laying ticks.

In recent years, Denmark had an outbreak of Bluetongue virus serotype 8 (2008) and an outbreak with Schmallenbergs virus (2012); both outbreaks were in ruminants and transmitted via biting midges (*Culicoides* sp.). Further, an ongoing spread of exotic invasive mosquito species has been observed in Europe and since 2000, and outbreaks of mosquito borne West Nile virus, Dengue fever, Chikungunya fever virus, malaria and *Dirofilaria* parasites in humans have been reported in Europe. As a response to the two outbreaks in ruminants in Denmark and due to the increasing risk of introduction of mosquito borne diseases in Denmark, two vector surveillance programs for the abundance of mosquitoes and biting midges were implemented in 2011 and 2012, respectively.

During transmission season the vector abundance of two species of biting midges (*Obsoletus* and *Pulicaris* groups) and five species of mosquitoes (*Aedes, Anopheles, Culex, Culiseta* and *Coquillettidia*) are monitored at sentinel sites. Weekly surveillance data are available at www.myggetal.dk with a three days delay throughout the season. Most diseases transmitted by mosquitoes and biting midges have epidemic potential, and monitoring of temperatures and vector abundance followed by a calculation of the disease transmission potential (R0 - the number of new cases arising from each case) forms an important component of the early warning system at the Technical Univerity of Denmark. This potential for disease transmission must be interpreted in parallel with the risk of introduction. The two main zoonotic introduction threats are presently considered to be the mosquito borne Usutu virus and the mosquito borne *Dirofilaria* worms, with outbreaks recorded in Germany in 2010 [1] and 2014 [2], respectively.

The surveillance of mosquitoes showed a low to medium abundance of *Aedes, Anopheles, Culex* and *Culiseta* for the 2014 season caused by the dry summer, and a normal

Table 6.1 Overview of vector borne diseases presented in the text

Vector	Pathogen		Transmission	Transmitted in Denmark
Ticks				
	Bacteria	*Rickettsia helvetica*	Zoonotic	Yes
		Neoehrlichia mikurensis	Zoonotic	Yes
		Anaplasma phagycytophilum	Zoonotic	Yes
		Borrelia sp.	Zoonotic	Yes (several species)
		Babesia sp.	Zoonotic	Yes (two species)
Mosquitoes				
	Virus	Chikungunya fever	Only between humans	No
		Dengue fever	Only between humans	No
		Usutu virus	Zoonotic	No
		West Nile virus	Zoonotic	No
	Parasite	Malaria	Only between humans	No
		Dirofilaria (worms)	Zoonotic	No
Biting Midges				
	Virus	Bluetongue	Only between ruminants	Yes (2007)
		Schmallenberg	Only between ruminants	Yes (2012)

Source: National Veterinary Institute, Technical University of Denmark.

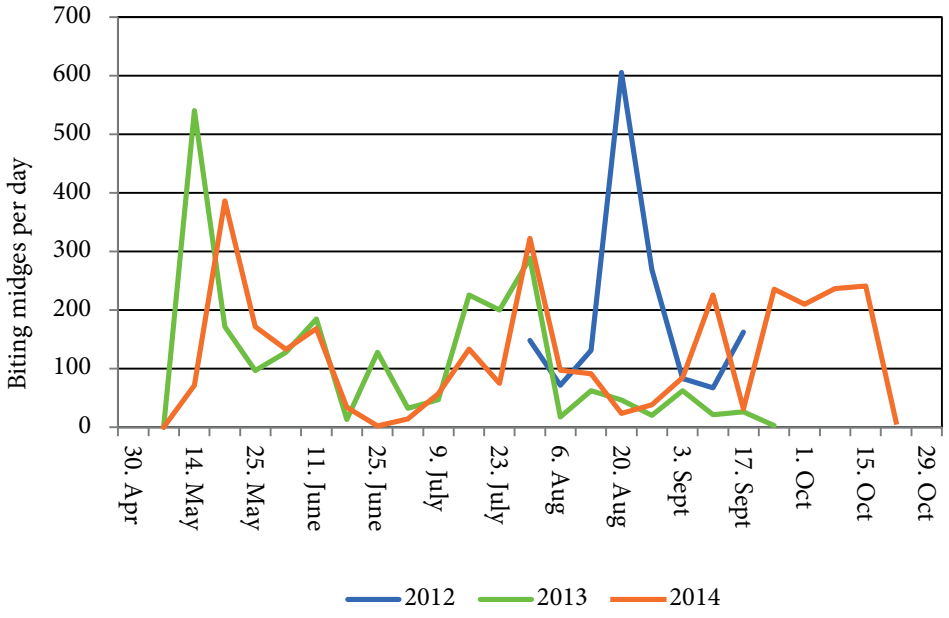
abundance of *Coquillettidia*, a species not affected by low rainfall in Denmark. However, the high temperatures in late summer gave rise to three generations of biting midges compared to the usual two generations, and resulting in midge abundances in mid-October comparable to peak abundances normally measured during summer (Figure 6.1). This illustrates that small increase in temperatures may have disproportional large impact on vector biology.

The mosquito surveillance program also identified a new mosquito species in Denmark, *Culex modestus* (*Cx. modestus*, 'nilfeber-myg' in Danish). This is the most northern recording of this species in Europe [3]. This species has in recent years been reported moving north in Europe and was in 2012 reported in large numbers south of London in United Kingdom [4]. *Cx. modestus* is a highly competent vector for zoonotic West Nile virus with reservoir in wild birds. Denmark has large populations of both *Cx. pipiens* and *Cx. torrentium* mosquitoes that effectively transmit the virus between birds. But these species do not bite humans. *Cx. modestus* however will bite both man and birds and thus act as an important bridge vector between wild birds and humans during outbreaks. *Cx. modestus* was discovered in a densely populated residential area just south of Copenhagen with local biting rates on humans reaching up to one per minute in the late afternoon during peak season. The species was however found to be confined to just a single small breeding site on the beach. It is important to note that Usutu and West Nile virus were never identified in Denmark, but migrating birds have been found seropositive when returning to Denmark in the spring. The large spread of West Nile virus and Usutu virus within Europe the last 15 years and the presence of seropositive migrating birds highlights why we need to be aware of the presence of a bridge vector like *Cx. modestus* in Denmark.

In 2013, the invasive exotic mosquito *Aedes japonicus* became established in Hannover just 200 km from the Danish border. Screening of water samples from private gardens in southern Jutland that where hatched to adult mosquitoes in the laboratory at the National Veterinary Institute, did not reveal this species in southern Denmark in 2014. But the rapid spread in Holland and Germany suggests that it may eventually spread across the border to Denmark.

Screening of pools of tick nymphs from Danish forests using a newly developed DNA screening chip revealed two new *Borrelia* pathogens in *Ixodes ricinus* [5]. *Borrelia spielmanii* and *Borrelia miyamotoi* which is different from known European *Borrelia* species as it causes a relapsing type of fever in humans. The screening also found the parasites *Babesia venatorum* and *Babesia divergens*. *Babesia divergens* is a well know infection in Danish cattle, but

Figure 6.1 Biting midges (Obsoletus *sp.) per day on cattle herds, May-October, 2012-2014. Monitoring starting July, 2012*



Source: National Veterinary Institute, technical University of Denmark.

this is the first time it is identified in Danish ticks. It is not known if *Babesia venatorum* causes disease in humans. Additionally, the screening confirmed the already known presence of *Borrelia burgdorferi, Borrelia garinii, Borrelia afzelii, Borrelia valaisiana, Anaplasma phagocytophilum* and *Rickettsia helvetica.* Further the screening found a widespread presence of *Neoehrlichia mikurensis* (figure 6.2). *Neoerlichia mikurensis* was newly discovered in Denmark [6] and has been associated with severe human disease in Sweden [7].

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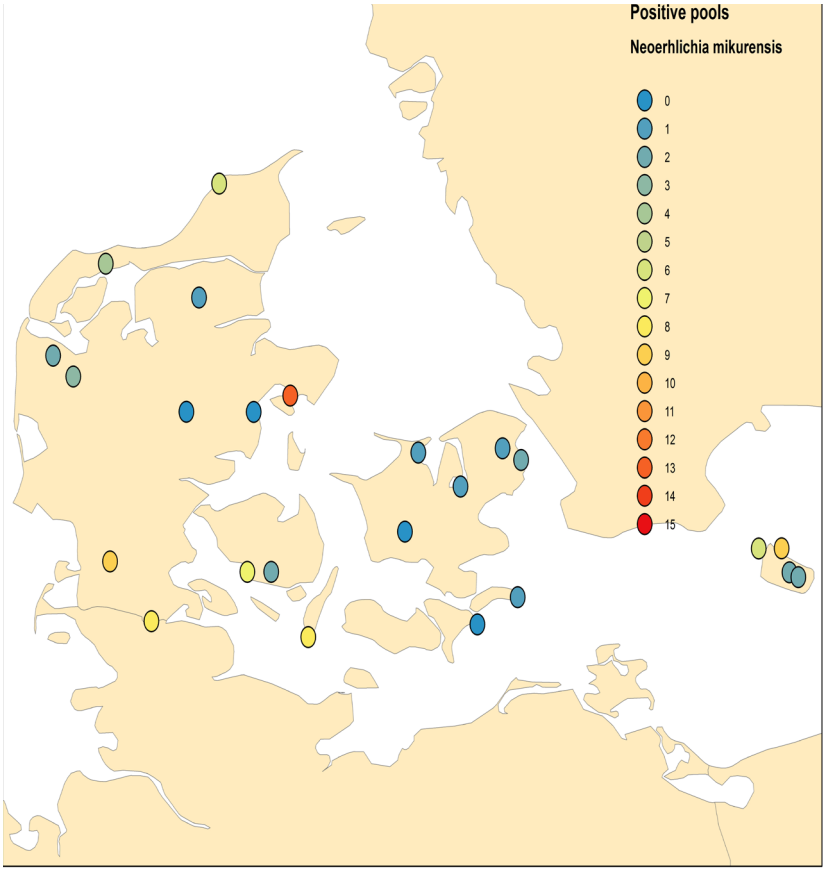
Figure 6.2 Neoehrlichia mikurensis in Denmark[a]



a) From each site 15 pools of 15 nymphs each was screened for tick borne pathogens, here *Neoehrlichia mikurensis*. The color indicates how many pools were positive at each site.
Source: National Veterinary Institute, Technical University of Denmark.

7. Status on national and EU targets

By Gudrun Sandø (gus@fvst.dk) and Pernille C.S. Tillisch (pes@fvst.dk)

In Denmark, action plans and programs on zoonoses have been in place for more than 25 years. The first plan targeted *Salmonella* in the broiler production and was developed as a response to an increase in the number of human cases related to eating chicken meat. Since then, plans have been developed for *Salmonella* in pigs and pork, *Salmonella* in layers (eggs), *Campylobacter* in broilers and *S.* Dublin in cattle and beef.

All plans have been outlined in cooperation between industry, research institutes and authorities, and are followed by a technical working group and a steering committee. This ensures progress, that new knowledge is incorporated in the plans, and an assessment of achievement of targets.

At EU level, harmonized surveillance programs and common targets have been set for the broiler and laying egg production.

An overview on the status on the targets can be seen in Table 7.1.

7.1 National targets

The first action plan for *Campylobacter* was initiated in 2008 and followed by the second plan in 2013 that covers the period until the end of 2016. The plan is targeting *Campylobacter* in broilers and chicken meat as well as other sources and food. Targets have been set for the reduction of the prevalence of *Campylobacter* positive flocks and of the relative risk related to eating chicken meat. At flock level the target is a 20% sum of reductions in 2011-2013 and 2014-2016. A reduction of 9.4% was obtained from 2011-2013, and the target for 2014-2016 is a reduction of 11%. At slaughterhouse level the target is a reduction of the relative risk by 25% in 2014 and by 50% in 2016 compared to 2013. A reduction of 28% was obtained in 2014.

