



Trends and sources in human salmonellosis

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Annual Report on Zoonoses in Denmark 2017



Annual Report on Zoonoses in Denmark 2017

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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 2017. 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.

Introduction

In 2017, *Campylobacter* was again the most common food-borne illness, with 4,257 confirmed cases. Two outbreaks of *Campylobacter* were caused by consumption of raw milk, one with 66 cases and one with six cases.

This year, results from a case-control study undertaken by Statens Serum Institut (SSI) and a source attribution model developed by the Technical University of Denmark (DTU) both shed new lights on sources of *Campylobacter* infection. Both studies found that cattle may be a source of *Campylobacter* infection, and these findings will be targeted in the new Danish Action Plan against *Campylobacter* 2018-2021.

Human infections with *Salmonella* were at similar level in 2017 with 1,067 cases compared to the 1,074 cases in 2016. The two most common strains were *S. Typhimurium*, including monophasic strains, and *S. Enteritidis*, with incidences of 5.0/100,000 inhabitants and 3.9/100,000 inhabitants, respectively.

The monophasic *S. Typhimurium* strains were reported in animals and humans more often than non-monophasic strains. In animals, *S. Typhimurium* was found in all types of sources except Danish and imported beef, while *S. Enteritidis* was found in Danish pork batches, broiler and layer flocks, as well as imported duck and broiler meat. In 2016, *S. Enteritidis* was only found in imported duck and broiler meat.

Foodborne outbreaks

In total, 63 foodborne outbreaks with 1,151 patients were reported in 2017. Compared to the last years, the number of outbreaks has increased, mainly due to an increase in the number of *Salmonella* outbreaks from 3 in 2015 to 12 in 2016 and now 25 in 2017. The increase of registered outbreaks is primarily attributable to the fact that SSI changed laboratory methods and from 2017 started using Whole Genome Sequencing (WGS) for routine typing of all human *Salmonella* isolates.

Norovirus accounted for 10 outbreaks affecting 298 persons. This is a large decrease compared to the 1,178 persons affected by Norovirus outbreaks in 2016.

The *Salmonella* source account

In 2017, the *Salmonella* source account, which links the number of human *Salmonella* infections to specific food items and animals reservoirs, by modelling the distribu-

tion of serovars, was for the first time based on results from WGS.

Domestic and imported pork were estimated to be the sources most commonly associated with human salmonellosis (approximately 87 and 74 cases respectively) after the number of human cases related to travel (~328 cases). This is similar to 2016, but imported pork is now more often attributed as source of infection than imported broilers (~37 cases). Domestic beef and table eggs were both estimated as sources of approximately 16 cases each. Domestic broiler meat was not associated with any human cases.

Burden of foodborne disease in Denmark

Burden of disease studies can help enable policy makers and other stakeholders carry out risk management, as they provide a ranking of diseases according to their health impact in the population.

Listeria monocytogenes is a gram-positive bacterium able to grow in food items with high salt concentration and low moisture. Manifestation is usually mild with gastroenteritis, but in vulnerable groups such as elderly, pregnant women and immunocompromised patients, infection can lead to severe disease. In total, 58 cases of *L. monocytogenes* infection and 12 deaths were reported in Denmark in 2017. Even though the number of cases with listeriosis is lower than e.g. salmonellosis, the burden of disease is high due to the serious nature of the disease when vulnerable groups are affected.

Infection with Norovirus is not notifiable in Denmark, but it is considered to be the most common food-born infection. The burden of disease study on Norovirus estimated approximately 185,060 cases of Norovirus and 26 deaths in Denmark in 2017.

Vector-borne zoonoses

With the recent migration of golden jackals and grey wolves to Denmark, the risk of introducing new tick species with new pathogens may increase. In 2017, a golden jackal was accidentally shot in Jutland, and the carcass was sent to the National Veterinary Institute at the DTU. At the carcass, 21 male ticks of the species *Dermacentor reticularis* were found and 18 of these were positive for *Rickettsia raoultii*. This finding shows that there is a risk that new tick-borne pathogens may establish in Denmark after migration of medium-sized carnivores.

1. Trends and sources in human salmonellosis

By Nanna Sophia Mucha Munck (nsmm@food.dtu.dk), Patrick Njage, Eva Litrup and Tine Hald

In 2017, a total of 1,067 human cases of Salmonellosis were reported in Denmark. This corresponds to an incidence of 18.5 cases per 100,000 inhabitants, which is slightly lower than last year (Figure 1.1). The incidence of *S. Enteritidis* was 3.9 per 100,000 inhabitants continuing the decreasing trend seen since 2013. The incidence of *S. Typhimurium* including the monophasic variant was 5.0 cases per 100,000 inhabitants, which is a decrease compared to last year (5.6 in 2016). The monophasic variant constituted 60%, which is slightly lower than last year (64%). The predominance of the monophasic variant of *S. Typhimurium* was observed for the first time in 2014¹.

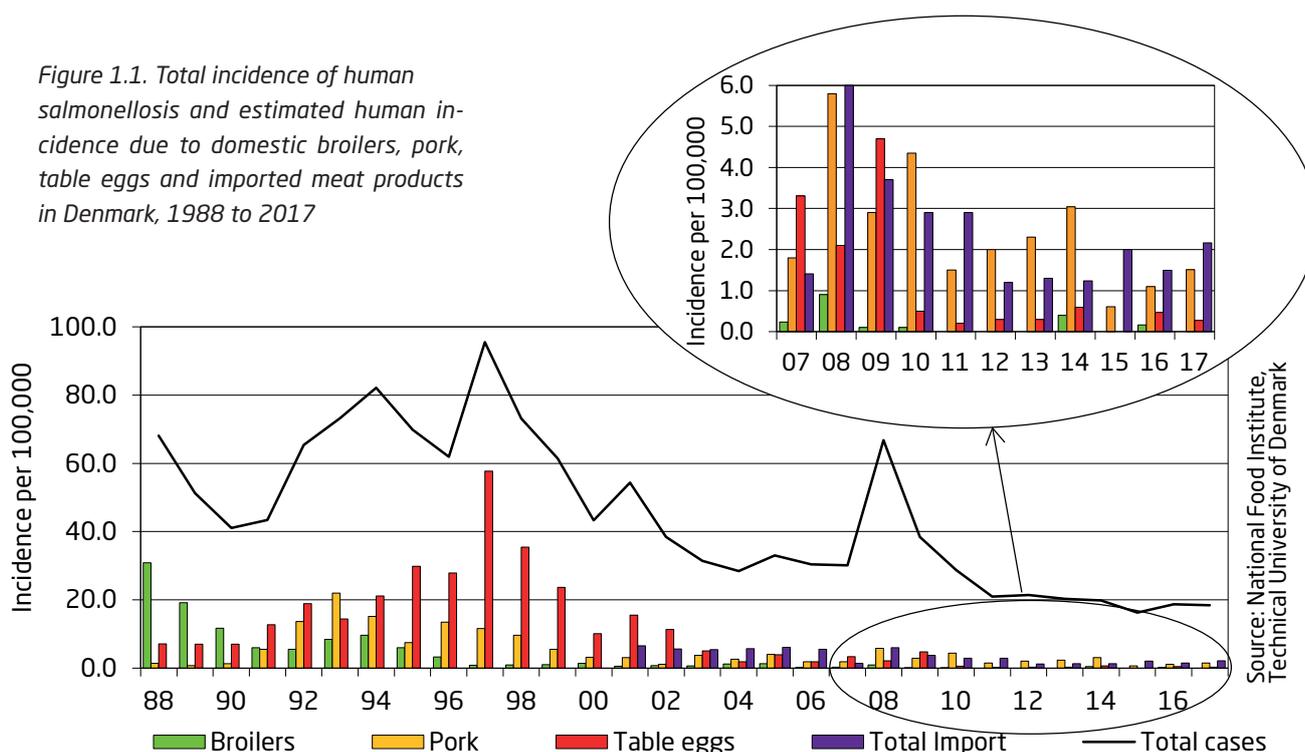
For the past two decades, the attribution of human salmonellosis cases to different sources has been based on a mathematical model² that compares the number of human cases caused by different *Salmonella* subtypes with the distribution of the same subtypes isolated from various animal-food sources using a Bayesian modelling approach that also accounts for the prevalence of the *Salmonella* types in the different sources and the amount of each food source consumed per year³. The subtypes have over the years been defined by serotyping, phage typing, Multiple Locus Variable

Tandem Repeat Analysis (MLVA) and/or resistance profiling. However, from January 1st 2017, serotyping and MLVA analysis were replaced by whole genome sequencing (WGS) of all isolates found as part of the national *Salmonella* surveillance programs for animals, food and humans.

The National Food Institute has consequently developed a new approach for source attribution applying WGS data. The new model is based on a machine-learning approach. Machine learning (ML) is a collective name for mathematical models that learn from data and improves with experience/more data⁴. The models are defined by algorithms capable of recognizing patterns in large and complex datasets making the method applicable for analyzing DNA sequence data⁴. The method identifies relevant features in the dataset enabling the ability to make strong predictions.

For *Salmonella* source attribution 2017, we applied core genome Multi-locus sequence typing (cgMLST), by which all core genes are used in the analysis, and strains are differentiated by their allelic variations. Statens Serum Institute provided the cgMLST profiles for each sequence using the Enterobase scheme⁵ in BioNumerics version 7.6 (Applied

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 2017



Source: National Food Institute, Technical University of Denmark

Source: National Food Institute, Technical University of Denmark

Maths, Sint-Martens-Latem, Belgium). The core genome of *Salmonella* consist of 3,002 loci with one single locus having several allele variations, thereby providing a high discriminatory power compared to previous methods used.

We applied a supervised classification ML model. The classification is supervised, because the machine is 'told' from which of the eight different animal reservoirs (classes) each of the specific isolates from food and animal originates, and the model then identifies those cgMLST that are able to differentiate between the sources based on their allelic variation. The ML model was built from a training dataset consisting of the majority (70%) of the animal and food isolates. The accuracy of the model was then determined from the models' ability to predict the origin of the remaining part (30%) of the animal and food isolates. As soon as a model with a satisfying accuracy is obtained, the probability of each human isolate to originate from a specific source can be predicted. The sum of these probabilities within each source equals the total number of human cases attributed per source. Human isolates whose source could not be predicted are referred to an unknown source category.

The previous Bayesian approach estimated uncertainties around the mean number of attributed cases per source. The new machine learning model does not compute uncertainty intervals per se, but takes the uncertainties into account when building the model by repeating the model building 10 times and applying a 7-fold cross validation for each model build. The uncertainty of the results is reflected in Figure 1.2, where the probability of each human case to belong to one of the eight sources is illustrated (see further description below).

1.1. *Salmonella* source account 2017

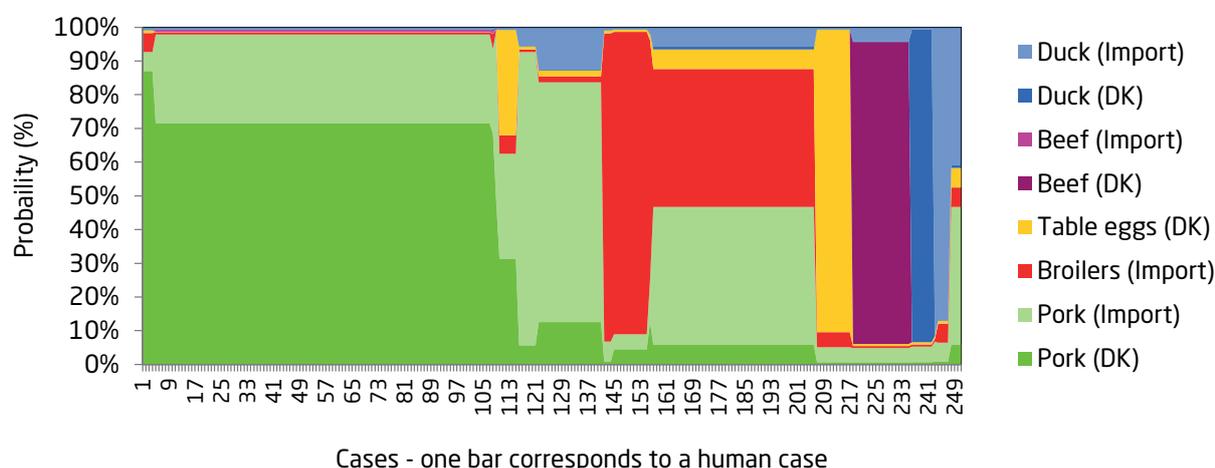
The ML model includes only domestic and sporadic cases, i.e. cases with no or unknown travel history as well as one case

from each domestic outbreak. Of the 1,067 human cases reported in 2017, 328 had reported travelling and 154 were outbreak related leaving 585 cases to be included in the model. However, for 51 cases, cgMLST was either not available or the quality of the sequencing was too poor to allow inclusion. This left 534 cases of which the model attributed 251 to known sources and 283 to unknown sources. The specific attribution of the 251 human cases is visualized in Figure 1.2., where the 251 predicted human cases are lined up along the x-axis and the source specific probabilities for each of the human cases are stacked along the y-axis. As it can be seen, many cases have a high probability (>70%) of originating from a single source, whereas other cases have a more or less equal chance of originating from two sources. The dominating colors in the graph refer to the main sources, namely domestic pork and imported pork followed by imported broilers.

The overall trend in human salmonellosis cases attributable to the major food-animal sources is presented in Appendix Table A1. Similar to last year, Danish produced pork was assessed to be the most important food source of human salmonellosis (87 cases corresponding to 8.2%), followed by imported pork and imported broiler meat. No positive samples were isolated from Danish produced broiler meat. This year, 16 cases (1.5%) were attributed to Danish produced table eggs, which is a reduction compared to 2016. The 16 cases (1.5%) attributed to Danish produced beef is in line with last year. Danish produced ducks was estimated to be responsible for 7 cases (0.7%), which is also comparable to last year (Appendix Table A1).

Imported sources were responsible for slightly more cases in 2017 (125 cases) compared to 2016 (114 cases). Imported pork was predicted to be the most important source with 74 cases followed by imported broiler meat (37 cases), imported duck meat (13 cases) and imported beef (1 case). Last year

Figure 1.2. Result of the predictive machine learning model. All the 251 predicted human cases are lined up along the x-axis and the source specific probabilities for each of the human cases are stacked along the y-axis. Human cases attributed to an unknown source not shown



Source: National Food Institute, Technical University of Denmark

imported broiler meat was the most important source among imported food followed closely by imported pork.

Two Danish turkey flocks were found positive for *Salmonella* in 2017, but the isolates were not analysed for cgMLST and therefore not included in the model. Sporadic and outbreak cases allocated to unknown sources may be associated with exposure to foods not included in the national surveillance programs, such as turkey, or by non-food sources such as direct contact with pet animals or person-to-person transmission.

Travels

In 2017, around one third of all cases reported travelling before disease onset. This appears as a major decrease compared to last year, where more than half of the cases was estimated to be travel related, but is in fact caused by the modeling approach. In contrast to previous years' models, the ML model does not predict 'extra' travelers among cases with an unknown travel history, but rather assume all these to be domestic, resulting in an overall lower number of travel cases.

Antibiotic resistance in *S. Typhimurium*

Information about resistance was determined by phenotypic analysis of the human cases. The panel of antimicrobial agents tested for was identical to last year. For the predicted cases, the resistance information was added to the list of human cases predicted from the machine learning model and the number of cases was obtained by summing over the probabilities. For travel cases, the number of resistant strains was calculated by summing the human travel-related cases. Only sporadic cases were reviewed for resistance.

Resistance information was available for all but one of the 129 *S. Typhimurium* and its monophasic variant cases attributed to Danish produced and imported food. The vast majority of the cases predicted to originate from Danish produced food were caused by resistant strains (62 cases), which is almost double the number of cases in 2016 (37 cases). Four cases

were caused by multi-resistant strains and two cases were caused by quinolone resistant strains. Quinolone-resistant human strains are observed for the first time since 2013. Six cases were caused by susceptible strains (Figure 1.3).

Resistant strains also dominated among the human cases predicted to origin from imported food sources (36 cases) and were higher than last year. Also here, quinolone resistance (two cases) was observed for the first time since before 2013. Twelve cases were caused by susceptible strains, which are significantly higher than in 2016 (5 cases) (Figure 1.3).

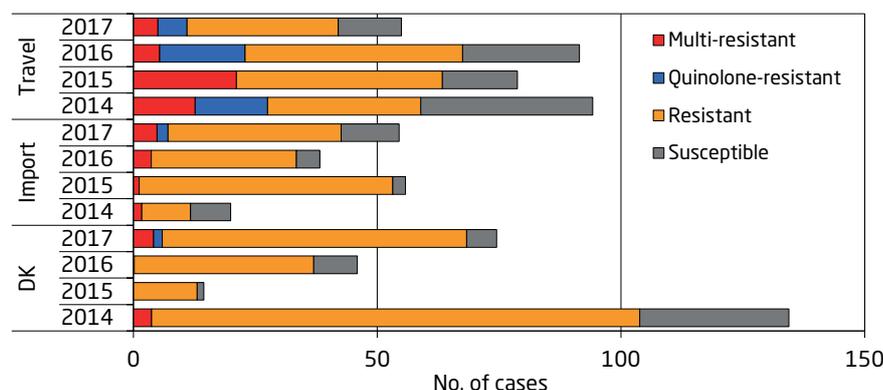
Resistance information was available for all 55 travel related *S. Typhimurium* cases. Among these resistant strains dominated (31 cases). Multi-resistant strains corresponded to the level observed in 2016 (5 cases), while a reduction from 17 cases to 6 cases was seen for quinolone resistant strains (Figure 1.3).

All quinolone resistant strains found in humans were also multi-resistant.

1.2 References

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3. Anonymous (2001). Annual Report on Zoonosis in Denmark 2000, National Food Institute, Technical University of Denmark.
4. Libbrecht MW & Noble WS (2015). Machine learning applications in genetics and genomics. *Nat. Rev. Genet.* 16, 321-332.
5. Alikhan N-F, Zhou Z, Sergeant MJ & Achtman M (2018) A genomic overview of the population structure of *Salmonella*. *PLoS Genet* 14(4): e1007261.

Figure 1.3. Distribution of antimicrobial resistance 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, 2014-2017



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, chloramphenicol, sulphamethoxazole, tetracycline, trimethoprim, ciprofloxacin, gentamicin, nalidixic acid, cefotaxime and ceftazidime.

Source: National Food Institute, Technical University of Denmark

Where do we acquire *Salmonella* infections?

By Luise Müller (lum@ssi.dk)

In 2017, 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 69.5% of the *Salmonella* cases in 2017. Among the cases with known travel history, 49.5% were infected abroad (Table 1.1). However, the proportion of travel-related cases varied greatly between the different serotypes, hence 75.6% of the *S. Enteritidis* cases, 30.5% of the *S. Typhimurium* cases, 24.0% of the monophasic *S. 1,4,[5],12:i:-* cases and 50.9% of cases with other serotypes were infected abroad. Similar to previous years, the majority of travel-related cases in 2017 travelled to Thailand and Turkey. Eight travel-related outbreaks were identified (see chapter 2 and Appendix Table A4).

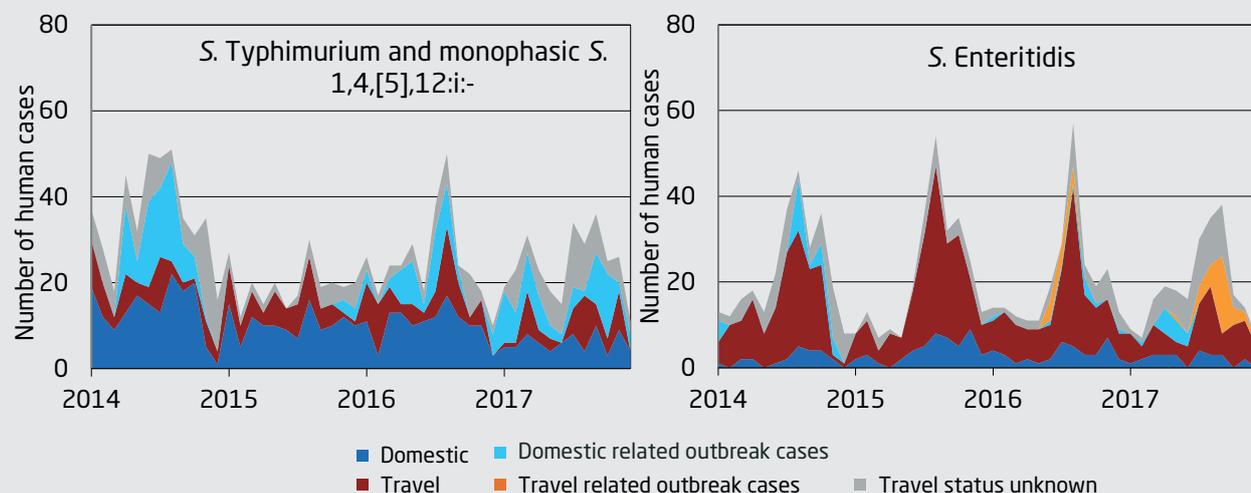
Table 1.1. Top 10 *Salmonella* serotypes in humans and information about travel abroad, 2016-2017

2017	Number of patients (%)	% of patients ^a infected		2016	Number of patients (%)	% of patients ^a infected	
		Abroad ^b	Domestically			Abroad ^b	Domestically
Enteritidis	226 (21.2)	75.6	24.4	Enteritidis	246 (22.9)	78.2	21.8
1,4,[5],12:i:-	175 (16.4)	24.0	76.0	1,4,[5],12:i:-	192 (17.9)	30.6	69.4
Typhimurium	115 (10.8)	30.5	69.5	Typhimurium	108 (10.1)	33.0	67.0
Stanley	33 (3.1)	91.7	8.3	Stanley	42 (3.9)	81.1	18.9
Dublin	25 (2.3)	8.3	91.7	Newport	29 (2.7)	52.2	47.8
Newport	25 (2.3)	41.2	58.8	Java	27 (2.5)	76.2	23.8
Agona	20 (1.9)	18.8	81.3	Infantis	23 (2.1)	57.9	42.1
Kentucky	20 (1.9)	94.4	5.6	Dublin	20 (1.9)	9.1	90.9
Virchow	17 (1.6)	100.0	0.0	Kentucky	19 (1.8)	94.1	5.9
Infantis	16 (1.5)	28.6	71.4	Saintpaul	19 (1.8)	69.2	30.8
Other serotypes	395 (37.0)	48.2	51.8	Other serotypes	349 (32.5)	53.2	46.8
Total	1,067	49.5	50.5	Total	1,074	55.1	44.9

a) Patients with unknown travel information (30.5% of all patients in 2017 and 18.7% in 2016) 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* and *S. Typhimurium* incl. monophasic *S. 1,4,[5],12:i:-* cases, 2014-2017

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 2017 are presented in Appendix Table A4. Figure 2.1 shows the relative distribution of these outbreaks by the different causative agents. Household outbreaks and clusters that could not be verified as common source outbreaks are not included. The outbreak investigation procedures in Denmark are described in further detail in Chapter 8.

