Annual Report on Zoonoses in Denmark 2019

DTU Food
National Food Institute

Annual Report on Zoonoses in Denmark 2019

Edited by:
Birgitte Helwigh and Channie Kahl Petersen
National Food Institute
Technical University of Denmark

Mia Torpdahl
Statens Serum Institut

This is an official publication from the National Food Institute, Technical University of Denmark, the Danish Veterinary and Food Administration, and Statens Serum Institut.

Suggested citation:
Anonymous, 2020. Annual Report on Zoonoses in Denmark 2019, National Food Institute, Technical University of Denmark.

Reprints can be ordered from:
National Food Institute
Technical University of Denmark
Kemitorvet
Building 202
DK - 2800 Kgs. Lyngby
Denmark
Phone: +45 35 88 74 95
E-mail: anga@food.dtu.dk

Layout: Birgitte Helwigh
Photos: Colourbox
Printing: www.STEPPRINTPOWER.dk

2. edition with minor corrections

ISSN: 1600-3837

The report is also available at:
www.food.dtu.dk

Contents

Introduction

Campylobacter

For the third year in a row, the number of human cases with *Campylobacter* infections continued to increase. However in 2019 the increase was larger than previously seen (18.5%), with 5,389 cases compared to 4,546 cases in 2018. This is probably a reflection of a large outbreak further described below. Previous investigations have pointed at chicken meat and cattle/beef (minced meat) as the two major sources to campylobacteriosis in humans. In 2019, Statens Serum Institut (SSI) and the Danish Veterinary and Food Administration (DVFA) initiated an investigation to obtain knowledge on the relative impact of these sources and trends over time. The DVFA initiated sampling of chicken meat and beef and SSI collected human clinical isolates from three regional laboratories to detect clusters and possible links to food samples as a continuous surveillance setup. Analysis of WGS data by cgMLST showed that almost a third of the human isolates matched chicken isolates and half of the human isolates clustered with other human isolates, i.e. half of the cases were probably infected by the same source. In total, 77 clusters were detected, most of them with less than 15 cases. One large outbreak with 88 cases in 2019 and 3 cases in 2018 was traced back to a specific slaughterhouse. A thorough investigation lead to an action plan for optimising procedures and equipment and after the initiation of these precautions, the human infection rate declined. The poultry industry has been trying to reduce the levels of *Campylobacter* for years, but this study and especially the large outbreak has led to extensive new initiatives and investments targeting *Campylobacter* throughout the production chain. In total, nine foodborne outbreaks were investigated and six of them had Danish produced chicken meat as the source. In broiler flocks, the prevalence was 22.7%, which is lower than in 2018 (24.6%) but higher than the previous three years where 16.6 to 20.8% of the flocks were positive.

In the EU, campylobacteriosis in humans has been the leading cause of bacterial infections and there is a lot of focus on this issue. In 2019, EFSA published an updated version of an opinion on control options for *Campylobacter* in broiler meat production at different stages of the food chain. The opinion provides novel estimates of the effectiveness of specific biosecurity measures to control *Campylobacter* at broiler farms, as well as updated estimates for control options aiming to reduce the concentrations in the caeca. A decision on implementation of a control option not only depends on effectivity, but also on other factors such as ease of application, cost and animal welfare impact. Denmark is at the forefront of *Campylobacter* risk science. Broiler flock prevalence is relatively low in Denmark (22.7% in 2019) compared to other European countries which suggests that the biosecurity system is already effective, although there is still room for improvement. The findings support the efforts in The National Action Plan and may contribute some of the many ongoing studies on *Campylobacter* control in Denmark.

Salmonella

Human infections with *Salmonella* remain at a level comparable to previous years with 1,120 cases in 2019, and 1,168 and 1,067 cases in 2018 and 2017, respectively. *S.* Enteritidis and *S.* Typhimurium including the monophasic variant (S. 4,[5],12:i:-) continue to be the most common serotypes with 310 and 272 cases. Nine national *Salmonella* outbreaks were registered. Three of them were caused by *S.* 4,[5],12:i:- and in two of these outbreaks Danish produced pork meat was the source. The third outbreak was the largest *Salmonella* outbreak in 2019 with 57 patients. This outbreak was related to an international investigation with more than 200 registered cases from 2018 to 2019 with a WGS profile that was very similar to the one reported for the Danish outbreak. However the suspected food vehicle for the international cluster of cases was pork meat products and the suspected source in the Danish outbreak was Danish produced minced beef meat.

The lowest *Salmonella* prevalence in broiler flocks ever reported was observed in 2019 with only 0.3% positive flocks. In Laying hen flocks the *Salmonella* prevalence was 1.9% which was lower than 2018, where the highest number of positive flocks in more than a decade was

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 2019. 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.

reported (2.6%), but still considerably higher than previous years (0 - 1.1%).

SiTTi - a decision support tool for safe temperature and time

In 2019, a free web based decision support tool for predicting safe temperature and time combinations for food processing was developed by the National Food Institute at the Technical University of Denmark for publication on the Danish Veterinary and Food Administration (DVFA) website (www.fvst.dk, in Danish). The tool is called SiTTi and determines safe temperature and time for processes involving heating and cooling of food as well as keeping food warm. Food business operators, consumers, industry associations as well as the competent authority and the official food control units are potential users of the tool. SiTTi is an acronym for "Sikker Temperatur og Tid" which in English means safe temperature and time.

Foodborne outbreaks

In 2019, 51 foodborne outbreaks were reported with 1,929 cases. The outbreaks were mainly regional or local (63%). Eighteen outbreaks were national outbreaks of which four were part of international outbreaks. The most frequent setting was "restaurants" (29%).

In 2019, an increased number of outbreaks due to *C. perfringens* was reported. Outbreaks involving *Bacillus cereus* and *Clostridium perfringens* are traditionally caused by insufficient cooling of large portions of food, like meat sauces or sous vide /slow roasted meats. The largest outbreak, involving 268 persons, was an outbreak of *Clostridium perfringens*, which was caused by insufficient cooling of an industrial sized portion of minced meat sauce.

Norovirus (NoV) was the most frequent cause of foodborne outbreaks in 2019 which is similar to 2018 but a substantial increase compared to 2017. One of the NoV outbreaks was caused by oysters harvested in Denmark by a private person in the shallows of a closed zone. The oysters were served raw at a private party. Further two NoV outbreaks were caused by oysters imported from other EU countries.

Vectorborne zoonoses

The *Hyalomma* vector has important zoonotic potential as the vector for Crimean Congo Hemorrhagic Fever virus. The overwintering and subsequent recording of an adult tick on a human suggest that the species and its exotic pathogens may have to be considered a zoonotic risk in a future warmer climate.

Three tick borne encephalitis (TBE) clinical cases were reported in the late summer of 2019. All cases had visited a forest area in Northern Zealand and a hot spot in Tisvilde forest at the northern coast of the island was identified. Sequencing revealed the virus was a strain different from the previous hot spot in Tokkekøb forest and instead grouping closely with a virus sequence from Norway. TBE is spreading geographically in Southern Scandinavia and there is now a risk of TBE establishing in Danish forests.

In the warm year of 2018, mosquito borne West Nile virus (WNV) spread north in Europe and reached the northern parts of Germany. The important vector *Culex modestus* has previously been identified on the island of Amager as well as on the nearby coast of Greve and on the coast of Sweden just a few kilometres away. In 2019, the vector was identified further south in the Solrød and Jersie municipalities along the Køge Bay area. However, it is important to note that neither WNV nor Usutu virus have ever been identified in Denmark.

Tick-borne pathogens are a frequent source of zoonotic infections in Danish forests. A screening for multiple infections in 1,000 *I. ricinus* ticks collected from the public Grib forest in 2016 and 2017 showed that 19.1% of the nymphs harboured at least one zoonotic pathogen while 3.5% were infected with two or more pathogens. In adult ticks, 52.2% harboured at least one pathogen while 12.3% harboured more than one pathogen. The results demonstrate that co-infections need to be considered in the diagnosis and treatment of tick-borne diseases in Denmark, as the identification of one pathogen in a patient does not exclude the presence of other and potentially more pathogenic species.

The *Salmonella* source account

In 2019, the *Salmonella* source account, which attribute human cases to food sources, was based on a machine learning source attribution model using cgMLST profiles of food isolates and human isolates.

Similar to previous years, the most important food source was Danish produced pork (8.0% of the cases). Surprisingly this was followed by imported duck (6.5%). This was the first time such a large proportion of cases has been attributed to imported duck. The third most common source was imported pork (3.7%) followed by Danish produced table eggs (3.1%). These findings are similar to previous years.

As always, more than 40% of the cases were related to travel and this is by far the most important risk factor for *Salmonella* infections in humans.

1. *Campylobacter* – surveillance in humans and food sources

By Gudrun Sandø (GUS@fvst.dk), Eva Møller Nielsen and Mette R. Gantzhorn

For more than a decade campylobacteriosis has been the most frequently reported bacterial zoonotic disease in Denmark as well as in the rest of Europe. In 2019, the number of registered cases in Denmark was 5,389, which is an increase from 2018. Previous studies have shown that the main route of transmission is food, in particular poultry meat, raw milk, contaminated vegetables and water. Other sources are contact with contaminated water during recreational activities and contact to animals. In Denmark approximately one third of all cases are infected when travelling abroad.

As described in Annual Report on Zoonoses in Denmark 2017, several studies on sources and their relative impact were carried out in 2015-17. A source attribution study, where human isolates were compared to food, animal and environmental isolates in conjunction with a case control study, pointed at chicken meat and cattle/beef (minced meat) as the two major sources. Furthermore, analysis of whole-genome sequencing (WGS) data revealed a large number of small clusters of human cases (comprising 47% of all cases) as well as genetic matching of 30% of the isolates from humans to isolates from food, primarily chicken meat [1].

To obtain knowledge on the relative impact of these sources and trends over time, the Danish Veterinary and Food Administration (DVFA) initiated WGS-analyses of samples of chicken meat and beef in 2019. Concurrently, Statens Serum Institute (SSI) collected human clinical isolates from three regional laboratories to detect clusters and possible links to food samples as a continuous surveillance setup.

1.1 Description

The DVFA sampled chilled chicken meat at retail in northern Jutland and at distribution centres covering the major retail chains in Denmark. The DVFA also collected samples of thigh skin deriving from organic and free range broilers at slaughterhouses as part of the on-going surveillance, as well as samples of minced beef of Danish and non-Danish origin at retail.

From the sampling of chilled chicken meat at retail and at slaughterhouses, 131 isolates were collected: 124 from chicken meat of Danish origin and 7 samples from chicken meat of non-Danish origin. *Campylobacter* was not detected in any of the 402 samples of minced beef that were analysed.

For the WGS-based human surveillance in 2019, 668 clinical *C. jejuni* and *C. coli* isolates were continuously collected and analysed. All available isolates from clinical cases in northern Jutland and a subsample of isolates from Funen and Zealand were included, covering 12% of all national cases.

WGS-data of human clinical isolates and food isolates were analysed and compared continuously over the year. Sequencing and analysis of WGS data by cgMLST (1341 loci) was performed as described in Joensen et al. (2020) [1]. Clusters of clinical isolates and matches to chicken isolates were defined on basis of cgMLST allele differences using a cluster threshold of four by the use of single-linkage clustering.

1.2 Surveillance of human cases and matches to chicken isolates

In line with the previous study in 2015-17, analysis of WGS data by cgMLST showed that almost a third of the clinical isolates matched chicken isolates and half of the clinical isolates clustered with other clinical isolates, i.e. half of the cases were likely to be part of common-source outbreaks. Most of the 77 detected clusters of human cases comprised less than 10 cases each and four clusters had 11-14 cases. In contrast to previous years, a very large cluster of 88 cases in 2019 and 3 cases in 2018 was also detected (FUD1782) (Table A3). Cases occurred during the whole year, peaking in May-August 2019. The cluster type belonged to the MLST sequence type ST122 and was designated ST122#1. *C. jejuni* ST122 has been detected occasionally in Denmark in previous years. The cluster type ST122#1 defined by cgMLST had not been identified before and was genetically distant from other ST122 strains. Retrospectively, clinical isolates from end of 2018 were also sequenced and three ST122#1 were detected.

1.3 Investigations and follow up at slaughterhouse

The ST122#1 cluster matched four chicken isolates obtained from the sampling of retail meat: two sampled in May 2019 and two sampled in August 2019. These isolates were traced back to one slaughterhouse, where extensive

sampling as part of the microbiological follow-up lead to the collection of further 30 ST122#1 isolates. Some of these were subjected to WGS retrospectively. Ten isolates deriving from two samples were collected from the slaughterhouse environment in late October.