The first action plan for reduction of *Salmonella* in the broiler production was developed by the industry in 1988. An official plan of action was adopted in 1996 and included the table-egg production as well. Both action plans have been adjusted several times over the years. The target was from the beginning an eradication of *Salmonella* from the broiler production. Today there is a zero-tolerance of *Salmonella* in Danish produced broiler meat. Meat from *Salmonella* positive flocks must be heat treated, and broiler meat tested positive for *Salmonella* after slaughter cannot be marketed as fresh meat. In the table-egg production, Denmark has achieved special guarantees for *Salmonella* in the EU (for further information see Annual Report 2012, Chapter 7). Both plans are now focused on maintaining the low prevalence.

In the pig production, the fifth *Salmonella* action plan was adopted at the end of 2013 and runs until the end of 2017. It continues the measures from former plans and points at new measures as well (See Annual Report 2013 for more information). The target is set for the prevalence of *Salmonella* positive carcasses at the slaughterhouse level and is a maximum of 1% positive carcasses. The target must be achieved in 2014 and maintained throughout the period. At the end of 2014 the prevalence was just below 1%, and the target for 2014 was reached (Figure 7.1). The trend in number of human *Salmonella* cases attributed to the pig reservoir in the *Salmonella* source account is evaluated annually. Since the large outbreaks in 2008-2010, the annual number of cases in outbreaks from domestic pork ranged between 0 and 65, and the estimated number of sporadic cases ranged between 63 and 120, resulting in an increasing overall number (Figure 7.2).

The first action plan for eradication of *S.* Dublin in cattle and beef was adopted in 2008 and has been changed several times since then. In recent years, the program has been tightened as the number of positive herds has been declining (for further information see Annual Report 2013, Chapter 5). The target is to eradicate *S.* Dublin in cattle herds by the end of 2016.

7.2 EU targets

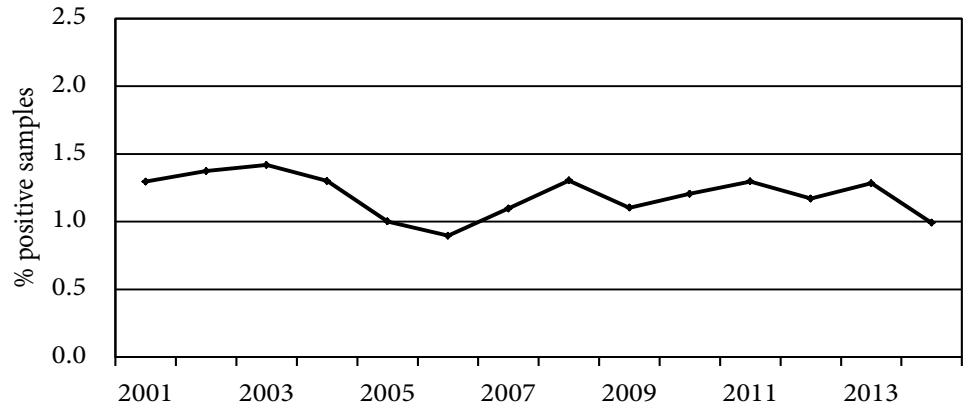
Based on the results of baseline studies in flocks of poultry (*Gallus gallus* and turkeys), harmonised regulation on targets and surveillance in the poultry production has been laid down by the Commission.

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 and micribiological criteria set on *Salmonella* have included these types as well.

The EU target for breeding and fattening turkey flocks is laid down in Regulation (EC) No 1190/2012. According to this regulation the target is maximum 1% flocks positive for *S.* Typhimurium and *S.* Enteritidis. In Denmark, no turkey flocks were positive with *Salmonella* in 2014 (Appendix Table A12).

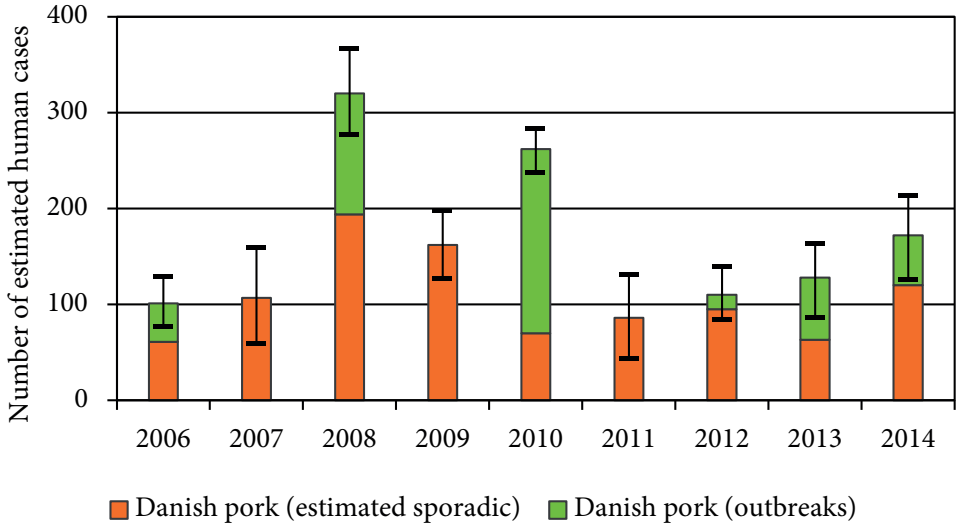
In breeding flocks of *Gallus gallus*, the target according to Regulation (EC) No 200/2010 is maximum 1% adult flocks positive for *S.* Typhimurium, including the monophasic *S.* 1,4,[5],12:i:- strains, *S.* Enteritidis, *S.* Hadar, *S.*

Figure 7.1. Salmonella *in pork based on swab samples from carcasses, estimated prevalence[a], 2001-2014*



a) Percent positive single samples, based on occurrence of *Salmonella* in pooled and single samples (see Appendix Table A5).
Source: Danish Veterinary and Food Administration.

Figure 7.2. Estimated number of human cases of salmonellosis and foodborne Salmonella *outbreaks due to Danish pork, 2006-2014*



Source: Annual Report on Zoonoses in Denmark from 2006 to 2014, Danish Zoonoses Centre, National Food Institute.

Infantis and *S.* Virchow. In the legislation, no distinction is made between breeding flocks from the table egg and broiler production lines. In Denmark, three breeding flocks from the broiler production were positive with *Salmonella*. The flocks were infected with *S.* Typhimurium DT41, *S.* 1,4,12:i:- DT120 and *S.* Bareilly. In total 1.3% of breeding flocks were positive for target *Salmonella* seotypes (Appendix Table A9 and A11), and the target has not been reached.

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, according to Regulation (EC) No 517/2011, are 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. The target is set for *S.* Typhimurium, including the monophasic S. 1,4,[5],12:i:- strains, and *S.* Enteritidis. For Denmark, the target is a maximum of 2% adult flocks positive for the target serotypes. The prevalence in Denmark has been below 2% since 2004. In 2014, two flocks (0.6%) were positive, both with target serotypes (one flock with *S.* Enteritidis FT21 and one flock with *S.* Typhimurium DT40) (Appendix Table A9).

In broiler flocks of *Gallus gallus*, the target is maximum 1% flocks positive for *S.* Enteritidis and *S.* Typhimurium, including the monophasic *S.* 1,4,[5],12:i:- strains, and is laid down in Regulation (EC) No 200/2012. Denmark has had intensive *Salmonella* control programs since the 1990's and the target of 1% was reached in 2000. In 2014, 0.7% of broiler flocks were positive with *Salmonella,* and 0.4% of the flocks were positive with target serotypes (Appendix Table A11).

Table 7.1. Status on targets for Campylobacter and Salmonella, 2014

National Action Plans	Target	Status
Campylobacter in broilers 2013-2016		
Flocks at farm	Reduction in prevalence of positive flocks: 20% (sum of reductions in two periods: 2011-2013 and 2014-2016)[a]	A reduction of 9.4% was obtained for the period 2011-2013
Fresh meat at slaughterhouse	Reduction of the relative human risk (RR) compared to the level in 2013[b] 2014: RR reduced by 25% 2016: RR reduced by 50%	A reduction of 28% was obtained in 2014 compared with 2013
Salmonella in poultry[c]		
Laying hen flocks of *Gallus gallus*	Initially eradication, later a reduction strategy in the table egg production	0.6% (two flocks) (Table A9-A10) Eggs from positive flocks are destroyed or heat treated
Carcasses at slaughterhouse	Initially eradication, later a reduction strategy in the broiler production Zero-tolerance in Danish broiler meat.	1.4% positive batches (Table A11) Positive batches are heat treated
Salmonella in pigs 2014-2017		
Carcasses at slaughterhouse	Max. 1% *Salmonella* at carcass level in 2014-2017	Overall 0.99% at carcass level in 2014 (Table A15)
Estimated human cases from pigs	No considerable increase in number of estimated human cases from pigs in the Danish *Salmonella* source account	In total 172 cases attributed to Danish pork in 2014 (CI95%: 126-214) vs. 128 cases in 2013 (CI95%: 86-163). (Chapter 1, Figure 7.2, and Table A1)
Salmonella Dublin in cattle 2012-2016		
Herds at farm	Eradication of *S.* Dublin in all herds by 2016., i.e. all herds in level 1[d] by the end of 2016	6.3% of milk-producing herds and 2.9% of non-milk producing herds are in level 2 or 3 (07.01.2015) (Table A17)
EU Regulations		
Regulation (EC) No. 1190/2012		
Breeding and fattening turkey flocks	Max. 1% positive for *S.* Enteritidis and *S.* Typhimurium[e]	No positive flocks (N=10) (Table A12)
Regulation (EC) No. 200/2010		
Breeding flocks of *Gallus gallus*	Max. 1% adult flocks positive for *S.* Typhimurium[e], *S.* Enteritidis, *S.* Hadar, *S.* Infantis and *S.* Virchow	2.0% (3 flocks) (Table A9 and A11) 1.3% (2 flocks) with target serovars
Regulation (EC) No. 1168/2006		
Laying hen flocks of *Gallus gallus*	MS specific targets, for Denmark: Max. 2% adult flocks positive for *S.* Typhimurium[e] and *S.* Enteritidis	0.6% (two positive flocks with target serovars) (Table A9)
Regulation (EC) No. 646/2007		
Broiler flocks of *Gallus gallus*	Max. 1% positive *S.* Typhimurium[e] and *S.* Enteritidis	0.7% (26 positive flocks) (Table A11) 0.4% (14 flocks) with target serovars

a) Data from 2013 and 2014 cannot be compared due to a change in sampling per 1 January, 2014 from AM testing of sock samples 7-10 days before slaughter to cloacal swabs at the point of slaughter. The target has therefore been changed to cover two periods: 2011-2013 and 2014-2016. The target is an overall 20% reduction (the sum of percent reductions in the two periods).
b) Data from 2013 has been agreed as the baseline since 2012-data are not comparable with data from 2013 and onwards due to a necessary improvement in the collection of data.
c) Supplementary to EU-regulations.
d) See Table A36 for explanation of the herd levels.
e) Including the monophasic strains *S.* 1,4,[5],12:i:-.
Source: Danish Veterinary and Food Administration.

8. International topics

By Inge-Lis Kyllesbæk Andersen (ilka@fvst.dk) and Hanne Rosenquist (hanro@fvst.dk)

8.1 *Trichinella*

In June 2014 an amendment to the EU Regulation on *Trichinella* came into force. This new provision included among other things a new sampling regime for *Trichinella* and introduction of the concept of "Controlled housing conditions". Slaughter pigs, sows and boars, which are kept under controlled housing conditions, are exempted testing. Free range pigs, horses, wild game (e.g. wild boar) and other species, susceptible to *Trichinella* must be tested.