In total, 63 foodborne outbreaks were reported to FUD in 2017. This is an increase in the number of outbreaks compared to last year. The increase is mainly seen in outbreaks caused by *Salmonella* (Figure 2.2).

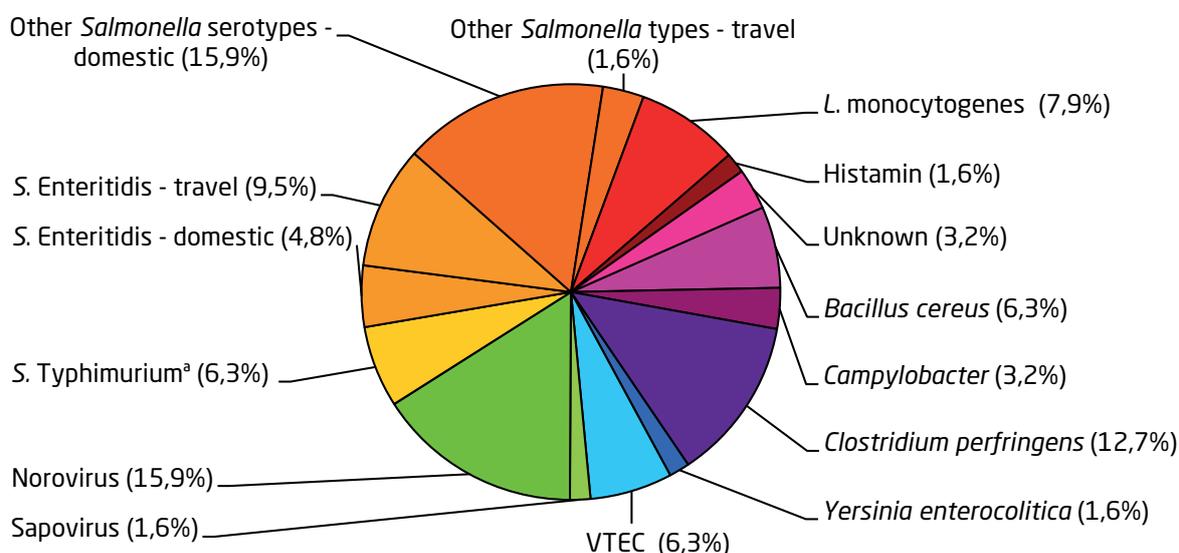
In total, the number of persons affected by foodborne outbreaks was 1,151, with a median of eight persons per outbreak (range 1-86). The outbreaks were mainly regional or local (57%). Seventeen outbreaks were classified as national and two were part of international outbreaks. The largest, involving 86 persons, was a local outbreak caused by *Clostridium perfringens* (FUD 1554).

In 2017, *C. perfringens* was associated with eight foodborne outbreaks affecting a total of 336 people compared to 7, 11, and 7 outbreaks caused by this agent in 2016, 2015, and 2014, respectively. Outbreaks involving *Bacillus cereus* and *C. perfringens* are traditionally caused by insuff-

icient cooling of large portions of food items like various meat sauces. This was also the case in 2017. However, the source of two outbreaks (FUD 1554 and 1566) appeared to be various pieces of meat prepared by cooking at low temperatures (<100 degrees Celsius) for many hours followed by cooling and reheating prior to serving. Cooking meat at low temperatures sous vide or in an oven for several hours is a method of preparation that is becoming more widely used also by smaller restaurants. If the process and temperature is not thoroughly monitored pathogens may survive or even grow to infectious levels. Furthermore larger amounts of meat tend to be cooked and stored for later when using this method adding another problematic element of insufficient cooling and reheating before serving.

When dividing the outbreaks by reported setting, the most frequent setting was "restaurants" (25%) with 16 outbreaks affecting 359 people (mean: 22 people per outbreak). Outbreaks taking place in workplace canteens and through catering (9 outbreaks) also affected a high number of people (348 people) and affected on average 39 persons per outbreak. "Composite meals" (18 outbreaks) and "buffet meals" (10 outbreaks) combined were the most frequently reported types of foods associated with outbreaks in 2017 and most often these outbreaks were caused by *B. cereus* or *C. perfringens* (Appendix Table A4).

Figure 2.1. Aetiology of the 63 foodborne disease outbreaks reported with a causative agent in the Food- and waterborne Outbreak Database (FUD), 2017. Percentage of total outbreaks indicated in brackets



A: Including the monophasic strains S. 1,4,[5],12:i:-.

Source: Food- and waterborne Outbreak Database (FUD)

2.1 Norovirus outbreaks

Norovirus (NoV) was the second most frequent cause of foodborne outbreaks in 2017 (10 outbreaks), and in total 298 persons were affected. This is a substantial decrease compared to previous years. 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 2015-17 are shown. The most common way of infection with NoV in 2017 was contamination from symptomatic or healthy carriers among kitchen staff. In 2017, this way of infection constituted 70% of the NoV outbreaks. In 2017 a NoV outbreak caused by commercially harvested Danish oysters was notified for the first time (FUD1623). The oysters were harvested in Northern Jutland and the contamination was possibly due to heavy rainfalls prior to harvest. Two restaurants in the Copenhagen area had received the oysters from the same lot and distributor. In total, 10 guests got ill. Samples from both patients and oysters were positive for NoV. The contaminated oysters were withdrawn from the market and the area was closed for commercial harvesting of oysters and mussels.

2.2 *Salmonella* outbreaks

The number of registered *Salmonella* outbreaks continued to increase in 2017 compared to previous years and was the most frequent cause of foodborne outbreaks. In 2017, 25 *Salmonella* outbreaks were reported compared to 12 in 2016 and three in 2015. The increase of registered outbreaks is primarily attributable to the fact that Statens Serum Institut (SSI) changed laboratory methods and from 2017 started using whole-genome sequencing (WGS) for routine typing of all human *Salmonella* isolates. The total number of registered *Salmonella* cases did not increase from 2016 to 2017 (Appendix Table A2).

Outbreaks of *Salmonella* in Denmark is primarily caused by the serotype *S. Typhimurium* or its monophasic variant: O:4,[5],12; H:i:-. In 2017, ten outbreaks were caused by these serotypes and the source was revealed for five of them - all were related to pork meat or pork meat products. The largest *S. Typhimurium* outbreak started in December 2016 and lasted until April 2017 (FUD1558). In all, 21 patients were registered. Extensive interviews pointed at a frozen meat loaf ready-to-eat dish from the supermarkets. This was confirmed by a case-control study, comparable analyses of patients' household supermarket receipts, and trace-back investigation. The product was recalled from consumers on 15 March 2017 following which the outbreak stopped. Among patients, fourteen were female and seven male, and they were aged from two months to 94 years. The median age was 71 years. Two patients with underlying disease died. The patients were primarily elderly people living alone and cooking simple food by themselves. Four patients were children under five years. Three of them were from the same day care center, and two most likely secondary cases.

In April 2017, an outbreak of *S. O:4,[5],12; H:i:-* was detected by WGS (FUD1577). In total, 13 patients were registered from March to November 2017; eight male and five female, aged 16-69 years old. Three patients worked in the same company and were most likely exposed in the company canteen. Human isolates were compared using WGS with recent food isolates. A match was found to fresh pork meat from a Danish slaughterhouse, which had been tested as part of the company's own check program. Interviews confirmed that the patients had eaten different dishes prepared with pork meat from the slaughterhouse and from the relevant production dates. The company canteen had received pork from that same slaughterhouse. It was however, not possible to point out a specific underlying pig herd since several farms had delivered pigs to the slaughterhouse in the same period.

Table 2.1. Norovirus outbreaks per route of transmission based on number of cases or number of outbreaks, 2015-2017

Transmission route/source	2017		2016		2015	
	No. of outbreaks	No. of persons ill	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	7	168	6	258	5	153
Kitchen staff tending to ill persons at home before entering the kitchen	1	42	2	40	4	96
Ill person/guest attending a buffet	1	78	4	355	4	108
Seafood (oysters)	1	10	3	92	1	22
Frozen raspberries/strawberries	0	0	0	0	1	9
Leafy greens / lettuce	0	0	3	433	0	0
Water	0	0	0	0	1	142
Total	10	298	18	1,178	16	530

Source: Food- and waterborne Outbreak Database (FUD)

A third *S. Typhimurium* outbreak – primarily affecting children – took place in September–October 2017 (FUD1603). In total, 13 patients were registered; nine male and four female. The age range was 2–63 years with a median age of 12 years. A case-control study where photos of different salami products were presented to cases and control persons pointed out Fuet Coins (thick slices of Fuet salami) produced in Spain as the source. The product had been sold with a limited time offer in a specific supermarket chain from week 37 when the outbreak began. The product was voluntarily recalled by the supermarket chain on 3 November 2017. In Sweden, a separate *S. Typhimurium* outbreak with a different subtype was notified in the same period caused by another Fuet salami product from the same producer. In Denmark four additional *Salmonella* patients were part of the Swedish outbreak (FUD1615).

Nine *Salmonella* outbreaks were caused by *S. Enteritidis* and six of these were found to be related to foreign travel. Two outbreaks were associated with travel to Turkey, one to Egypt and one to Germany. In two outbreaks, defined by WGS (FUD1647, FUD1650), patients had been travelling to multiple European countries. These outbreaks were most likely a part of the pan-European outbreak in 2016–17 linked to eggs from Poland¹ (see also AR2016 chapter 2). In 2017, two domestic outbreaks with *S. Enteritidis* were detected (FUD1581 and FUD1582) with seven and six cases, respectively. For both outbreaks, the suspected source was almonds – however, this could not be verified.

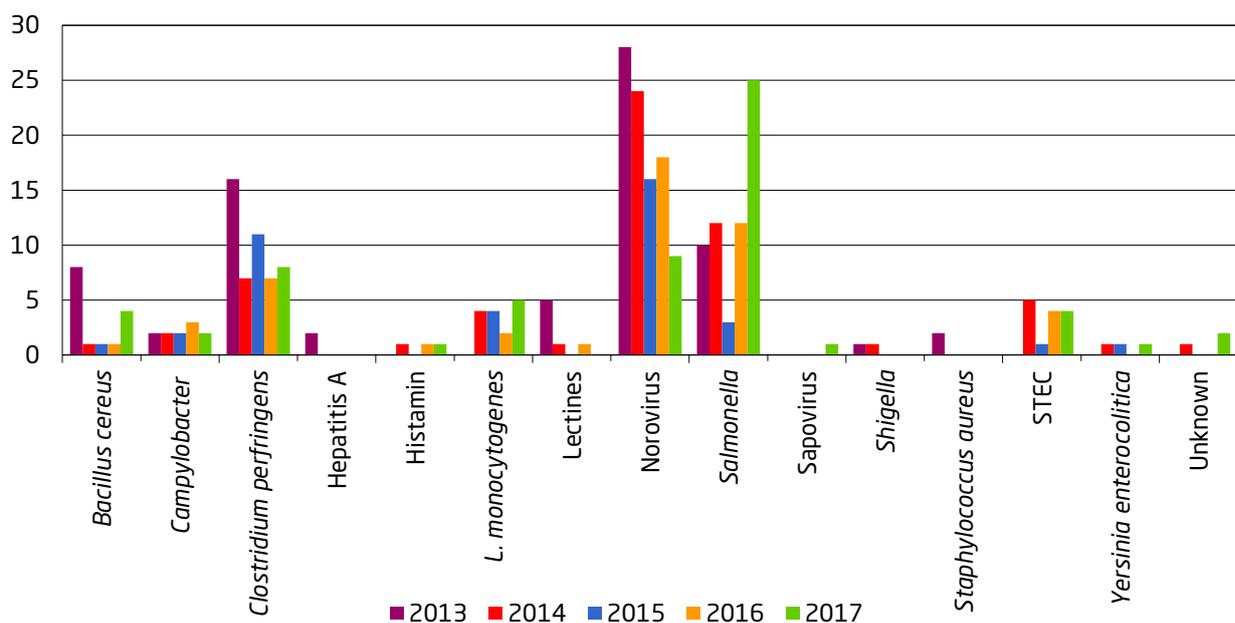
2.3 Other outbreaks of interest

One long-lasting outbreak of listeriosis with six patients was investigated in 2017 (FUD1597). Five patients fell ill from

May to August 2017 and an additional patient from October 2015 could also be included by WGS. The patients were four females and two males aged 59–96 years – all with underlying disease. One patient died. The outbreak vehicle was identified as cold-smoked salmon produced in Poland. A food isolate from the product and interviews with patients confirmed this. The product was recalled from consumers on 30 August 2017. On 31 August 2017, the outbreak was notified internationally via the European Centre for Disease Prevention and Control (ECDC) epidemic intelligence information system (EPIS) and through the Rapid Alert System for Food and Feed (RASFF). Exchange of genome sequences allowed identification in France of a food isolate from a salmon-derived product and a human isolate from 2016, as well as three human isolates identified in Germany from 2017 all genetically linked to the Danish outbreak². Figure 2.3a shows the comparison of Danish and French outbreak isolates with other ST8 isolates from Danish patients in the period 2012–2017. The outbreak isolates clearly formed a distinct cluster using core genome multilocus sequence typing (cgMLST). Both SSI and the National Food Institute, Technical University of Denmark (DTU Food) performed a single-nt polymorphisms (SNP) analysis using different pipelines leading to the same conclusion of maximum nine SNPs between any two isolates in the cluster shown in Figure 2.3b.

In 2017, two notified outbreaks were caused by *Campylobacter* – both of them caused by intake of raw unpasteurised cow's milk. The first outbreak took place in June 2017 where 245 primary school children, aged 6–10 years visited a farm and drank raw milk (FUD1586). In total 66 persons became ill and a cohort study confirmed that intake of raw cow's milk was

Figure 2.2. Foodborne outbreaks reported in Denmark by pathogen, 2013–17



Source: Food- and waterborne Outbreak Database (FUD)

the source. *C. jejuni* was found in samples from 19 patients. One patient not associated with the school had also consumed milk from the farm, and was also tested positive for *Campylobacter*. The second outbreak also took place during summer when a group of 40 people visited another farm and drank raw milk (FUD1642). Six persons fell ill with gastroenteritis and three were confirmed positive with *C. jejuni*.

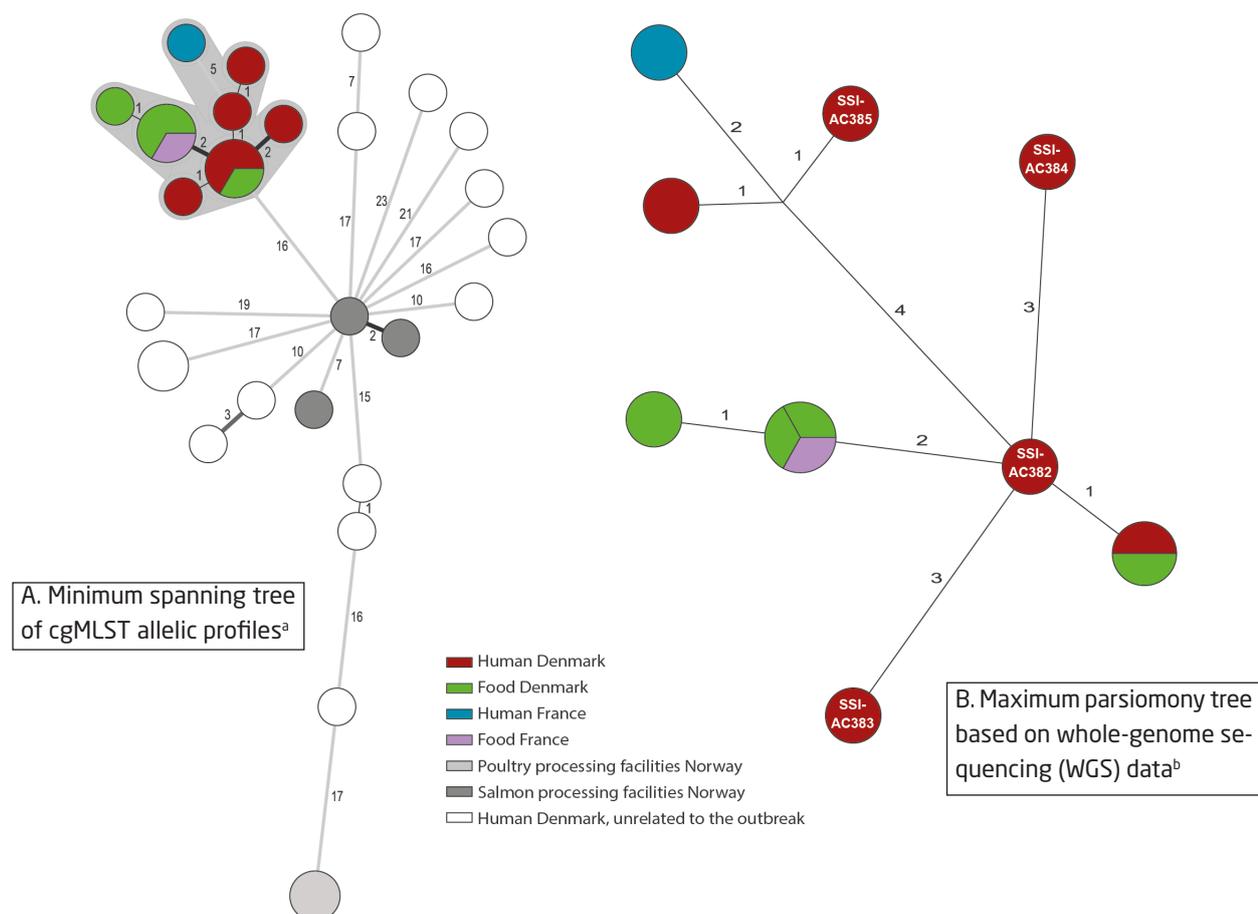
In March 2017, a number of gastroenteritis cases - some with bloody diarrhoea - were reported from a boarding school for lower secondary school students in Jutland (FUD1575). One student tested positive with *Yersinia enterocolitica*. Investigations showed that six students over a period of two weeks had undergone surgery for acute appendicitis and a further three students were observed for the same. A questionnaire survey was initiated among the students from the school in order to investigate the extent of the outbreak and the source. The results showed that 80 students had been ill with gastroenteritis. Four tested positive for *Y. enterocolitica*. The cohort study further showed an association between

illness and participation in activities held 11-12 March 2017 and the dinner served on this occasion. The dinner consisted of pork cutlets in tomato sauce with rice and salad. Improper heating or possible cross contamination of the pork meat was suspected to be the mechanism of the outbreak.

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Figure 2.3. Whole genome sequencing based typing of *Listeria monocytogenes* ST8 isolates as part of a cross-border listeriosis outbreak investigation, Denmark and France, 2015-2017



Note: The size of nodes corresponds to the number of isolates. Branch lengths represent the SNP difference.

A: Minimum spanning tree of core genome multilocus sequence typing (cgMLST) allelic profiles of different *L. monocytogenes* ST8 isolates

B: Maximum parsimony tree of single-nt polymorphisms (SNPs) within the *L. monocytogenes* ST8 isolates in the cluster. SSI-AC382, ERS2039635) as the reference in the analysis.

Source: Schjørring et al (2017)

Four cases of leptospirosis after a sports event including running and swimming in fresh water in September 2017

By Charlotte Kjelsø (jel@ssi.dk)

In September 2017, Statens Serum Institut (SSI) registered four patients infected with leptospirosis after contact with freshwater during a swim-run race in Jutland. A total of 28 athletes attended the race, which lasted for approximately two hours and was composed of a total of 2.6 km of swimming in a fresh water stream and 9 km of running on a meadow track next to the stream. The facilities for the race were outdoor stalls serving food, tents for changing clothes by the stream, and a number of toilet cars. There were no shower facilities in close proximity of the races, but it was possible to shower after the races in a nearby sports arena.