The slaughter dates for the batches, where ST122#1 was initially identified, led to the connection of the outbreak clone to one specific farm. It is possible that other farms harbour the same strain, however no other match was found at that point in time. Investigations are however still ongoing.

The slaughterhouse and the DVFA investigated the likely source, both at flock and at slaughterhouse level. Shortly after the outbreak became apparent, the slaughterhouse decided to initiate extensive sampling especially of batches deriving from the suspected farm. For positive batches, the National Food Institute at the Technical University of Denmark evaluated the risk and several batches were considered unsafe. These batches were not marketed. From August 2019, the slaughterhouse decided that fresh meat from this farm should be frozen. Based on results from the investigations, the slaughterhouse outlined an action plan for optimising procedures and equipment. After the initiation of these precautions, the human infection rate declined.

The outbreak has highlighted chicken meat as a important food source. The poultry industry has been working on ways to reduce the levels of *Campylobacter* for years. This study and especially the large outbreak has led to extensive new initiatives and investments targeting *Campylobacter* throughout the production chain.

1.4 References

1. Joensen KG, Kiil K, Gantzhorn MR, Nauerby B, Engberg J, Holt HM, Nielsen HL, Petersen AM, Kuhn KG, Sandø G, Ethelberg S & Nielsen EM (2020). Whole-Genome Sequencing to Detect Numerous *Campylobacter jejuni* Outbreaks and Match Patient Isolates to Sources, Denmark, 2015–2017. Emerg Infect Dis. 26(3):523-532. doi: 10.3201/eid2603.190947

2. Control of *Campylobacter* in broilers at primary production in the EU

By Maarten Nauta (maana@food.dtu.dk) and Johanne Ellis-Iversen

Campylobacter has been the most frequently reported foodborne zoonosis in the EU for more than a decade. Therefore, EFSA published an opinion on control options for *Campylobacter* in broiler meat production at different stages of the food chain in 2011 [1]. The opinion was updated in 2019-20 [2]. The new opinion identifies and ranks possible control options at the primary production level and was based on scientific literature published since 2010. Literature was reviewed and the potential relative risk reductions that may be achieved were calculated and assessed by experts. The outcome was a risk reduction and was defined as "*The percentage reduction in human campylobacter cases in the EU associated with the consumption of broiler meat that could be achieved by implementing control options at primary production of broilers*". After the assessments based on research data and models, an expert knowledge elicitation (EKE) was performed to translate the science into estimates reflecting EU-wide effects in field conditions and to account for the overall uncertainty of the estimates [3, 4].

2.1 Control options

2.1.1 Biosecurity

One class of control options reduces the prevalence of contaminated broiler flocks. These options are usually related to biosecurity, aiming to prevent introduction of *Campylobacter* into the flock. Their effect was estimated by calculating population attributable fractions (PAF) from epidemiological risk factor studies performed in the EU. Based on the available data from a large variety of EU countries, PAFs were calculated for six control options, which had sufficient evidence: "hygienic anteroom"; "effective rodent control"; "having no animals in close proximity to the broiler house"; "addition of disinfectant to drinking water"; "employing few and well-trained staff" and "avoiding drinkers that allow standing water". The variation in PAFs was greater between the different control options than for the same control options in individual studies, which increased the confidence in extrapolation of the results to the whole EU.

2.1.2 Reduction of concentration in the caeca

Another class of control options reduces the concentration of *Campylobacter* in the broiler caeca. Examples of these are vaccination and the application of feed or water additives. The effect of these control options was estimated by using a linear regression model associating concentrations in the caeca and on skin samples, combined with a microbiological risk assessment model combining a consumer phase and a dose-response model.

Further to the 2011 opinion, the data and models were updated. The updated model resulted in a flatter regression line describing the relation between concentrations in caecal contents and on skin. This resulted in lower estimates for the effectiveness of this class of control options than in 2011. For example, a 2-log10 reduction in caecal concentrations yielded a relative risk reduction of 42% (95% CI 11-75%), whereas in the previous opinion, this relative risk reduction was 76 - 98%. Similarly, a 3-log10 reduction in broiler caecal concentrations was estimated to reduce the risk by 58% (95% CI 16-89%), compared to more than 90% in in the previous opinion [1].

2.2 Ranking of control options

An Expert Knowledge Elicitation was performed, using the experts involved in drafting the opinion to rank the control options, based on the scientific data and modelling results. Control options that could not be analysed by PAF or modelling were also included, based on evidence from literature. This resulted in twenty control measures and eight of these were selected for a quantitative expert opinion assessment of their effectiveness, based on the quality of evidence available as well as the practical feasibility of their implementation.

Figure 2.1 shows the EKE estimates of the effectiveness, expressed as the risk reduction i.e. *The percentage reduction in human Campylobacter cases in the EU associated with the consumption of broiler meat that can be achieved, if a control option is correctly implemented by all broiler farms in the EU, taking into account the current level of implementation.* As the boxplot shows, the uncertainty of the estimates was large. Apart from these eight control options, other promising control options were "no animals in close proximity of the broiler houses"; "effective cleaning and disinfection between flocks"; "reduced slaughter age" and "application of bacteriophages".

2.3 Discussion

Compared to the 2011 opinion, the 2020 opinion provides novel estimates of the effectiveness of specific biosecurity measures to control *Campylobacter* at broiler farms, as well as updated estimates reduction of the concentrations in the caeca. Still, the uncertainty associated with these estimates is large, which illustrates the complexity of research into effective *Campylobacter* control.

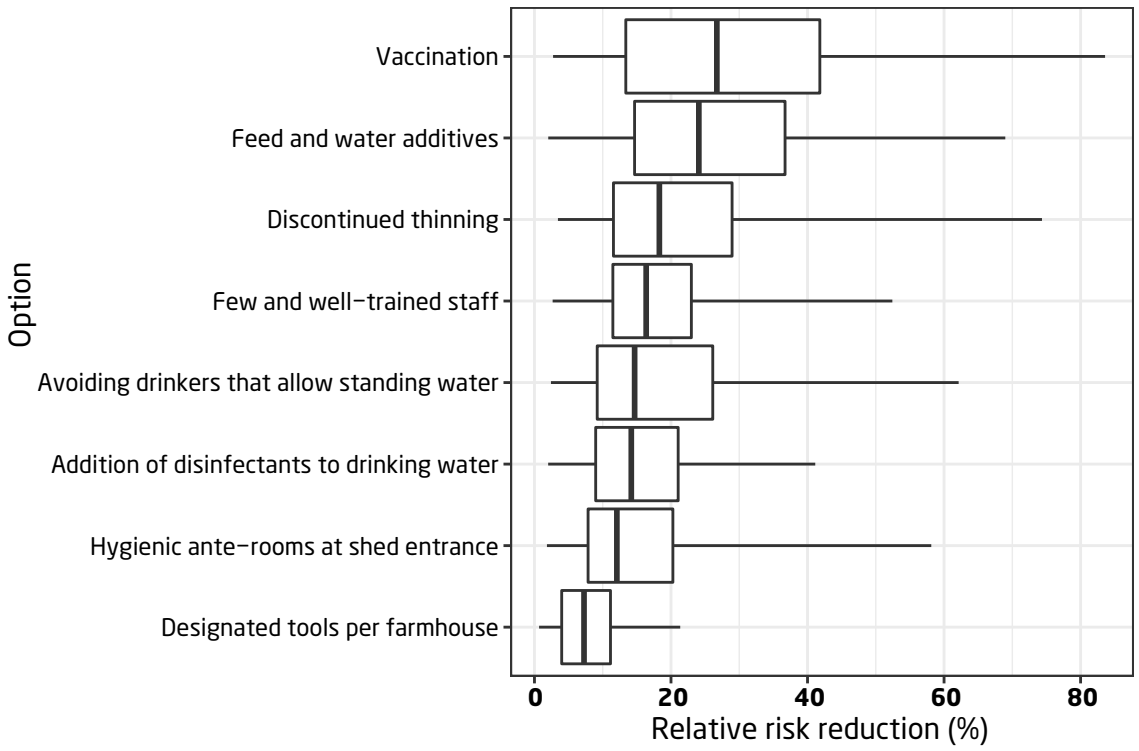
A decision on implementation of a control option does not only depend on effectivity, but also on other factors such as ease of application, cost and animal welfare impact. These advantages and disadvantages of control options are also discussed in the opinion [2].

The main message is that strict biosecurity to prevent introduction of *Campylobacter* into broiler flocks remains paramount. The estimates in the opinion relate to the EU as a whole and do not directly apply to individual countries. Nonetheless, a large study from Denmark was used in the PAF calculations and with two national experts in the working group, Denmark is at the forefront of *Campylobacter* risk science. Broiler flock prevalence is relatively low in Denmark (22.7% in 2019, Table A9) compared to other European countries, suggesting that biosecurity already is effective, although we still have room for improvement. The findings support the efforts in The National Campylobacter Action Plan and may contribute to some of the many ongoing initiatives on *Campylobacter* control in Denmark.

2.4 References

1. EFSA BIOHAZ Pane (2011). Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal 9(4): 2105.
2. EFSA BIOHAZ Panel (2020). Update and review of control options for Campylobacter in broilers at primary production. EFSA Journal 2020;18(4):6090, 89 pp. https://doi.org/10.2903/j.efsa.2020.6090
3. EFSA (2014). Guidance on Expert Knowledge Elicitation in Food and Feed Safety Risk Assessment. EFSA Journal 12(6): 278pp.
4. EFSA Scientific Committee (2018). Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 16(1):5123, 39 pp.

Figure 2.1. Ranking of eight selected control options for reduction of Campylobacter *in broilers. The horizontal axis is the relative risk reduction for each control option, assessed by expert judgement and expressed as % relative risk reduction in EU* Campylobacter *cases, if the control option was implemented by all EU broiler producers. For each control option, the horizontal line shows the 95% probability interval for the estimated risk reduction (P2.5 and P97.5), the box shows the interquartile range (P25 and P75) and the vertical line shows the median (P50). Note that there is a large degree of overlap in the effect estimates between options [2].*



3. SiTTi – a decision support tool for predicting safe temperature and time

By Tina Beck Hansen (tibha@food.dtu.dk), Zanne Dittlau, Niels Ladefoged Nielsen, Cristina Galliano and Ulrich Pinstrup

3.1 Introduction

A collaboration between the Danish Veterinary and Food Administration (DVFA) and the National Food Institute at the Technical University of Denmark (DTU Food) constitutes a comprehensive development of a decision support tool for predicting safe temperature and time combinations for food processing in small and medium-sized enterprises (SMEs). Since 2015, an initiative from DVFA has formed the basis for the development of a tool called SiTTi. SiTTi is an acronym for "Sikker Temperatur og Tid" which in English means safe temperature and time. It was the ambition of the working group to follow the principles and guidelines for the conduct of microbiological risk assessment and risk management published by Codex Alimentarius Commission [1,2].

SiTTi is a tool for determining safe temperature and time for processes involving heating and cooling of food as well as keeping food warm. Food business operators, consumers, industry associations as well as the competent authority and the official food control units are potential users of the tool. SiTTi is a web-based freeware and are expected to be published on the website of the DVFA during the fall 2020 (www.fvst.dk In Danish)

3.2 A tool for food business operators

Food business operators (FBO) must heat, cool and keep their products warm so that food safety is ensured. They are free to use any tools or guidelines to determine safe heat treatments, safe cooling processes and safe procedures for keeping products warm.

SiTTi can be used to determine and document the safe time and temperature combination of these processes as part of the FBO own-control programme. They can also use SiTTi to document that they have chosen e.g. a heat treatment method ensuring food safety. SiTTi operates with two intrinsic food conditions that influence bacterial growth, namely salt and pH. Other tools or guidelines may provide results different from those provided by SiTTi as they may have additional growth inhibiting principles built-in.

Industry associations can also use SiTTi to guide their members and to provide guidelines for heat treatment, cooling and keeping food warm in national standards.

3.3 A tool for the food control units

In Denmark, FBOs are free to use the method they want for heat treatment and cooling, and for keeping food warm as long as they can document that food safety is not compromised. The official food control units must assess the methods and documentation when auditing the establishments. SiTTi is an aid for this assessment; e.g. the official food control units can use SiTTi if there is any doubt about a method of heat treatment or if the documentation of the method used by the FBO is not sufficient. The official food control units can also guide FBOs in using SiTTi as needed. As mentioned above, FBOs can use various other available tools or guidelines where results may differ from those provided by SiTTi. In these situations, the official food control units are advised to look further into the basis for the deviation.