8.2 Ebola and imported foods

In 2014, an outbreak of Ebola virus in West Africa, primarily in the countries Guinea, Liberia and Sierra Leone, increased dramatically in size, and by the end of 2014 more than 20.000 confirmed, probable, and suspected cases of Ebola virus disease with around 8000 deaths were reported by WHO (http://apps.who.int/ebola/en/current-situation/ebola-situation-report). Due to the seriousness of the outbreak, WHO has declared the outbreak a Public Health Emergency of International Concern.

Ebola is transmitted through direct contact with blood or other bodily fluids from infected people. Concerns have been raised about the risk of transmission through illegal import of bush meat from Africa into Europe, since several animal species, mainly primates and fruit bats, have been found to harbor Ebola virus, and illegal import of bush meat from Africa into e.g. France has shown to be large (estimated to around five tonnes per week in personal baggage through Paris Roissy-Charles de Gaulle airport) [1].

The risk from handling and consuming bush meat was assessed by EFSA [2]. EFSA concluded that the potential for introducing and transmitting Ebola virus via bush meat to Europe is currently categorised as low risk. However, due to lack of data and knowledge, which results in very high uncertainty, the exact risk could not be estimated. In conclusion, the low risk is explained by (i) the limited number of outbreaks confirmed to date in Africa in spite of the routine consumption of bush meat on that continent, (ii) the handling of bush meat in Europe not involving high risk practices such as hunting and butchering, and (iii) the assumed low overall consumption of bush meat in Europe. The probability of transmission of Ebola via bush meat to Denmark is considered to be lower than for Europe in general. This is because illegal import of bush meat to Denmark is considered to be negligible. Further, the border control is aware of the risk.

EFSA and ECDC have also assessed the risks related to dogs and cats having been in contact with people infected with Ebola virus [3]. In Europe, this event is assumed to be very rare. If it happens, the probability of the pet becoming infected may range from very low to high. A similar range for the probability (low-high) was assessed for human exposure to the virus through infected pets, as it will depend on the specific circumstances. EFSA and ECDC therefore recommend that the risk in each case is assessed jointly by veterinary and public health authorities.

8.3 References

1. Chaber A-L, Allebone-Webb S, Lignereux Y, Cunningham AA and Rowcliffe JM (2010). The scale of illegal meat importation from Africa to Europe via Paris. Conservation Letters, 3, 317-323.

2. EFSA (European Food Safety Authority) (2014). An update on the risk of transmission of Ebola virus (EBOV) via the food chain. EFSA Journal;12(11):3884, 25 pp.

3. EFSA (European Food Safety Authority) (2014). Risk related to household pets in contact with Ebola cases in humans. EFSA Journal;12(12):3930, 12 pp.

9. Surveillance and control programmes

The collaboration on zoonoses between national and regional authorities, the industry and non-governmental organizations in Denmark is presented in Figure 9.1. 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 A30 and Table A31, respectively, including reference to the relevant legislation.

9.1 Surveillance of human disease

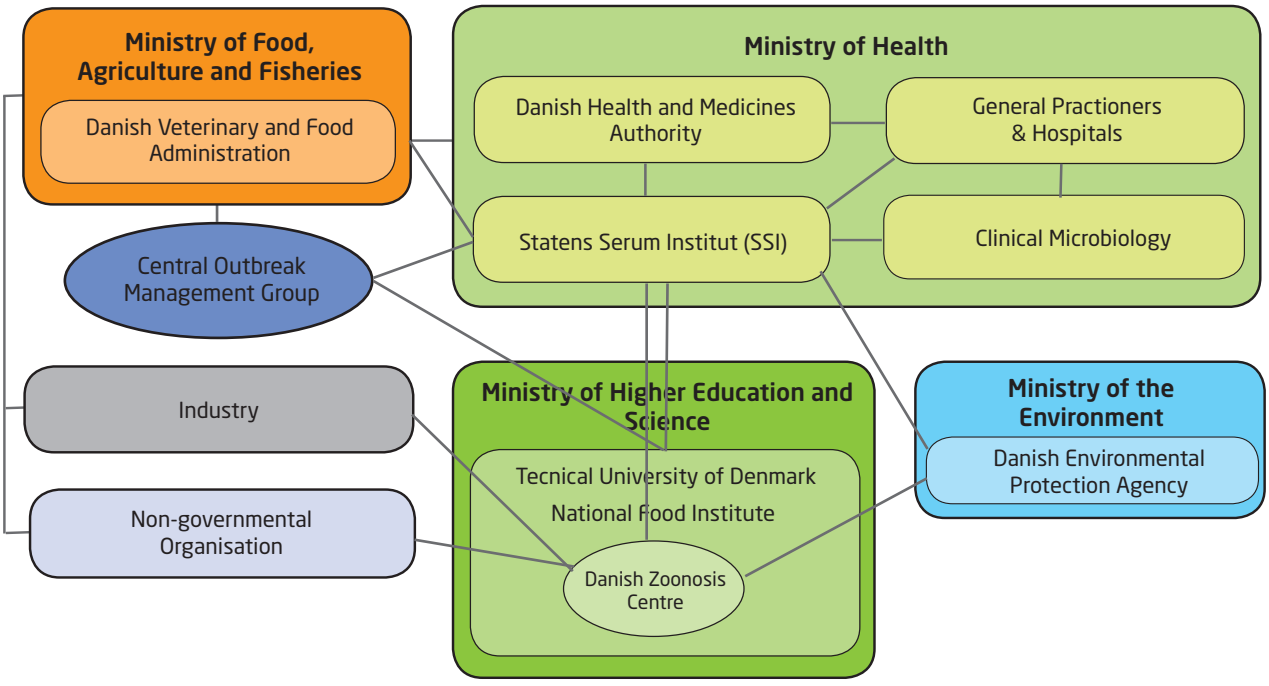
Information on human cases due to zoonotic pathogens presented in this report is reported to Statens Serum Institut through different channels depending on the disease:

- Notifiable through the laboratory surveillance system: *Salmonella, Campylobacter, Yersinia*, Verotoxin-producing *E. coli* (VTEC) and *Listeria.*
- Individually notifiable zoonotic pathogens: *Chlamydia psittacci* (ornithosis), *Leptospira* (Weils disease), *Mycobacterium*, Bovine Spongieform Encephalopathy (BSE) prions (var. Creutzfeldt-Jakob Disease), Verotoxin-producing *E. coli* (VTEC) and Lyssavirus (rabies).
- Non-notifiable zoonotic pathogens: *Brucella*, *Cryptosporidium*, *Echinococcus* 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. 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 and MLVA distribution is presented in Appendix Table A5-A8.

Figure 9.1. Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feedstuffs in Denmark, 2013



9.2 Outbreaks of zoonotic gastrointestinal infections

In Denmark, local and regional foodborne outbreaks are typically investigated by the Food Control Offices[1] 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, react upon outbreak alerts and coordinate investigations. The formal responsibility of food- or waterborne outbreak investigations is divided between three ministeries based on the outbreak source: the Ministry of 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. Further, clusters of cases may be noted in local laboratories or identified through the laboratory surveillance system of gastrointestinal bacterial infections or subtyping of bacterial isolates from patients, both at Statens Serum Institut.

A list of verified outbreaks (not including household outbreaks) reported to the Food- and waterborne Outbreak Database (FUD) is presented in Appendix Table A4 and some of the outbreaks from 2014 are outlined in Chapter 2.

9.3 Surveillance and control of animals and animal products

Salmonella surveillance and control programmes for poultry, pigs and cattle are presented in Appendix Tables A32-A37. 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 the 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 A38.

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 and A9-A10.
- Results from the broiler production are presented in Appendix Tables A5-A7, A11 and A18.
- Results from the duck and turkey productions are presented in Appendix Table A5-A6, A12 and A18.
- Results from the pig production are presented in Appendix Tables A5-A6, A15, A18 and Figures A1-A3.
- Results from the cattle production are presented in Appendix Tables A5-A6, A8, A16-A17 and Figure A4.
- Results from the feeding stuff production are presented in Appendix Tables A19-A20.
- Results from the rendering plants are presented in Appendix Table A21.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Table A23-A24.

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

- Results from the broiler production are presented in Appendix Tables A13-A14 and A18.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Table A23-A24.

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 A15-A16.

Results from the surveillance for Bovine Spongiform Encephalopathy (BSE) in cattle, and Transmissible Spongiform Encephalopathy (TSE) in sheep/goat are presented in Appendix Tables A25-A27.

Results from the monitoring of *Coxiella burnetii* (Q fever) in cattle are presented in Appendix Table A16.

Results based on suspicion of diseases with *Chlamydia*

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.

psittacci, Cryptosporidium, Trichinella, classical rabies and European Bat *Lyssavirus* in zoo animals, pets and wild life are presented in Appendix Table A23-A24.

9.4 Official testing of zoonotic pathogens in foodstuffs

In Denmark, control of zoonotic microorganisms in foodstuffs is mainly carried out as projects which are co-ordinated 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 A28 provides information on the centrally coordinated studies conducted in 2014.

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

New legislation on *Salmonella* and *Campylobacter* in broilers and *Salmonella* in pigs and pork

By Gudrun Sandø (gus@fvst.dk)

The changes of Order no. 1512 of 13/12/2013, regulating the control of *Salmonella* and *Campylobacter* in poultry, include

- Change of testing for *Campylobacter.* 12 cloacal swabs from 24 animals from each flock are to be analyzed in one pool instead of, as in 2010-2013, sock samples in the flock.
- *Salmonella* control in ducks is no longer included in the programme, as no action is taken on isolation of *Salmonella* in ducks, and the prevalence is known to be high.
- The *Salmonella* control after slaughter now includes more samples at the small slaughterhouses.

The changes of Order no. 1183 of 06/11/2014, regulating the control of *Salmonella* in pigs and pork, include

- A new way to calculate the index, that divides the herds into the categories 1,2 or 3 (footnote g) Table A37).

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, 2012-2014

Source	2014		2013		2012	
	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	172 (126-214)	15.4	128 (86-163)	11.3	110 (84-139)	8.0
Domestic beef	25 (20-31)	2.2	21 (4-58)	1.9	85 (72-100)	7.1
Domestic table eggs	33 (22-47)	3.0	17 (7-31)	1.5	15 (1-35)	1.3
Domestic broilers	22 (1-69)	2.0	0[c]	0	0[c]	0
Domestic ducks	No data	-	9 (0-21)	0.8	10 (1-23)	0.8
Imported pork	13 (0-44)	1.1	30 (15-50)	2.6	3 (0-10)	0.2
Imported beef	3 (0-7)	0.2	22[d] (13-33)	2.0	11 (4-20)	0.9
Imported broilers	33 (14-53)	2.9	12 (2-26)	1.1	21 (3-47)	1.8
Imported turkey	0[b]	0	7 (0-18)	0.6	13 (1-28)	1.1
Imported duck	22 (11-34)	2.0	No data	-	22 (13-34)	1.6
Travels	538 (528-549)	48.0	458 (445-471)	40.3	539 (527-550)	45.0
Unknown source	216 (178-252)	19.2	228 (191-264)	20.1	332 (293-369)	27.7
Outbreaks, unknown source	45	4.0	204	18.0	37	4.3
Total	1,122		1,136		1,198	

a) The model is based on a Bayesian framework which gives 95% credibility intervals.
b) No samples from imported turkey meat were found positive for *Salmonella* in 2014.
c) No samples from domestic broiler meat were found positive for *Salmonella* in 2012 and 2013.
d) No data from imported beef in 2013. The number of cases attributed to this source was modelled from previous years' data.
Source: Danish Zoonosis Centre, National Food Institute.