The persons that contracted leptospirosis either had small cuts on their hands before the race or got scratches during the run. Furthermore, the majority did not change clothes or shower until they arrived at their homes approx. three to four hours after the end of the race. The participants are believed to have been exposed to *Leptospira* either from the water in which they swam, or from running at the meadow track which was flooded with freshwater of unknown origin due to a previous rainfall and possibly overflow from the nearby stream resulting in participants running in ankle-deep water. The organizers of the race and several participating clubs have subsequently received descriptions of the disease and its symptoms. Furthermore, they have received preventive advice, such as e.g. to cover any wounds and scratches with water repellent material and to change and wash clothes as well as shower in clean water right after a swim and run race.

Facts about leptospirosis

Leptospirosis is a rare disease caused by infection with bacteria of the species *Leptospira*. In 2017, 22 cases of leptospirosis were notified. Compared to previous years this is a 100% increase and was connected to the swim-run race¹.

Infected animals excrete the bacterium in large amounts through their urine. The bacteria are found in many animals, but in Denmark it is usually bacteria in urine from mice or more often rats that cause disease. Humans are infected by the bacteria penetrating through small wounds and scratches either via direct skin contact to urine or indirectly via contact to freshwater, sewage or similar².

The symptoms of leptospirosis are often uncharacteristic in the beginning of the disease and may resemble influenza symptoms with high fever, headache, muscle aches and red eyes (conjunctival suffusion). In the severe cases, called Weil's disease, mortality is 5-15% and a relatively rapid onset of lung infection and organ failure is seen, in which the function of the liver, kidney and the coagulation system is particularly affected. Leptospirosis can be treated with penicillin or other antibiotics. The best effect is achieved if the treatment is initiated within the first four days of illness.

In connection with contact with freshwater or sewage, e. g. after flooding, it is advisable to use protective equipment such as rubber boots and long-handed rubber gloves or otherwise to cover any wounds and scratches with water repellent material. When the contact is over it is advisable to immediately shower, to dry thoroughly with a clean towel, and to dress in clean clothes. The clothes used during the water contact should immediately be washed at high temperature - preferably above 80 degrees. Alternatively, discard the clothes in a sealed black plastic bag and dispose it as ordinary waste³. Persons with influenza-like symptoms and a history of unprotected contact to freshwater or floodwater during the previous month should consult a physician for further examination.

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3. Status on *Campylobacter* in Denmark

By Mette R. Gantzhorn (merga@fvst.dk), Sara M. Pires, Johanne Ellis-Iversen, Katrin Kuhn and Eva Møller Nielsen

Campylobacter spp. is the most common bacterial cause of food borne illness in Denmark, with 4,257 confirmed cases of Campylobacteriosis in 2017. The true number of cases is estimated to 50,000, because only a small proportion of cases seek medical care and is registered in the system. According to IFRO report no. 2621 each registered case of campylobacteriosis costs the Danish society about 250,000 DKK on average, which is a significant economic burden on society. Even though, approximately one third of cases are infected abroad, it is still essential to reduce the number of human cases by controlling *Campylobacter* in every step of the Danish food production chain.

Campylobacter is a Gram negative curved, spiral or S-shaped motile bacteria. There are several different species, but *C. jejuni* and to some extent *C. coli* are the species most commonly associated with food borne infections. *Campylobacter* is a natural inhabitant of the gastrointestinal tract of many animals, including broilers, cattle, pigs and dogs.

Contaminated broiler meat is the most common source of campylobacteriosis acquired in Denmark, but other sources also play a role. These include consumption of contaminated water and unpasteurized milk as well as contact to pets. A rather large proportion of cases remains without a known source.

In Denmark, there has been action plans to reduce *Campylobacter* since 2008, and surveillance of the prevalence of *Campylobacter* in broilers started back in 1996, as a collaboration between the Danish Veterinary and Food Administration (DVFA) and the industry. The action plans have focused on reducing *Campylobacter* in the broiler reservoir to prevent transmission to broiler meat.

To continue targeted and effective control measures in Denmark, a new national case-control study was undertaken by the Statens Serum Institut (SSI), and The National Food Institute, Danish Technical University (DTU Food), developed a source attribution model. The projects were part of the action plan against *Campylobacter* 2013-2016, and the DVFA supported the projects financially and contributed with data from food and environmental sources.

3.1 Surveillance and control of *Campylobacter* Control of Danish and imported broiler meat

Since 2007, batches of Danish and imported broiler meat has been examined for the presence of *Campylobacter* as

part of the Case-by-case Control Programme. Based on the results of the examinations, DTU Food carries out risk assessments. The DVFA decides if a batch is safe or unsafe based on several different factors, including the results of the risk assessment, antimicrobial resistance and knowledge of the intended use of the batch. If a batch is declared unsafe, it is removed from the market.

In 2017, *Campylobacter* was found in 14% of the batches of Danish broiler meat and in 50% of the tested, imported batches of broiler meat. One batch of Danish meat (0.8%) and 10 batches of imported meat (6.6%) were determined unsafe. An overview of the results in the case-by-case control can be found in Appendix table A15.

Surveillance of *Campylobacter* status in broiler flocks

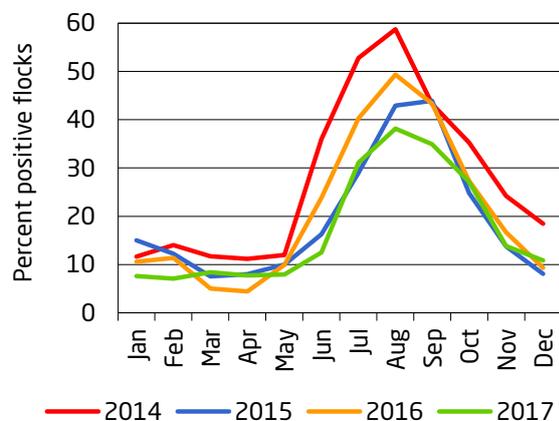
Campylobacter status of broiler flocks is monitored through samples obtained at the slaughterhouses where all flocks are sampled. The surveillance of *Campylobacter* in broiler flocks show more positive flocks in the late summer months (Figure 3.1), and that flocks with access to the outdoors are more often positive for *Campylobacter* than flocks kept inside (data not shown). A 38% reduction in positive flocks has been observed since 2014 and in 2017, only 16.6% of broiler flocks were tested positive for *Campylobacter* (Appendix Table A10). This is a very low level and might be a result of the measures taken at farms to prevent introduction of *Campylobacter* combined with a rather cold summer.

Surveillance of *Campylobacter* in fresh broiler meat at the slaughterhouses

More than 90% of Danish broilers are slaughtered at the two largest slaughterhouses in Denmark, where the DVFA has carried out surveillance for *Campylobacter* in fresh meat for more than a decade. In 2013, the surveillance sample type was changed to include thigh skin only. The results feed into a model that predicts the risk to humans from *Campylobacter* in Danish broiler meat relative to the risk in 2013, yielding a relative risk measure (RR). The RR is monitored to assess progress of the national action plan.

Similar to the observations of *Campylobacter* in broiler flocks, the prevalence of *Campylobacter* in Danish broiler meat is higher in the late summer months (Figure 3.2) and organic meat is more often positive for *Campylobacter* than meat from conventional produced broilers (data not shown).

Figure 3.1: Percentage of broiler flocks positive for *Campylobacter* per month, 2014-2017



Source: Danish Agriculture and food Council

Over the years, a reduction in the prevalence of *Campylobacter* in samples obtained at the two largest slaughterhouses has been observed, and the concentration of *Campylobacter* in positive samples has decreased (Appendix Table A11). In combination, these reductions have led to a decrease in the RR from Danish produced broiler meat compared to 2013 by 43% (Table 3.1).

3.2 Source attribution of domestically-acquired

Table 3.1. Relative risk estimates 2014-2017

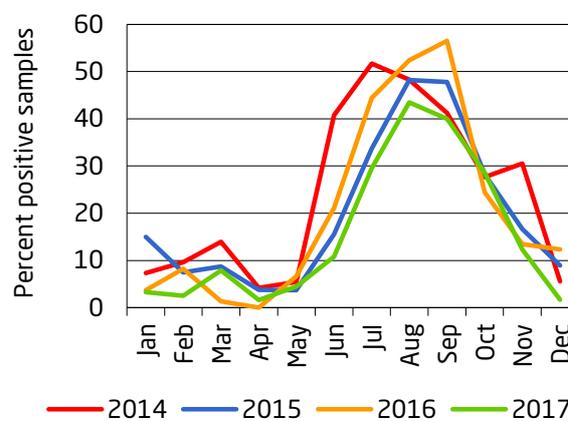
	2013	2014	2015	2016	2017
Relative risk	1.0	0.72	0.64	0.64	0.57

Campylobacter infections

The implemented interventions in the broiler production did not result in the expected reduction in number of human cases. This may be related to other factors counterbalancing the effect of these interventions, particularly with the role of other sources of exposure. To investigate this, a *Campylobacter* source attribution model was developed to identify the relative contribution of other *Campylobacter* sources in order to provide additional information to the authorities when prioritizing food safety intervention strategies for *Campylobacter*.

To obtain sufficient data for the model the DVFA broadened the surveillance of *Campylobacter* in 2015- March 2017. During this period, samples were taken from a range of possible sources; broiler, turkey and duck meat at retail, unpasteurized milk, and different kinds of salad. Furthermore, samples from bathing water in lakes and seas around Denmark, and intestinal content samples from cattle, broilers, pigs and dogs were collected. During the period September 2015 to March 2017, isolates from a representative subset of

Figure 3.2. Prevalence of *Campylobacter* in the surveillance on thigh skin samples from conventionally produced broilers, 2014-2017



Source: Danish Veterinary and Food Administration

domestically acquired human infections were collected from four clinical microbiology laboratories. The DVFA subtyped part of the food and animal isolates by Multi Locus Sequence Typing (MLST) and SSI subtyped all the human isolates. Only *C. jejuni* were included.

A source attribution approach using microbial subtyping data from human *Campylobacter* cases and isolates from food, animal and environmental sources was applied. The principle is to match the subtypes of the human isolates with the most likely subtypes of isolates from different sources (e.g., animals, food). This method is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source, providing a heterogeneous distribution of subtypes among the sources. Subtypes exclusively or almost exclusively isolated from one source are regarded as indicators for the human health impact of that particular source, assuming that all human infections with these subtypes originate only from that source. Human infections caused by subtypes found in several reservoirs are then distributed relative to the prevalence of the indicator types. This approach utilizes a collection of temporally and spatially related isolates from various sources. The applied model was developed by Hald et al. (2004)² and adapted by Boysen et al. (2013)³. The model is built in a Bayesian framework and estimates a set of unknown parameters that account for the differences in the ability of different subtypes to cause infection and of different sources to act as a vehicle for infection. More details about the model can be found in Pires & Christensen (2017)⁴.

The model attributed 731 MLST typed human isolates to eight food, animal and environmental sources for which MLST type distribution was available (Table 3.2).

Results showed that the most important source of *C. jejuni* infections was domestic broilers (338 cases), followed by

cattle (139 cases) and imported broilers (69 cases) (Table 3.3); these estimates correspond to attribution proportions of 46%, 19% and 9%, respectively. They also show that contact with dogs and bathing in contaminated recreational waters are relevant sources (4% of cases attributed to each of these sources). This was the first time the contribution of these sources for campylobacteriosis in Denmark were quantified, and several studies from other countries have found these to be important sources for human campylobacteriosis. Imported turkey and duck were responsible for less than 2% of cases, and pigs were estimated to be the least important source, with an attribution proportion below 1%. No cases were estimated to be attributed to domestic duck, and 13% of cases could not be attributed to any source. In summary, broiler meat and the cattle reservoir remains to be the most important sources of *C. jejuni* infections, but other sources, like sea and lake water and dogs are also relevant sources.

3.3 Case-control study

Although *Campylobacter* is the most prevalent bacterial gastrointestinal infection in Denmark, many aspects of its basic epidemiology and transmission cycle remain poorly understood - particularly concerning the importance of non-food related exposures as risk factors. In order to gain better understanding of campylobacteriosis in Denmark, a national case-control study was undertaken during 2016 with the purpose of quantifying the major sources of infection, focusing particularly on those in the environment. The study was aimed at young adults and children as these are at highest risk of disease and least affected by partial acquired

immunity. Cases included all persons aged older than 1 and younger than 31 years of age with a laboratory confirmed *Campylobacter* infection and a valid Danish address at the time of diagnosis. Frequency matched control persons were selected from The Danish Civil Registration System.

All participants were recruited using a postal letter-based invitation, containing an introduction to the study and a personalised link to the online questionnaire. The questionnaire collected information on a range of exposures including: medical history, demographic information, and overseas travel, recreational activities, dining locations, food and drink, kitchen hygiene and animal contact in a 5-day exposure period prior to disease onset/interview. Both cases and controls reporting travel abroad were asked to provide further information about their travels, including exposures, after which they were excluded from completing the rest of the questionnaire.

1,366 persons aged 1-30 years with a confirmed *Campylobacter* infection were included in the study of which 887 (65%) responded to the questionnaire. Of the 4,418 approached controls, 2,935 (66%) completed the questionnaire.

In total, 309 cases (35%) and 298 controls (10%), who answered the questionnaire, reported foreign travel in the 14-day exposure period, making travelling abroad the single most important risk factor for infection in the study. Risk of infection was significantly higher for visitors to Asian countries and Turkey while in comparison visiting Northern Europe and Scandinavia in particular was inversely associated with infection. Whilst travelling, eating food from street kitchens and having close contact to water, sand and mud were associated with an increased risk of infection.

Table 3.2. Overview of the data used for the microbial subtyping model (Model 1)

Source	Number of MLST types in source (Number of isolates)	Number of MLST types matched to human (Number of isolates)
Human	136 (731)	136 (731)
Broilers*	42 (132)	32 (121)
Cattle*	38 (208)	21 (191)
Danish Pig*	6 (22)	4 (16)
Broilers**	46 (176)	35 (161)
Duck**	1 (1)	0 (0)
Dogs	19 (25)	16 (22)
Bathing water	2 (2)	1 (1)
Imported Vegetables	1 (1)	0 (0)
Broilers**	46 (90)	25 (65)
Duck**	16 (20)	8 (8)
Turkey**	7 (9)	6 (7)

*Fecal samples. **Meat samples.

Source: Danish Veterinary and Food Administration and Statens Serum Institut

For persons, who did not report travelling abroad, a range of determinants was identified in a univariate analyses. These were not surprisingly related to consumption of certain foods and drinks but also the environment and animals as well as other exposures such as use of medication (antibiotics and proton pump inhibitors, PPIs). In general, univariate results are used to indicate the significance of single variables without adjustment for possible interactions between and within variables. In order to describe the results in a biologically more feasible way, multivariate modelling is used which combines statistically significant exposures and adjustment for confounding effects. In this study, the multivariate models showed that consumption of broiler meat remains an important risk factor for campylobacteriosis in Denmark. As a slightly more surprising result, the models also suggested that minced beef might potentially be a source of *Campylobacter* infections. This will be further examined as part of the new action plan against *Campylobacter*. The results additionally showed that eating barbecued meat is a risk, but also indicated that environmental factors, mainly contact to water and sand, could play a more significant role in the transmission of *Campylobacter* than previously assumed. Lastly, eating fresh strawberries, contact to dogs and in particular dog faeces was shown to be a significant independent risk factor, again especially for children.

In summary, this case-control study, the largest undertaken on *Campylobacter* in Denmark, re-confirmed well-known domestic routes of infection but also highlighted new possible risk factors, some of which point to transmission of infection through the environment. By including extended answers from persons who had travelled abroad, it was

possible to confirm that visiting specific countries and exposure to certain potential sources of infection carries a significantly higher risk of infection whilst travelling. These results are important as they not only strengthen our current knowledge of *Campylobacter* epidemiology but also point towards new areas of research and not least improved guidelines for prevention of infection.

3.4 Source attribution and case-control combined

The case-control study (CC) and the source attribution (SA) study provided valuable information on current sources and transmission routes for *Campylobacter*. Even though they did not investigate the exact same things; the results were pointing in the same direction and provided some guidance to the new *Campylobacter* Action Plan. Figure 3.3 compares the results by looking at relative importance of different sources or pathways identified by each study.

Both studies agree that broilers and cattle were the main reservoirs for domestically acquired *Campylobacter* cases in Denmark. In the CC study, the transmission route from the cattle reservoir was minced beef and in the SA model cattle isolates rather than meat isolates were used, suggesting that cattle isolates may also have other transmission routes than minced beef. Dogs contributed to human cases and the environment through different vehicles also play a role. The SA model attributed a few cases to duck, turkey, and pork meat and the CC study identified barbecuing, proton pump inhibitors (PPI's) and contact with animal faeces as risks for human *Campylobacter*. The last three were not investigated in the SA study.

Both studies agree that broilers remain the most important single source suggesting that handling and/or cooking of

Table 3.3. Number and proportion of *Campylobacter jejuni* cases attributed different sources (mean, median and 95% Confidence Interval (CI))

	Number of cases			Attribution proportion (%)	
	Mean	Median	95% CI	Mean	95% CI
Broiler DK ¹	338.1	338	[261.3, 411.6]	46.3	[35.7, 56.3]
Cattle DK	139.4	136.9	[83.5, 199.8]	19.1	[11.4, 27.3]
Pig DK	5.1	4.3	[0.7, 14.1]	0.7	[0.1, 1.9]
Duck DK	0			0	
Broiler IMP ¹	69.1	67.8	[43.0, 100.6]	9.5	[5.9, 13.8]
Duck IMP	11.9	11.1	[4.3, 23.6]	1.6	[0.6, 3.2]
Turkey IMP	14.2	12.8	[3.1, 32.9]	1.9	[0.4, 4.5]
Dog	30.1	28.2	[8.5, 62.1]	4.1	[1.2, 8.5]
Bathing seawater	27.6	28.4	[4.0, 46.4]	3.7	[0.6, 6.3]
Unknown	95.7	98.3	[47.8, 146.3]	13.1	[6.5, 20.0]
Total	731				

1) DK: Danish meat, IMP: Imported meat

Source: National Food Institute, Technical University of Denmark

raw broilers still is problematic for consumers, as no tradition of eating raw broiler meat exists in Denmark. It illustrates the difficulties in avoiding cross contamination in the kitchen and of ensuring that the meat is properly cooked irrespectively of shape and form. Sufficient cooking is more complicated for some pieces of meat than others, depending on a variety of factors such as uniformity of shape, presence of bones and type of cooking.

In the CC study, the only identified transmission route from the cattle reservoir, identified in the SA study, was minced beef. In both studies, the cattle reservoir or minced beef contributed to a large proportion of cases in Denmark. That minced beef could be the transmission route, was an unexpected finding, since only 0.1% of 3,046 samples of minced meat were positive of *Campylobacter* in 2001. However, the strength of the risk factor in the case control study was convincing both for the whole study population and for the very young children. It is possible that something in the production chain of minced meat has changed since 2001, increasing the risk to humans. Modified atmosphere packaging (MAP) is increasingly used and extends the shelf life for minced beef from 24 hours to eight days⁷. High levels of oxygen are used to maintain the red meat colour and it also causes the meat to turn brown at 55°C, before the meat reaches a temperature that eliminates *Campylobacter* (55-60°C). Minced beef as a source of campylobacteriosis will be examined further as part of the new *Campylobacter* action plan.

Other possible *Campylobacter* transmission routes from cattle to humans are direct contact, through milk and through environmental contamination, but no evidence was found of these indirect links. However, the current studies were

not ideal to identify such associations and different studies are needed to investigate reservoirs for environmental contamination.

Manure from all farm animals including poultry and cattle is used for fertilising crops, fruits and vegetables⁵. The common practice in industrialised farming of fruit and vegetables is to fertilise the soil beneath the surface before sowing. There are recommendations to the time gap between fertilizing and sowing. This practice decreases the risk of contamination of the finished crops from the manure. Nevertheless, in very wet weather, heavy rain may bring manure to the surface and contaminate very fast growing fruits and vegetables that grow close to the ground. This could explain why eating fresh strawberries was identified as a risk in the case-control study and why lettuce rank highly in DTU Foods risk ranking model for vegetables and fruit^{5,8}. Nothing is known about how manure is used as fertiliser in private gardens, but it is common practice to use fresh manure from close by farms or small-holdings. Fresh strawberries as a source will be investigated further as part of the new action plan against *Campylobacter*.

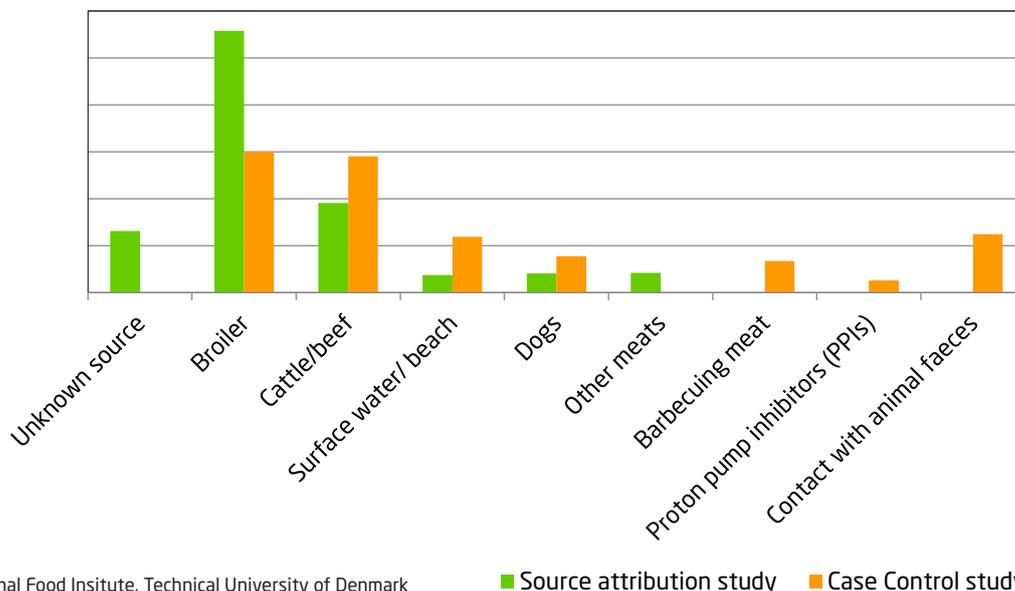
3.5 Action plans against *Campylobacter*

Denmark has had National Action Plans against *Campylobacter* since 2008. The first action plan was focusing solely on broilers and broiler meat, but the focus has been broadened in the later action plans.