In collaboration with DTU Food, the DVFA has established the limits for pathogenic microorganisms within which SiTTi operates. If an FBO uses tools or guidelines where these limits are different, it may be relevant for the food control units to look more closely into the underlying conditions.

3.4 What can SiTTi do?

SiTTi provides a wide range of time-temperature combinations for safe heat treatment of foods. The lowest heat treatment temperature in SiTTi is 53 °C, the highest is 100 °C. The longest heat treatment time is 24 hours.

The tool provides safe cooling times for heat-treated foods during cooling from 53 °C to 10 °C. The cooling times are between four and eight hours depending on the product type, salt content and pH-value. The provided time-temperature combinations refer to the warmest spot in the foods.

In the case of keeping foods warm, the instructions in SiTTi have so far been limited to foods with a firm texture, e.g. meats and vegetables, for a period of three hours at temperatures from 20 °C to 65 °C. For liquid foods, such as soups, sauces and casseroles, data have not been sufficient to establish microbiological limits on a safe basis. For some foods, SiTTi imposes certain restrictions on keeping food warm for three hours. It is typically for low salt foods and high pH foods and typically in the temperature range from 30 °C to 50 °C.

3.5 Microbiological limits in SiTTi

3.5.1 Microbiological hazards

SiTTi operates with the following hazards of concern for processes involving heating, cooling and keeping foods warm: *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*, and Norovirus.

L. monocytogenes is essential to control during heat treatment of foods due to its capacity of being cold-tolerant as well as being among the most heat tolerant vegetative pathogens. In SiTTi, the heat tolerance of *L. monocytogenes* determines the safe time-temperature combinations for heat treatment of foods with pH < 4.5 or water-phase-salt > 10% independent of the desired shelf-life at max 5 °C. For foods, with higher pH and lower water-phase-salt, and with a desired shelf-life up to 10 days at max 5 °C, it is also primarily *L. monocytogenes* that determines the safe time-temperature combinations. Although, the safety of low-temperature-long-time (LTLT) heat treatment of red meats, may be determined by *C. perfringens* when the temperature rises very slowly and it takes more than 90 minutes to get to a product temperature of 53 °C. For foods with a desired shelf-life longer than 10 days at max 5 °C, SiTTi predicts safe time-temperature combinations based on the heat tolerance of endospores from cold-tolerant *C. botulinum* for shelf-life up to 21 days, and from cold-tolerant *Bacillus cereus* for shelf-life up to 90 days. Finally, Norovirus is the determining factor when predicting safe heating processes of frozen berries.

For safe cooling processes and safe procedures for keeping food warm, the safe time-temperature combinations in SiTTi are based on the growth potential of sporeforming pathogens, which have survived the heat treatment and, therefore can germinate and grow in the food if the conditions allow it. *C. perfringens*, *B. cereus* and *C. botulinum* are all relevant to control under these processes. Exactly which one of them that determines the safe time-temperature combinations depends on the specific process and food. In SiTTi, the growth potential of *C. perfringens* determines the safe time-temperature combinations for cooling profiles. Whereas safe processes for keeping food warm are determined either by *C. perfringens* or by *B. cereus* depending on their growth potential for specific foods and facilities used for keeping the food warm.

3.5.2 Critical levels for microorganisms in food

For promoting the protection of consumers against foodborne diseases, Codex Alimentarius Commission has defined a set of risk-based metrics to aid the competent authority in reaching that objective [2]. As a starting point, a food safety objective (FSO) has to be defined for the particular food safety issue (see 3.8 Glossary for explanation). This implies setting critical levels of pathogenic microorganisms in food.

As critical levels for *L. monocytogenes* are already set out in EU Regulation (EC) No. 2073/2005 on Microbiological criteria for food [3], these are applied in SiTTi. Critical levels for *C. perfringens* and *B. cereus* are not provided in EU regulations. SiTTi applies critical levels of 10^5 per g for both. These levels have been decided by DVFA based on scientific knowledge collected and presented by DTU Food. For *C. botulinum* the critical levels are related to their formation of botulinum toxins and a precautionary principle has been taken that no toxins should be present in the food. Due to the low infectious dose for norovirus (NoV) the critical limit is defined as absence of NoV genome copies pr. sample.

3.5.3 Performance criteria

A performance criterion is another of the risk-based metrics suggested by Codex Alimentarius as a risk management principle with the potential to relate directly to consumer protection [2]. It expresses the effect that should be attained by a control measure, e.g. a heat treatment. The competent authority as advice can set performance criteria to FBOs

Table 3.1. Performance criteria for Listeria monocytogenes *for heat processing steps*

Safe shelf-life at max 5 °C	All foods with	All foods with WPS > 10 %[a]	Fish products	Meat products	Other foods
pH < 4.5	All foods with	1 log reduction	Not established[b]	3 log reduction	1 log reduction
WPS[a] > 10%	Fish products	Meat products	Other foods	4 log reduction	2 log reduction
Served directly after heat treatment	1 log reduction	1 log reduction	Not established[b]	3 log reduction	1 log reduction
Up to 5 days	1 log reduction	1 log reduction	2 log reduction	4 log reduction	2 log reduction
More than 5 days	1 log reduction	1 log reduction	5 log reduction	6.5 log reduction	5 log reduction

a) WPS is short for water phase salt

b) Until more data is available, the heat treatment of at least 60 °C for 1 min is recommended [4,5]

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

Table 3.2. Performance criteria for other hazards for heat processing steps

Microbiological hazard	Type of foods	Performance criteria
Clostridium perfringens (vegetative cells)	Meat products, heated slowly, where a temperature of 53 °C is reached after 90 min	3 log reduction
Bacillus cereus (cold-tolerant, spores)	Foods with pH < 6.0 and safe shelf-lives from 22 to 90 days at max 5 °C	1 log reduction
	Foods with pH ≥ 6.0 and safe shelf-lives from 22 to 90 days at max 5 °C	3 log reduction
Clostridium botulinum (cold-tolerant, spores)	Foods with pH > 4.4, < 10% salt-in-water, and safe shelf-lives longer than 10 days at max 5 °C	6 log reduction
Norovirus	Frozen berries	Not established[a]

a) Until more data is available, heat treatments equivalent to 1 min at 100 °C are recommended [6]
Source: National Food Institute, Technical University of Denmark & Danish Veterinary and Food Authority

Table 3.3. Performance criteria for cooling steps and process steps where foods are kept warm

Microbiological hazard	Cooling	Keeping firm foods warm for 3 hours at 20 °C to 65 °C
Clostridium perfringens	0.7 log increase	2 log increase
Bacillus cereus	0.3 log increase	1 log increase
Clostridium botulinum	No growth	No growth

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

that are not capable of establishing performance criteria themselves, e.g. SMEs.

In SiTTi, performance criteria are defined as the minimum reduction of the hazards required by heat treatment and the maximum increase of the hazards allowed under cooling and when foods are kept warm. The performance criteria applied in SiTTi are shown in the Tables 3.1 and 3.2 for heat processing steps and in Table 3.3 for cooling steps and for steps keeping foods warm.

SiTTi translates these performance criteria into the specific temperature and time combination(s) needed to achieve a safe process.

3.6 Conclusion

Development of SiTTi was initiated as a part of DVFAs process of changing the national regulations in this area from fixed rules to flexibility taking into consideration the overarching priority not to compromise food safety in any way. The flexibility comes with an obligation of documenting that food safety is ensured. For many SMEs, the requirement for documentation is difficult to meet and this is why DVFA has embarked on developing a digital tool for predicting and documenting temperature and time combinations for safe heating procedures, safe cooling procedures and safe procedures for keeping foods warm. With SiTTi, FBOs will have both the needed food safety assessment and the needed food safety documentation in place if they follow the instructions given in the tool.

3.7 References

1. Codex Alimentarius commission (2014). Principles and guidelines for the conduct of microbiological risk assessment (CAC/GL 30-1999 Adopted 1999. Amendments 2012, 2014).
2. Codex Alimentarius Commission (2007). Principles and guidelines for the conduct of microbiological risk management (MRM) (CAC/GL 63-2007).
3. Commission Regulation (EC) No 2073/2005 of 15/11/2005 on microbiological criteria for foodstuffs.
4. Order of Food Hygiene no 1354 of 28/11/2017, § 25, article 3 point 4.
5. Guideline of Food Hygiene no 9613 of 05/06/2019, Chapter 27.1.
6. Order of Food Hygiene no 1354 of 11/28/2017, § 26, article 1.

3.8 Glossary

	Description
Appropriate Level of Protection (ALOP)	The level of protection deemed appropriate by the WTO? Member Country establishing a sanitary and phytosanitary measure to protect human, animal or plant life or health within its territory
Food Safety Objective (FSO)	The maximum frequency and/or concentration of a microbiological hazard in a food – at the time of consumption – that provides or contributes to the ALOP
Performance Objective (PO)	The maximum frequency and/or concentration of a microbiological hazard in a food - at a specific point in the food chain – that provides or contributes to the FSO or ALOP
Performance Criterion (PC)	The effect in frequency and/or concentration of a microbiological hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or the FSO
Process Criterion (PcC)	A PcC specifies the conditions of treatment that a food must undergo at a specific step in its manufacture to achieve a desired level of control of a microbiological hazard (e.g. time and temperature of heat treatment)
Product Criterion (PdC)	A PdC specifies a chemical or physical characteristic of a food (e.g. pH or water activity) that, if met, contributes to food safety

Source: World Trade Organization (WTO) & Codex Alimentarius Commission (CAC)

4. 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). Appendix Table A3 contain the outbreaks that occurred in 2019. Figure 4.1 shows the relative distribution of these outbreaks by the different causative agents. Household outbreaks and clusters not verified as common source foodborne outbreaks are excluded. Outbreak investigation procedures in Denmark are described in Chapter 8.

In 2019, 51 foodborne outbreaks were reported in FUD and the total number of persons affected by foodborne outbreaks was 1,929 with a median of seventeen persons per outbreak (range 3-268). The outbreaks were mainly regional or local (63%). Eighteen outbreaks were national outbreaks of which four were part of international outbreaks. The largest outbreak, involving 268 persons, was an outbreak caused by *Clostridium perfringens* (FUD 1784).

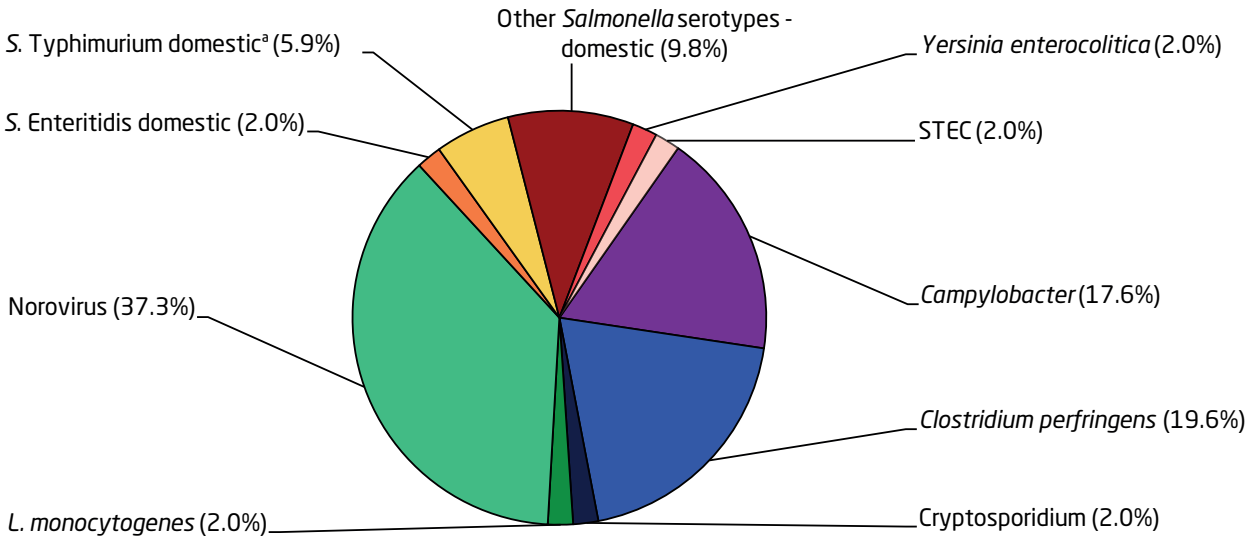
When dividing the outbreaks by reported setting, the most frequent setting was "restaurants" (29%) with 15 outbreaks affecting 534 people (mean 38 people per outbreak). Outbreaks taking place in workplace/school canteens and through catering (10 outbreaks) also affected a high number of people (723 people) and affected on average 72 persons per outbreak. "Composite meals" (11 outbreaks) and "buffet meals" (8 outbreaks) combined were the most frequently reported types of foods associated with outbreaks in 2019 and most often these outbreaks were caused by Norovirus (NoV) (Appendix Table A3).