Human disease and outbreak data

Table A2. Zoonoses in humans, number of laboratory-confirmed cases, 2009-2014

	Incidence per 100,000 inhabitants	Reported no. of cases					
Zoonotic pathogen	2014	2014	2013	2012	2011	2010	2009
Bacteria							
Brucella abortus/melitensis[a,d]	-	4	4	2	7	6	7
Campylobacter coli/jejuni[b]	67.1	3,782	3,766	3,728	4,068	4,035	3,352
Chlamydia psittaci[b]	0.3	16	12	12	7	9	14
Leptospira spp.[b]	0.2	10	3	7	11	10	12
Listeria monocytogenes[b]	1.6	92	50	50	49	62	97
Mycobacterium bovis[b]	0.02	1	0	0	1	2	0
Salmonella total[b]	19.9	1,122	1,136	1,198	1,166	1,598	2,129
S. Enteritidis[b]	4.8	268	346	242	293	388	600
S. Typhimurium[b,c]	7.6	427	337	415	386	521	767
Other serotypes[b]	7.6	427	453	541	487	689	762
VTEC total[b]	4.4	248[f]	186	190	224	184	165
O157	0.7	37	23	36	27	25	24
Other O-groups or non-typeable	3.4	192	163	154	197	159	141
Yersinia enterocolitica[b]	7.7	432	345	291	224	192	238
Parasites[g]							
Cryptosporidium spp.[a,d]	-	5	6	8	31	25	35
Echinococcus multilocularis[a,e]	-	0	3	7	4	1	0
Echinococcus granulosus[a,e]	-	10	9	20	31	10	11
Trichinella spp.[a,e]	-	1	0	0	0	0	0
Viruses							
Lyssavirus[b]	0	0	0	0	0	0	0

a) Not notifiable, hence the incidence cannot be calculated.
b) Notifiable.
c) *S.* Typhimurium and the 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.
f) Includes 19 cases verified by PCR only (see Table A3).
g) The nation-wide neonatal screening of congenital toxoplasmosis stopped in 2007
Source: Statens Serum Institut.

Table A3. VTEC *O-group distribution in humans[a], 2014*

O-group	Number of episodes	O-group	Number of episodes
O157	37	O91	5
O103	29	O-rough	6
O26	24	Other O-groups or non-typeable	95
O146	13	Isolates total	229
O145	13	Confirmed by PCR only	19
O117	7	Notified[b]	32
Continued in the next column		Total	280

a) All O-groups that resulted in five or more episodes are listed.
b) The cases are reported through the notification system, isolates or DNA not available for verification.
Source: Statens Serum Institut.

Table A4. Food- and waterborne disease outbreaks[a] reported in the Food- and Waterborne Outbreak Database (FUD) (n=60), 2014

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
Bacillus cereus	7	-	Restaurant	Composite meal	1403
Campylobacter	3	3	Restaurant	Chicken (imp/dk)	1437
Campylobacter	2	1	Private party	Chicken	1375
Clostridium perfringens	45	-	Sports event	Composite meal	1420
Clostridium perfringens	6	-	Restaurant	Composite meal	1417
Clostridium perfringens	4	-	Restaurant	Composite meal	1400
Clostridium perfringens	8	-	Restaurant	Composite meal	1394
Clostridium perfringens	4	-	Restaurant	Composite meal	1393
Clostridium perfringens	391	11	Catering	Composite meal	1390
Clostridium perfringens	70	-	Restaurant	Buffet meal	1359
E. coli (not typed)	18	-	Restaurant	Herbs (imp)	1418
E. coli (AEEC) O171:H19	19	7	Restaurant	Composite meal	1387
Histamine	3	-	Restaurant	Fish	1401
L. monocytogenes, MLST224[d,e]	41	41	National	Cold cuts	1373
L. monocytogenes, MLST391	8	8	National	Unknown	1376
L. monocytogenes, MLST399	6	6	Hospital	Composite meal	1384
L. monocytogenes, MLST6	6	6	National	Fish[f]	1385
Lectines	4	-	Canteen	Dried beans	1407
Norovirus	27	-	Institution	Buffet meal	1439
Norovirus	3	-	Restaurant	Composite meal	1435
Norovirus	11	-	School	Strawberries (imp)	1433
Norovirus	147	1	Shop	Composite meal	1432
Norovirus	23	-	Restaurant	Composite meal	1419
Norovirus	57	-	Conference Center	Composite meal	1412
Norovirus	35	4	Restaurant	Unknown	1411
Norovirus	42	3	Canteen	Buffet meal	1405
Norovirus	53	3	Canteen	Buffet meal	1404
Norovirus	22	1	Restaurant	Composite meal	1402
Norovirus	22	-	Restaurant	Oysters (imp)	1398
Norovirus	9	3	Restaurant	Oysters (imp)	1397
Norovirus	4	-	Restaurant	Composite meal	1389
Norovirus	31	3	Restaurant	Composite meal	1388
Norovirus	9	1	Private party	Rasberries (imp)	1383
Norovirus	21	-	Restaurant	Buffet meal	1371
Norovirus	9	-	Institution	Buffet meal	1369
Norovirus	153	-	Canteen	Composite meal	1367
Norovirus	50	-	Canteen	Buffet meal	1365
Norovirus	16	-	Restaurant	Composite meal	1364
Norovirus	430	7	Canteen	Buffet meal	1363
Norovirus	9	-	Restaurant	Oysters (imp)	1362
Norovirus	22	-	Private party	Buffet meal	1353
Norovirus	134	-	Canteen	Composite meal	1352

Continued on the next page

Table A4. Food- and waterborne disease outbreaks[a] reported in the Food- and Waterborne Outbreak Database (FUD) (n=60), 2014 (Continued from previous page)

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
S. Agona[b]	8	8	National	Unknown	1317
S. Enteritidis, MLVA0206[c,g]	5	5	National	Unknown	1327
S. Enteritidis, MLVA0017	4	4	National	Chicken (imp)	1391
S. Enteritidis, MLVA0019	18	18	National	Eggs	1379
S. Infantis	8	8	Restaurant	Chicken (imp)	1380
S. Infantis	5	5	Regional	Unknown	1370
Salmonella 1,4,5,12:i:-, MLVA0201	25	25	National	Pork	1372
S. Typhimurium, MLVA1788	12	12	National	Unknown	1410
Salmonella 1,4,12:i:-, MLVA0007	22	22	Shop	Pork	1368
Salmonella 1,4,5,12:i:-, MLVA1277	19	19	National	Minced beef	1374
Salmonella 1,4,5,12:i:-, MLVA0008	38	15	Sports event	Unknown	1378
Salmonella 1,4,5,12:i:-, MLVA0334	5	5	Private party	Pork	1446
Shigella sonnei	5	3	Canteen	Sugar snaps (imp)	1408
Unknown	11	-	Restaurant	Composite meal	1431
VTEC O103:H2, *eae* and *ehxA, vtx1a*	5	5	National	Unknown	1377
VTEC O157:H-, eae, *vtx1a*, *vtx2a*	4	4	Institution	Unknown	1392
VTEC O157:H7, eae, *vtx1a*, *vtx2a*	7	4	Restaurant	Beef	1409
Yersinia enterocolitica O:3, biotype 4	24	24	National	Unknown	1354
Total	2,209	295			

Note: (imp)= imported product. (imp/dk)= uncertain origin of product.
a) In addition to the above mentioned outbreaks, 1 household outbreak (FUD 1350) with 2 persons involved was registered in 2014. The cause of the otbreak was possible presence of histamine in tunaseaks.
b) Outbreak FUD No 1317 - Additional 13 cases had onset in 2013. However, these cases were not reported in Annual Report 2013.
c) Outbreak FUD No 1327 referred to in Chapter 2 concerning, in total, 12 cases of *Salmonella* Enteritidis, MLVA0206 was already mentioned in the report for 2013. Seven of these cases are therefore not presented in this table.
d) Multi-Locus Sequence Type (see chapter 3).
e) Four cases in FUD No. 1373 had onset in 2014.
f) Cold smoked fish.
g) MLVA profiles for the most common human MLVA-types can be found in tables A6, A7 and A8.
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 meat, 2014. N=number of culture positive units[a].*

	Human	Pig[b]	Pork[c]	Beef[d]	Layer[e]	Broiler[e]	Broiler[f]	Imported meat (batches)			
	cases	animals	batches	batches	flocks	flocks	batches	Pork[g]	Beef[h]	Broiler[g]	Ducks[h]
Serotype	N=1,122	N=170	N=108	N=11	N=2	N=26	N=6	N=20	N=4	N=9	N=24
Enteritidis	23.9	0	0	0	50.0	3.8	0	0	0	8.3	0
1,4,[5],12:i:-	20.5	22.4	22.2	0	0	30.8	66.7	31.8	0	0	4.2
Typhimurium	17.6	18.2	15.7	0	50.0	19.2	16.7	22.7	25.0	0	41.7
Infantis	3.4	1.2	6.5	0	0	26.9	16.7	4.5	0	25.0	0
Dublin	1.9	0	0	80.8	0	0	0	0	25.0	0	0
Stanley	1.9	0	0	0	0	0	0	0	0	8.3	0
Newport	1.7	0	0	0	0	3.8	0	0	0	0	20.8
Virchow	1.6	0	0	0	0	0	0	0	0	0	0
Agona	1.4	0	0	0	0	0	0	0	0	0	0
Kentucky	1.4	0	0	0	0	0	0	0	0	0	0
Chester	1.0	0	0	0	0	0	0	0	0	0	0
Paratyphi B var Java	1.0	0	0	0	0	0	0	0	0	25.0	0
Oranienburg	0.9	0	0	0	0	0	0	0	0	0	0
Derby	0.8	55.9	45.4	9.1	0	0	0	22.7	0	0	0
Muenchen	0.8	0	0	0	0	0	0	0	0	0	0
Others	18.0	2.4	1.9	0	0	15.4	0	13.6	50.0	33.3	33.3
Unknown	2.3	0	8.3	9.1	0	0	0	4.5	0	0	0
Total	100	100	100	100	100	100	100	100	100	100	100

a) One isolate per serotype per unit is included, thus the number of isolates may exceed the number of units. Thus, in 2014 more isolates were included from imported pork and broiler meat.
b) Isolates collected from coecum samples taken randomly at slaughter. Where more than one *Salmonella* positive pig with different serotypes was randomly selected from a herd, one pig per serotype was included.
c) Sampling of pork carcasses at slaughterhouses according to the surveillance programme (Table A37).
d) Data are from sampling of beef carcasses at slaughter houses according to the surveillance programme (10 positive samples) (Table A36) and from a centrally coordinated study (one positive sample) (see section 9.4 and Table A28 for description).
e) Sampling of production flocks prior to slaughter according to surveillance programmes (Tables A33).
f) Sampling of broiler meat (neck skin) at slaughterhouses according to the surveillance programme (4 positive batches) (Table A33), and case-by-case control of Danish meat (2 positive batches).
g) Case-by-case control of imported meat. One batch of imported pork with three serotypes: *S.* Typhimurium (3), *S.* 1,4,[5],12:i:- (2) and *S.* Derby (1). One batch of imported broiler meat with four serotypes: *S.* Paratyphi B var Java (1), *S.* 1,4,12:b:- (1), *S.* 6,7:r:- (1) and *S.* Infantis (1). For further information regarding case-by-case control programme and Annual Report on Zoonoses in Denmark, 2007.
h) Centrally coordinated study (see section 9.4 and Table A28 for description).
Source: Danish Veterinary and Food Administration, Statens Serum Institut, and National Food Institute.