Action plan against *Campylobacter* 2013-2017

The second action plan was adopted in 2013 and is described by Anonymous (2013)⁹. Initially, it ran until 2016, but it was prolonged until the end of 2017.

Figure 3.3. The relative importance of each source or risk factor for *Campylobacter* assigned by a source attribution study and a case-control study



Source: National Food Institute, Technical University of Denmark

The main goal in the action plan was to reduce the number of human cases. Due to changes in both diagnostic methods and the registration of human cases, it is not possible to compare results from 2016 to previous data. There was a decrease of 9% in the number of human cases in 2017 compared to 2016.

In broiler meat, the target was to reduce the risk of illness from *Campylobacter* to one-half by 2017 relative to the risk in 2013. By the end of 2017, the risk of getting *Campylobacter* from broiler meat was 57 % of the risk in 2013, nearly reaching the target of the action plan.

In broiler flocks, the prevalence in positive flocks was reduced by 38% during the action plan, which was considerably higher than the target reduction of 20%.

Action plan against *Campylobacter* 2018-2021

The initiatives in the new action plan from 2018-2021 are based on the experiences, results and knowledge obtained during the previous action plan. The action plan was prepared by the DVFA in collaboration with Danish Agriculture and Food Council, Confederation of Danish Industry, FødevarerDanmark and DTU Food. The action plan is available at the DVFA website (fvst.dk).

The main target is to obtain a 5% reduction in registered human cases each year, which is the first time a specific target on the reduction of human cases is included in the *Campylobacter* Action Plan. Furthermore, it is an aim to maintain the low prevalence of approximately 17% in broiler flocks. Reducing the relative risk of *Campylobacter* from Danish broiler meat compared to 2013 also remains a target in the new action plan. Many efforts and measures from the previous action plan are continued and efforts to include more slaughterhouses and production systems e.g. organic and free range production of broilers are part of the measures in the new action plan.

The source attribution and case-control projects have pointed at some new and interesting possible sources of infection with *Campylobacter*, including minced beef and strawberries. The significance of these sources will be examined further. As the case-control has also confirmed, some of the largest risk factors of *Campylobacter* infections is travelling and barbecuing. Hence, it is a measure in the action plan to prepare and disseminate information material to the public on how to avoid and minimize these risks.

3.6 Conclusion

The risk of getting ill from *Campylobacter* in Danish broiler meat has decreased since 2013. Nevertheless, broiler meat remains to be the most important source of campylobacteriosis.

New sources of *Campylobacter* have been identified and more knowledge on the significance and possible interventions will be obtained. Therefore, the new action plan against *Campylobacter* includes further examinations of these possible sources, and contains initiatives that will contribute to reducing the number of human cases in Denmark. The action plan is flexible and dynamic, meaning that new targets can be set and new measures can be included, depending on the results obtained during the period.

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Clustering of human isolates

By Eva Møller Nielsen

Outbreaks of *Campylobacter* are traditionally considered to be rare; however, rather than that being the true nature of the disease, this may reflect our present inability to detect them. In relation to the above-mentioned source attribution study based on MLST of *C. jejuni* isolates, a specific analysis of human clinical isolates was carried out with the objective to determine the genetic and epidemiological degree of clustering among *C. jejuni* isolates from Danish patients¹. Whole-genome sequencing (WGS) was applied to 245 *C. jejuni* isolates from patients with domestically acquired infection over a 9-month period from October 2015 to June 2016 in four regions of Denmark. 7-locus MLSTs were extracted and for isolates within the same ST, and represented by more than two isolates, a core-genome SNP analysis was performed. A genetic cluster was defined as the clustering - and clear separation from other genomes - of two or more genomes. A SNP cut-off was not predefined, but clustered genomes were <5 SNPs apart.

A geographical cluster was defined by patients from the same (or adjacent) municipality and a temporal cluster by laboratory dates within 12 days.

Eighty-three STs were identified among the 245 patient isolates; six included more than 10 isolates and 50 included only one isolate. SNP analysis demonstrated that 62 (25%) isolates clustered genetically. In total, 21 genetic clusters were identified of which four (18%) consisted of five isolates or more. Seventeen (81%) of the 21 genetic clusters were clustered in space and/or time. Of the 245 isolates, 49 (20%) were part of a temporal and/or geographical cluster¹. The identified clusters included two outbreaks; one which had not been identified through the existing surveillance system. Using WGS, we show that *Campylobacter* case clustering and even outbreaks appear to occur more frequently than previously assumed, providing important new insight into the relatively poorly understood epidemiology of the most important cause of bacterial gastroenteritis in the industrialized world.

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4. The Danish Laboratory based surveillance on foodborne bacteria

By Mia Torpdahl (mtd@ssi.dk), Anne Mette Seyfarth, Gitte Ortved Bjerager and Jette Sejer Kjeldgaard

Whole-genome sequencing (WGS) is increasingly used for surveillance of foodborne pathogens worldwide, replacing both the phenotypic methods and the molecular subtyping methods like pulsed-field gel electrophoresis (PFGE) and multi-locus variable number of tandem repeats analysis (MLVA). In Denmark, the transition started in 2013 introducing WGS as the primary and only subtyping method for molecular surveillance of *Listeria*, followed by other foodborne pathogens. The current surveillance practice is outlined below for the public health sector and the food and veterinary sector.

4.1 Foodborne bacteria isolated from humans

Statens Serum Institut (SSI) is responsible for the laboratory surveillance of foodborne bacteria in Denmark, whereas diagnostics of foodborne infections are done locally at clinical departments at hospitals in Denmark. *Campylobacter*, *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria*, *Shigella* and *Yersinia* are all laboratory notifiable infections in Denmark and the clinical departments have an obligation to provide information on disease and patient to SSI. The clinical departments are furthermore sending all isolates, when possible to recover, from patients diagnosed with either a *Salmonella*, STEC, *Shigella* or *Listeria* infection to SSI. Local agreements are also in place ensuring that a selected number of *Campylobacter* and *Yersinia* isolates are sent to SSI each year. A surveillance system is set up at SSI based on combining patient information with the isolate information after further characterization of isolates in the laboratory.

At present, molecular surveillance on *Salmonella*, STEC and *Listeria* at SSI are based on real-time whole-genome sequencing (WGS) using the Illumina platform and read length of 150 bp. Furthermore *Campylobacter* and *Shigella* have been sequenced for project purposes and might be included at a later stage. Upon receipt of isolates in the SSI laboratory, isolates are registered in a central database where isolate information is connected with the patient information provided by the clinical department. The isolates are saved at -80 degrees °C and DNA purification for sequencing started. A central sequencing unit has been set-up at SSI and all isolates are sequenced within one week of receipt. Sequences are

provided back with a comparison of expected/detected species, a sequence type (ST) and a QC report providing quality scores on the sequence. The actual laboratory surveillance is performed in the commercial software BioNumerics (Applied Maths), where species-specific databases have been set up and patient information imported. Sequence links are imported to BioNumerics where data are uploaded to the species-specific BioNumerics calculation engine, and alleles are called according to public cg/wgMLSTdatabases. For both *Salmonella* and STEC the enterobase schemes (<https://enterobase.warwick.ac.uk>)¹ are used, and for *Listeria* the Institut Pasteur cgMLST scheme² is used. Sequences are further used to assign serotypes of *Salmonella* with an in-house developed script combining the serotype derived from enterobase based on ST with the seqsero in silico serotyping (<http://www.denglab.info/SeqSero>)³. In STEC, serogroup and virulence genes are derived using a local copy of the virulence finder and serotype finder databases from CGE tools (<http://www.genomicepidemiology.org/>). Resistance genes are extracted using a local copy of the ResFinder database from CGE tools (<http://www.genomicepidemiology.org/>).

The initial cluster detection is done using cgMLST for all species, using single linkage clustering in BioNumerics. Clusters are assigned using locally developed species-specific allelic differences and all clusters are named using arbitrary numbers. When relevant, clusters (number of cases depending of species) are further confirmed with a SNP analysis, using a local version of the NASP pipeline (<http://tgennorth.github.io/NASP/>). After verification, clusters are reported to our local epidemiological department and decisions on action needed are taken in close collaboration between microbiologists and epidemiologists and are based on both patient information and strain characterization. When it is decided to initiate an outbreak investigation, this is based on number of patients, date of onset of disease and interviewing patients. If an outbreak has been identified, we inform members of the Danish Central Outbreak Management Group (DCUG), which include members from SSI, Danish Veterinary and Food Administration (DVFA) and National Food Institute, Danish Technical University (DTU Food). If decided relevant, we share our sequences with DTU Food for further comparison with sequences from food

and animals. Furthermore, sequences are made publically available at the European nucleotide archive (ENA) and knowledge on patients, strain characterization and a link to ENA are shared with the European public health community using the Epidemic Intelligence Information System (EPIS) developed by European Centre for Disease Prevention and Control (ECDC). Annual aggregated data derived from the sequences are reported to Annual Report on Zoonoses in Denmark (<http://www.food.dtu.dk/english/publications/disease-causing-microorganisms/zoonosis-annual-reports>), DANMAP (<http://www.food.dtu.dk/english/publications/antimicrobial-resistance/danmap>) and to the European surveillance system (TESSy) developed by ECDC.

4.2 Foodborne bacteria isolated from production animals and food

DVFA performs a number of official samplings, monitoring and control campaigns as well as laboratory based projects, collecting microbiological samples from food and veterinary establishments throughout the year. The campaigns are directed towards sources and reservoirs where there is a suspicion of biohazards or high risks of contaminants. The official samples collected derive from EU- or national legislation, national monitoring programmes or targeted control projects.

The samples are processed including isolation, identification and whole genome sequencing (WGS) at the DVFA laboratory in Ringsted. Sequencing is performed using the Illumina platform and read lengths of 2x250 bp (paired-end sequencing). All isolates are stored at -80°C and all sequences are kept at the laboratory.

The *Salmonella* isolates, including the ones submitted to the laboratory for serotyping, are routinely sequenced, except for isolates deriving from imported fish meal for feed. For *Listeria monocytogenes* only a fraction of the detected isolates are selected for sequencing (one isolate per batch of meat or four isolates per company in case of environmental sampling). In cases of human disease or outbreak situations, more samples/isolates from relevant sources of both *Listeria* and *Salmonella* are selected for sequencing. At the moment, isolates of *Campylobacter* and STEC are only sequenced in relation to specific projects, but the sequence data is stored and can be investigated further, if needed.

The classical antigen serotyping of *Salmonella* isolates has been replaced by WGS based serotyping using the bioinformatics pipeline *Salmonella*TypeFinder web tool, developed by DTU Food (<https://bitbucket.org/genomicepidemiology/salmonellatypefinder>). The use of this pipeline was implemented at DVFA in 2016 and subsequently validated by DTU Food and DVFA. Since January 2017, it has been routinely performed at DVFA. The results and sequencing data are used in surveillance, in research projects and for outbreak detection of foodborne pathogens by the DCUG.

For outbreak investigations, both the *Salmonella* serotypes and sequence types, and the *Listeria* sequence types (MLST) are identified along with QC parameters using the *Salmonella*TypeFinder web tool. The sequencing and typing results are shared with DTU Food through upload of sequences and metadata for the isolates in a common DVFA Storage database. For cluster analysis, DTU Food applies an in-house developed bioinformatics pipeline (CSI Phylogeny version 1.4)⁴, to identify clusters of bacteria with the same or related contamination sources based on SNP differences. These results are linked with disease outbreak information from SSI in the DCUG.

4.3 Conclusion

The last five years has seen an ongoing transition of real-time surveillance of selected foodborne pathogens to the routine use of WGS on clinical, food and veterinary isolates. The method has already proven superior in detecting clusters of identical isolates, supporting epidemiological investigations, defining outbreaks and comparing isolates between sectors. To ensure optimal real-time surveillance, rapid detection of outbreaks and timely comparison between sectors, developments are still ongoing for an easier, uniform and standardized extraction of data from WGS.

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5. Vectorborne zoonoses

By René Bødker (rebo@vet.dtu.dk), Lene Jung Kjær, Mette Frimodt Hansen, Andreas Petersen, Louise Lohse and Kirstine Klitgaard Schou

The National Veterinary Institute, Technical University of Denmark (DTU Vet) monitors vectors and vector-borne diseases in Denmark on behalf of the Danish Veterinary and Food Administration (DVFA). DTU Vet is responsible for the national surveillance of key vector species and for quantifying and mapping ticks and tick-borne pathogens. The surveillance is focused on endemic vectors but also screens for exotic vectors.

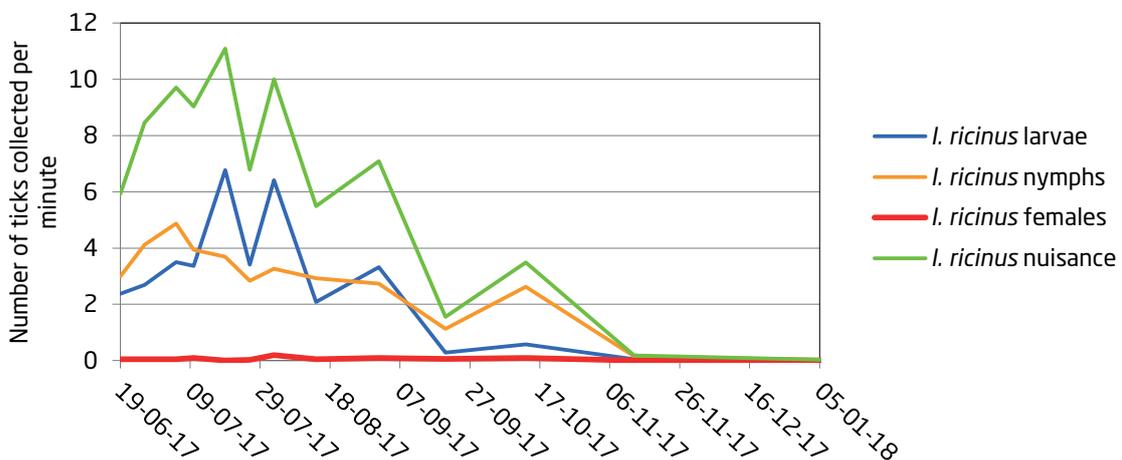
Mosquitoes and biting midges have been monitored weekly during the vector season since 2011 and 2012, respectively, as a part of the national vector surveillance program. These weekly data are available at the vector surveillance website www.myggetal.dk. In 2017, the national vector surveillance was expanded to include regular surveillance of *Ixodes ricinus* tick larvae, nymphs and adults throughout the year as well as weekly surveillance of mechanical biting flies of the Tabanidae family. Prevention of tick-borne infections in humans, pets and production animals relies solely on personal protection including, prevention of tick bites and vaccination for Tick Borne Encephalitis (TBE). Therefore, the surveillance of tick bite risk was added to the surveillance website. The rising threat of Lumpy Skin Disease in cattle spreading from the Balkans, outbreaks of Infectious Equine Anemia in horses in Germany and the continuous spread of African Swine Fever in pigs in the Baltic countries and Poland, prompted the vector surveillance to add surveillance of Tabanidae biting flies in 2017 and to include the running surveillance results on the website. Tabanidae biting flies can be a nuisance to humans

but they are at present not considered to be vectors of any zoonotic pathogen in Denmark.

The abundance of mosquitoes and biting midges in the relatively cool summer of 2017 can be classified as 'within normal range'. No exotic mosquitoes were found at the five national surveillance sites, at an urban surveillance site or at the cargo terminal at Copenhagen Airport.

In the last 4-5 years Denmark has received a wave of migrating grey wolves (*Canis lupus*) and golden jackals (*Canis aureus*). These are long-distance migrating animals originating in Eastern Europe or Germany and now establishing in Denmark. Because these medium sized carnivores migrate rapidly over long distances, they constitute a risk of introducing both new ticks and tick-borne pathogens to Denmark. All ticks collected during vector surveillance activities are routinely screened for exotic tick pathogen species using PCR. In 2017, a golden jackal was accidentally shot in Jutland in the western part of Denmark. On arrival at DTU Vet, 21 ticks were removed from the carcass. These were all male *Dermacentor reticulatus*, a tick species not previously recorded from wild life in Denmark. The ticks were routinely tested for pathogens using high-throughput real-time PCR (Fluidigm DNA chip) able to detect 40 different European tick-borne pathogens. Of the 21 ticks, 18 were positive for *Rickettsia raoultii*, a zoonotic pathogen not previously recorded in Scandinavia¹. Human infections with *R. raoultii* may lead to eschar (known as black wounds) or may affect the lymph system (TIBOLA). Both *D. reticulatus* and *R. raoultii*

Figure 5.1. Number of *Ixodes ricinus* tick larvae, nymphs and adults, 2017



Source: National Veterinary Institut, Technical University of Denmark

Figure 5.2. The tick species *Dermacentor reticulatus* was for the first time recorded from Danish wildlife in 2017. The ticks introduced a new zoonotic tick borne pathogen to Denmark



Foto: National Veterinary Institut, Technical University of Denmark

are presently spreading in Western Europe² and there is a potential risk, they may become established in Denmark. A likely mechanism of introduction is by migrating grey wolves or golden jackals or by domestic dogs accompanying their owners on vacations or hunting trips further south in Europe without proper protection against tick bites.

Two other tick species are considered a potential risk for introduction, *Ixodes persulcatus* (a close relative of the endemic *I. ricinus*) and *Haemaphysalis punctata*. All collected tick pools are routinely examined for *I. persulcatus* and the species has never been recorded in Denmark. A more targeted approach was used to screen for *H. punctata*, which in Sweden is found on grazing sheep on the island of Öland. Sheep from three Danish sheep flocks in Jutland, on Zealand and on the island of Bornholm were screened for ticks. In total 1,242 ticks were removed from the sheep and none of the ticks were visually found to be *H. punctata*, but they will all be tested with PCR during 2018.

In 2009, a small transmission area for TBE virus was discovered at the forest Tokkekøb Hegn just north of Copenhagen after two persons fell ill. Since then, TBE positive ticks have been found several times at the same hotspot. However 1,200 ticks collected in 2016 and additional 2,350 ticks collected in 2017 from the same area were all negative for TBE virus. If TBE is still present in this area it is therefore with a much lower prevalence than recorded previously³.

West Nile virus (WNV) and Usutu virus (USUV) are circulating in Europe and outbreaks have been reported as far north as Austria and the Netherlands, respectively. A national surveillance program has been in place since 2012, and samp-

Figure 5.3. In 2017 three sheep holdings from different parts of the country were screened for exotic *Haemaphysalis punctata* ticks. The tick species is endemic in Sweden on the island of Öland, but remains absent from Denmark



Foto: National Veterinary Institut, Technical University of Denmark

les have been collected from domestic free ranging poultry, migratory birds as well as the *Culex* mosquito. To ensure a wide geographical representation, samples originate from different locations in Denmark. In 2017, 410 serum samples from Danish outdoor poultry and 250 serum samples from migratory birds were analysed for WNV-specific antibodies, using ELISA and confirmatory neutralization test. All samples from Danish poultry were negative for WNV, whereas 5.6% of the migratory birds had antibodies against WNV/USUV. The prevalence of seropositive migratory birds has increased compared to the previous years where 1% to 4% of the birds were positive. All seropositive birds came from Africa, south of Sahara. The *C. pipiens* mosquitoes (275 individuals) were tested in pools for WNV and USUV RNA using RT-PCR. All pools were negative.

Results from the WNV surveillance program 2017 indicates that WNV/USUV, so far, have not been introduced to Denmark.

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6. Burden of foodborne disease in Denmark

By Sara Pires (smpi@food.dtu.dk)

Burden of disease studies have been recognized as a powerful tool to enable policy makers and other stakeholders to set appropriate, evidence-based priorities in the area of food safety. At The National Food Institute, Danish Technical University (DTU Food) and under the Metrix Project^a, we are developing methods to estimate the burden of a range of foodborne diseases caused by microbial agents, chemical hazards and diet-associated risk factors. These estimates will then be used to rank diseases in Denmark according to their overall health impact in the population, and ultimately to inform risk management strategies in the area of food and health.

6.1 Total incidence of disease by foodborne pathogens

It is widely recognized that cases of foodborne infections are largely under-reported, and that for many pathogens the true incidence of disease in the population is largely unknown. The gap between the true number of cases caused by contaminated foods and what is captured by public health surveillance is larger for diseases with mild and short-duration symptoms; on the contrary, severe diseases or infections that particularly affect vulnerable groups of society are more likely to be diagnosed and reported. To address knowledge gaps, we estimate the true number of cases of different foodborne diseases in the population.