In 2019, *Clostridium perfringens* was associated with ten foodborne outbreaks affecting 551 people compared to five, eight, and seven outbreaks caused by this agent in 2018, 2017 and 2016, respectively. This is an increase in numbers of outbreaks and affected persons due to this agent. Outbreaks involving *Bacillus cereus* and *Clostridium perfringens* are traditionally caused by insufficient cooling of large portions of food like various meat sauces or sous vide /slow roasted meats. This was also the case in 2019. A large outbreak with 268 registered cases (FUD 1784) was caused by insufficient cooling of an industrial sized portion of minced meat sauce packaged with other food items into chilled ready-to-heat meals and delivered to approximately 3,500 subscribers of a meal box delivery scheme.

4.1 Norovirus outbreaks

Norovirus (NoV) was the most frequent cause of foodborne outbreaks in 2019 (19 outbreaks), and in total 932 persons were affected. This is a substantial increase compared to 2017 and is unfortunately at the same level as in 2018 (Table 4.1). The transmission routes for NoV causing foodborne

Figure 4.1. Aetiology of the 51 foodborne disease outbreaks reported with a causative agent in the Food- and waterborne Outbreak Database (FUD), 2019. Percentage of total outbreaks indicated in brackets



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

Source: Food- and waterborne Outbreak Database (FUD)

outbreaks were multiple. In Table 4.1, a breakdown of the number of outbreaks and the number of people affected per route of transmission for 2017-19 is presented.

The most common way of infection with NoV in 2019 was contamination from symptomatic or healthy carriers among kitchen staff. In 2019, this way of infection constituted 63% of the NoV outbreaks. This too is an unfortunate increase compared to the previous years.

One of the NoV outbreaks was caused by oysters harvested in Denmark by a private person in the shallows of a closed zone. The oysters were served raw at a private party (FUD 1719). Further two NoV outbreaks were caused by oysters imported from other EU countries.

4.2 *Salmonella* outbreaks

In 2019, nine *Salmonella* outbreaks were registered. Outbreaks related to travelling abroad are not included in the report for 2019. Three of the nine outbreaks of *Salmonella* in 2019 were caused by the monophasic variant of *S.* Typhimurium (*S.* 4,[5],12:i:-). The source of two of the outbreaks was Danish produced pork meat. The third outbreak was the largest *Salmonella* outbreak in 2019 (FUD 1728) with 57 patients registered between January and November. The patients were 18 female and 39 male in the age range of 6 months to 84 years. The median age was 49 years. This outbreak was related to an international investigation with more than 200 registered cases from 2018 to 2019 with a monophasic variant of *S.* Typhimurium WGS profile very similar to the one reported for the Danish outbreak. The suspected food vehicle for the international cluster of cases was pork meat products. Finland and Sweden also reported cases as part of this cluster. However, investigations based solely on the Nordic cases pointed towards minced beef meat sold via a Danish establishment as clustering isolates from minced beef batches from that production site were found in both Finland and Sweden. An investigation at the production site did not reveal a possible contamination (raw material or environmental). It was not possible to conclude whether the Danish outbreak was caused by multiple batches of contaminated raw material (beef) being processed over time or if it was due to the *Salmonella* establishing itself in the equipment for a period of time contaminating some batches of minced meat.

An outbreak with *S.* Derby was investigated (FUD 1787). The outbreak lasted from April to June 2019 and involved 11 cases, aged 45 to 79 years. Comparison with isolates from food showed clustering with *S.* Derby found in samples from raw pork meat sausage from one producer and swab samples from slaughtered pigs from a Danish slaughterhouse. No definite connection between the two establishments was found. This was a national outbreak most probably caused by pork meat or pork meat products produced from pork from the Danish slaughterhouse.

A larger outbreak with *S.* Coeln took place from May to August with the majority of cases becoming ill in the weeks 22 and 23 (FUD 1790). The outbreak involved 26 cases, 14 female and 12 male aged 8-87 years. Interviews did not reveal the source of the outbreak. The outbreak was also investigated as part of an international outbreak and compared to a larger outbreak in the Czech Republic from 2018 suspected to have been caused by poultry meat.

4.3 Other outbreaks of interest

From February to April Denmark and Sweden experienced simultaneous outbreaks with *Yersinia enterocolitica* O3, biotype 4 (FUD 1773) [1]. In Sweden, 37 persons became ill. In Denmark, the outbreak involved 20 cases, 11 female and 9 male aged 2-74 years and predominantly young adults.

Table 4.1. Norovirus outbreaks per route of transmission based on number of cases or number of outbreaks, 2017-2019

	2019		2018		2017	
Transmission route/source	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	12	691	10	408	7	168
Kitchen staff tending to ill persons at home before entering the kitchen	2	80	1	30	1	42
Ill person/guest attending a buffet	2	89	4	193	1	78
Seafood (oysters)	3	72	4	146	1	10
Frozen raspberries/strawberries	0	0	1	50	0	0
Leafy greens / lettuce	0	0	1	12	0	0
Water	0	0	0	0	0	0
Total	19	932	21	839	10	298

Source: Food- and waterborne Outbreak Database (FUD)

Hypothesis generating interviews pointed to a vegetable or leafy green as a possible source, and a case-control study pointed out fresh spinach as the possible source of the outbreak in Denmark. An extensive trace-back investigation of the spinach on the market at the time of the outbreak was performed in both countries. The result showed that both countries had been supplied with spinach from the same two initial lots from one producer in Italy. The spinach was delivered to different packaging facilities and thus was packaged in different sites to the Danish and Swedish markets respectively, supporting that the contamination of the spinach took place before entering the packaging facilities. No other countries reported cases related to this outbreak.

A large increase in registered outbreaks with *Campylobacter* jejuni was seen in 2019. The reason for this and a more detailed description of the largest of these outbreaks are reported in Chapter 1.

One of the *Campylobacter* outbreaks (FUD 1831) however was a local outbreak with cases on Bornholm. The outbreak was initially reported by the local medical practitioners. Further investigations revealed that the outbreak consisted of 31 cases, 10 female and 21 male aged 1 to 85 years. The majority of cases became ill during week 47 (18-21 November 2019) indicating a food item produced and distributed locally and with a short shelf life. Hypothesis generating interviews pointed to milk as a possible source. A case-control study was performed. The result supported the hypothesis and showed that the odds of becoming ill increased with increased intake of milk.

Tracing and information provided from the dairy on Bornholm concerning the processes etc. performed when processing the milk did not reveal a possible cause of contamination or insufficiency in the pasteurization processes. The source of the outbreak could not be verified.

Finally, an outbreak of *Listeria monocytogenes* ST1 with cases reported from the year 2016 and onwards was resolved. The outbreak counted a total of 11 cases, three from 2019. Based on interviews with these three cases it was possible to identify a possible common source of Listeria. Salads including hummus from a small retail enterprise in Jutland seemed to be the common food source. Swab samples and samples of the products from the establishment were analysed and *L. monocytogenes* was found. Further comparison showed that the isolates clustered with the isolates from cases in the outbreak.

4.4 References

1. Espenhain L, Riess M, Müller L, Colombe S, Ethelberg S, Litrup E , Jernberg C, Kühlmann-Berenzon S, Lindblad M, Hove NK, Torpdahl M & Mörk MJ (2019). Cross-border outbreak of Yersinia enterocolitica O3 associated with imported fresh spinach, Sweden and Denmark, March 2019. Euro Surveill. 24(24):pii=1900368. https://doi.org/10.2807/1560-7917.ES.2019.24.24.1900368.

5. Vector-borne zoonoses

By René Bødker (rebo@sund.ku.dk), Louise Lohse and Lene Jung Kjær

The Danish Veterinary Consortium at University of Copenhagen monitors vectors and vector-borne diseases in Denmark on behalf of the Danish Veterinary and Food Administration. The surveillance focuses on endemic vectors but also screens for exotic vectors. Mosquitoes and biting midges in Denmark have been monitored weekly during the vector season since 2011 and 2012, respectively. Mechanical vectors (Tabanidae) and tick vectors (*Ixodes ricinus*) have been monitored regularly since 2017. Surveillance data are continuously updated at www.myggetal.dk.

After the unusually dry and warm summer in 2018 and the resulting record low vector abundance, the summer of 2019 can be classified as a normal year with an average vector abundance. The warm summer of 2018 resulted in the first findings of adult ticks in Denmark belonging to the exotic species *Hyalomma marginatum*. These are likely introduced as larvae by migrating birds every year, but are only able to develop into the adult stage when summers are warm and dry. Interestingly, some of these adult ticks survived the Danish winter as a single specimen was recorded crawling on a person returning from a nature walk in the spring of 2019. The *Hyalomma* vector has important zoonotic potential as the vector for Crimean Congo Hemorrhagic Fever virus, but it is considered unlikely that the species will be able to establish a population in Scandinavia. However, the overwintering and recording of an adult tick on a human suggest that the species and its exotic pathogens may have to be considered a zoonotic risk in a future warmer climate.

Tick borne encephalitis (TBE) has historically only been found on the Danish island of Bornholm [1]. However, in 2008 we identified a small hot spot established in the Tokkekøb forest just north of Copenhagen [2]. The hot spot resulted in two human cases of encephalitis before it disappeared again in 2016 [3]. In 2019, three new human TBE clinical cases were reported in late summer. All cases had visited a forest area in Northern Zealand [4]. By flagging for ticks, we identified a new hot spot in Tisvilde forest at the northern coast of the island. All collected ticks (n=1,067) were tested for TBE virus at Statens Serum Institut in pools. The hot spot appeared to be centered on a large playground area within the forest (Photo 5.1). We found TBE virus in *I. ricinus* nymphs in various sections flagged around and along 200 meter transects radiating from the playground. The prevalence decreased rapidly away from the playground area starting with very high prevalence of 8.0% (95% CI: 4.0 – 14.0) close to the playground. However, the prevalence could not be determined at the very edge of

Photo 5.1. In 2019, record high TBE virus prevalence was found in ticks in an emerging hot spot close to a public playground in Denmark.

Photo: Danish Veterinary Consortium

the playground where 44 nymphs were tested in five pools, as all five pools were TBE positive indicating a minimum prevalence of 11% [4]. Sequencing revealed the virus was a strain different from the previous hot spot in Tokkekøb forest and instead grouping closely with a virus sequence from Norway. TBE is spreading geographically in Southern Scandinavia and there is now a risk of TBE establishing in Danish forests.

In the warm year of 2018, mosquito borne West Nile virus (WNV) spread north in Europe and reached the northern parts of Germany. There is now a real risk of the virus spreading further north and reaching Denmark in the coming years, and therefore the national vector surveillance has a specific surveillance focus on the distribution of the important WNV vector *Culex modestus* in Denmark. The vector was identified on the island of Amager as well as on the nearby coast of Greve and on the coast of Sweden just a few kilometers away. In 2019, the vector was identified further south in the Solrød and Jersie municipalities along the Køge Bay area. In both municipalities, the vector was found in a narrow band along the coast and always close to shallow ponds. However, the vector was not found in similar areas further south e.g. Køge and Vallø or at Møn. It has been speculated that the vector may have been recently introduced to Denmark. However, this year a study of haplotypes of individual *C. modestus* specimens collected in Greve in 2014, revealed substantial genetic diversity suggesting the population is not simply the result of a recent accidential introduction of a gravid female mosquito [5]. In 2019, a total of 125 *C. modestus* and an additional 206 other *Culex pipiens/torrentium* collected in the regular five sentinel mosquito surveillance traps were tested for WNV and Usutu virus at Statens Serum Institut and were all found virus negative. It is important to note that neither WNV nor Usutu virus have ever been identified in Denmark.

Tick-borne pathogens are a frequent source of zoonotic infections in Danish forests. The prevalence of different *Borrelia* species and other zoonotic bacteria and parasites in ticks are high (Table 5.1). It has been suggested that tick bites may constitute a greater risk to humans if a tick bite is received from a tick infected with more than one pathogen. This is because some infections e.g. *Anaplasma* has a local immunosuppressive effect facilitating the establishment of other more pathogenic species introduced at the same time. We therefore screened for multiple infections in 1,000 *I. ricinus* ticks collected by flagging from the public Grib forest over two consecutive years (2016-17).