Table A6. Top 10 (humans) MLVA[i] distribution (%) of Salmonella *Typhimurium including the monophasic S. 1,4,[5],12:i:- from humans, animals, carcasses and imported meat, 2014. N= number of isolates*

MLVA type[i]		Human	Pork[b]	Layer[c]	Broiler[c]	Broiler[d]	Imported meat (batches)		
		cases	batches	flocks	flocks	batches	Pork[e]	Beef[a]	Ducks[f]
STTR 9|5|6|10|3	Danish	N=423	N=40	N=1	N=13	N=3	N=11	N=1	N=11
3|12|9|NA|0211	0007	9.2	2.5	0	0	0	0	0	0
3|14|9|NA|0211	0201	7.8	12.5	0	0	0	0	0	0
3|13|9|NA|0211	0008	5.7	0	0	0	0	20.0	0	0
3|12|10|NA|0211	0005	4.3	5.0	0	0	0	6.7	0	0
3|13|10|NA|0211	0192	4.3	7.5	0	15.4	33.3	6.7	0	0
3|12|17|NA|0211	1277	3.8	0	0	0	0	0	0	0
5|19|NA|NA|0201	1788	2.8	0	0	0	0	0	0	0
3|14|8|12|0311	1690	2.1	0	0	0	0	0	0	0
3|14|12|NA|0211	0334	1.9	0	0	15.4	0	0	0	0
3|14|10|NA|0211	0126	1.4	2.5	0	0	0	0	0	0
Other		56.7	70.0	100	69.2	66.7	66.7	100	100
Total		100	100	100	100	100	100	100	100

For footnotes a-h see Table A5.
i) The isolates are analysed for the following loci: STTR9|STTR5|STTR6|STTR10|STTR3 and the results are reported in the same order in the table. "Danish" is the Danish MLVA-number for the MLVA profile. "NA"= locus missing.
Source: Danish Veterinary and Food Administration, Statens Serum Institut, and National Food Institute.

Table A7. Top 10 (humans) MLVA[i] distribution (%) of Salmonella *Enteritidis from humans, animals and imported meat, 2014. N= number of isolates*

MLVA type[i]		Human	Layer[c]	Broiler[c]	Imported meat (batch)
		cases	flocks	flocks	Broiler[e]
SE 1|5|2|9|3	Danish	N=268	N=1	N=1	N=1
4|10|5|3|1	0019	27.7	100	0	0
3|10|7|2|2	0004	16.4	0	0	0
4|9|5|3|1	0017	15.4	0	0	100
4|11|5|3|1	0020	12.3	0	0	0
4|11|4|3|1	0034	7.7	0	0	0
3|11|7|2|2	0029	7.2	0	0	0
4|9|8|2|2	0206	4.1	0	0	0
3|10|8|2|2	0005	3.6	0	0	0
3|13|9|2|2	0022	3.1	0	0	0
4|12|5|3|1	0031	2.6	0	0	0
Other		27.2	0	100	0
Total		100	100	100	100

For footnotes a-h see Table A5.
i) The isolates are analysed for the following loci: SE1|SE5|SE2|SE9|SE3 and the results are reported in the same order in the table. "Danish" is the Danish MLVA-number for the MLVA profile.
Source: Danish Veterinary and Food Administration, Statens Serum Institut, and National Food Institute.

Table A8. Top 10 (humans) MLVA[i] distribution (%) of Salmonella *Dublin from humans, carcasses and imported meat, 2014. N= number of isolates*

MLVA type[i,j]		Human	Beef[b1]	Imported meat (batch)
		cases	batches	Beef[f]
SE1|SE2|SE5|SD1	Danish	N=21	N=9	N=1
2|3|14|4	0043	14.3	0	0
2|4|11|6	0104	14.3	0	0
Other		71.4[k]	100	100
Total		100	100	100

For footnotes a-h see Table A5.
i) The isolates are analysed for the following loci: SE1|SE2|SE5|SD1 and the results are reported in the same order in the table. "Danish" is the Danish MLVA-number for the MLVA profile.
j) Kjeldsen MK, Torpdahl M, Campos J, Pedersen K, Nielsen EM (2014). Multiple-locus variable-number tandem repeat analysis of *Salmonella enterica subsp. enterica* serovar Dublin. J Appl Microbiol. 116(4):1044-54.
k) One isolate of each MLVA-type.
Source: Danish Veterinary and Food Administration, Statens Serum Institut, and National Food Institute.

Table A9. Occurrence of Salmonella *in the table egg production[a], 2005-2014*

	Rearing period[c] (parent flocks)		Adult period[d] (parent flocks)		Pullet-rearing flocks		Table egg layer flocks	
	N	Positive	N	Positive	N	Positive	N	Positive
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
2014	22	0	8	0	150	0	347	2[b]

a) See Tables A32 and A34 for description of the surveillance programmes.
b) One flock positive with *S.* Enteritidis PT21, and one flock positive with *S.* Typhimurium DT40. For information on MLVA types see Tables A6-A7.
c) *Salmonella* was not detected in grandparent flocks during rearing period (7 flocks).
d) *Salmonella* was not detected in grandparent flocks during adult period (8 flocks).
Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration.

Table A10. Occurrence of Salmonella *in the table egg layer flocks sorted by type of production, 2005-2014*

	Deep litter		Free range		Organic		Battery	
	N	Positive	N	Positive	N	Positive	N	Positive
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	137	3	94	0
2014	97	0	30	0	125	1[a]	95	1[b]

a) One flock positive with *S.* Typhimurium DT40. For information on MLVA types see Table A6.
b) One flock positive with *S.* Enteritidis FT21. For information on MLVA types see Table A7.
Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration.

Table A11. Occurrence of Salmonella *in the broiler production[a], 2005-2014*

	Rearing period[i] (parent flocks)		Adult period[j] (parent flocks)		Broiler flocks		Slaughterhouse (flocks/batches)	
	N	Positive	N	Positive	N	Positive	N	Positive
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	3,448	27	368	0
2013	128	0	152	1	3,498	34	288	0
2014	121	2[e]	131	3[f]	3,470	26[g]	277	4[h]

a) See Tables A32-A33 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) Both isolates were *S.* Typhimurium DT120, one MLVA 3|7|11|15|0311 and one with both 3|7|11|15|0311 and 3|7|12|14|0311. For more information on *S.* Typhimurium MLVA types see Table A6.
f) *S.* Typhimurium DT41 MLVA 2|12|12|8|0212 (1), *S.* 1,4,12:i:- DT120 MLVA 3|15|8|NA|0211 (1), *S.* Bareilly (1). For more information on *S.* Typhimurium MLVA types see Table A6.
g) *S.* Typhimurium (DT170 (2), DT41 (2), RDNC (1)), *S.* Enteritidis (PT1B (1)), *S.* 1,4,5,12:i:- (DT193 (7), Not phage typed (1)), *S.* Senftenberg (1), *S.* Infantis (7), *S.* Indiana (1), *S.* Newport (1), *S.* Manhattan (1), *S.* Tennessee (1).
h) *S.* 1,4,5,12:i:- and *S.* 1,4,12:i:- DT193 (1), *S.* 1,4,5,12:i:- DT193 (2), *S.* Infantis (1).
i) *Salmonella* was not detected in grandparent flocks during rearing period (13 flocks).
j) *Salmonella* was not detected in grandparent flocks during adult period (6 flocks).
Source: Danish Agriculture and Food Council, and Danish Veterinary and Food Administration.

Table A12. Occurrence of Salmonella *in turkey and duck flocks, 2006-2014*

	Duck flocks		Turkey flocks[a]	
	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[b]	20.3	56	3.6
2014	0[b]	-	10	0

a) See Table A35 for description of the surveillance programme for turkey flocks. 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) Since 20/09/2013 samples from ducks were no more taken.
Source: Danish Agriculture and Food Council.

Table A13. Occurrence of Campylobacter *in broiler flocks, 2005-2014[a] UKLART om N er swabs/socks eller flocks*

	Cloacal swabs at slaughter		Sock samples at farm	
	N	% pos	N	% pos
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	-	-	3,132	16.5
2011	-	-	3,379	14.4
2012	-	-	3,376	11.6
2013	-	-	3,508	13.1
2014	3,474	27.7	-	-

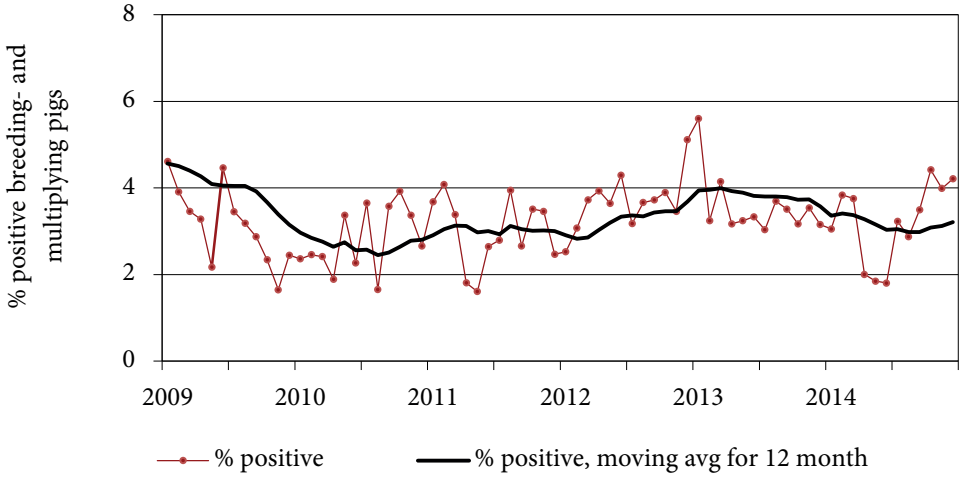
a) See Tables A33 for description of the surveillance programmes. In 2014 the sampling method changed back from boot swabs collected in the stable 7-10 days before slaughter to cloacal swabs at slaughter according to Regulation no. 1512 of 13/12/2013.
Source: Danish Agriculture and Food Council, Danish Veterinary and Food Administration, and National Veterinary Institute, Technical University of Denmark (until 2009).

Table A14. Occurrence of Campylobacter *in non-heat treated broiler meat at slaughter and retail[a], 2012-2014*

		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
2014	Conventional	927	25.7	-	-	-	-
	Organic/free-range	108	75.0	-	-	-	-
	In total	-	-	-	-	-	-

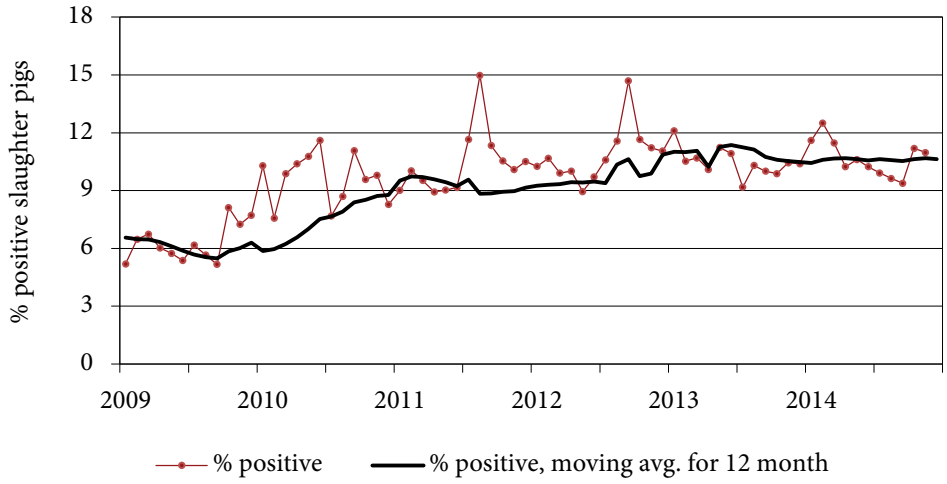
a) Centrally coordinated studies (see Table A28 and section 9.4 for description). Limit of quantification: 10 cfu/g.
b) The prevalence is calculated as a mean of quarterly prevalences., except orgnanic/free-range results.
c) Leg-skin samples only.
d) Included are 238 leg-skin samples, prevalence = 24,4%.
Source: National Food Institute.