6.2 Disability Adjusted Life Years (DALYs)

To be able to compare diseases with different causes, incidence and symptoms, we apply a harmonized health metric that assesses the impact of diseases in terms of incidence, severity, duration, and mortality. DALYs are the sum of years lived with disability (YLD), and the years of life lost due to premature death caused by the disease (YLL). We here present estimates for listeria and norovirus infections, while *Salmonella*, *Campylobacter*, *STEC*, *Toxoplasma* and *Yersinia* infections have been covered in chapters in the two previous years' Annual Reports of Zoonoses in Denmark^{1,2}.

6.3 Burden of disease of listeriosis in Denmark

Listeriosis is caused by the gram-positive bacterium *Listeria monocytogenes*, an ubiquitous pathogen that can grow in

food with low moisture content, high salt concentration, and at refrigeration temperatures. This ability to persist and multiply in the food environment makes *L. monocytogenes* especially difficult to control³. Infection with *L. monocytogenes* can lead to mild disease in otherwise healthy people, manifesting with usually mild and self-limiting gastroenteritis. However, in risk groups such as the elderly, immunocompromised or unborn babies and neonates (which can be infected by vertical transmission) clinical disease can be severe, with possible symptoms including sepsis, meningitis, or encephalitis, abortion and death⁴. In these cases, disease often leads to hospitalization of patients and intensive health care, which results in a high societal and economic burden.

Denmark has reported the highest incidence rates in the European Union in recent years⁵ and one of the highest incidences globally⁴. Common sources of *L. monocytogenes* include ready-to-eat food products such as smoked fish, processed meats, soft cheeses and pre-packed sandwiches^{1,4,6}.

In 2014, an increase in the number of human infections by *L. monocytogenes* that resulted from several foodborne outbreaks motivated a critical review of the Danish efforts to control the pathogen by the Danish Ministry of Food, Agriculture and Fisheries. The review led to the development of recommendations to improve the general control and management of *Listeria* in the food production¹. These included increased information on *Listeria* risk to food handlers preparing food to vulnerable groups, e.g. in hospitals, to risk groups, to food companies and food control, and optimized procedures for sampling and source tracing.

In 2017, 58 cases of listeriosis and 12 associated deaths were reported in Denmark, with no substantial gender differences observed and the majority of cases occurring in people over 65 years of age.

Underreporting of listeriosis infections

Because invasive listeriosis is a severe illness, we assumed that all cases occurring in the population are diagnosed and notified to the public health surveillance system. Age and gender-specific incidence of listeriosis in 2017 was collected from the National Surveillance data base (Statens Serum Institute (SSI), <http://www.ssi.dk/Smitteberedskab/Sygdomsovervaagning.aspx>). Three pregnancy-associated cases were reported to SSI.

a) The Metrix Project is funded by the National Food Authorities and aims at performing integrated analyses of the health risks and benefits of food, nutrients, and food diets, as well as ranking foodborne diseases on the basis of their health and economic burden in Denmark.

Health outcomes

Listeriosis can be divided into two types of disease: acquired listeriosis, which can be mild or invasive (complicated) and occurs in people exposed through consumption of contaminated foods; and perinatal listeriosis, which occurs in unborn babies due to exposure of the mother to *L. monocytogenes*. We have here focused on the burden of acquired infections, i.e. disregarded health outcomes of perinatal infections. To estimate the incidence of all potential health outcomes of invasive listeriosis in the population, we followed the disease model, BCode, defined by the European Center for Disease Prevention and Control. For acquired invasive (complicated) symptomatic cases, the model accounted for the probability that patients would present with meningitis and for the probability of death following infections. For perinatal cases, the disease burden for health outcomes of early- and late-onset listeriosis are combined into one category.

Incidence and DALY estimation of listeriosis

We estimated that the 58 reported cases of listeriosis and 12 deaths resulted in 196 DALYs. YLD contributed with 14 years and YLL with 186 years for this burden (Table 6.1). The estimated DALY per case was 3.

Discussion

Compared with other pathogens commonly transmitted through foods (e.g. *Campylobacter*, *Salmonella*), *L. monocytogenes* causes fewer cases in Denmark. However, the severity of its invasive form means that the burden of disease at the population level is high, as is the burden per case. We have chosen to focus our estimates on the burden of acquired listeriosis.

Because symptoms are typically severe, we have assumed that all cases of invasive *L. monocytogenes* infections are diagnosed and reported to the public health authorities. This may lead to an underestimation of the overall disease burden, either due to unreported severe cases or due to the occurrence of mild but potentially frequent cases.

Even though it has been difficult to identify the most important sources of *L. monocytogenes* and define effective control strategies in the food chain, recent developments in typing methods have allowed for improvements in surveillance of both foods and human cases, and consequently in tracking the most important sources of infection. Whole-genome sequencing (WGS) was introduced in routine typing for surveillance of listeriosis in Denmark in September 2013 and has increased the discrimination of isolates. Procedures with parallel, on-time, analysis of clinical and food isolates have been introduced the following year, which has increased the ability to find the link between human cases and food sources⁷. This had led to the identification and resolution of several outbreaks that would previously not have been identified, which again has led to a better understanding of how these infections can be prevented.

6.4 Burden of disease of norovirus infections in Denmark

Norovirus is recognized as one of the most important causes of foodborne disease worldwide^{8,9}. A large number of reported outbreaks has led to an increased awareness of the public health impact of norovirus infections in the last decades, and recent studies suggest that the burden of sporadic, endemic illnesses is even greater¹⁰. The recent reports from WHO's Foodborne Disease Burden Epidemiol-

Table 6.1. Cases, incidence and disability adjusted life years of listeriosis in Denmark, 2017

	Incidence	Incidence per 100,000	YLD ^a	YLL ^b	DALY ^c
Acute symptomatic infection					196 [194-199] ^d
Cases	58	1.03	10 [7-12]		10 [7-12]
Deaths	12	0.2		186	186
Permanent disability due to meningitis					5 [4-6]
Cases	1.1 [1.04-1.15]	0.02 [0.02-0.029]	5 [4-6]		5 [4-6]
Deaths	0				0
Total health outcomes			14 [11-17]	139 [139-141]	154 [152-156]

a) YLD: Years lived with disability

b) YLL: Years of life lost due to premature death caused by the disease

c) DALY: Disability adjusted life years

d) [xx-xx]: Confidence interval

ogy Reference Group (FERG) showed that norovirus is the leading cause of foodborne gastroenteritis (GE) incidence and mortality globally, with a particularly high relative contribution in developed countries, where other diarrheal agents play a minor role¹¹.

Even though a large proportion of norovirus illnesses are mild, leading to short-duration vomiting, diarrhea or acute GE, infection can in rare cases develop with severe symptoms, hospitalization and even death¹². Furthermore, the high incidence of disease results in a high social and economic burden due to large productivity losses at population level. Still, few studies have thus far estimated the overall burden of norovirus disease at country-level. Robust assessment of such burden requires evidence on the population incidence of illness, which is subject to a high degree of underreporting and underdiagnosis, linked to low health-care seeking rates, absence of notification requirement for individual cases, and lack of widely used, rapid and sensitive clinical assays¹⁰.

Noroviruses are highly infectious, environmentally stable, and able to utilize different transmission routes¹². Transmission occurs through the fecal-oral route, either person-to-person by ingestion of aerosolized vomit, or by exposure via contaminated environmental surfaces, food and water. Most often, foodborne transmission in outbreak-related cases occur by contamination from food handlers with gastrointestinal symptoms. However, contamination earlier in the food production chain with human waste or polluted irrigation water has been demonstrated frequently as well.

In Denmark, norovirus caused 16% of the registered foodborne-disease outbreaks in 2017, with 10 outbreaks reported implicating over 298 cases (Table 2.1). There is no reporting system for norovirus infections or for gastroenteritis outbreaks in the country, except if it is a suspected foodborne outbreak. Because reported outbreak-related cases are known to represent a small fraction of total cases, we assume that

available data are not sufficient to describe the epidemiology, public health or economic impact of the disease.

Underreporting of norovirus infections

To estimate the total incidence and mortality of norovirus infections in Denmark, we adapted a previously described approach¹³. In brief, we attributed total national diarrhea incidence and mortality envelopes as published by the Global Burden of Disease Study (available at <http://ghdx.healthdata.org/gbd-results-tool>) and the WHO (available at http://www.who.int/healthinfo/mortality_data/en/), respectively, to norovirus disease based on data extracted from a systematic review¹⁴. To account for asymptomatic carriage, we defined a norovirus attributable factor based on prevalence studies with healthy controls¹⁴. Finally, to account for norovirus illnesses and deaths that were not associated with diarrhea, but were vomiting-only, we defined a vomiting inflation factor based on prevalence data from the same study.

We accounted for the following clinical outcomes: acute severe, moderate and mild diarrhea; acute vomiting-only; and death.

Incidence and DALY estimation of norovirus infections

We estimated that 185,060 cases of norovirus occurred in Denmark in 2017, resulting in an estimated 26 deaths and 485 DALYs. The incidence per 100,000 inhabitants was highest in children under five years of age; however, due to the associated mortality in older age groups, the DALY per 100,000 was substantially higher in the older age category (Table 6.2 and Figure 6.1). The estimated DALY per case was 0.002.

Discussion

Norovirus is not notifiable in Denmark, and only outbreak-related cases are reported; thus, our estimates could not be based on surveillance data. Building our model on estimated

Table 6.2 Cases, death and disability adjusted life years (DALYs) of norovirus in Denmark, 2017

Age Group	Cases	Cases per 100,000	Deaths	DALYs	DALYs per 100,000
0-4	64,620 (54,290-75,082)	21,658	0 (0-0)	65 (50-85)	3
5-14	21,218 (17,174-25,341)	3,194	0 (0-0)	11 (9-14)	2
15-49	39,598 (32,085-47,251)	1,548	0 (0-0)	21 (17-25)	1
50-69	23,242 (18,777-27,779)	1,615	2 (2-3)	71 (58-86)	5
70+	36,382 (29,462-43,331)	5,203	24 (19-29)	317 (252-383)	45
Total	185,060 (156,506-212,627)		26 (20-32)	485 (398-573)	

total diarrhea incidence and mortality and on a systematic review of prevalence studies leads to a high degree of uncertainty. Still, the 298 to 1,178 outbreak related cases reported in 2015-2017 represent less than 1% of the total estimates cases in 2017, demonstrating a very high level of underdiagnosis and underreporting. Moreover, our estimates are in line with other national burden of disease studies conducted in Europe and United States^{10,15}, as well as with European estimates derived in a global study¹¹.

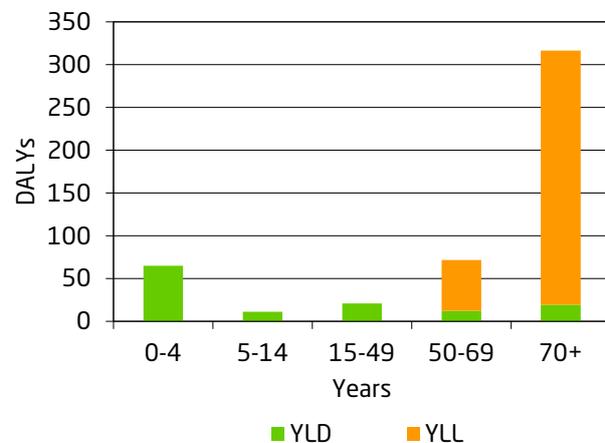
The very mild and short duration nature of disease mean that burden of disease caused by disability is low. However, we estimated some mortality in the older age group, which lead to a contribution to the burden estimates caused by years of life lost. Norovirus-associated deaths are not reported in Denmark, and these estimates are based on WHO's mortality statistics and prevalence of norovirus among inpatient data in other countries; consequently, they need to be interpreted with care. Nevertheless, these estimates may be plausible because elderly people may be more vulnerable and have more severe manifestations of acute gastrointestinal disease, including mortality.

It is important to highlight that the large proportion of the very high incidence of norovirus infections cannot be attributed to foodborne transmission. WHO have estimated that only around 26% of infections in the EU are caused by consumption of contaminated foods, the remaining burden being explained by person-to-person or environmental transmission¹⁶. While controlling norovirus in the food chain and reducing the burden of foodborne disease is imperative, other measures (non-food safety related) are also needed to limit the impact of this disease in society.

6.5 References

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Figure 6.1 Estimated disability adjusted life years (DALY), years of life lost due to disability (YLD) and years of life lost (YLL) due to norovirus infections by age group in Denmark, 2017



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7. International topics

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EU targets

Harmonised regulation on targets and surveillance in the poultry production has been laid down by the Commission. An overview is presented in Appendix Table A28.

According to Regulation (EC) No 1190/2012, the EU target for *Salmonella* in breeding and fattening turkey flocks is 1% positive for *S. Typhimurium* or *S. Enteritidis*. In Denmark, no turkey flocks were positive with *S. Typhimurium* or *S. Enteritidis* in 2017 (Appendix Table A9).

In breeding flocks of *Gallus gallus*, Regulation (EC) No 200/2010 lays down a target of maximum 1% adult flocks positive for *S. Typhimurium* including the monophasic *S. 1,4,[5],12:i:-* strains, *S. Enteritidis*, *S. Hadar*, *S. Infantis* and *S. Virchow*. In the legislation no distinction is made between breeding flocks from the table egg and broiler production lines. In Denmark, two breeding flocks were positive for target serovars in 2017; one with *S. Typhimurium* and one with *S. Enteritidis* (Appendix Table A6 and A8). Thereby 0.8% of the breeding flocks of *G. gallus* in Denmark were positive for target serovars.

Regulation (EC) No 517/2011 lays down targets for the reduction of *Salmonella* in laying flocks. The targets are Member States specific and are set either as an annual 10-40% reduction of positive adult flocks dependent on the prevalence of adult flocks in the Member State the previous year or a maximum of 2% adult flocks positive. For Denmark, the target is a maximum of 2% adult flocks positive for *S. Typhimurium* (including the monophasic *S. 1,4,[5],12:i:-* strains) and *S. Enteritidis*. The prevalence in Denmark has been below 2% since 2004. In 2017, two flocks (0.4%) were found positive with target serovars (Appendix table A6).

In broiler flocks of *G. gallus*, Regulation (EC) No 200/2012 lays down a target at a maximum of 1% flocks positive for *S. Enteritidis* and *S. Typhimurium* including the monophasic *S. 1,4,[5],12:i:-* strains. Denmark has had intensive *Salmonella* control programmes since the 90's and the target of 1% was reached in 2000. In 2017, 0.3% of broiler flocks was positive with target serovars (Appendix Table A8).

8. 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 8.1. According to the Danish legislation, 41 infectious diseases are clinically notifiable in Denmark. An overview of the notifiable and non-notifiable human and animal diseases, presented in this report, is provided in Appendix Table A29 and Table A30, respectively, including reference to the relevant legislation.

8.1 Surveillance of human disease

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

- Notifiable through the laboratory surveillance system: *Salmonella*, *Campylobacter*, *Yersinia*, Shiga toxin-producing *E. coli* (STEC) and *Listeria*.
- Individually notifiable zoonotic pathogens: *Chlamydia psittacci* (ornithosis), *Leptospira* (Weils disease), *Mycobacterium*, Bovine Spongiform Encephalopathy (BSE) prions (var. Creutzfeldt-Jakob Disease), Shiga toxin-producing *E. coli* (STEC) and Lyssavirus (rabies).
- Non-notifiable zoonotic pathogens: *Brucella*.

In Denmark, the physicians report individually notifiable zoonotic diseases to the Danish Health Authority and the Department of Infectious Disease Epidemiology and prevention at SSI. 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 Department of Bacteria, Parasites and Fungi at SSI. The laboratories must report positive results to SSI within one week. Furthermore, all *Salmonella* and STEC isolates are sent to the reference laboratory at SSI for further sero- and genotyping. The results are recorded in the Register of Enteric Pathogens maintained by SSI. 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.
- STEC O-group distribution in humans is presented in Appendix Table A3.
- The *Salmonella* serovar distribution is presented in Appendix Table A5.

8.2 Outbreaks of zoonotic gastrointestinal infections

In Denmark, local and regional foodborne outbreaks are typically investigated by the Food Inspection Unit in collaboration with the Public Health Medical Officers at the Danish Patient Safety Authority, and the regional clinical microbiology laboratories. Larger regional and national outbreaks are investigated by SSI, the National Food Institute,

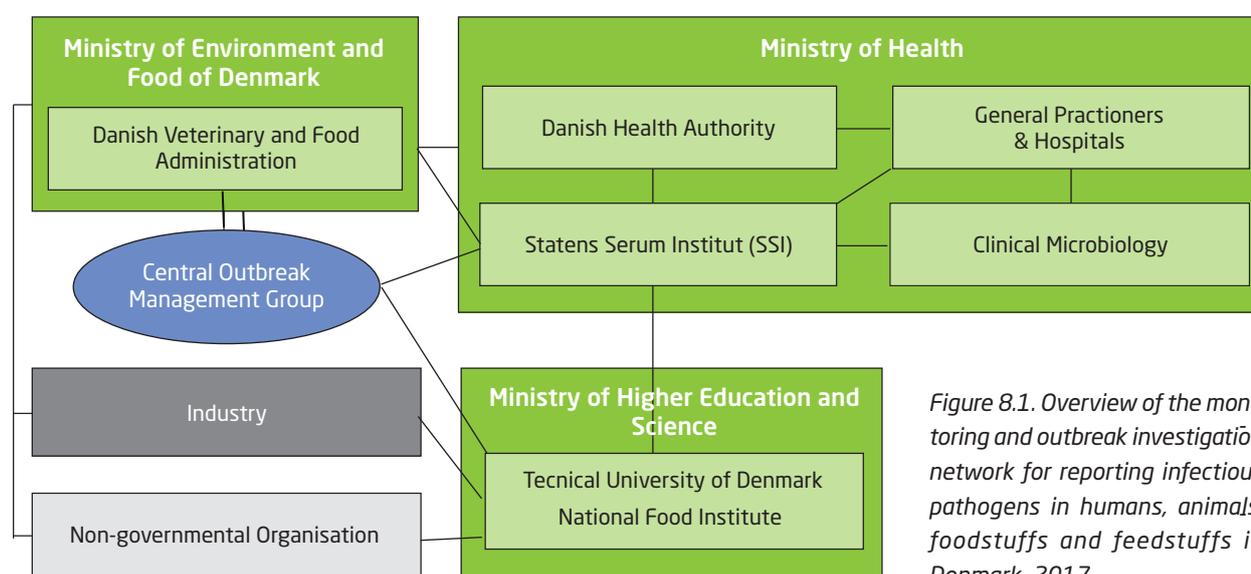


Figure 8.1. Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feedstuffs in Denmark, 2017

Technical University of Denmark (DTU Food) and the Danish Veterinary and Food Administration (DVFA) in collaboration. These institutions may also aid in the investigation of local outbreaks. Representatives from these institutions meet regularly in the Central Outbreak Management Group to discuss surveillance results, compare the reported occurrence of zoonotic agents in animals, food and feedstuffs with that in humans, and coordinate the investigation of outbreaks. The formal responsibility of investigating food- or waterborne outbreaks is currently divided between two ministries based on the outbreak source: the Ministry of Health for infectious diseases; the Ministry of Environment and Food for foodborne and animal related diseases, and for waterborne diseases. The latter are investigated in collaboration with the municipalities.

Outbreaks may be detected in various ways. Clusters of cases may be noted in the local clinical laboratory or identified at SSI through the laboratory surveillance system of gastrointestinal bacterial infections through subtyping of bacterial isolates from patients. Food handlers are obliged to contact the DVFA if the food they served are suspected to have caused illness. 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 Inspection Unit. General practitioners and hospitals are obliged to report all suspected water- and foodborne infections to the Danish Patient Safety Authority and to Statens Serum Institut.

A list of verified outbreaks (not including household outbreaks) reported to the Food- and waterborne Outbreak Database (FUD) are presented in Appendix Table A4 and some of the outbreaks from 2017 are outlined in Chapter 2.

8.3 Surveillance and control of animals and animal products

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 A28.

Salmonella surveillance and control programmes for poultry, pigs and cattle are presented in Appendix Tables A31-A36. Sample analysis is performed at the DVFA laboratory for all isolates except poultry. *Salmonella* isolates are forwarded to the DTU Food for serotyping, some isolates are also subtyped by WGS as well as tested for antimicrobial resistance. An overview of the methods used for subtyping is presented in Appendix Table A37.

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

- Results from the table egg production are presented in Appendix Tables A6-A7.
- Results from the broiler production are presented in Appendix Tables A5, A8 and A15.
- Results from the duck and turkey productions are presented in Appendix Tables A5 and A9.
- Results from the pig production are presented in Appendix Tables A5, A12, A15 and Figures A1-A3.
- Results from the cattle production are presented in Appendix Tables A5, A13-A14 and Figure A4.
- Results from the feed production are presented in Appendix Tables A16-A17.
- Results from the rendering plants are presented in Appendix Table A18.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Tables 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 A10-A11 and A15.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Tables A23-A24.

Pig and cattle carcasses are screened for *Mycobacterium* and *Echinococcus* during meat inspection at the slaughterhouse. Although swine kept under controlled housing conditions in Denmark are exempted from examination for *Trichinella* at slaughter, all slaughter pigs, sows and boars are still examined at slaughter. Free range pigs, horses, wild game (e.g. wild boar) and other species susceptible to *Trichinella* must still be tested. 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 DVFA. Results are presented in Appendix Table A12-A13.

a) The Danish Veterinary and Food Administration (DVFA) is one authority that 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 Inspection Unit followed by the location synonymous with the location in question.