Overall, 19.1% of the nymphs harbored at least one pathogen while 3.5% were infected with two or more pathogens [6]. Infection levels were higher in adult ticks, where 52.2% harbored at least one zoonotic pathogen while 12.3% harbored more than one pathogen [6]. On average, 21% of all the ticks that carried a pathogen carried more than one pathogen (18% of infected nymphs and 24% of the infected adult ticks) [6]. Hence, if a tick is infected there is 21% risk that it is infected with more than one pathogen. The results demonstrate that co-infections need to be considered in the diagnosis and treatment of tick-borne diseases in Denmark, as the identification of one pathogen in a patient does not exclude the presence of other and potentially more pathogenic species.

Table 5.1. Individual prevalence of tick borne pathogens in 509 nymphs and 504 adult Ixodes ricinus *ticks collected from Grib forest, 2016-2017*

Pathogen	Prevalence in nymphs (%)	Prevalence in adults (%)
Borrelia s.l.	8.6	24.8
B. afzelii	1.4	2.8
B. valaisiana	2.0	1.2
B. miyamotoi	0	2.6
B. burgdorferi	2.0	3.6
B. garinii	2.9	3.0
B. spielmanii	1.2	10.1
Borrelia miyamotoi	0	2.6
Rickettsia helvetica	5.7	13.3
Anaplasma phagocytophilum	6.1	14.3
Neoehrilichia mikurensis	0	0.4
Babesia venatorum	0.2	0.8

Source: Copenhagen University

References

1 Andersen NS, Bestehorn M, Chitimia-Dobler L, Kolmos HJ, Jensen PM, Dobler G, Skarphédinsson S. (2019). Phylogenetic characterization of tick-borne encephalitis virus from Bornholm, Denmark. Ticks and Tick-borne Diseases 10(3):533-539. DOI: 10.1016/j.ttbdis.2018.12.008

2 Fomsgaard A, Christiansen C, Bødker R (2009). First identification of tick-borne encephalitis in Denmark outside of Bornholm, August 2009. Euro Surveill. 14(36):19325.

3 Petersen A, Worsøe Rosenstierne M, Rasmussen M, Fursted K, Nielsen HV, Andersen LO, Bødker R, Fomsgaard A. (2019). Field samplings of Ixodus ricinus ticks from a tick-borne encephalitis virus micro-focus in Northern Zealand, Denmark. Ticks and Tick-borne Diseases 10(5):1028-103. DOI: 10.1016/j.ttbdis.2019.05.005

4 Agergaard CN, Rosenstierne MW, Bødker R, Rasmussen M, Andersen PHS, Fomsgaard A (2019). New tick-borne encephalitis virus hot spot in Northern Zealand, Denmark, October 2019. Euro Surveill. 24(42).

5 Hernández-Triana LM, Brugman VA, Pramual P, Barrero E, Nikolova NI, Ruiz-Arrondo I, Kaiser A, Krüger A, Lumley S, Osório HC, Ignjatovic-Cupina A, Petric D, Laure Setier-Rio M, Bødker R, Johnson N (2020). Genetic diversity and population structure of Culex modestus across Europe: does recent appearance in the United Kingdom reveal a tendency for geographical spread? Med Vet Entomol 34(1):86-96. DOI: 10.1111/mve.12412

6 Klitgaard K, Kjær L, Isbrand A, Hansen MF, Bødker R (2019). Multiple infections in questing nymphs and adult female Ixodes ricinus ticks collected in a recreational forest in Denmark. Ticks and Tick-borne Diseases 10 (5): 1060-1065. DOI: 10.1016/j.ttbdis.2019.05.016

Photo 5.2. Adult tick Ixcodes ricinus

Photo: Danish Veterinary Consortium

6. Trends and sources in human salmonellosis

By Birgitte Helwigh (bhel@food.dtu.dk), Nanna Munck and Eva Litrup

In 2019, cgMLST profiles were generated for 1,024 isolates from human cases and included in the *Salmonella* source attribution model. The human isolates were attributed to food sources applying a machine learning source attribution model on cgMLST profiles of food isolates from three consecutive years, namely 2017, 2018 and 2019. Human *Salmonella* cases from 2019 were predicted by the model and attributed to ten different food and animal sources. The main source was Danish produced pork followed by imported ducks and imported pork. This chapter describes the human cases in more detail followed by a description of the food data used as model input, the method and results.

6.1 Isolates from human *Salmonella* cases included in the model

Of the 1,024 human *Salmonella* isolates, 879 cases were sporadic and 145 cases were from 9 domestic outbreaks (of which 13 cases were associated with an outbreak initiated in 2018). The sporadic cases included 419 travel related cases, 182 domestic cases and 278 with unknown travel history. The source attribution model were used to allocate sporadic cases with no or unknown travel history and the index cases for the nine domestic outbreaks (469 cases in total). Travel related cases were directly attributed to travel without using the model.

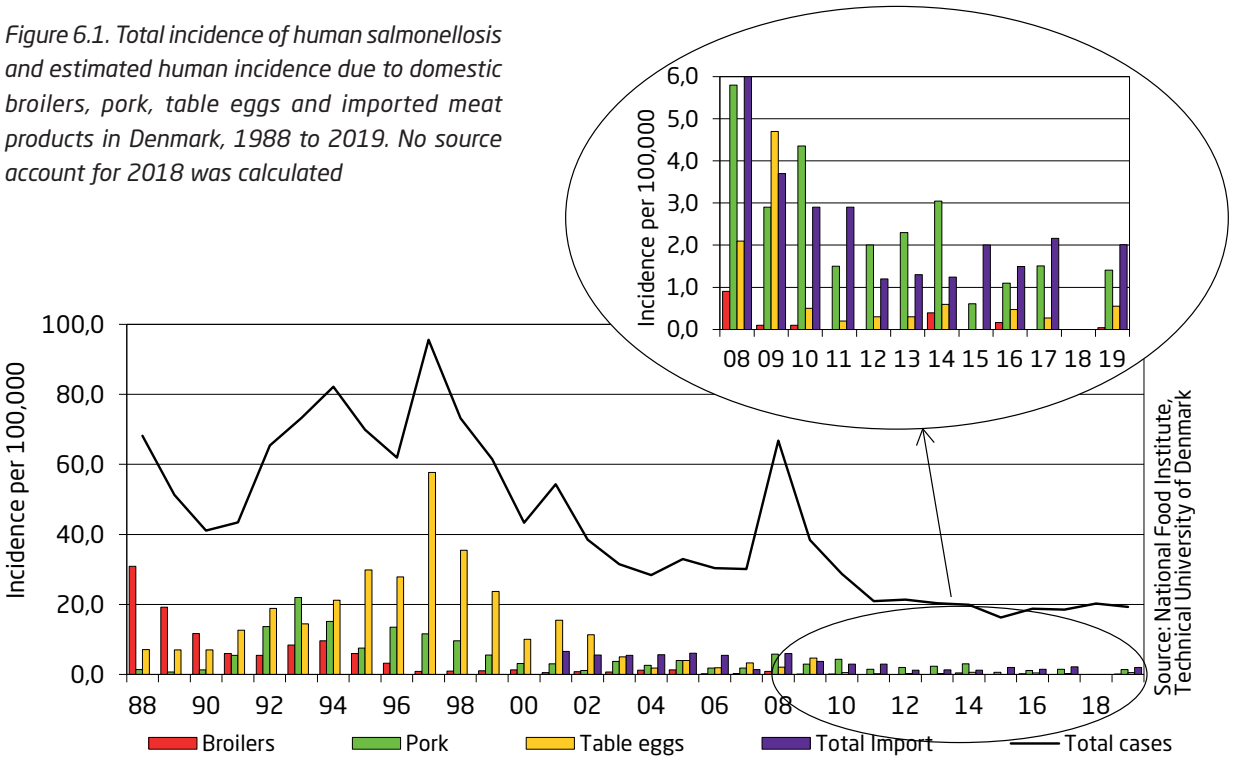
6.2 Isolates from food and animal included in the model

Salmonella isolated from animal and food were collected as part of the Danish National *Salmonella* surveillance programmes for animals and food and the source attribution model was based on associated core genome Multi-locus sequence typing profile (cgMLST). From 2017, 144 isolates were included, 182 were included from 2018 and 184 were included from 2019. The isolates originated from ten different food sources (Figure 6.2).

6.3 Method

In 2017, serotyping and Multiple Locus Variable Tandem Repeat Analysis (MLVA) were replaced by whole genome

Figure 6.1. Total incidence of human salmonellosis and estimated human incidence due to domestic broilers, pork, table eggs and imported meat products in Denmark, 1988 to 2019. No source account for 2018 was calculated



Source: National Food Institute, Technical University of Denmark

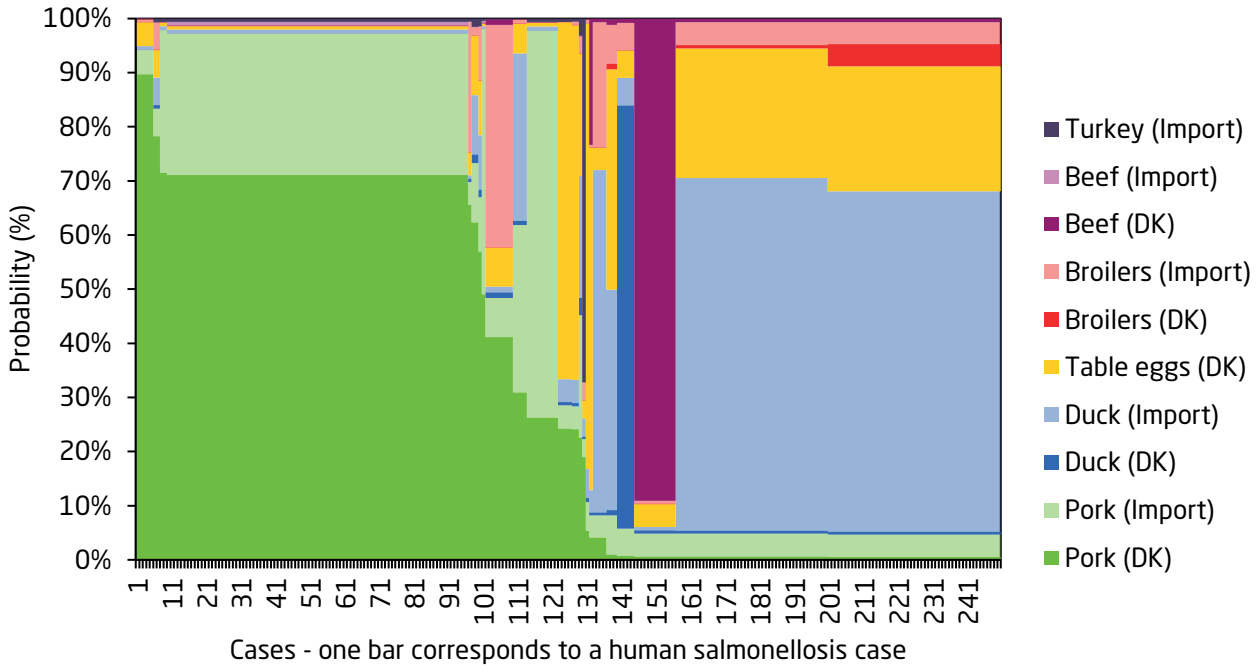
sequencing (WGS) of all isolates found as part of the National *Salmonella* surveillance programmes for animals and food, and the National surveillance of human *Salmonella* infection. Consequently, the Bayesian source attribution model was replaced by a machine learning model, developed for the purpose [1]. Machine learning (ML) is a collective name for mathematical models that learn from data and improves with experience/more data [2]. The models are defined by algorithms capable of recognizing patterns in large and complex datasets making the method applicable for analysing DNA sequence data [2]. The method identifies relevant features in the dataset enabling the ability to make strong allocations.

For the *Salmonella* source attribution 2019, we applied cgMLST, by which all core genes are used in the analysis, and strains are differentiated by their allelic variations. Statens Serum Institut provided the cgMLST profiles for each sequence using the Enterobase scheme [3] in BioNumerics version 7.6 (Applied 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. A 'feature reduction' step identified which 20 loci (of the 3,002) that provided most information about the source-patterns and these were then used in the model while the remaining loci were excluded. The final model was thus constructed from 510 food isolates and associated allelic values of the 20 loci.

We applied a supervised classification ML model. The classification is supervised, because the machine is 'told' from which of the different animal sources (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 alleleic variation. The ML model was constructed from a training dataset consisting of the majority (70%) of the food isolates. The accuracy of the model was then determined from the models' ability to allocate the origin of the remaining part (30%) of the animal and food isolates. As soon as a model with a satisfying accuracy was obtained, a final model using the entire (100%) of the food isolates was constructed. The probability of each human isolate to originate from a specific source was allocated from the final model. 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 allocated are referred to an unknown source category.