Figure A1. Serological surveillance of Salmonella in breeding and multiplying pigs[a] based on monthly testing of blood samples, 2009-2014



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], 2009-2014. Percentage of seropositive meat juice samples (first sample per herd per month)[b]*



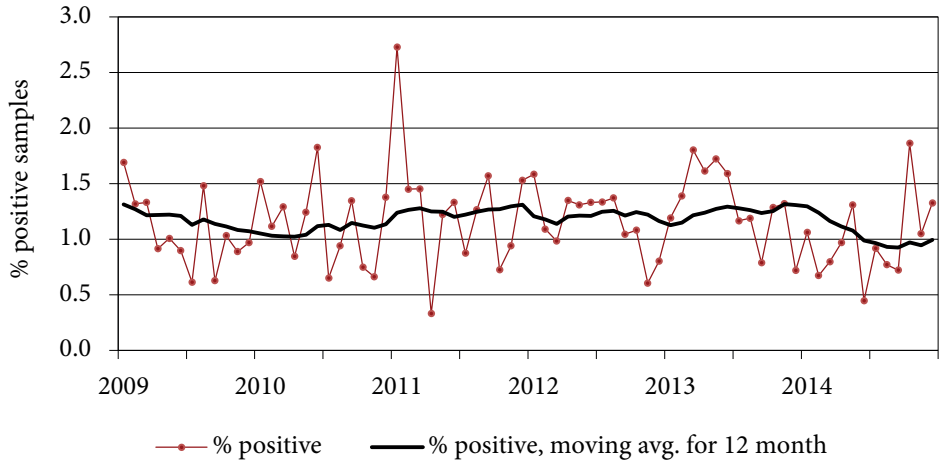
a) For more information about the surveillance programme, see Table A37.
b) The peaks in January 2010 and August 2011 were due to data transfer problems. The reason for the increase in late summer 2012 is unknown.
Source: Danish Agriculture and Food Council.

Table A15. Occurrence of zoonotic pathogens in pigs and pork in Denmark, 2014

	Herds		Animals/Samples		
Zoonotic pathogen	N	Pos	N	Pos	% pos
At farm					
Brucella abortus[a]	-	-	20,163	0	-
Leptospira[b]	81	3	121	6	5.0
At slaughterhouse (slaughter pigs)					
Salmonella spp.[c,d]	6,761	337[e]	-	-	-
Salmonella spp.[c,f] (slaughtering >50 pigs/month)	-	-	17,250	-	0.98[g]
Salmonella spp.[c,f] (slaughtering 50 or less pigs/month)	-	-	537	-	1.30
Salmonella spp.[c,h]	-	-	801	173	21.6
Trichinella spp.[i]	-	-	18,250,233	0	0
Mycobacterium bovis[j]	-	-	18,407,939	0	0
Echinococcus granulosis/multilocularis[j]	-	-	18,407,939	0	0

a) Including samples from boars (examined at pre-entry, every 18 month, and prior to release from semen collection centres) (17,007 samples), samples collected in connection with export (3,011), import (no samples this year) or diagnostic samples (145 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 immunoflourescence techniques.
c) See Table A37 for describtion of the *Salmonella* surveillance programme.
d) Data are from December 2014. Slaughter pig herds monitored using serological testing of meatjuice samples collected at slaughter.
e) Includes herds belonging to *Salmonella* 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 cm^2. 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, Technical University of Denmark.

Figure A3. Salmonella *in pork, monitored at slaughterhouses[a], 2009-2014*



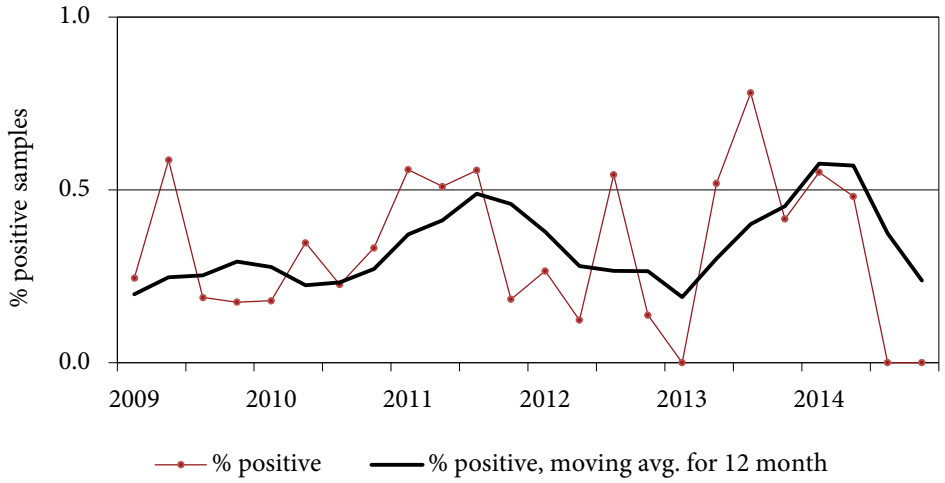
a) For more information about the surveillance programme, see Table A37.
Source: Danish Veterinary and Food Administration.

Table A16. Occurrence of zoonotic pathogens in cattle and beef in Denmark, 2014

	Herds		Animals/Samples		
Zoonotic pathogen	N	Pos	N	Pos	% pos
At farm					
Brucella abortus[a]	-	-	1,452	0	-
Mycobacterium bovis[b, c]	-	-	1,150	0	-
Coxiella burnetii	26[d]	15	168[e]	6	-
At slaughterhouse					
Salmonella spp.[f] (slaughtering >50 cattle/month)	-	-	5,350	-	0.2[g]
Salmonella spp.[f] (slaughtering 50 or less cattle/month)	-	-	969	-	0.4
Mycobacterium bovis[b, h]	-	-	485,147	0	0
VTEC O157[i]	228	16	-	-	-
Echinococcusus granulosis/multilocularis[h]	-	-	485,147	0	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) (1,197 samples), samples collected in connection with export (206), import (43) or diagnostic samples (6). 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 interdermal 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 (41 samples, 6 pos), export (85 samples, no pos), import (2 samples, no pos) and breeding (40 samples, no pos) and analysed using an ELISA method. An additional 9 samples from placenta was analysed using the FISH method, none were positive.
f) See Table A36 for describtion 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 cm^2. 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, Technical University of Denmark.

Figure A4. Salmonella *in beef, monitored at slaughterhouses[a], 2009-2014*



a) For more information about the surveillance programme, see Table A36.
Source: Danish Veterinary and Food Administration.

Table A17 Cattle herds in the S. Dublin *surveillance programme[a], January 2014*

Salmonella Dublin level			Non-milk producing herds N	Non-milk producing herds %	Milk producing herds N	Milk producing herds %
Level 1		On the basis of milk samples	-	-	3,205	93.7
		On the basis of blood samples	14,070	97.1	-	-
	Total	Probably *Salmonella* Dublin free	14,070	97.1	3,205	93.7
Level 2		Titer high in blood- or milk samples	87	0.6	169	4.9
		Contact with herds in level 2 or 3	241	1.7	19	0.6
		Other causes	83	0.6	8	0.2
Level 3		Salmonellosis, official supervision	5	0	20	0.6
	Total	Non *Salmonella* Dublin free	416	2.9	216	6.3
Total number of herds			14,486		3,421	

a) See Table A36 for description of the surveillance programme.
Source: Seges, Cattle.

Table A18 Results from the intensified control of Salmonella *and* Campylobacter *in fresh meat based on case-by-case risk assessments 2014*

		Batches tested	No. of batches positive	No. of batches deemed unsafe based on a risk assessment	Batches deemed unsafe based on other criteria[a]	Mean prevalence in batches[b,d]	Mean relative human risk in batches[c,d]
Campylobacter							
Danish	Broiler	124	32	2	-	47.1[d]	3.5[d]
Imported	Broiler	149	66	7	-	46.3[d]	4.5[d]
Salmonella							
Danish	Pork	144	14	5	-	26.6	16.8
	Broiler	105	2	0	2	-	-
Imported	Pork	153	20	2	-	9.4	5.6
	Broiler	155	9	1	3	28.1	13.3
	Turkey	27	0	0	0	-	-

a) Microbiological criteria specified in regulation (EC) No 2073/2005 as amended. For Danish broiler meat there is a zero-tolerance for *Salmonella* and all positive batches must be heat treated before being put on the marked (Order no. 1512 of 13/12/2013).
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.
d) In 2014, a lower limit for when batches contaminated with *Campylobacter* were sent for risk assessment was introduced. Therefore these figures are not comparable to previous years.
Source: Danish Veterinary and Food Administration, and National Food Institute.

Table A19. Feed business operators own sampling of Salmonella *in compound feeds, feed processing and feed material (batch-based data), 2012-2014*

	2014		2013		2012	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control)[a]:						
Ordinary inspections - clean zone	7,557	17[d]	7,132	2	7,105	11
Ordinary inspections - unclean zone	456	63[e]	577	88	736	82
Compound feed, farm animals	858	0	375	0	316	0
Feed materials, farm animals[b]	1,656	28[f]	1,295	11	1,369	25
Transport vehicles, clean zone/hygiene samples[c]	1,143	1[g]	973	4	884	0
Transport vehicles, unclean zone/hygiene samples[c]	235	7[h]	255	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.* Falkensee, *S.* Idikan, *S.* Isangi, *S.* Rissen, *S.* Tennessee,
e) *S.* Agona, *S.* Derby, *S.* Falkensee, *S.* Putten, *S.* Rissen, *S.* 1,4,5,12:i:-.
f) *S.* Cubana, *S.* Havana, *S.* Infantis, *S.* Livingstone, *S.* Liverpool, *S.* Mbandaka, *S.* Senftenberg, *S.* Tennessee, *S.* 13,23:-:-.
g) *S.* Derby.
h) *S.* Derby, *S.* Rissen.
Source: Danish Veterinary and Food Administration and the feed business operators.

Table A20. Control of Salmonella *in compound feeds, feed processing and feed material (batch-based data), 2011-2014*

	2014		2013		2012		2011	
	N	Positive	N	Positive	N	Positive	N	Positive
Feed processing plants (process control)[a]:								
Ordinary inspections[b]	402	10[d]	333	7	311	11	377	12
Feed materials, farm animals[c]	90	4[e]	99	2	99	4	68	3

a) Presence of *Salmonella* in compound feed is indirectly monitored by environmental samples collected during feed processing. Companies are sampled one to four times per year.
b) Primarily findings of *Salmonella* in the dirty zone.
c) Predominantly soy bean meal and rapeseed cake.
d) *S.* Infantis (3 dirty, 1 clean), *S.* Rissen (1 dirty, 1 clean), *S.* 13,23:-:- (1 clean), *S.* Agona (1 clean), *S.* Falkensee (1 dirty), *S.* Senftenberg (1 dirty).
e) *S.* Infantis (2), *S.* Idikan (1), *S.* Mbandaka (1).
Source: Danish Veterinary and Food Administration.

Table A21 Salmonella *in three categories of meat and bone meal by-products not intended for human consumption[a], 2014*

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	210	2	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	450	2	1,717	1
	Total	725	4	1,786	1

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: Daka Denmark A/S.