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-A26.

Results from the monitoring of *Coxiella burnetii* (Q fever) in cattle are presented in Appendix Table A13.

Results based on suspicion of diseases with *Chlamydia psittacci*, *Cryptosporidium*, *Trichinella*, classical rabies and European Bat *Lyssavirus* in zoo animals, pets and wild life are presented in Appendix Table A23-A24.

8.4 Official testing of zoonotic pathogens in foodstuffs

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

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

Appendix Table A27 provides information on the centrally coordinated studies conducted in 2017.

For further information, consult the website of the DVFA, www.fvst.dk

What is a microbiological criteria?

Microbiological criteria give guidance on the acceptability of foodstuffs and their manufacturing processes. Preventative actions, such as the application of Good Hygiene and Manufacturing Practices (GHP, GMP) and the Hazard Analysis Critical Control Point (HACCP) principles contribute to achieving food safety. Microbiological testing alone cannot guarantee the safety of a foodstuff tested, but these criteria provide objectives and reference points to assist food businesses and competent authorities in their activities to manage and monitor the safety of foodstuffs respectively.¹

A microbiological criterion (MC) is defined in EU Regulation 2073/2005 that lays down food safety criteria for relevant foodborne bacteria, their toxins and metabolites, such as *Salmonella*, *Listeria monocytogenes*, *Enterobacter sakazakii*, staphylococcal enterotoxins and histamine in specific foods. These criteria define the acceptability of a product or a batch of food applicable to products placed on the market. In addition, this Regulation lays down certain process hygiene criteria to indicate the correct functioning of the production process. The microbiological criteria have been developed in accordance with internationally recognised principles, such as those of Codex Alimentarius.¹

The criteria support the hygiene legislation and apply to food business operators (FBO). The FBO's use the criteria to validate and verify the correct functioning of their food safety management procedures based on HACCP principles and GMP.

There are two types of MC - food safety criteria and process hygiene criteria.

- The food safety criteria are used to assess food safety of a product or process. The microorganism in question is mainly pathogens, e.g. *Salmonella* or *Listeria*. If the food safety criteria are not met the food must be withdrawn /recalled from the market.
- The process hygiene criteria apply to a stage in the production and do not apply to products on the market and are an indication of the performance of the food production process. No market restrictions apply if process hygiene criteria are not met.

The Danish Veterinary and Food Administration verify that FBO's comply with the microbiological criteria defined in the legislation in context of a number of annual sampling projects. Every year approximately 2,500-3,000 samples are collected for control of compliance to criteria.

References:

1. https://ec.europa.eu/food/safety/biosafety/food_hygiene/microbiological_criteria_en

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, 2015-2017

Source	2017		2016		2015	
	Estimated no. of reported cases	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	87	8.2	64 (46-91)	6.0	35 (16-60)	3.7
Domestic beef	16	1.5	17 (9-27)	1.6	13 (3-23)	1.4
Domestic table eggs	16	1.5	22 (1-55)	2.0	0 ^d	0
Domestic broilers	0 ^b	-	9 (1-21)	0.9	0 ^d	0
Domestic ducks	7	0.7	5 (0.2-17)	0.5	No data	-
Imported pork	74	6.9	40 (12-70)	3.7	61 (35-86)	6.6
Imported beef	1	0.1	0 ^c	0	0 ^d	0
Imported broilers	37	3.5	43 (24-64)	4.0	27 (9-49)	2.9
Imported turkey	0 ^b	-	3 (0-9)	0.3	11 (1-28)	1.2
Imported duck	13	1.2	4 (0-11)	0.4	15 (5-27)	1.6
Travels	328	30.7	573 (566-579)	53.3	522 (516-528)	56.5
Unknown source	283	31.3	233 (196-267)	21.7	220 (185-254)	23.8
Outbreaks, unknown source	154	14.4	61	5.7	21	2.3
Total	1,067		1,074		925	

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

b) No samples from domestic broilers and imported turkey were found positive for *Salmonella* in 2017

c) No samples from imported beef were found positive for *Salmonella* in 2016

d) No samples from domestic table egg layers, domestic broiler meat and imported beef were found positive for *Salmonella* in 2015

Source: National Food Institute, Technical University of Denmark

Human disease and outbreak data

Table A2. Zoonoses in humans, number of laboratory-confirmed cases, 2012-2017

Zoonotic pathogen	Incidence	Reported no. of cases					
	per 100,000 inhabitants	2017	2016	2015	2014	2013	2012
Bacteria							
<i>Brucella abortus/melitensis</i> ^{a,b}	-	3	3	6	4	4	2
<i>Campylobacter coli/jejuni</i> ^f	73.9	4,257	4,677	4,348	3,782	3,766	3,728
<i>Chlamydia psittaci</i> ^f	0.2	14	24	25	16	12	12
<i>Leptospira</i> spp. ^c	0.4	22	10	5	10	3	7
<i>Listeria monocytogenes</i> ^c	1.0	58	39	43	92	50	50
<i>Mycobacterium bovis</i> ^c	0.03	2	2	1	1	0	0
<i>Salmonella</i> total ^c	18.5	1,067	1,074	925	1,122	1,136	1,198
<i>S. Enteritidis</i> ^c	3.9	226	246	258	268	346	242
<i>S. Typhimurium</i> ^{c,d}	5.0	290	320	233	427	337	415
Other serotypes ^c	9.6	551	508	434	427	453	541
STEC total ^c	6.0	346	269	228	248 ^e	186	190
O157	0.9	50	37	33	37	23	36
Other O-groups or non-typeable	3.7	215	204	195	192	163	154
<i>Yersinia enterocolitica</i> ^f	6.1	354	573	539	432	345	291
Viruses							
<i>Lyssavirus</i> ^c	0,0	0	0	0	0	0	0

a) Not notifiable, hence the incidence cannot be calculated.

b) 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.

c) Notifiable.

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

Source: Statens Serum Institut

Table A3. STEC O-group distribution in humans^a, 2017

O-group	Number of episodes	O-group	Number of episodes
157	50	128	7
103	31	113	7
146	21	187	6
26	21	111	5
63	17	2	5
145	11	Notification ^b	81
91	11	Other O-groups or not-typed	64
27	8	Isolate not available but presence of vtx genes confirmed by PCR	1
Continued in the next column		Total	346

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=63), 2017

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
<i>Bacillus cereus</i>	26		Restaurant	Composite meal	1566
<i>Bacillus cereus</i>	28	4	Hotel	Composite meal	1579
<i>Bacillus cereus</i>	20		Regional	Composite meal	1609
<i>Bacillus cereus</i>	21		Catering	Composite meal	1654
<i>Campylobacter jejuni</i>	6	3	Farm	Raw milk	1642
<i>Campylobacter jejuni</i> , ST19 and ST21 ^a	66	18	Farm	Raw milk	1586
<i>Clostridium perfringens</i>	86		Restaurant	Composite meal	1554
<i>Clostridium perfringens</i>	53		Canteen	Composite meal	1568
<i>Clostridium perfringens</i>	6		Restaurant	Composite meal	1569
<i>Clostridium perfringens</i>	50		Canteen	Buffet meal	1595
<i>Clostridium perfringens</i>	53		Restaurant	Composite meal	1607
<i>Clostridium perfringens</i>	55		Catering	Composite meal	1617
<i>Clostridium perfringens</i>	20		Restaurant	Composite meal	1635
<i>Clostridium perfringens</i>	13		Restaurant	Composite meal	1656
Histamine	2	2	Restaurant	Fish (imp)	1653
<i>L. monocytogenes</i> , ST1 ^b	1	1	National	Unknown	1592
<i>L. monocytogenes</i> , ST1247	6	6	National	Unknown	1559
<i>L. monocytogenes</i> , ST55 ^c	2	2	National	Unknown	1632
<i>L. monocytogenes</i> , ST6 ^c	2	2	International	Sweet corn (imp)	1618
<i>L. monocytogenes</i> , ST8 ^d	6	6	National	Smoked salmon (imp)	1597
Norovirus	20	7	Restaurant	Buffet meal	1570
Norovirus	12		Restaurant	Composite meal	1576
Norovirus	50		Private party	Buffet meal	1611
Norovirus	24	1	Restaurant	Composite meal	1612
Norovirus	42	1	Restaurant	Buffet meal	1616
Norovirus	10		Restaurant	Oysters (DK)	1623
Norovirus	16		Restaurant	Open sandwiches	1627
Norovirus	78	6	Canteen	Buffet meal	1637
Norovirus	29	1	Canteen	Buffet meal	1638
Norovirus	17	5	Canteen	Buffet meal	1663
<i>Salmonella Agona</i> , ST13	10	10	National	Unknown	1589
<i>Salmonella Bovismorbificans</i> , ST1499 ^b	7	7	National	Unknown	1593
<i>Salmonella Enteritidis</i> , ST11	7	7	National	Unknown	1581
<i>Salmonella Enteritidis</i> , ST11	6	6	National	Unknown	1582
<i>Salmonella Enteritidis</i> , ST11	7	3	Private party	Composite meal	1585
<i>Salmonella</i> O:4,5,12;H:i:-, ST 34	10	7	Catering	Composite meal	1601
<i>Salmonella</i> O:4,5,12;H:i:-, ST 34 ^c	8	8	National	Unknown	1645
<i>Salmonella</i> O:4,[5],12:i:-, ST34	5	5	National	Unknown	1596
<i>Salmonella</i> O:4,12;H:i:-	13	13	National	Pork meat	1577
<i>Salmonella</i> O:4,5,12;H:i:-, MLVA0617 ^b	16	16	National	Composite meal	1558
<i>Salmonella</i> O:4,5,12;H:i:-, MLVA0617, ST34	4	4	Regional	Unknown	1624

Continued on the next page

Table A4. Food- and waterborne disease outbreaks^a reported in the Food- and waterborne Outbreak Database (FUD) (n=63), 2017 (Continued from previous page)

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
<i>Salmonella Tennessee, ST3288^c</i>	8	8	Regional	Unknown	1600
<i>Salmonella Typhimurium</i>	6	4	Regional	Unknown	1580
<i>Salmonella Typhimurium, MLVA1919</i>	5	5	National	Unknown	1572
<i>Salmonella Typhimurium, ST19</i>	13	13	National	Dried pork sausage (imp)	1603
<i>Salmonella Typhimurium, ST2212</i>	3	3	International	Dried pork sausage (imp)	1615
<i>Salmonella Worthington, ST592</i>	4	4	National	Unknown	1599
<i>Sapovirus</i>	35	4	Canteen	Buffet meal	1598
<i>STEC O103:H2, ST17</i>	4	4	National	Unknown	1621
<i>STEC O131:H5</i>	15	3	Restaurant	Buffet meal	1584
<i>STEC O157:H7, ST11</i>	3	3	National	Unknown	1620
<i>STEC O157:H7, ST11</i>	2	2	Regional	Unknown	1622
Unknown	4		Restaurant	Composite meal	1556
Unknown	10		Restaurant	Buffet meal	1583
<i>Yersinia enterocolitica</i>	80	4	School	Composite meal	1575
Outbreaks related to travel					
<i>Salmonella Enteritidis, ST11</i>	5	5	Travel (Germany)	Unknown	1604
<i>Salmonella Enteritidis, ST11^c</i>	6	6	Travel (Egypt)	Unknown	1628
<i>Salmonella Enteritidis, ST11^e</i>	7	7	Travel (EU)	Eggs	1647
<i>Salmonella Enteritidis, ST11</i>	5	5	Travel (Turkey)	Unknown	1648
<i>Salmonella Enteritidis, ST11</i>	6	6	Travel (Turkey)	Unknown	1649
<i>Salmonella Enteritidis, ST11^e</i>	7	7	Travel (EU)	Eggs	1650
<i>Salmonella Kottbus</i>	4	4	Travel (Germany)	Pork meat product	1594
<i>Salmonella Stanley, ST29</i>	6	6	Travel (Thailand)	Unknown	1651
Total	1,151	254			

Note: (imp)= imported product.

a) ST=MLST Sequence Type.

b) Additional outbreak cases in 2016; FUD1558: 5 cases, FUD1592: 2 cases, FUD1593: 3 cases

c) Additional outbreak cases in 2018 at time of reporting; FUD1600: 1 case, FUD1618: 2 cases, FUD1628: 2 cases, FUD1632: 1 case, FUD1645: 1 case

d) Additional outbreak cases in 2015; FUD1597: 1 case

e) Possibly a part of multi-national outbreak caused by Polish eggs 2016-2017 see also description in Annual Report on Zoonoses in Denmark 2016, chapter 2.

Source: Food- and waterborne Outbreak Database (FUD)

Monitoring and surveillance data

Table A5. Top 15 (humans) serotype distribution (%) of *Salmonella* from humans, animals, carcasses, Danish and imported meat, 2017. N=number of culture positive units^a

Serotype	Human	Pig ^b	Pork ^c	Beef ^d	Broiler ^e	Layer ^e	Ducks ^f	Imported meat (batches)			
	cases N=1,067	animals N=44	batches N=75	batches N=8	flocks N=25	flocks N=4	flocks N=4	Pork ^g N=12	Broiler ^g N=17	Ducks ^f N=8	Beef ^f N=1
Enteritidis	21.2	0	1.3	0	8.0	50.0	0	0	5.9	25.0	0
1,4,[5],12:i:-	16.4	31.8	40.0	0	40.0	0	0	50.0	0	0	0
Typhimurium	10.8	15.9	20.0	0	0	25.0	50.0	43.8	0	37.5	0
Stanley	3.1	0	0	0	0	0	0	0	0	0	0
Dublin	2.3	0	0	100	0	0	0	0	0	0	0
Newport	2.3	0	0	0	0	0	50.0	0	0	0	0
Agona	1.9	0	0	0	0	0	0	0	0	0	0
Kentucky	1.9	0	0	0	0	0	0	0	0	0	0
Virchow	1.6	0	0	0	0	0	0	0	0	0	0
Infantis	1.5	2.3	2.7	0	8.0	0	0	0	41.2	0	0
Bovismorbificans	1.4	0	0	0	0	0	0	0	0	0	0
Oranienburg	1.3	0	0	0	0	0	0	0	0	0	0
Java	1.2	0	0	0	0	0	0	0	0	0	0
Derby	1.1	47.7	29.3	0	24.0	25.0	0	12.5	0	0	0
O:4,5,12; H:b:-	1.1	0	0	0	0	0	0	0	0	0	0
Saintpaul	1.1	0	0	0	0	0	0	0	0	0	0
Other	21.7	2.3	1.3	0	12.0	0	0	18.8	52.9	37.5	100
Unknown	8.1	0	5.3	0	8	0	0	0	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.

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 A36).

d) Sampling of beef carcasses at slaughterhouses according to the surveillance programme (Table A35).

e) Sampling of production flocks prior to slaughter according to surveillance programmes (Tables A32).

f) Centrally coordinated study (see section 7.4 and Table A27 for description).

g) Case-by-case control of imported meat. For further information regarding case-by-case control programme see Annual Report on Zoonoses in Denmark, 2007. 16 serovars in 12 samples of imported pork and 17 serovars in 15 samples of imported broiler meat.

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

Table A6. Occurrence of Salmonella in the table egg production^a, 2007-2017

	Rearing period ^b (parent flocks)		Adult period ^c (parent flocks)		Pullet-rearing flocks		Table egg layer flocks	
	N	Positive	N	Positive	N	Positive	N	Positive
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
2015	15	0	8	0	123	0	344	0
2016	15	0	10	0	132	0	426	3
2017	7	0	8	1 ^d	138	1 ^e	446	3 ^f

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

b) *Salmonella* was not detected in grandparent flocks during rearing period (1 flock).

c) *Salmonella* was not detected in grandparent flocks during adult period (5 flocks).

d) *S. Typhimurium*

e) *S. Enteritidis*

f) *S. Derby*, *S. Typhimurium*, *S. Enteritidis*

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

Table A7. Occurrence of Salmonella in the table egg layer flocks sorted by type of production, 2007-2017

	Deep litter		Free range		Organic		Battery	
	N	Positive	N	Positive	N	Positive	N	Positive
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	95	1
2015	108	0	29	0	172	0	86	0
2016	125	1	31	0	196	1	74	1
2017	126	0	42	1 ^a	217	2 ^b	61	0

a) *S. Derby* (1).

b) *S. Enteritidis* (1), *S. Typhimurium* (1)

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

Table A8. Occurrence of *Salmonella* in the broiler production^a, 2007-2017

	Rearing period ^b (parent flocks)		Adult period ^c (parent flocks)		Broiler flocks		Slaughterhouse (flocks/batches)	
	N	Positive	N	Positive	N	Positive	N	Positive
2007	152	0	258	3	3,703	60	884	10
2008	146	0	293	2	3,845	43	518 ^g	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	131	3	3,470	26	277	4
2015	91	0	289	1	3,631	23	148	0
2016	184	0	182	3	3,606	21	203	1
2017	170	2 ^d	250	1 ^e	4,290	25 ^f	259	0

a) See Tables A30-A31 for description of the surveillance programmes.

b) *Salmonella* was not detected in grandparent flocks during rearing period (12 flocks).

c) *Salmonella* was not detected in grandparent flocks during adult period (10 flocks).

d) *S. Typh.* DT 193 (2)

e) *S. Enteritidis* (1)

f) *S. 4.5.12:l:-* (6), *S. 4.12:l:-* (4), *S. Give* (1), *S. Derby* (6), *S. Yoruba* (1), *S. Enteritidis* (2), *S. Infantis* (2), *S. Anatum* (1), *Salmonella. sp.* (2)

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

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

Table A9. Occurrence of *Salmonella* in turkey flocks, 2007-2017

	Turkey flocks ^a	
	N	Positive
2007	13	0
2008	10	1
2009	15	0
2010	24	1
2011	38	1
2012	23	0
2013	56	3
2014	10	0
2015	80	1
2016	76	0
2017	24	1

a) See Table A34 for description of the surveillance programme for turkey flocks. The major turkey slaughterhouse in Denmark closed down in 2004. Therefore, most commercially reared turkey flocks are transported abroad for slaughter.

Source: Danish Veterinary and Food Administration

Table A10. Occurrence of *Campylobacter* in broiler flocks, 2007-2017^a

	Cloacal swabs at slaughter		Sock samples at farm	
	N (Flocks)	% pos	N (Flocks)	% pos
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	-	-
2015	3,274	19.6	-	-
2016	3,184	20.8	-	-
2017	3,316	16.6	-	-

a) See Table A31 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 and National Veterinary Institute (until 2009)

Table A11. Occurrence of *Campylobacter* in non-heat treated chilled broiler meat samples at slaughter and retail^a, 2012-2017

		At slaughter		At retail			
		Denmark		Denmark		Import	
		N (samples)	% pos	N (samples)	% pos ^b	N (samples)	% pos ^b
2012	Conventional	1,044 ^d	21.5	-	-	-	-
	Organic/free-range	-	-	-	-	-	-
	In total	-	-	521	9.7	154	28.2
2013	Conventional	870 ^e	28.2	849	12.1	170	12.8
	Organic-free-range	93 ^e	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	-	-	-	-
2015	Conventional	960	20.1	-	-	-	-
	Organic/free-range	115	78.2	-	-	-	-
2016	Conventional	999	21.3	1,339	12.8	232	37.9
	Organic/free-range	117	87.2	93	71.0	245	78.8
	In total	-	-	1,432	17.4	477	57.5
2017	Conventional ^c	1,258	25.0	-	-	-	-
	Organic/free-range ^c	203	79.0	-	-	-	-

a) Centrally coordinated studies (see Table A27 and section 7.4 for description). Limit of quantification: 10 cfu/g.

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

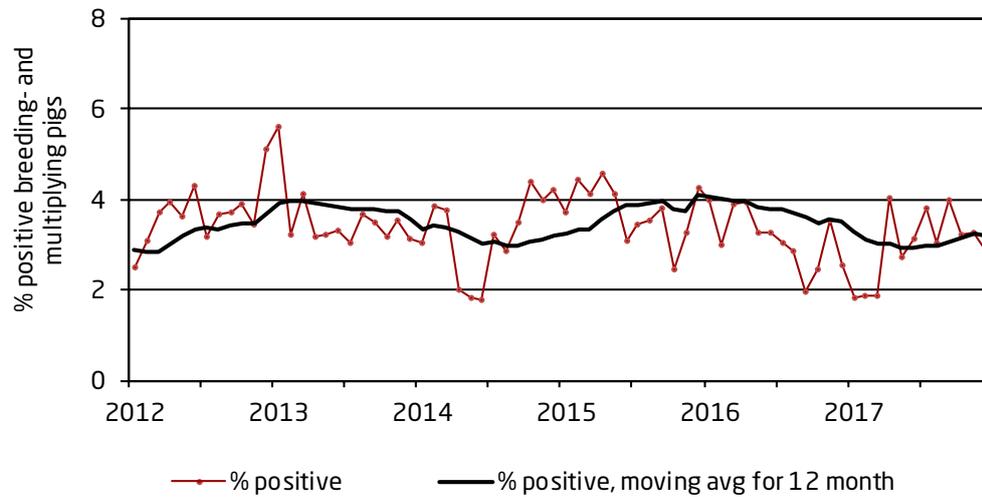
c) In 2017, data from additional slaughterhouses (one conventional and one organic) were included, which influenced the overall percentage of positive samples. Using data from the slaughterhouses included in previous year, the number of samples and % positive were: conventional N=1005, 15% positive; organic N=98, 86% positive.

d) Included are 238 leg-skin samples, prevalence = 24.4%.

e) Leg-skin samples only.