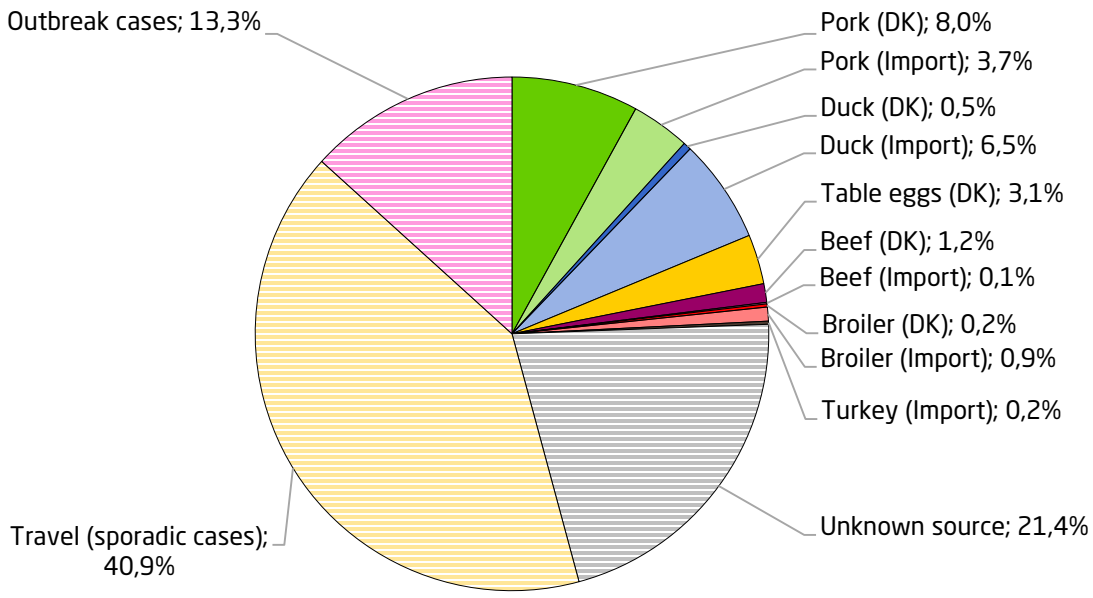
The previous Bayesian approach estimated uncertainties around the mean number of attributed cases per source. The ML model does not compute uncertainty intervals per se, but takes the uncertainties into account when building the model by repeating the model building 10 times and

Figure 6.2. Probability of sources attributed to each sporadic case (incl. outbreak index cases) by the ML model. The 250 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

Figure 6.3. Relative attribution (%) of the 1,024 human salmonellosis cases in 2019.



Note: The striped categories were not attributed by the ML model but case information alone.

Source: National Food Institute, Technical University of Denmark

applying a 7-fold cross validation for each model build. The uncertainty of the results is reflected in Figure 6.2 where the probability of each human case to belong to one of the included sources is illustrated. This source account attributed sporadic human *Salmonella* cases including index cases from nine foodborne outbreaks to food sources included in the source attribution model.

6.4 Results

The model attributed 250 (53%) of the 469 human cases to a food source (Figure 6.1). Most of the cases had a high probability (>70%) of originating from a single source, whereas other cases had a more or less equal chance of originating from two or three sources.

Similar to previous years, the most important food source was Danish produced pork (82 cases corresponding to 8.0% of the 1,024 human cases) followed by imported duck (6.5%), imported pork (3.7%), Danish produced table eggs (3.1%) and Danish produced beef (1.2%) (Figure 6.3). Few cases were also attributed to imported broilers, Danish produced duck, Danish produced broilers, imported turkey and imported beef. In total, 133 (28%) cases was attributed to Danish produced food, 117 (25%) cases attributed to imported food and 219 (47%) cases attributed to an unknown source.

The most surprising observation was the large proportion of cases attributed to imported duck. This has not been seen previously. In the source account including 2017 and 2018 cases (not published), imported duck was the sixth most important source. The prevalence of *Salmonella* isolated from imported duck was particularly high in 2019, with 18 *Salmonella* positive batches originating from four different countries, compared to only 8 positive batches in the last survey in 2017.

This year, the source attribution model is based on food data from 2017, 2018 and 2019 and allocated human cases from 2019 only. Multiple years of source data was included to enhance the robustness of the source data. A model using only 2018 and 2019 source data was also constructed (data not shown). The model with two years sources allocated 44% 2019 cases, whereas the model containing three years 2017-19 allocated 53% of the 2019 cases, and the three most important sources remained the same, whether or not 2017 was included. Based on these observations, we decided to continue with the model including all three years in the 2019 source attribution.

One of the advantages with the ML model is that it improves when more data is introduced as the variability of *Salmonella* strains in the different sources is captured to a higher extent. On the other hand, if specific *Salmonella* types are present in given sources occasionally and disappear again, including these in following years could potentially be misleading.

The ML model is an additional tool to investigate the relative importance of sources of human salmonellosis. Like all other methodologies it has uncertainties, but the outputs supplement the other methodologies currently used for similar purposes e.g. outbreak investigations to aid decision-making. Furthermore, the model has potential for further expansion and development to account for factors such as prevalence and human consumption patterns.

6.5 References

1. Munck N,, Njage PMK, Leekitcharoenphon P, Litrup E. & Hald T (2020). Application of Whole Genome Sequences and Machine Learning in Source Attribution of Salmonella Typhimurium. Risk Anal. risa.13510 (2020). doi:10.1111/risa.13510.
2. Libbrecht MW & Noble W S (2015). Machine learning applications in genetics and genomics. Nat. Rev. Genet. 16, 321–332.
3. Alikhan NF, Zhou Z, Sergeant M J & Achtman M A. (2018). genomic overview of the population structure of Salmonella. PLoS Genetics 14, e1007261.

6.6 Software

R version 3.6.1 (2019-07-05)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows >= 8 x64 (build 9200)
Specific package versions:
e1071_1.7-2, purrr_0.3.3, caret_6.0-84, Boruta_6.0.0

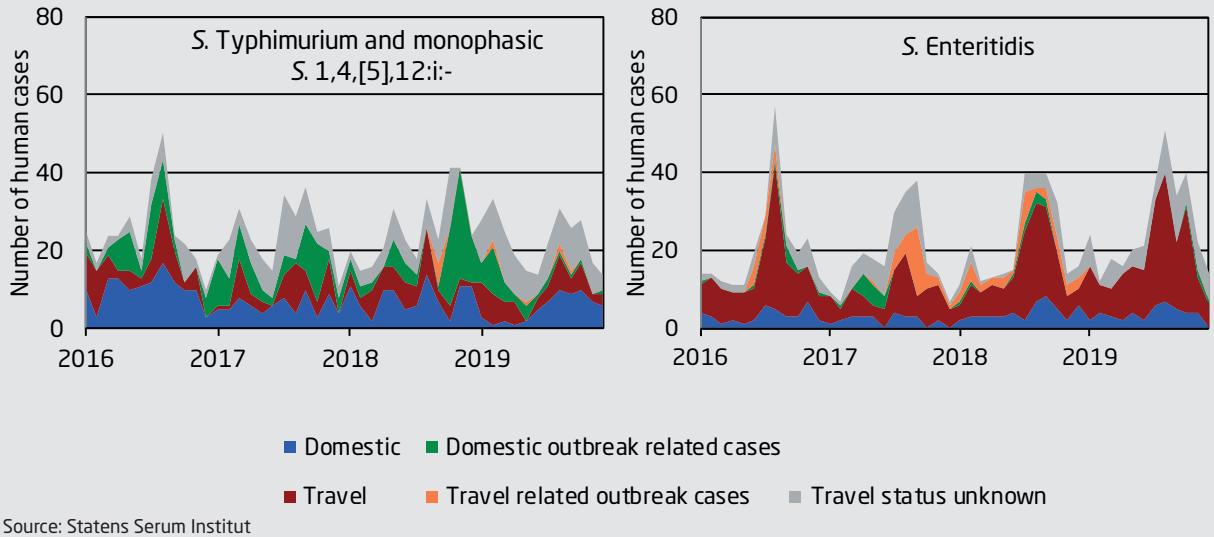
In 2019, Statens Serum Institut extracted all registered *Salmonella* cases including the available travel information from the Danish Microbiology Database (MiBa) that receives copies of reports from all Danish departments of clinical microbiology. This information was complemented with information from interviews performed by Statens Serum Institut of some of the *Samonella* cases. Travel information was available from 66.0% of the *Salmonella* cases in 2019. Among the cases with known travel history, 64.1% were infected abroad (Table 6.1). However, the proportion of travel-related cases varied greatly between the different serotypes, hence 79.7% of the *S.* Enteritidis cases, 53.4% of the *S.* Typhimurium cases, 33.0% of the monophasic *S.* Typhimurium (*S.* 1,4,[5],12:i:-) cases and 72.7% of cases with other serotypes were infected abroad (Figure 6.4). Similar to previous years, the majority of travel-related cases in 2019 travelled to Turkey, Thailand and Egypt.

Table 6.1. Top 10 Salmonella *serotypes in humans and information about travel abroad, 2018-2019*

2019	Number of patients (%)	% of patients[a] infected Abroad[b]	Domestically	2018	Number of patients (%)	% of patients[a] infected Abroad[b]	Domestcally
Enteritidis	310 (27.7)	79.7	20.3	Enteritidis	268 (22.9)	75.0	25.0
1,4,[5],12:i:-	184 (16.4)	33.0	67.0	1,4,[5],12:i:-	196 (16.7)	22.6	77.4
Typhimurium	88(7.9)	53.4	46.6	Typhimurium	110 (9.4)	45.6	54.4
Coeln	30 (2.7)	23.5	76.5	Stanley	32 (2.7)	84.6	15.4
Stanley	25 (2.2)	72.2	27.8	Newport	30 (2.6)	58.3	41.7
Paratyphi B var. Java	24 (2,1)	84.2	15.8	Dublin	26 (2.2)	6.7	93.3
Dublin	24 (2.1)	0.0	100.0	Kottbus	21 (1.8)	35.7	64.3
Infantis	22 (2.0)	47.1	52.9	Virchow	20 (1.7)	94.1	5.9
Newport	22 (2.0)	31.3	68.7	Java	18 (1.5)	100.0	0
Derby	21 (1.9)	28.6	71.4	Mikawasima	16 (1.4)	20.0	80.0
Other serotypes	281 (25.1)	72.7	27.3	Other serotypes	431 (36.9)	53.3	46.7
Total	1,120	64.1	35.9	Total	1,168	54.8	45.2

a) Patients with unknown travel information (34.0% of all paitents in 2019 and 24.2% in 2018) 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 6.4. Monthly distribution of S. Enteritidis *and* S. Typhimurium *incl. the monophasic variant* S. 1,4,[5],12:i:- *cases, 2016-2019*



Source: Statens Serum Institut

7. International topics

By Mette Rørbæk Gantzhorn (merga@fvst.dk)

7.1 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 A25.

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 2019 (Appendix Table A8).

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:- variant, *S.* Enteritidis, *S.* Hadar, *S.* Infantis and *S.* Virchow. In the legislation no distinction is made between breeding flocks from the table egg and broiler production lines. In Denmark, one breeding flock was positive for target serovars in 2019 with *S.* Hadar. (Appendix Table A5 and A7). Thereby, 0.7% 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 State 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:- variant and *S.* Enteritidis. The prevalence in Denmark has been below 2% since 2004, except for 2018, where 2.2% of flocks were found positive with target serovars. In 2019 the prevalence was again below 2%; three flocks (0.7%) were positive with target serovars (Appendix Table A5).

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:- variant. Denmark has had intensive *Salmonella* control programmes since the 90's and the target of 1% was reached in 2000. In 2019, 0.2% of broiler flocks was positive with target serovars (Appendix Table A7).

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 A26 and Table A27, 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 extracted from the Danish Microbiology Database (MiBa) or 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 Spongform 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 Patient Safety Authority and SSI. Physicians send specimens from suspected cases to one of the clinical microbiology laboratories depending on the geographical region. A copy of the results of the diagnostic analysis from regional clinical microbiology laboratory is transmitted to MiBa. All cases of infections with laboratory notifiable pathogens are collected in the Register of Enteric Pathogens maintained by SSI. *Campylobacter*, *Salmonella* and *Yersinia* cases are extracted from MiBa and STEC and *Listeria* are reported to SSI directly from the clinical microbiology laboratories. Furthermore, all *Salmonella* and STEC and a subset of Yersinia and *Campylobacter* isolates are sent to SSI for further characterisation and the results are recorded in the Register of Enteric Pathogens. 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 A1.
- STEC O-group distribution in humans is presented in Appendix Table A2.
- The *Salmonella* serovar distribution is presented in Appendix Table A4.