Table A22. Pathogens in batches[a] of ready-to-eat vegetables, herbs and fruits[b], 2014

	Salmonella		*Campylobacter*		*E. coli*	
Type of sample	N	Pos	N	Pos	N	>100 cfu/g[i]
Vegetables						
Baby corn	6	0	6	0	6	1[c]
Cucumber	4	0	4	0	4	0
Pepper	6	0	6	0	6	0
Salad/leafy green	33	0	33	0	33	1[d]
Sprouts	6	0	6	0	6	1[e]
Sugar peas	10	0	10	0	10	0
Tomato	13	0	13	0	13	0
Other vegetables[f]	14	0	14	0	14	0
Herbs						
Parsley	8	0	8	0	8	0
Spearmint	2	0	2	0	2	0
Spring onions	7	0	7	0	7	0
Other herbs[g]	5	0	5	0	5	1[h]
Fruit and berries						
Apples and pears	4	0	4	0	4	0
Grapes	3	0	3	0	3	0
Rasberries	7	0	7	0	7	0
Strawberries	12	0	12	0	12	0
Other berries	11	0	11	0	11	0
Other fruits	2	0	2	0	2	0
Total	153	0	153	0	153	4

a) Five samples per batch.
b) Centrally coordinated study (See section 9 for description) to control and investigate *Salmonella, Campylobacter* and *E. coli* in Danish and imported ready-to-eat vegetables, sprouts and herbs.
c) 1 batch of babycorn from Thailand.
d) 1 batch of baby leaves from Denmark.
e) 1 batch of azuki sprouts from Denmark.
f) Including aubergine, broccoli, spinach, zucchini.
g) Including chives, oregano, thyme, dill.
h) 1 batch of dill from Italy.
i) Batches with >100 cfu/g in one or more samples.
Source: Danish Veterinary and Food Administration.

Table A23. Occurrence of zoonotic pathogens in pets and zoo animals in Denmark[a], 2014

	Pet animals						Zoo animals			
	Dogs		Cats		Others		Mammals & reptiles		Birds	
Zoonotic pathogen	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
Salmonella spp.	1	1[b]	0	-	0	-	7[c]	0	16	0
Chlamydia psittaci	0	-	0	-	46	6	0	-	14	-
Cryptosporidium spp.	7	1	2	0	0	-	11[d]	0	0	-
Lyssavirus (classical)	1	0	1	0	0	-	0	-	0	-
European Bat *Lyssavirus*	1	0	1	0	0	-	0	-	0	-

a) All samples are analysed based on suspicion of disease, and does not reflect the country prevalence.
b) *S.* Dublin.
c) Two chimpansees, one alpaca, one reindeer, one camel, one tiger, one zebra (four of these animals were tested due to export).
d) Three ring-tailed lemurs, two chimpansees, one alpaca, one tiger, one zebra, one antilope, one monkey, one seacow (three of these animals were tested due to export).
Source: National Veterinary Institute, Technical University of Denmark, and Danish Veterinary and Food Administration.

Table A24. Occurrence of zoonotic pathogens in wild and farmed wildlife in Denmark[a], 2014

	Farmed wildlife				Wildlife			
	Wild boar		Minks & chincillas		Mammals		Birds	
Zoonotic pathogen	N	Pos	N	Pos	N	Pos	N	Pos
Salmonella spp.	8	0	14	5[b]	41[c]	4[d]	10[e]	0
Campylobacter spp.	0	-	16	11	0	-	0	-
Chlamydia psittaci	0	-	0	-	0	-	8	8
Cryptosporidium spp.	0	-	0	-	99[f]	6[g]	0	-
Echinococcus multilocularis	0	-	0	-	477[h]	9[i]	0	-
Trichinella spp[j]	482[k]	0	0	-	446[l]	0	0	-
Lyssavirus (classical)	0	-	0	-	25[m]	0	0	-
European Bat *Lyssavirus*	0	-	0	-	25[m]	0	0	-

a) All samples are analysed based on suspicion of disease or risk based and does not reflect the country prevalence, except for animals analysed for *Echinococcus multilocularis*. These animals are collected as part of a survey.
b) *S.* Typhimurium (1), *S.* Derby (1), *S.* Enteritidis (1), *S.* 1,4,12:i:- (1), *S.* 1,4,5,12:i:- (1).
c) 41 badgers.
d) *S.* Typhimurium (1), *S.* Newport (1), not serotyped (2)
e) 10 starlings
f) 3 mice, 24 raccoon dogs, 59 roe deer, 13 seals.
g) 1 raccoon dogs and 5 roe deer.
h) 344 foxes, 20 badgers, 112 raccoon dogs, 1 raccoon.
i) 7 foxes and 2 raccoon dogs.
j) In 2007, Denmark achived official status as region with negligible risk of *Trichinella*, according to EU regulation (EC) No 2075/2005.
k) Samples reported from slaughterhouses. An additional 133 samples were reported from the laboratory. Double registrations from slaughterhouses and laboratory may occur.
l) 50 mammals from the sea, 46 minks, 53 raccoon dogs, 273 foxes, 1 raccoon and 23 badgers.
m) 16 bats, 3 foxes, 2 badgers, 1 marten, 1 mink, 1 roe deer, 1 seal.
Source: National Veterinary Institute, Technical University of Denmark, and Danish Veterinary and Food Administration.

Table A25. The Bovine Spongiform Encephalopathy (BSE) surveillance programme[a] for cattle, 2014

Type of surveillance	N[b]	Positive
Active surveillance		
Healthy slaughtered animals	43	0
Risk categories:		
Emergency slaugthers	1,122	0
Slaughterhouse antemortem inspection revealed suspicion or signs of disease	0	-
Fallen stock	20,392	0
Animals from herds under restriction	0	-
Passive surveillance		
Animals suspected of having clinical BSE	2	0
Total	21,559	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 histopathology or immunohistochemistry. Further confirmation on autolysed material is performed at the European Union TSE reference laboratory.
Source: National Veterinary Instistute, Technical University of Denmark, and Danish Veterinary and Food Administration.

Table A26. The Transmissible Spongiform Encephalopathy (TSE) surveillance programme[a] for sheep and goats, 2014

Type of surveillance	N[b]	Positive
Active surveillance		
Fallen stock (>18 months)	701	0
Animals from herds under restriction	0	-
Passive surveillance		
Animals suspected of having clinical TSE	2	0
Total	703	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 histopathology or immunohistochemistry. Further confirmation on autolysed material is performed at the European Union TSE reference laboratory.
Source: National Veterinary Institute, Techncal University of Denmark, and Danish Veterinary and Food Administration.

Table A27. Distribution[a] (%) of prion protein genotype of sheep randomly selected, 2014

	Genotype	Sheep n=100
NSP 1	ARR/ARR	27.0
NSP 2	ARR/AHQ, ARR/ARH, ARR/ARQ	26.0
NSP 3 (ARQ/ARQ)	ARQ/ARQ	31.0
NSP 3 (Other)	AHQ/AHQ, AHQ/ARH, AHQ/ARQ, ARH/ARQ, ARH/ARH, ARQ/ARH, ARH/AHQ, ARQ/AHQ	10.0
NSP 4	ARR/VRQ	1.0
NSP 5	ARH/VRQ, ARQ/VRQ, VRQ/VRQ, AHQ/VRQ	5.0
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, Technical University of Denmark, and Danish Veterinary and Food Administration.

Table A28. Centrally coordinated studies conducted in 2014

Title of project	No. of samples	Pathogen surveyed	Further information
Campylobacter spp. in fresh, chilled Danish broiler meat (conventional)	927	*Campylobacter* spp.	Appendix Table A14
Campylobacter spp. in fresh, chilled Danish broiler meat (organic)	108	*Campylobacter* spp.	Data are being processed[a]
Campylobacter spp. in fresh, chilled and frozen Danish and imported broiler meat (processed meat)	248	*Campylobacter* spp.	Appendix Table 14
Intensified control for *Salmonella* spp. and *Campylobacter* in fresh Danish and imported meat (poultry and pig)	7,622 (639 batches)	*Campylobacter* spp., *Salmonella* spp.	Appendix Table A18
Official verification of microbiological criteria (EU 2073/2005)	2440	*Listeria monocytogenes*, *Salmonella* spp., staphylococci, *Escherichia coli,* Aerobic colony count, *Enterobacteriaceae*	Data are being processed[a]
ESBL in Danish poultry production	114	ESBL	Data are being processed
Import control - processed products of animal origin (not fish)	5	*Listeria monocytogenes, Salmonella* spp., staphylococci	Data are being processed[a]
Import control - fish, fish products and bivalve molluscan shellfish	100	*Listeria monocytogenes, Salmonella* spp., *Escherichia coli*, staphylococci	Data are being processed[a]
Import control - food of non animal origin	90	*Salmonella* spp. (herbs) Norovirus (frozen straberries) Hepatitis A (frozen strawberries)	Data are being processed
Listeria monocytogenes in cold smoked halibut	215	*Listeria monocytogenes*	Data are being processed[a]
Listeria monocytogenes, *Salmonella* spp., *Escherichia coli*, staphylococci in fish goods from Greenland	100	*Salmonella* spp., *Listeria monocytogenes, Escherichia coli*, staphylococci	Data are being processed[a]
Microbiological classification of mussel production areas in Denmark	100	*Salmonella* spp., *Escherichia coli*	Data are being processed
MRSA in pigs in the top of the breeding pyramid	350	Methicillin resistant *Staphylococcus aureus*	Data are being processed
MRSA in slaughterpig herds	1,008	Methicillin resistant *Staphylococcus aureus*	Data are being processed
Pathogens in Danish and imported ready-to-eat vegetables	815	*Salmonella* spp., *Campylobacter* spp., *Escherichia coli*	Appendix Table A22
DANMAP - Antibiotic resistance in poultry, pigs and cattle, and in Danish and imported broiler, beef and pork meat	1,505	*Campylobacter* spp., *Enterococcus faecium, E. faecalis, ESC Escherichia coli*	Results are presented in the 2014 DANMAP report
Salmonella Dublin in beef	375	*Salmonella* spp., *Escherichia coli, Enterobacteriaceae,* enterococci	Data are being processed
Salmonella in animal feed	409	*Salmonella* spp.	Results are published on the DVFA website www.fvst.dk (In Danish)
Salmonella in pigs at slaughter	804	*Salmonella* spp.	Appendix Tables A5 and A15
Salmonella spp. and *Escherichia coli* in raw, frozen scallop from Greenland	35	*Salmonella* spp., *Escherichia coli*	Data are being processed[a]
Salmonella spp. antibiotic resistance in fresh, chilled and frozen imported beef and duck meat incl. ESBL in duck meat	364	*Salmonella* spp., ESC *Escherichia coli*	Appendix Tables A5-A6 and A8[a]

a) Results are published on the DVFA website www.fvst.dk (in Danish).

Source: Danish Veterinary and Food Administration.

Table A29. Listeria monocytogenes *in Danish produced ready-to-eat (RTE) foods[a], 2014*

		Samples analysed by a qualitative method[b]				Samples analysed by a quantitative method			
		Batches[c]		Single samples		Batches[c]		Single samples	
Food category	Sampling place	N	Pos	N	Pos	N	Pos	N	Pos
Cheese, RTE	At processing	24	0	120	0	15	0	75	0
Milk and dairy products, RTE	At processing	33	0	170	0	21	0	110	0
Products made from broiler meat, RTE	At processing	-	-	-	-	1	0	5	0
Products made from other poultry meat, RTE	At processing	1	0	5	0	-	-	-	-
Products made from pork, RTE	At processing	35	2	175	2	22	0	110	0
Products made from beef, RTE	At processing	7	1	35	3	7	0	40	0
Fruit, RTE	At processing	1	0	5	0	4	0	20	0
Vegetables, RTE	At processing	22	1	125	3	10	0	50	0
Fish and Fishery products, RTE	At processing	57	6	284	24	59	1	295	1
Shellfish and products thereoff, RTE	At processing	6	2	30	2	10	0	55	0
Other RTE products	At processing	66	5	330	25	49	0	245	0

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) Five samples from each batch, analysed individually.
Source: Danish Veterinary and Food Administration.