Source: National Food Institute and Danish Veterinary and Food Administration

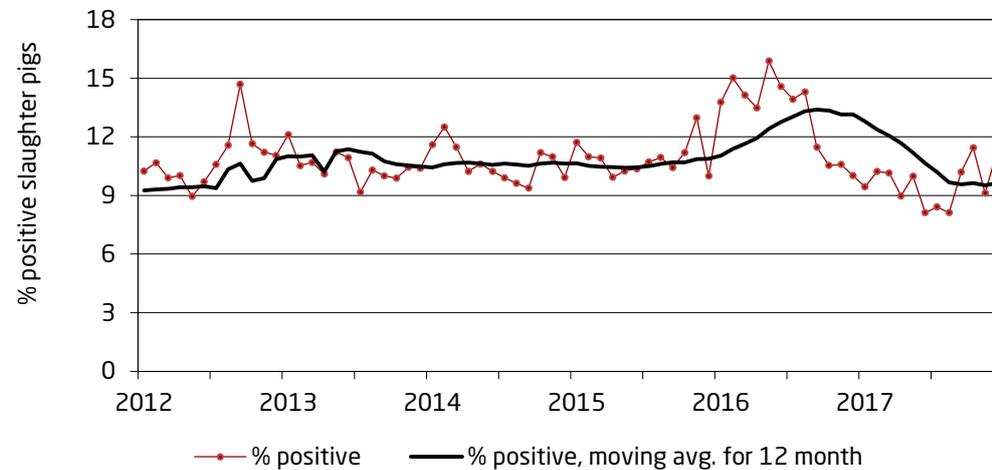
Figure A1. Serological surveillance of Salmonella in breeding and multiplying pigs^a based on monthly testing of blood samples, 2011-2017



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

Source: Danish Agriculture and Food Council

Figure A2. Serological surveillance of Salmonella in slaughter pigs^a, 2011-2017. Percentage of seropositive meat juice samples (first sample per herd per month)^b



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

b) The peak in 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 A12. Occurrence of zoonotic pathogens in pigs and pork in Denmark, 2017

Zoonotic pathogen	Herds		Animals/Samples		
	N	Pos	N	Pos	% pos
At farm					
<i>Brucella abortus</i> ^a	-	-	33,423	0	-
<i>Leptospira spp.</i> ^b based on suspicion	43	1	-	-	-
<i>Leptospira spp.</i> ^c	113	75	-	-	-
At slaughterhouse (slaughter pigs)					
<i>Salmonella spp.</i> ^{d,e}	6,097	268 ⁱ	-	-	-
<i>Salmonella spp.</i> ^d (slaughtering >30.000 pigs/year)	-	-	18,174 ^j	-	0.7 ^l
<i>Salmonella spp.</i> ^d (slaughtering 1.000 or more and less than 30.000 pigs/year)	-	-	251 ^k	-	0
<i>Salmonella spp.</i> ^{d,f}	-	-	295	44	14.9
<i>Trichinella spp.</i> ^g	-	-	16,858,136	0	-
<i>Mycobacterium bovis</i> ^h	-	-	16,987,437	0	-
<i>Echinococcus granulosus/multilocularis</i> ^g	-	-	16,987,437	0	-

a) 5-8 ml blood samples were analysed using either the SAT, RBT or ELISA methods.

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

c) Serological analyses were performed for different serotypes (*Leptospira bataviae*, *L. bratislava*, *L. grippityphosa*, *L. hardjo*, *L. tarassovi*, *L. icterohaemorrhagiae*, *L. pomona* and *L. sejroe*) depending on the purpose of sampling. Antibodies were detected against *L. bratislava* in 72 herds, against *L. bratislava* and *L. icterohaemorrhagiae* in 2 herds, and against *L. grippityphosa* in 1 herd.

d) See Table A36 for description of the *Salmonella* surveillance programme.

e) Data are from December 2017. Slaughter pig herds monitored using serological testing of meat juice samples collected at slaughter.

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

g) Samples collected from slaughter pigs at slaughter were examined using the method described in Regulation (EU) 2015/1375. In 2014, an amendment to EU regulation (EC) No 2075/2005 came into force stating that slaughter pigs, sows and boars kept under "controlled housing conditions" in Denmark are exempted testing for *Trichinella*. Free range pigs must be tested for *Trichinella*.

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

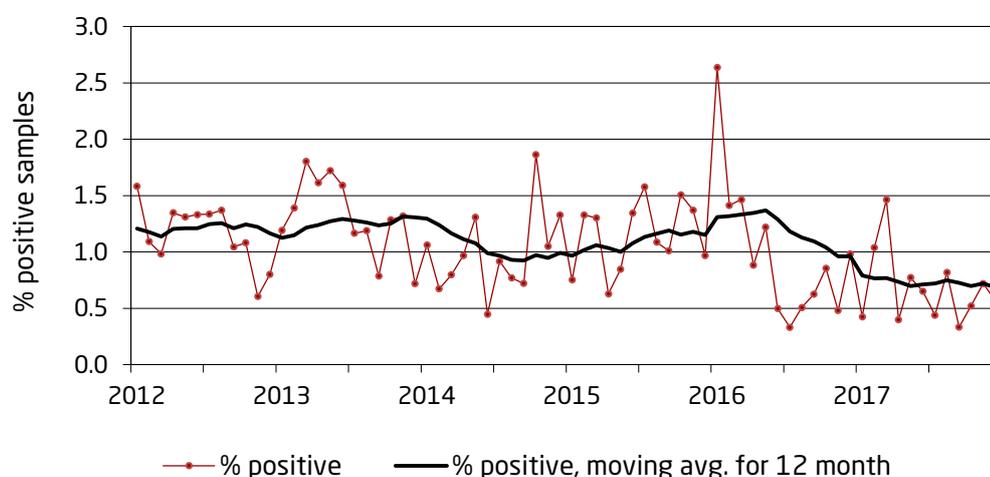
i) Includes herds belonging to *Salmonella* level 2 and 3 only (See Table A36).

j) Samples from five animals were pooled. In 2017, 4 single samples were included.

k) Samples were analysed individually. In 2017 one pooled sample was included.

l) 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.

Source: Danish Veterinary and Food Administration, National Veterinary Institute and National Food Institute, Technical University of Denmark



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

Source: Danish Veterinary and Food Administration

Table A13. Occurrence of zoonotic pathogens in cattle and beef in Denmark, 2017

Zoonotic pathogen	Animals/Samples		
	N	Pos	% pos
At farm			
<i>Brucella abortus</i> ^a	1,007	0	-
<i>Mycobacterium bovis</i> ^{b, c}	847	0	-
<i>Coxiella burnetii</i>	138 ^f	6	-
At slaughterhouse			
<i>Salmonella</i> spp. ^d (slaughtering \geq 7.500 cattle/year)	6,940 ^g	-	0.2 ⁱ
<i>Salmonella</i> spp. ^d (slaughtering 250 or more and 7.500 or less cattle/year)	291 ^h	-	0
<i>Mycobacterium bovis</i> ^{b, e}	475,700	0	-
<i>Echinococcus granulosus/multilocularis</i> ^e	475,700	0	-

a) Denmark has been declared officially brucellosis free since 1979. The last outbreak was recorded in 1962. 5-8 ml blood samples were analysed using either the SAT or CFT methods. In addition 55 aborted fetuses were tested, none were positive

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) See Table A35 for description of the surveillance programme.

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

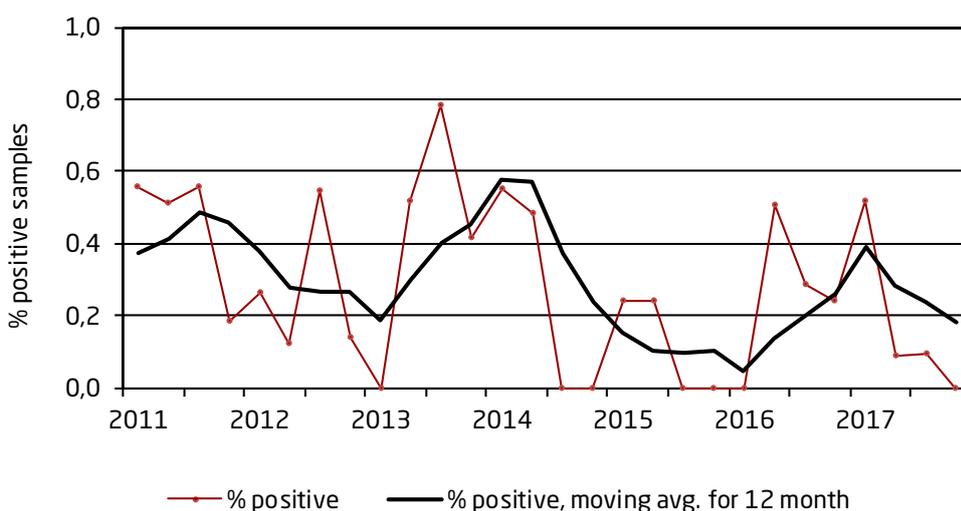
f) Samples analysed using an ELISA method. Animals were tested by blood samples. In 2017, 9 herds were tested, 7 were positive (milk samples).

g) Samples from five animals were pooled. In 2017, 80 single samples were included

h) Samples were analysed individually. In 2017, 13 pooled sample were included.

i) 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.

Source: Danish Veterinary and Food Administration, National Veterinary Institute, and National Food Institute, Technical University of Denmark

Figure A4. Salmonella in beef, monitored at slaughterhouses^a, 2011-2017

a) For more information about the surveillance programme, see Table A35.

Source: Danish Veterinary and Food Administration

Table A14 Cattle herds in the Salmonella Dublin surveillance programme^a, December 2017

Salmonella Dublin level		Non-milk producing herds		Milk producing herds	
		N	%	N	%
Level 1	On the basis of milk samples			2,755	92.5
	On the basis of blood samples	13,404	97.8		
	Total	13,404	97.8	2,755	92.5
Level 2	Titer high in blood- or milk samples	131	1.0	181	6.1
	Contact with herds in level 2 or 3	127	0.9	20	0.7
	Other causes	38	0.3	11	0.4
Level 3	Salmonellosis, official supervision	11	0.1	10	0.3
	Total	307	2.2	222	7.5
Total number of herds		13,711		2,977	

a) See Table A35 for description of the surveillance programme.

Source: SEGES

Table A15 Results from the intensified control of Salmonella and Campylobacter in fresh meat based on case-by-case risk assessments, 2017

		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 positive batches
<i>Campylobacter</i>						
Danish	Broiler	121	17	1	-	32 ^b
Imported	Broiler	152	76	10	-	33 ^b
<i>Salmonella</i>						
Danish	Pork	151	18	1	-	-
	Broiler	96	0	-	-	17
Imported	Pork	153	12	1		51 ^{c,d}
	Broiler	126	15	-	2	-
	Turkey	25	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 market (Order no. 77 of 20/01/2017).

b) The *Campylobacter* prevalence in each batch of broiler meat is based on the proportion of positive samples (12 samples per batch). Include all positive batches.

c) Only batches subjected to risk assessment (n) have been included.

d) The *Salmonella* prevalence in each batch of pork is based on the proportion of positive pooled samples (1-4 subsamples per pool, 10 pools per batch). Includes all positive batches sent to risk assessment.

e) The *Salmonella* prevalence in each batch of broiler meat and turkey meat is based on the proportion of positive samples (5 samples per batch). Include all positive batches.

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

Table A16 Feed business operators own sampling of *Salmonella* in compound feeds, feed processing and feed material (batch-based data), 2015-2017

	2017		2016		2015	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control):						
Ordinary inspections - clean zone	7,263	7 ^c	7,062	9	7,307	6
Ordinary inspections - unclean zone	1,130	26 ^d	10,009	30	602	29
Compound feed, farm animals	657	0	700	0	1,148	1
Feed materials, farm animals ^a	1,445	22 ^e	1,386	13	1,416	17
Transport vehicles, clean zone/hygiene samples ^b	1,216	0	1,166	1	1,190	5
Transport vehicles, unclean zone/hygiene samples ^b	123	4 ^f	144	4	63	10

Note: Data are from one feed and grain trade organisation only, representing a proportion of feed at the Danish market.

a) Predominantly products of soy (e.g. soybean meal) but also products of rape (e.g. rapeseed cake) and sunflower (e.g. sunflower meal).

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

c) *S. Idikan*, *S. Goettingen*, *S. Falkensee*

d) *S. Putten*, *S. Rissen*, *S. Idikan*, *S. Enterica*, *S. Kedougou*, *S. Falkensee*, *S. Anatum*, *S. Tsevie*

e) *S. Enterica* subspecies *enterica* (I), *S. Mbandaka*, *S. Panama*, *S. Rissen*, *S. Senftenberg*, *S. Havana*, *S. Yoruba*, *S. Soerenga*, *S. Agona*, *S. Infantis*, *S. Muenster*

f) *S. Putten*

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

Table A17. Control of *Salmonella* in feed processing and feed material (batch-based data), 2015-2017

	2017		2016		2015	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control) ^a :						
Ordinary inspections ^b	277	8 ^d	278	7	319	17
Feed materials, farm animals ^c	62	3 ^e	64	1	71	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 unclean zone.

c) Predominantly soybean meal and rapeseed cake.

d) *S. Derby*, *S. Infantis*, *S. Falkensee*, *S. Senftenberg*, *S. Havana* and *S. Meleagridis*.

e) *S. Infantis*, *S. Agona* and *S. Idikan*. 2 samples of rapeseed cake and 1 of fish meal.

Source: Danish Veterinary and Food Administration

Table A18. *Salmonella* in three categories of meat and bone meal by-products not intended for human consumption^a, 2017

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	420	14	49	1
2: By-product of this material may be used for feed for fur animals	95	0	201	2
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	2,862	32	1,447	40
Total	3,377	46	1,697	43

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 A19. Pathogens in batches^a of ready-to-eat vegetables^b, 2017

Type of sample	<i>Salmonella</i>		<i>Campylobacter</i>		<i>E. coli</i>	
	N	Pos	N	Pos	N	>100 cfu/g ^c
Vegetables						
Iceberg	10	0	9	0	9	0
Romaine	13	0	11	0	11	0
Lolo Bionda	4	0	2	0	2	0
Rucola	6	0	6	0	6	0
Spinach	3	0	3	0	3	0
Various salads	15	0	4	0	4	0
Sprouts	3	0	3	0	3	0
Micro greens ^d	10	0	3	0	3	0
Herbs						
Basil	4	0	4	0	4	0
Chives	1	0	1	0	1	0
Coriander	4	0	4	0	4	0
Dill	3	0	3	0	3	0
Oregano	1	0	1	0	1	0
Parsley	9	0	9	0	9	0
Spearmint	6	0	6	0	6	0
Thyme	2	0	2	0	2	0

a) Five samples per batch.

b) Centrally coordinated study (See section 7.4 for description) to control and investigate *Salmonella*, *Campylobacter* and *E. coli* in Danish and imported ready-to-eat vegetables, sprouts and herbs.

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

d) Eg. Pea "shoot", Beetrot "shoot"

Source: Danish Veterinary and Food Administration

Table A20. *Listeria monocytogenes* in Danish produced ready-to-eat (RTE) foods^a, 2017

Food category	Sampling place	Samples analysed by a qualitative method ^b		Samples analysed by a quantitative method	
		Single samples	Single samples	Single samples	Single samples
		N	Positive	N	Positive
Egg and egg products	At processing	10	0	20	0
Cheese, RTE	At processing	85	0	90	0
Milk and dairy products excluding cheeses), RTE	At processing	85	0	100	0
Products made from broiler meat, RTE	At processing	5	0	10	0
Products made from turkey meat, RTE	At processing	5	0	-	-
Products made from pork, RTE	At processing	75	1	80	0
Products made from beef, RTE	At processing	15	0	35	0
Meatproducts, unspecified, RTE	At processing	50	0	35	0
Fruit, RTE	At processing	5	0	5	0
Vegetables, RTE	At processing	55	1	40	0
Fish and Fishery products, RTE	At processing	275	15	15	1
Other RTE products	At processing	170	3	345	1
Babyfood and food for special medical purpose, RTE	At processing	10	0	-	0
Fruit juice	At processing	25	0	5	0
Dried fruits	At processing	-	-	15	0
Total		870	20	795	2

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.

Source: Danish Veterinary and Food Administration

Table A21. Histamine in Danish and imported fish products^a, 2017

Food category	Danish		Imported ^b	
	N	Positive	N	Positive
Canned herring	9	0	0	0
Canned herring	18	0	0	0
Canned tuna	27	0	504	1
Fresh fish, unspecified	9	0	0	0
Fresh garfish	9	0	0	0
Fresh herring	36	0	0	0
Fresh mackerel	90	0	0	0
Fresh salmon	9	0	0	0
Fresh tuna	9	0	0	0
Total	216	0	504	1

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

b) Samples from Colombia, Ecuador, El Salvador, Ghana, Mauritius, Papua New Guinea, Philippines, Seychelles, Thailand and Vietnam. The positive sample was imported from Vietnam

Source: Danish Veterinary and Food Administration

Table A22. Salmonella in Danish produced food items^a, 2017

Food category	N	positive
Egg and egg products	30	0
Cheese, RTE	40	0
Products made from broiler meat, RTE	5	0
Products made from pork, RTE	100	0
Products made from beef, RTE	30	0
Fruit, RTE	10	0
Vegetables	95	0
Babyfood and food for special medical purpose, RTE	30	0
Fruit juice	30	0
Products made from pork, intended to be cooked	225	9 ^b
Products made from beef, intended to be cooked	220	1 ^c
Products made from sheep, intended to be cooked	5	0
Meatproducts, unspecified, intended to be cooked	40	0
Total	860	10

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

b) S. 4, [5], 12:i:-

c) S. Montevideo

Source: Danish Veterinary and Food Administration

Table A23. Occurrence of zoonotic pathogens in pets and zoo animals in Denmark^a, 2017

Zoonotic pathogen	Pet animals						Zoo animals			
	Dogs		Cats		Others		Mammals & reptiles		Birds	
	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
<i>Salmonella</i> spp.	0	-	0	-	3 ^b	0	0	-	0	-
<i>Chlamydia psittaci</i>	0	-	0	-	18 ^c	3 ^c	0	-	1 ^f	0
<i>Cryptosporidium</i> spp.	3	0	0	-	0	-	3 ^e	0	0	-
<i>Lyssavirus</i> (classical)	1	0	4	0	2 ^d	-	0	-	0	-
European Bat <i>Lyssavirus</i>	1	0	4	0	2 ^d	0	0	-	0	-

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

b) Pigeon (3)

c) Pooled samples of birds

d) Sheep (1), cow (1)

e) Elephant (1), Southern pig-tailed macaque (1), Ring-tailed lemur (1)

f) Parrot (1)

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, 2017

Zoonotic pathogen	Farmed wildlife						Wildlife			
	Wild boar		Mink and chin-chillas		Birds		Mammals		Birds	
	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
<i>Salmonella</i> spp.	1 ^c	0	-	-	0	-	8 ^e	2 ^e	1 ^j	0
<i>Chlamydia psittaci</i>			-	-	2 ^d	0	0	-	1 ^j	0
<i>Cryptosporidium</i> spp.			-	-	0	-	164 ^f	25 ⁱ	0	-
<i>Echinococcus multilocularis</i>	-	-	-	-	-	-	1 ^g	1 ^g	-	-
<i>Trichinella</i> spp ^b	437	0	34	0	-	-	47	0	0	-
<i>Lyssavirus</i> (classical)	0	-	0	-	0	-	13 ^h	0	0	-
European Bat <i>Lyssavirus</i>	0	-	0	-	0	-	13 ^h	0	0	-

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

b) In 2014, an amendment of EU regulation (EC) No 2075/2005 came into force stating that slaughter pigs, sows and boars kept under "controlled housing conditions" in Denmark are exempted testing for *Trichinella*. Free range pigs, horses and wild game and other species susceptible to *Trichinella* must be tested

c) Wild boar

d) Ducks

e) Badger

f) Fallow deer (14), raccoon dog (18), red deer (14), western roe deer (118)

g) Fox

h) Bats (12), fox (1)

i) Western roe deer (19), raccoon dog (5), fallow deer (1)

j) Great comorant

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, 2017

Type of surveillance	N ^b	Positive
Active surveillance		
Healthy slaughtered animals	59	0
Risk categories:		
Emergency slaughters	1,295	0
Slaughterhouse antemortem inspection revealed suspicion or signs of disease	0	0
Fallen stock	21,036	0
Animals from herds under restriction	0	0
Passive surveillance		
Animals suspected of having clinical BSE	1	0
Total	22,391	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. 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, Technical University of Denmark, and Danish Veterinary and Food Administration

Table A26. The Transmissible Spongiform Encephalopathy (TSE) surveillance programme^a for sheep and goats, 2017

Type of surveillance	N ^b	Positive
Active surveillance		
Slaughtered for human consumption	1	0
Fallen stock (>18 months)	609	0
Animals from herds under restriction	0	0
Passive surveillance		
Animals suspected of having clinical TSE	1	0
Total	611	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. 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, Technical University of Denmark, and Danish Veterinary and Food Administration