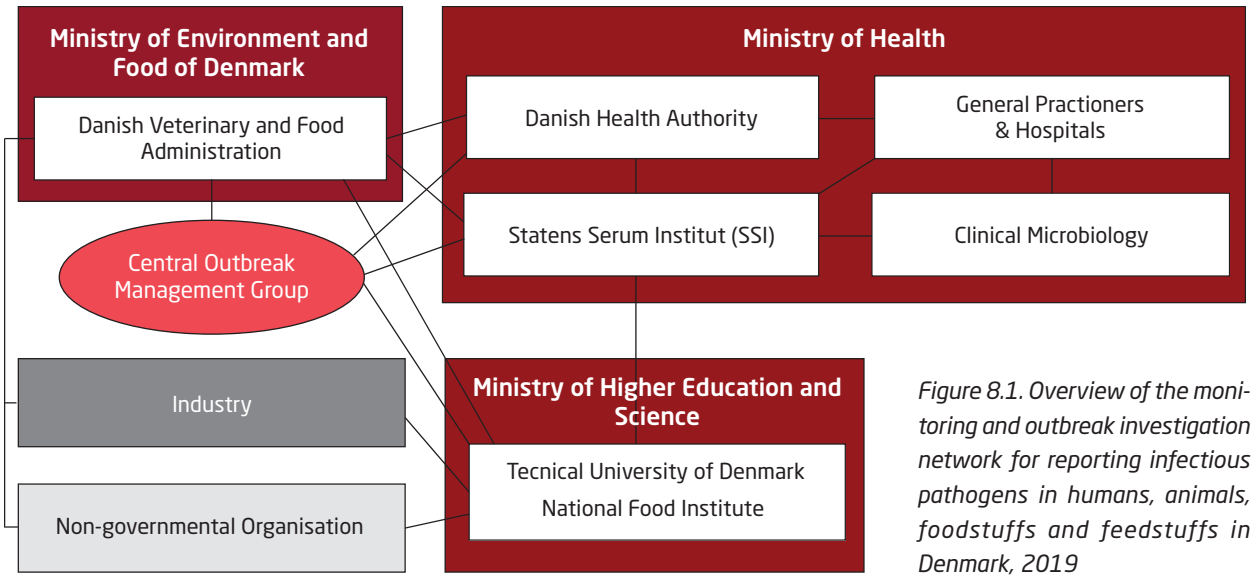


Figure 8.1. Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feedstuffs in Denmark, 2019

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. National outbreaks are investigated by SSI, the National Food Institute at the 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 regional or 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 food- and waterborne infections to the Danish Patient Safety Authority and to SSI.

A list of verified outbreaks (not including household outbreaks) reported to the Food- and waterborne Outbreak Database are presented in Appendix Table A3 and some of the outbreaks from 2019 are outlined in Chapter 4.

8.3 Surveillance and control of animals and animal products

In Denmark, action plans and programmes 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, harmonised surveillance programmes 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 A25.

Salmonella surveillance and control programmes for poultry, pigs and cattle are presented in Appendix Tables A28-33. 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 A34.

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

- Results from the table egg production are presented in Appendix Tables A5-A6.
- Results from the broiler production are presented in Appendix Tables A4 and A7.
- Results from the duck and turkey productions are presented in Appendix Tables A4 and A8.
- Results from the pig production are presented in Appendix Tables A4, A11 and Figures A1-A3.
- Results from the cattle production are presented in Appendix Tables A4, A12-A13 and Figure A4.
- Results from the rendering plants are presented in Appendix Table A14.
- Results from the feed production are presented in Appendix Tables A15-A16.
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in Appendix Tables A20-A21.

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

- Results from the broiler production are presented in Appendix Tables A9-A10.

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.

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 A11-A12.

Results from the surveillance for Bovine Spongiform Encephalopathy (BSE) in cattle, and Transmissible Spongiform Encephalopathy (TSE) in sheep/goat are presented in Appendix Tables A22-A23.

Results from the monitoring of *Coxiella burnetii* (Q fever) in cattle are presented in Appendix Table A12.

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 A20-A21.

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 A24 provides information on the centrally coordinated studies conducted in 2019.

For further information, consult the website of the DVFA, www.foedevarestyrelsen.dk (in Danish).

Human disease and outbreak data

Table A1. Zoonoses in humans, number of laboratory-confirmed cases, 2014-2019

	Incidence per 100,000 inhabitants	Reported no. of cases					
Zoonotic pathogen	2019	2019	2018	2017	2016	2015	2014
Bacteria							
Brucella abortus/melitensis[a,b]	-	7	3	3	3	6	4
Campylobacter coli/jejuni[c,e]	92.7	5,389	4,546	4,257	4,677	4,348	3,782
Chlamydia psittaci[c]	0.6	32	16	14	24	25	16
Leptospira spp.[c]	0.2	14	19	22	10	5	10
Listeria monocytogenes[c]	1.1	62	47	58	39	43	92
Mycobacterium bovis[c]	0.0	0	1	2	2	1	1
Salmonella total[c,e]	19.3	1,120	1,168	1,067	1,074	925	1,122
S. Enteritidis[c,e]	5.3	310	268	226	246	258	268
S. Typhimurium[c,d]	4.7	272	306	290	320	233	427
Other serotypes[c]	7.7	449	594	551	508	434	427
STEC total[c,e]	10.8	630	495	346	269	228	248[e]
O157	1.0	60	43	50	37	33	37
Other O-groups or non-typeable	6.2	359	259	215	204	195	192
Yersinia enterocolitica total[c,e]	6.4	374	366	354	573	539	432
Yersinia enterocolitica (Biotype 2,3 and 4)	2.4	139	-	-	-	-	-
Viruses							
Lyssavirus[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) Including the monophasic variant of *S.* Typhimurium (*S.* 1,4,[5],12:i:-).
e) Includes also only notified cases.

Source: Statens Serum Institut

Table A2. STEC O-group distribution in humans[a], 2019

O-group	Number of episodes	Proportion of total (%)	O-group	Number of episodes	Proportion of total (%)
O157	60	9.5	O111	7	1.1
O26	32	5.1	O174	5	0.8
O103	32	5.1	O8	5	0.8
O146	31	4.9	O121	5	0.8
O63	26	4.1	O54	5	0.8
O145	19	3.0	Other	84	13.3
O27	19	3.0	Unknown O-group	51	8.1
O128	13	2.1	Not verifyed[b]	114	18.1
O117	13	2.1	Notification[c]	97	15.4
O91	12	1.9			
Continued in the next column			Total	630	

a) All O-groups that resulted in five or more episodes are listed.
b) Cases sent for verification at SSI but not possible to verify and/or determine O-group.
c) Cases not sent for verification at SSI and/or only notified through the clinical notification system.

Source: Statens Serum Institut

Table A3. Food- and waterborne disease outbreaks reported in the Food- and waterborne Outbreak Database (FUD) (n=51), 2019

Pathogen[a]	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.[f]
Campylobacter jejuni, ST42[b]	31	31	Regional	Unknown	1831
Campylobacter jejuni, ST122[c]	88	88	National	Chicken meat	1782
Campylobacter jejuni, ST19	6	6	National	Chicken meat	1819
Campylobacter jejuni, ST2079	13	13	National	Chicken meat	1817
Campylobacter jejuni, ST257[c]	5	5	Regional	Unknown	1818
Campylobacter jejuni, ST257[c]	7	7	National	Chicken meat	1799
Campylobacter jejuni, ST3628	14	14	National	Unknown	1797
Campylobacter jejuni, ST42[b]	11	11	National	Chicken meat	1783
Campylobacter jejuni, ST7355	13	13	National	Chicken meat	1816
Clostridium perfringens	17	1	Restaurant	Buffet meal	1854
Clostridium perfringens	26	-	Canteen	Composite meal	1839
Clostridium perfringens	36	1	Restaurant	Composite meal	1835
Clostridium perfringens	52	-	Restaurant	Buffet meal	1829
Clostridium perfringens	17	2	Restaurant	Sandwiches	1804
Clostridium perfringens	9	2	Private party	Slow roasted beef meat	1802
Clostridium perfringens	21	2	Restaurant	Beef meat sous vide prepared	1800
Clostridium perfringens	268	-	Producer	Composite meal	1784
Clostridium perfringens	101	-	Canteen	Buffet meal	1770
Clostridium perfringens	4	-	Restaurant	Composite meal	1750
Cryptosporidium	87	3	Canteen	Buffet meal	1803
Listeria monocytogenes, ST1[e]	3	3	Retail, delicatessen	Salads	1592

Continued on the next page

Table A3. Food- and waterborne disease outbreaks reported in the Food- and waterborne Outbreak Database (FUD) (n=51), 2019 (Continued from previous page)

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.[e]
Norovirus	8	-	Restaurant	Composite meal	1870
Norovirus	15	-	Canteen	Buffet meal	1863
Norovirus	26	5	Restaurant	Composite meal	1833
Norovirus	6	5	Restaurant	Sushi, fish	1832
Norovirus	14	2	Retail bakery	Cakes	1826
Norovirus	31	5	Restaurant	Oysters (imp)	1825
Norovirus	19	4	Restaurant	Composite meal	1823
Norovirus	84	2	Canteen	Open sandwiches	1815
Norovirus	80	11	Canteen	Buffet meal	1814
Norovirus	66	6	Canteen	Buffet meal	1810
Norovirus	14	-	Retail, delicatessen	Sandwiches	1808
Norovirus	17	6	Catering	Open sandwiches	1806
Norovirus	33	3	Restaurant	Oysters (imp)	1805
Norovirus	205	5	Restaurant	Composite meal	1781
Norovirus	50	1	Restaurant	Composite meal	1776
Norovirus	180	5	Canteen	Composite meal	1775
Norovirus	9	4	Restaurant	Composite meal	1756
Norovirus	67	7	School	Buffet meal	1754
Norovirus	8	-	Private party	Oysters	1719
Salmonella Coeln, ST1995	26	26	National	Unknown	1790
Salmonella Derby, ST682	11	11	National	Pork meat	1787
Salmonella Enteritidis, ST11	8	8	National	Unknown	1845
Salmonella London, ST155	4	4	National	Unknown	1820
Salmonella Mikawasima, ST1815	3	3	International	Vegetables, lettuces	1828
Salmonella Muenchen, ST82	4	4	International	Unknown	1801
Salmonella 4,[5],12:i:-, ST34#79	14	14	National	Pork meat	1772
Salmonella 4,[5],12:i:-, ST34#107	5	5	National	Pork meat	1771
Salmonella 4,[5],12:i:-, ST34#34	57	57	International	Minced beef / beef meat	1728
STEC O157:H7, ST11	13	13	National	Unknown	1791
Yersinia enterocolitica O3:B4	20	20	International	Fresh spinach (imp)	1773
Total	1,929	441			

Note: (imp)= imported product.
a) ST= Sequence Type
b) Two distinct clusters of ST42 - FUD 1831: ST42#2 and FUD 1783: ST42#1.
c) Three additional outbreak cases in 2018
d) Two distinct clusters of ST257 - FUD 1818: ST257#5 and FUD 1799: ST257#2.
e) This outbreak consists of 11 cases from 2016 until 2019 - the last three cases in 2019 is reported. Interview with these cases revealed the common source of the outbrreak.
f) Additional outbreak cases in 2019 to outbreaks reported in previous years: FUD 1525: 2 cases; FUD 1559: 2 cases; FUD 1652: 1 case; and FUD 1713: 6 cases.