Monitoring and surveillance programmes

Table A30. Overview of notifiable and non-notifiable human diseases presented in this report, 2014

Patogen	Notifiable	Notification route
Bacteria		
Brucella spp.	no	-
Campylobacter spp.	1979[a]	Laboratory[b]
Chlamydophila psittaci (Ornithosis)	1980[a]	Physician[c]
Listeria monocytogenes	1993[a]	Physician
Leptospira spp.	1980[a]	Physician
Mycobacterium bovis/ tuberculosis	1905[a]	Physician (and laboratory[d])
Coxiella burnetii	no	-
Salmonella spp.	1979[a]	Laboratory
VTEC	2000[a]	Physician and laboratory
Yersinia enterocolitica	1979[a]	Laboratory
Parasites		
Cryptosporidium spp.	no	-
Echinococcus multilocularis	no	-
Echinococcus granulosus	no	-
Trichinella spp.	no	-
Viruses		
Lyssavirus (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 A31. Overview of notifiable and non-notifiable animal diseases presented in this report, 2014

Patogen	Notifiable	EU legislation	Danish legislation
Bacteria			
Brucella 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
Campylobacter spp.	no	-	-
Chlamydophila psittaci	1920	-	
Birds and poultry			Order no. 871 of 25/8 2011
Listeria monocytogenes	no	-	-
Leptospira spp. (only in production animals)	2003	-	Act no. 432 of 09/06/2004
Mycobacterium bovis/tuberculosis	1920[a]		
Cattle	OTF in 1980[d]	Decision 2003/467/EC	Order no. 1417 of 11/12 2007
Coxiella burnetii	2005	-	Act no. 432 of 09/06/2004
Salmonella 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	-	-
Yersinia enterocolitica	no	-	-
Parasites			
Cryptosporidium spp.	no	-	-
Echinococcus multilocularis	2004	Council Directive 64/433/EC	Act no. 466 of 15/05/2014
Echinococcus granulosus	1993	Council Directive 64/433/EC	Act no. 466 of 15/05/2014
Trichinella spp.	1920[a]	Regulation 2075/2005/EC	Order no. 412 of 28/05/2008
Viruses			
Lyssavirus (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 Commision Decision 2003/467/EC. No cases in since 1962.
c) Officially *Brucella melitensis* Free (ObmF) according to Council Directive 91/68/EC and Commision 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 neglible risk for BSE at World Organisation for Animal Health (OIE) general session in May 2011.
Source: Danish Veterinary and Food Administration.

Table A32. Salmonella *surveillance programme for the rearing flocks and adult flocks of the grandparent and parent generation of the broiler and table egg production, 2014*

Time	Samples taken	Material	Material
Rearing flocks		*Grandparent generation*	*Parent generation*
Day-old[a,b]	Per delivery	5 transport crates from one delivery: crate liners (>1 m^2 in total) or swab samples (>1 m^2 in total). Analysed as one pool	5 transport crates from one delivery: crate liners (>1 m^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 60 g
4th week[a,c]	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150 g	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 g
8th week[b,c]	Per unit	2 pairs of boot swabs (analysed as one pooled sample). Cage birds: 60x1 g samples of fresh droppings. Analysed as one pool	2 pairs of boot swabs (analysed as one pooled sample). Cage birds: 60x1 g 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 2x150 g	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 g
Adult flocks		*Grandparent generation*	*Parent generation*
Every two weeks[a,b] (Every 16th week)[e]	Per flock	Hatcher basket liners from 5 baskets (>1 m^2 in total) or 10 g of broken eggshells from each of 25 hatcher baskets (reduced to 25 g sub-sample). Analysed as one pool	Hatcher basket liners from 5 baskets (>1 m^2 in total) or 10 g of broken eggshells from each of 25 hatcher baskets (reduced to 25 g 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 60 g
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 2x150 g	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150 g
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 A33. Salmonella *and* Campylobacter *surveillance programme for the broiler flocks, 2014*

Time	Samples taken	Material
Salmonella		
15 - 21 days before slaughter[a,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	300x1 g 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
Campylobacter[f]		
After slaughter	Per flock	12 cloacal swabs from 24 animals, analysed in one pool[g]

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, laboratorium or the Danish Veterinary and Food Administration.
f) For flocks to be slaughtered outside Denmark 1 pair of boot swabs is collected by the owner 10 days before slaughter at the latest.
g) If the flock is slaughtered over several days, the last batch is sampled.
Source: Danish Veterinary and Food Administration.

Table A34. Salmonella *surveillance programme for the pullet-rearing, table egg layer and barnyard/hobby flocks in the table egg production, 2014*[a]

Time	Samples taken	Material
Pullet-rearing		
Day-old[a,c]	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[a,c]	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples of 60 gram
2 weeks before moving[a,b]	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples of 60 gram. 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,c,d, e]	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 g.
After positive serological findings[d]	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faecal samples consisting of 60 gram each
After positive findings of other serotypes than *S.* Enteritidis, *S.* Hadar, *S.* Infantis, *S.* Virchow or *S.* Typhimurium including the monophasic strains *S.* 1,4,[5],12:i:-[b]	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[f]		
Every 18 weeks[a,c,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) Samples collected by the Danish Veterinary and Food Administration.
c) Samples collected by the food business operator.
d) According to Regulation (EC) 2160/2003 sample collection must be carried out every 15 weeks as a minimum.
e) Until 01/10/2013 sampling every 9th week only.
f) Voluntary for hobby flocks.
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 A35. Salmonella *surveillance programmes for turkey flocks, 2014*

Time	Samples taken	Material
Turkey production		
Max. 21 days before slaughter[a,b]	Per flock	2 pairs of boot swabs. Analysed individually

a) Sampling requirements set out by Regulation (EC) 584/2008.
b) Samples collected by the food business operator or the local food control offices.
Source: Danish Veterinary and Food Administration.

Table A36. Salmonella *surveillance programme[a] for the cattle production, 2014*

No. of samples	Samples taken	Purpose/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 3rd 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 3rd month	Swab samples from 4 designated areas after 12 hours chilling (4x100cm^2)	Slaughterhouses slaughtering less than 50 cattle per month

a) Order no. 886 of 02/07/2014 as ammended. In 2013 and 2014, 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 milk samples (milk producing herd) or blood samples (non-milk producing herd or milk producing herd assumed free from 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 A37. Salmonella *surveillance programme[a] for the pig production, 2014*

Time	Samples taken	Purpose/Comment
Breeding and multiplier herds		
Every month	10 blood samples per epidemiological unit	Calculation of *Salmonella*-index based on the mean seroreaction from the last three months with more weight to the results from the more recent months (1:3:6)[b]
Max. twice per year	Herds with *Salmonella*-index 5 or above: Pen-faecal samples	Clarify distribution and type of infection in the herd[c]
Sow herds		
When purchaser of piglets is assigned to level 2 or 3, max. twice per year	Pen-faecal samples	Clarify distribution and type of infection in the herd, and possible transmission from sow herds to slaughter pig herds
Herds positive with *S.* Typhimurium, *S.* Infantis, *S.* Derby and *S.* Choleraesuis are considered positive for the following 5 years[d]	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 [e]: one meat juice sample per month	Calculation of slaughter pig index based on the mean proportion of positive samples from the last three months with most weight to the result from the most recent month (1:1:3)[f]. Assigning herds to level 1-3 and assigning herds to risk-based surveillance (RBOV)[e,g]
Slaughter pigs, animals		
At slaughter[h]	Coecum samples, avg. 73 samples per month, 12 months per year	Random collection of samples for monitoring of the distribution of serotypes and antimicrobial resistance.
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 3rd 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 3rd 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. 1280 of 4/12/2014.
b) Herds with index above 10 have to pay a penalty for each pig sold.
c) The herd owner must inform buyers of breeding animals about the infection level and type of *Salmonella*.
d) These serotypes are primarily spread by live trade, and are known to persist in herds. *S.* Typhimurium includes the monophasic *S.* 1,4,[5],12:i:-.
e) RBOV: risk-based surveillance in herds with a slaughter pig index of zero (no positive samples in the previous three months) the sample size is reduced to one sample per month. Increasing seroprevalence from level 1 to level 3.
f) Since November 2014: based on the proportion of seropositive samples from the last three months. Both the number of seropositive and total number of samples are weighted with most weight to samples from the most recent month (1:1:5).
g) Pigs from herds with highest level of infection (Level 3) must be slaughtered under special hygienic precautions.
h) Centrally coordinated study (Table A28)
Source: Danish Veterinary and Food Administration.

Table A38. Typing methods used in the surveillance of foodborne pathogens in Denmark, 2014

Methods	Human	Food	Animal
Salmonella enterica			
Serotype	All	All	All
Phage type	None	Few *S.* Typhimurium and *S.* Enteritidis	Few *S.* Typhimurium and *S.* Enteritidis, all isolates from poultry
Antimicrobial resistance	All *Salmonella* except *S.* Enteritidis	Almost all isolates	Almost all isolates
MLVA	*S.* Typhimurium[a], *S.* Enteritidis and *S.* Dublin	*S.* Typhimurium[a], *S.* Enteritidis and *S.* Dublin for the *Salmonella* source account, outbreak investigations and research	*S.* Typhimurium[a], *S.* Enteritidis and *S.* Dublin for the *Salmonella* source account, outbreak investigations and research
PFGE	Outbreak investigations	Outbreak investigations	Outbreak investigations
WGS	Outbreak investigations	Some for outbreak investigation and research	Some for outbreak investigation and research
Campylobacter coli/jejuni			
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, WGS	Outbreaks investigations, research	None	None
VTEC			
Serotype	All	None	All (O157)
Virulence profile	All	None	All (O157)
PFGE	All	None	Outbreak investigations
WGS	Outbreak investigations	None	None
Listeria			
Serogroup	All	None	None
PFGE	All	All	All
WGS	All	All	All
Yersinia enterocolitica			
O-group	All isolates send to SSI	None	None

a) Including the monophasic strains *S.* 1,4,[5],12:i:-.
Source: Statens Serum Institut, and Danish Zoonosis Laboratory, National Food Institute.

Population and slaughter data

Table A39. Human population, 2014

Age groups (years)	Males	Females	Total
0-4	153,001	145,367	298,368
5-14	340,301	323,978	664,279
15-24	373,323	356,621	729,944
25-44	713,218	701,478	1,414,696
45-64	752,353	748,946	1,501,299
65+	478,818	572,311	1,051,129
Total	2,811,014	2,848,701	5,659,715

Source: Statistics Denmark, 1 January 2015.

Table A40. Number of herds/flocks, livestock and animals slaughtered, 2014

	Herds/flocks	Livestock (capacity)	Number slaughtered
Slaughter pigs (>30 kg)	7,012	6,471,342	18,407,939
Cattle	18,577	1,563,933	485,147
Broilers	556	n/a	102,941,054
Layers (excl. barnyard)	214	3,240,000	-
Turkeys	37	360,670	3,825
Sheep & lambs	6,975	142,558	80,475
Goats	3,179	20,821	1,451
Horses	-	-	1,328

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

Table A41. Number of farms in the broiler production, 2014

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (grandparent)	3	13	50,000
Adult period (grandparent)	4	7	90,000
Rearing period (parent)	19	95	250,000
Adult period (parent)	40	141	700,000
Hatcheries	4	-	-
Broilers	234	556	n.a.

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

Table A42. Number of farms in the table egg production, 2014

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (grandparent)	2	2	50,000
Adult period (grandparent)	2	7	70,000
Rearing period (parent)	8	12	20,000
Adult period (parent)	8	9	50,000
Hatcheries	4	-	-
Pullet-rearing	57	94	1,050,000
Layers (excl. Barnyard)	154	214	3,240,000

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

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