Table A27. Centrally coordinated studies conducted in 2017

Title of project	No. of planned samples	Pathogen surveyed	Further information
<i>Campylobacter</i> spp. in fresh, chilled Danish broiler meat (conventional)	1250	<i>Campylobacter</i> spp.	Appendix Table A11
<i>Campylobacter</i> spp. in fresh, chilled Danish broiler meat (organic)	200	<i>Campylobacter</i> spp.	Appendix Table A11
<i>Campylobacter</i> spp. in neck skin and thigh-skin at slaughterhouses	800	<i>Campylobacter</i> spp.	Appendix table A11
Intensified control for <i>Salmonella</i> spp. and <i>Campylobacter</i> in fresh Danish and imported meat (poultry and pig)	up to 6,590 samples (640 batches)	<i>Campylobacter</i> spp., <i>Salmonella</i>	Appendix Table A15
<i>Campylobacter</i> surveillance in several matrices for defining source of attribution	640	<i>Campylobacter</i> spp.	Available at www.food.dtu.dk
Pathogens in Danish and imported ready-to-eat vegetables	500	<i>Salmonella</i> , <i>STEC</i> , <i>E. coli</i> , <i>Listeria</i>	Appendix Table A19
<i>Salmonella</i> in pigs at slaughter including antibiotic resistance	300	<i>Salmonella</i> spp.	Appendix Table A12
Official verification of microbiological criteria	2,500	<i>Listeria monocytogenes</i> , <i>Salmonella</i> spp., <i>staphylococci</i> , <i>Escherichia coli</i> , aerobic plate count, <i>Enterobacteriaceae</i> .	Data are being processed ^{a)}
ESBL in Danish poultry production	500	ESBL, AmpC, carbapenemase-producing <i>E. coli</i>	Project continues in 2018 and will be published in 2019
Antibiotic resistance in pork meat production	630	<i>Enterobacteriaceae</i> , <i>E. coli</i> , <i>Enterococcus</i> spp., ESBL, AmpC, carbapenemase-producing <i>E. coli</i>	Project continues in 2018 and will be published in 2019
DANMAP - Antibiotic resistance in poultry	165	<i>Campylobacter</i> spp., <i>Escherichia coli</i>	Results are presented in the 2017 DANMAP report
Surveillance of antibiotic resistance in broiler, pork and beef meat at retail (DANMAP and EU surveillance)	600	ESBL, AmpC, carbapenemase-producing <i>E. coli</i>	Results are presented in the 2017 DANMAP report
Surveillance of antibiotic resistance in pig and cattle (DANMAP and EU surveillance)	600	<i>Escherichia coli</i> , <i>Campylobacter</i> spp., <i>Enterococcus faecalis</i> , <i>Salmonella</i> , ESBL, AmpC, carbapenemase-producing <i>E. coli</i>	Results are presented in the 2017 DANMAP report
Official verification of process hygiene criteria at small slaughterhouses	150	<i>E. coli</i> , <i>Salmonella</i> and aerobic count	Project continues until mid 2018
<i>Salmonella</i> spp. and antibiotic resistance in fresh, chilled and frozen Danish and imported duck meat and imported beef incl. ESBL in duck meat	300	<i>Salmonella</i> spp., ESBL	Results are presented in the 2017 DANMAP report
<i>Salmonella</i> in intratraded shell eggs	50	<i>Salmonella</i> spp.	Data are available at fvst.dk ^a
Import control - fish, fish products and bivalve molluscan shellfish	140	<i>Listeria monocytogenes</i> , <i>Salmonella</i> spp, <i>Escherichia coli</i> , <i>staphylococci</i>	Data are being processed ^a
Import control - processed food products of animal origin	50	<i>Listeria monocytogenes</i> , <i>Salmonella</i> spp., <i>Escherichia coli</i> , <i>staphylococci</i>	Data are being processed ^a

Continued on the next page

Table A27. Centrally coordinated studies conducted in 2017 (Continued from previous page)

Title of project	No. of planned samples	Pathogen surveyed	Further information
Import control - food of non animal origin	100	<i>Norovirus (frozen raspberries)</i>	Data are being processed ^a
<i>Listeria monocytogenes</i> , <i>Salmonella spp.</i> , <i>Escherichia coli</i> and staphylococci in fish and fish products from Greenland	100	<i>Listeria monocytogenes</i> , <i>Salmonella spp.</i> , <i>Escherichia coli</i> , staphylococci	Data are being processed ^a
<i>Salmonella spp.</i> and <i>Escherichia coli</i> in raw frozen scallops from Greenland	50	<i>Salmonella spp.</i> , <i>Escherichia coli</i>	Data are being processed ^a
Microbiologic classification of mussel production areas in Denmark	45	<i>Salmonella spp.</i> , <i>Escherichia coli</i>	Data are being processed ^a
<i>Salmonella</i> in animal feed	340	<i>Salmonella spp.</i>	Table A17 ^a
<i>Listeria</i> in ready-to-eat meals	100	<i>Listeria monocytogenes</i>	Data are being processed ^a
<i>Listeria</i> in meatproducts and cheese in retail	500	<i>Listeria monocytogenes</i>	Data are being processed ^a
Fish products	390	<i>Listeria monocytogenes</i>	Data are being processed ^a
Hygienic quality of eatable seed weed	100	<i>E. coli</i> and <i>Salmonella spp.</i>	Data are being processed ^a
Germcell forming bacteria ind precooked food	2,000	<i>Clostridium perfringens</i> and <i>Bacillus cereus</i>	Data are being processed ^a
Baseline <i>Norovirus</i> in oysters (continued in 2018).	75	<i>Norovirus</i> og <i>E. coli</i>	Data will be processed together with 2018 data.
Antibiotic resistance in shrimps and <i>Pangasius</i> from Asia	180	<i>E. coli</i> , <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i>	Project continues in 2018 and will be published in 2018
<i>Salmonella</i> and STEC in beef meat at cutting facilities	400	<i>Salmonella</i> , <i>E. coli</i> , STEC.	Data are being processed ^a

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

Source: Danish Veterinary and Food Administration

Table A28. Status on targets for *Campylobacter* and *Salmonella*, 2017

National Action Plans	Target	Status
<i>Campylobacter</i> in broilers 2013-2017 ^a		
Flocks at farm	20% reduction in prevalence of positive flocks in 2016 compared to 2012. The target was maintained for 2017.	A reduction of 9.4% was obtained for the period 2011-2013 ^b A reduction of 25% was obtained for the period 2014-2016 ^b
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% 2017: RR reduced by 50%	A reduction of 28% was obtained in 2014 compared to 2013 ^c A reduction of 37% was obtained in 2016 compared to 2013 ^c A reduction of 43% was obtained in 2017 compared to 2013 ^c A reduction of 38% was obtained for the period 2014-2017 ^b
<i>Salmonella</i> in poultry ^d		
Laying hen flocks of <i>Gallus gallus</i>	Initially eradication, later a reduction strategy in the table egg production	3 positive flocks (Table A6-A7) 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.	No positive batches (Table A8) Positive batches are heat treated
<i>Salmonella</i> in pigs 2014-2017		
Carcasses at slaughterhouse	Max. 1% <i>Salmonella</i> at carcass level in 2014-2017	0.7% (Table A12)
<i>Salmonella</i> Dublin in cattle 2017-2020		
Herds at farm	Eradication of <i>S. Dublin</i> in all herds, i.e. all herds in level 1 ^e . Restrictions on slaughter procedures for herds in <i>S. Dublin</i> level 2 from 2020	7,5% of milk-producing herds and 2,2% of non-milk producing herds are in level 2 or 3 (December 2016) (Table A14)
EU Regulations		
Regulation (EC) No. 1190/2012		
Breeding and fattening turkey flocks	Max. 1% positive for <i>S. Enteritidis</i> and <i>S. Typhimurium</i> ^e	No fattening flocks positive with target serovars (N=24) (Table A9)
Regulation (EC) No. 200/2010		
Breeding flocks of <i>Gallus gallus</i>	Max. 1% adult flocks positive for <i>S. Typhimurium</i> ^f , <i>S. Enteritidis</i> , <i>S. Hadar</i> , <i>S. Infantis</i> and <i>S. Virchow</i>	0,8% (2 flocks) ^h (Table A6 and A8)
Regulation (EC) No. 1168/2006		
Laying hen flocks of <i>Gallus gallus</i>	MS specific targets, for Denmark: Max. 2% adult flocks positive for <i>S. Typhimurium</i> ^f and <i>S. Enteritidis</i>	0.4% (2 flocks) (Table A6)
Regulation (EC) No. 646/2007		
Broiler flocks of <i>Gallus gallus</i>	Max. 1% positive <i>S. Typhimurium</i> ^e and <i>S. Enteritidis</i>	0.3% (13 flocks) positive with target serovars (Table A8)

a) The duration of the action plan has been prolonged to the end of 2017, to ensure that new knowledge from ongoing projects is included.

b) As a consequence of a change in sampling from AM testing 7-10 days before slaughter to cloacal swabs at the point of slaughter, 2013 and 2014 data cannot be compared. The target has therefore been changed to cover firstly the period 2011-2013 and secondly the period 2014-2016. For the two periods as a whole, the target is 20%.

c) 2013 is agreed as the baseline since 2012 data are not comparable with data from 2013 and onwards due to a necessary improvement in the data collection.

d) Supplementary to EU-regulations.

e) See Table A33 for explanation of the herd levels.

f) Including the monophasic strains *S. 1,4,[5],12:i:-*.

h) One flock positive for *S. Enteritidis*, one flock positive for *S. Typhimurium*.

Source: Danish Veterinary and Food Administration

Monitoring and surveillance programmes

Table A29. Overview of notifiable and non-notifiable human diseases presented in this report, 2017

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

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

b) The regional microbiological laboratories report confirmed cases.

c) The physician report individually notifiable infections.

d) The laboratories voluntarily report confirmed cases.

Source: Statens Serum Institut

Table A30. Overview of notifiable and non-notifiable animal diseases presented in this report, 2017

Patogen	Notifiable	EU legislation	Danish legislation
Bacteria			
<i>Brucella</i> spp.	1920 ^a		
Cattle	Obf in 1979 ^b	Decision 2003/467/EC	Order no 305 of 3/5 2000
Sheep and goats	ObmF in 1995 ^c	Decision 2003/467/EC	Order no. 739 of 21/8 2001
Pigs	No cases since 1999	Directive 2003/99/EC	Order no. 205 of 28/3 2008
<i>Campylobacter</i> spp.	no	-	-
<i>Chlamydophila psittaci</i>		-	
Birds and poultry	1920		Order no. 575 of 30/05/2017
<i>Listeria monocytogenes</i>	no	-	-
<i>Leptospira</i> spp. (only in production animals)	2003	-	Order no. 132 of 18/11 2016
<i>Mycobacterium bovis/tuberculosis</i>	1920 ^a		
Cattle	OTF in 1980 ^d	Decision 2003/467/EC	Order no. 1417 of 11/12 2007
<i>Coxiella burnetii</i>	2005	-	Order no 1332 of 18/11 2016
<i>Salmonella</i> spp.	1993 ^e	-	
Cattle			Order no. 1326 of 29/11/2017
Swine			Order no. 604 of 01/06/2017
Eggs for consumption			Order no. 1413 of 04/12 2017
Hatching eggs			Order no. 1355 of 29/11/2017
Poultry for slaughter			Order no. 77 of 20/01/2017
STEC	no	-	-
<i>Yersinia enterocolitica</i>	no	-	-
Parasites			
<i>Cryptosporidium</i> spp.	no	-	-
<i>Echinococcus multilocularis</i>	2004	Council Directive 64/433/EC	Order no. 1332 of 18/11/2016
<i>Echinococcus granulosus</i>	1993	Council Directive 64/433/EC	Order no. 1332 of 18/11/2016
<i>Trichinella</i> spp.	1920 ^a	Regulation (EU) 2015/1375	Order no. 1714 af 15/12/2015
Viruses			
<i>Lyssavirus</i> (Rabies)	1920	-	Order no. 330 of 14/04/2011
Prions			
TSE			
Sheep and goats	yes	Regulation 999/2001/EC (as amended)	Order no. 1288 of 20/12/2011
BSE			
Cattle	yes ^f	Regulation 999/2001/EC (as amended)	Order no. 1326 of 26/11/2015

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

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

c) Officially *Brucella melitensis* Free (ObmF) according to Council Directive 91/68/EC and Commission Decision 2003/467/EC. the disease has never been detected in sheep or goat.

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

e) Only clinical cases notifiable.

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

Source: Danish Veterinary and Food Administration

Table A31. Salmonella surveillance programme for the rearing flocks and adult flocks of the grandparent and parent generation of the broiler and table egg production, 2017

Time	Samples taken	Material	Material
Rearing flocks		<i>Grandparent generation</i>	<i>Parent generation</i>
Day-old ^{a,b,c}	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,b,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) or 1 faeces sample of 60 g	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 g
2 weeks prior to moving ^{a,c,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		<i>Grandparent generation</i>	<i>Parent generation</i>
Every two weeks ^{a,b,c,e} (Every 16th week) ^d	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,c}	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,c}	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	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 ^{c,d,f}	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 200/2010.

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.

f) If samples are negative, sampling is repeated 14 days later.

Source: Danish Veterinary and Food Administration

Table A32 Salmonella and Campylobacter surveillance programme for the broiler flocks, 2017

Time	Samples taken	Material
<i>Salmonella</i>		
15 - 21 days before slaughter ^{a,b,c}	Per flock	5 pairs of boot swabs. Analysed in subsamples of 2 and 3 pairs
7 - 10 days before slaughter ^{d,e}	Per flock	5 pairs of boot swabs. Analysed in subsamples of 2 and 3 pairs
After slaughter ^{b,c,f}	Per batch	From slaughterhouses slaughtering 1,000 chickens or hen pr day or more: 300 neck skin samples of 1 gram, pooled into subsamples of 60 gram. From slaughterhouses slaughtering less than 1,000 chickens or hen pr day: 15 neck skin samples of approx. 10 gram pooled into 5 subsamples of 25 gram
<i>Campylobacter</i> ^g		
After slaughter	Per flock	12 cloacal swabs from 24 animals, analysed in one pool ^h

a) Sampling requirements set out by Regulation (EC) 200/2012.

b) Samples collected by the food business operator.

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

d) Sampling requirements set out by Order no. 77 of 20/01/2017 replacing 1644 of 14/12/2016 replacing 1512 of 13/12/2013 replacing 1105 of 18/09/2013 replacing 1462 of 16/12/2009.

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

f) sampling requirements set out by Regulation (EC) 2073/2005.

g) For flocks to be slaughtered outside Denmark, 1 pair of boot swabs is collected by the owner 10 days before slaughter at the latest.

h) If the flock is slaughtered over several days, the last batch is sampled.

Source: Danish Veterinary and Food Administration

Table A33. Salmonella surveillance programme for the pullet-rearing, table egg layer and barnyard/hobby flocks in the table egg production, 2017^a

Time	Samples taken	Material
Pullet-rearing		
Day-old ^{a,b}	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,b}	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples of 60 gram
2 weeks before moving ^{a,c}	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 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,b,d}	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 g.
After positive serological findings ^c	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 <i>S. Enteritidis</i> , <i>S. Hadar</i> , <i>S. Infantis</i> , <i>S. Virchow</i> or <i>S. Typhimurium</i> including the monophasic strains <i>S. 1,4,[5],12:i:-</i> ^c	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples consisting of 60 gram each, 2 dust samples (250 ml) and 5 birds (analysed for antimicrobial substances)
Barnyard and hobby flocks^e		
Every 18 weeks ^{a,b,f}	Per flock	Egg samples (serology)

a) Sampling requirements set out by Order no 1137 af 29/08/2016 replacing 227 of 02/03/2015 replacing 517/2011 .

b) Samples collected by the food business operator.

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

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

e) Voluntary for hobby flocks.

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

Source: Danish Veterinary and Food Administration

Table A34. *Salmonella* surveillance programmes for turkey flocks, 2017

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 and Order no. 77 of 20/01/2016.

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

Source: Danish Veterinary and Food Administration

Table A35. *Salmonella* surveillance programme^a for the cattle production, 2017

No. of samples	Samples taken	Purpose/Comment
Milk producing herds		
4 samples distributed over 18 months	Bulk tank samples	Calculation of herd level ^b
Non-milk producing herds		
1 sample every 3 months at slaughter ^c	Blood samples	Calculation of herd level ^b
1 sample every 6 months in farms with only heifer herds	Blood samples	Calculation of herd level ^b
4-8 samples depending on herd size ^d	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 7.500 or more cattle per year
5 samples every second month, analyzed individually	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering 2.500 or more and less than 7.500 cattle per year
5 samples every 6th month, analyzed individually	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering 250 or more and less than 2.500 cattle per year
No sampling		Slaughterhouses slaughtering less than 250 cattle per year

a) Order no. 537 of 01/06/2016 as amended. 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),

Level 2: Herd not assumed free of infection,

Level 3: Herd infected based on culture and clinical signs or bacteriological findings in the intensified sampling.

c) No samples are taken, if the herd has been tested for *S. Dublin* within the last 3 months.

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

Source: SEGES

Table A36. *Salmonella* surveillance programme^a for the pig production, 2017

Time	Samples taken	Purpose/Comment
Breeding and multiplier herds		
Every month	10 blood samples per epidemiological unit	Calculation of <i>Salmonella</i> -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 <i>Salmonella</i> -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 <i>S. Typhimurium</i> , <i>S. Infantis</i> , <i>S. Derby</i> and <i>S. Choleraesuis</i> 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, f}
Slaughter pigs, animals		
At slaughter ^g	Coecum samples, avg. 25 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 30.000 pigs per year
5 samples every second month	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering 10.000 or more pigs and less than 30.000 pigs per year
10 samples per year, 5 each 6 month	Swab samples from 4 designated areas after 12 hours chilling (4x100cm ²)	Slaughterhouses slaughtering 1.000 or more pigs and less than 10.000 pigs per year
No sampling		Slaughterhouses slaughtering less than 1000 pigs per year

a) Sampling requirements set out by Order no. 539 of 03/06/2016, replaced by Order no. 604 of 01/06/2017.

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 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.

f) Pigs from herds with highest level of infection (Level 3) must be slaughtered under special hygienic precautions.

g) Centrally coordinated study (Table A27).

Source: Danish Veterinary and Food Administration

Table A37. Typing methods used in the surveillance of foodborne pathogens in Denmark, 2017

Methods	Human	Food	Animal
<i>Salmonella enterica</i>			
Serotyping	All isolates (mainly WGS)	All isolates (by WGS)	All isolates (by WGS)
Phage typing	None	None	Few
Antimicrobial resistance testing	All <i>Salmonella</i> except <i>S. Enteritidis</i>	Almost all isolates	Almost all isolates
MLVA	In relation to International outbreak	None	None
WGS	All isolates	All outbreaks	All isolates
<i>Campylobacter coli/jejuni</i>			
Antimicrobial resistance testing	Isolates from 4 districts for DANMAP surveillance	Isolates for DANMAP, EU surveillance and the case-by-case program	Isolates for DANMAP, EU surveillance and the case-by-case program
WGS	Outbreaks investigations, research	Research and outbreaks	Research
STEC			
Serotype	All isolates (mainly WGS)	All isolates (by PCR & WGS)	All O157 isolates
Virulence profile	All isolates (mainly WGS)	All isolates (by PCR & WGS)	All O157 isolates
PFGE	None	None	Outbreak investigations
WGS	All isolates	All isolates	None
<i>Listeria</i>			
WGS	All isolates	Selected isolates (ST typing and outbreak investigations)	None
<i>Yersinia Enterocolitica</i>			
O-group	All isolates sent to SSI	None	None

a) Including the monophasic strains *S.* 1,4,[5],12:i:-.

Source: Statens Serum Institut and the Laboratory of the Danish Veterinary and Food Administration

Population and slaughter data

Table A38. Human population, 2017

Age groups (years)	Males	Females	Total
0-4	154,359	146,439	300,798
5-14	338,262	321,510	659,772
15-24	377,218	360,334	737,552
25-44	729,947	711,750	1,441,697
45-64	764,224	761,084	1,525,308
65+	512,463	603,600	1,116,063
Total	2,876,473	2,904,717	5,781,190

Source: Statistics Denmark, 1 January 2018

Table A39. Number of herds/flocks, livestock and animals slaughtered, 2017

	Herds/flocks (capacity)	Livestock (capacity)	Number slaughtered
Slaughter pigs	6,208	5,755,654	16,987,437
Cattle	18,031	1,561,756	475,700
Broilers	624	23,434,435	102,922,600
Layers (excl. barnyard)	277	4,464,691	-
Turkeys	28	337,525	1,200
Sheep & lambs	6,434	146,705	77,385
Goats	2,900	18,365	1,855
Horses	-	-	1,361

Source: Statistics Denmark and Danish Veterinary and Food Administration, June 2018

Table A40. Number of holdings, houses/flocks and livestock capacity in the broiler production, 2017

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (grandparent)	2	10	50,000
Adult period (grandparent)	3	9	82,500
Rearing period (parent)	19	109	139,100
Adult period (parent)	42	140	745,940
Hatcheries	5	0	0
Broilers	253	624	-

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

Table A41. Number of holdings, houses/flocks and livestock capacity in the table egg production, 2017

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (grandparent)	2	2	47,500
Adult period (grandparent)	2	7	75,000
Rearing period (parent)	7	7	37,500
Adult period (parent)	7	8	44,556
Hatcheries	6	32	0
Pullet-rearing	46	37	1,073,639
Layers (excl. barnyard)	179	277	4,464,691

Source: Danish Veterinary and Food Administration, and Danish Agriculture and Food Council, June 2018

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