Source: Food- and waterborne Outbreak Database (FUD)

Monitoring and surveillance data

Table A4. Top 15 (humans) serotype distribution (%) of Salmonella *from humans, animals, carcasses, Danish and imported meat, 2019. N=number of culture positive units*[a]

	Human	Pig[b]	Pork[c]	Beef[d]	Broiler[e]	Layer[e]	Duck[g]	Imported meat (batches)	
	cases	animals	batches	batches	flocks	flocks	batches	Pork[f]	Broiler[f]
	N=1,120	N=118	N=133	N=5	N=12	N=8	N=4	N=24	N=18
Enteritidis	27.7	-	-	-	-	-	-	-	61.1
4,[5],12:i:-	16.4	26.3	36.1	-	25.0	12.5	-	29.2	-
Typhimurium	7.9	11.0	8.3	-	50.0	25.0	-	37.5	5.6
Coeln	2.7	-	-	-	-	12.5	-	-	-
Stanley	2.2	-	-	-	-	-	-	-	-
Paratyphi B var. java	2.1	-	-	-	-	-	-	-	-
Dublin	2.1	-	-	80.0	-	-	-	-	-
Newport	2.0	-	-	-	8.3	-	100	-	-
Infantis	2.0	4.2	2.3	-	8.3	-	-	4.2	16.7
Derby	1.9	54.2	42.1	-	8.3	12.5	-	20.8	-
Mikawasima	1.1	-	-	-	-	-	-	-	-
Agona	1.0	-	-	-	-	-	-	-	-
Braenderup	1.0	-	-	-	-	-	-	-	-
Bareilly	0.9	-	-	-	-	-	-	-	-
Virchow	0.9	-	-	-	-	-	-	-	-
Oranienburg	0.9	-	-	-	-	-	-	-	-
Other	19.4	4.2	5.3	-	-	37.5	-	8.3	16.7
Unknown	7.9	-	5.9	20.0	-	-	-	-	-

a) One isolate per serotype per unit is included, thus the number of isolates may exceed the number of units.
b) Isolates collected from caecum 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 A33).
d) Sampling of beef carcasses at slaughterhouses according to the surveillance programme (Table A32).
e) Sampling of production flocks prior to slaughter according to surveillance programmes (Tables A29).
f) Centrally coordinated study (see section 8.4 and Table A24 for more information)

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

Table A5. Occurrence of Salmonella *in the table egg production[a], 2009-2019*

	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
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	138	1	446	3
2018	7	0	6	0	124	1	454	12
2019	7	0	6	0	101	0	411	8[d]

a) See Tables A28 and A30 for description of the surveillance programmes.
b) *Salmonella* was not detected in grandparent flocks during rearing period (3 flock).
c) *Salmonella* was not detected in grandparent flocks during adult period (4 flocks).
d) *S.* 4.12:I:- (1), *S.* Coeln (1), *S.* Derby (1), *S.* Give (1), *S.* Kottbus (1), *S.* Liverpool (1), *S.* Typhimurium (2).

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

Table A6. Occurrence of Salmonella *in the table egg layer flocks sorted by type of production, 2009-2019*

	Deep litter		Free range		Organic		Cage	
	N	Positive	N	Positive	N	Positive	N	Positive
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	217	2	61	0
2018	139	4	46	1	227	4	42	3
2019	135	1[a]	34	2[b]	220	5[c]	22	0

a) *S.* Typhimurium.
b) *S.* Give (1), *S.* Kottbus(1).
c) *S.* 4.12:I:- (1), *S.* Coeln (1), *S.* Derby (1), *S.* Liverpool(1), *S.* Typhimurium (1).

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

Table A7. Occurrence of Salmonella *in the broiler production[a], 2009-2019*

	Rearing period[b] (parent flocks)		Adult period[c] (parent flocks)		Broiler flocks		Slaughterhouse[d] (flocks/batches)	
	N	Positive	N	Positive	N	Positive	N	Positive
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	250	1	4,290	25	259	0
2018	184	1	149	1	4,245	35	249	1
2019	210	0	137	1[e]	4,012	12[f]	254	0

a) See Tables A28-A29 for description of the surveillance programmes.
b) *Salmonella* was detected in 2 out of 9 grandparent flocks during rearing period (*S.* Enteritidis, *S.* Stanley).
c) *Salmonella* was not detected in grandparent flocks during adult period (5 flocks).
d) 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.
e) *S.* Hadar (1).
f) *S.* 4.5.12:I:- (2), *S.* 4.12:I:- (1), *S.* Derby (1), *S.* Infantis (1), *S.* Newport (1), *S.* Typhimurium (6).

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

Table A8. Occurrence of Salmonella *in turkey flocks, 2009-2019*

	Turkey flocks[a]	
	N	Positive
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
2018	13	0
2019	85[b]	0

a) See Table A31 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.
b) The increase in number of tested flocks is primarily based on a change of registration.

Source: Danish Veterinary and Food Administration

Table A9. Occurrence of Campylobacter *in broiler flocks, 2009-2019*[a]

	Cloacal swabs at slaughter		Sock samples at farm	
	N (Flocks)	% pos	N (Flocks)	% pos
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	-	-
2018	3,411	24.6	-	-
2019	3,327	22.7	-	-

a) See Table A29 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 Danish Order no. 1512 of 13/12/2013.

Source: Danish Agriculture and Food Council and National Veterinary Institute (until 2009)

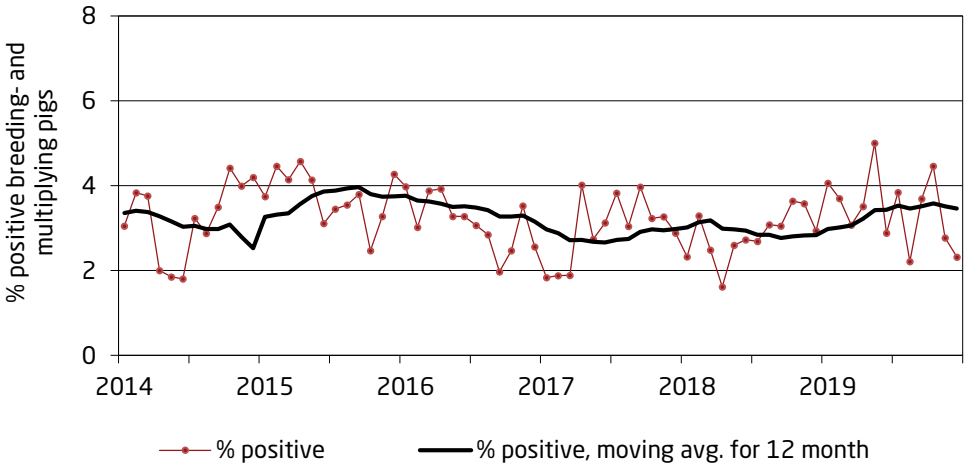
Table A10. Occurrence of Campylobacter *in non-heat treated chilled broiler meat samples at slaughter and retail*[a]*, 2014-2019*

		At slaughter[b]		At retail			
		Denmark		Denmark		Import	
		N (samples)	% pos	N (samples)	% pos[c]	N (samples)	% pos[c]
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
2017	Conventional	1,258	25.0	-	-	-	-
	Organic/free-range	203	79.0	-	-	-	-
2018	Conventional	1,250	31.0	-	-	-	-
	Organic/free-range	199	91.0	-	-	-	-
2019	Conventional	1,248	32.6	697	12.4	28	36.1
	Organic-free-range	123	68.3	155	31.6	28	82.1

a) Centrally coordinated studies (see Table A24 and section 8.4 for description). Limit of quantification: 10 cfu/g.
b) Leg-skin samples.
c) The prevalence is calculated as a mean of quarterly prevalences, except organic/free-range results.

Source: National Food Institute and Danish Veterinary and Food Administation

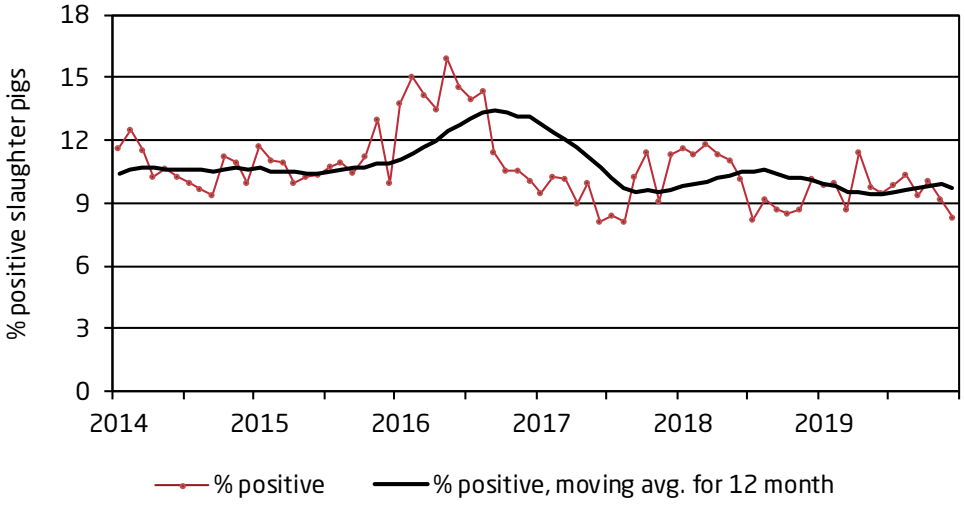
Figure A1. Serological surveillance of Salmonella *in breeding and multiplying pigs[a] based on monthly testing of blood samples, 2014-2019*



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

Source: Danish Agriculture and Food Council

Figure A2. Serological surveillance of Salmonella *in slaughter pigs[a], 2014-2019. Percentage of seropositive meat juice samples (first sample per herd per month)*



a) For more information about the surveillance programme, see Table A33.

Source: Danish Agriculture and Food Council

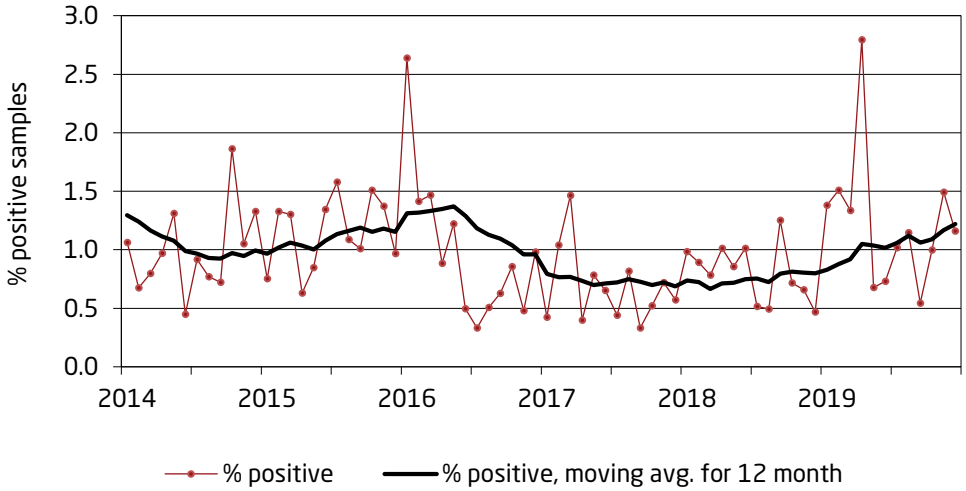
Table A11. Occurrence of zoonotic pathogens in pigs and pork in Denmark, 2019

	Herds		Animals/Samples		
Zoonotic pathogen	N	Pos	N	Pos	% pos
At farm					
Brucella spp.[a]	-	-	27,132	0	0
Leptospira spp.[b] based on suspicion	38	0	-	-	-
Leptospira spp.[c]	15	13	-	-	-
At slaughterhouse (slaughter pigs)					
Salmonella spp.[d,e]	5,465	202[j]	-	-	-
Salmonella spp.[d,f] (slaughtering >30.000 pigs/year)	-	-	17,905	-	1.2[k]
Salmonella spp.[d,f] (slaughtering 1.000 or more and less than 30.000 pigs/year)	-	-	151	-	0
Salmonella spp.[d,g]	-	-	765	118	15.4
Trichinella spp.[h]	-	-	16,146,201	0	-
Mycobacterium spp.[i]	-	-	16,754,410[l]	0	-
Echinococcus granulosis/multilocularis[h]	-	-	16,754,410[l]	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 immunoflourescence techniques.
c) Serological analyses were performed for *L. bratislava.*
d) See Table A33 for description of the *Salmonella* surveillance programme.
e) Data are from December 2019. Slaughter pig herds monitored using serological testing of meat juice samples collected at slaughter.
f) Swab samples from 4 designated areas after 12 hours chilling (4x100cm^2).
g) Caecum samples are randomly collected from slaughter pigs at slaughter.
h) 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 extempted testing for *Trichinella.* Free range pigs must be tested for *Trichinella.*
i) Slaughter pigs were examined by meat inspectors at slaughter.
j) Includes herds belonging to *Salmonella* level 2 and 3 only (See Table A33).
k) 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.
l) Includes sows and boars slaughtered.

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

Figure A3. Salmonella *in pork, monitored at slaughterhouses[a], 2014-2019*



a) For more information about the surveillance programme, see Table A33.

Source: Danish Veterinary and Food Administration

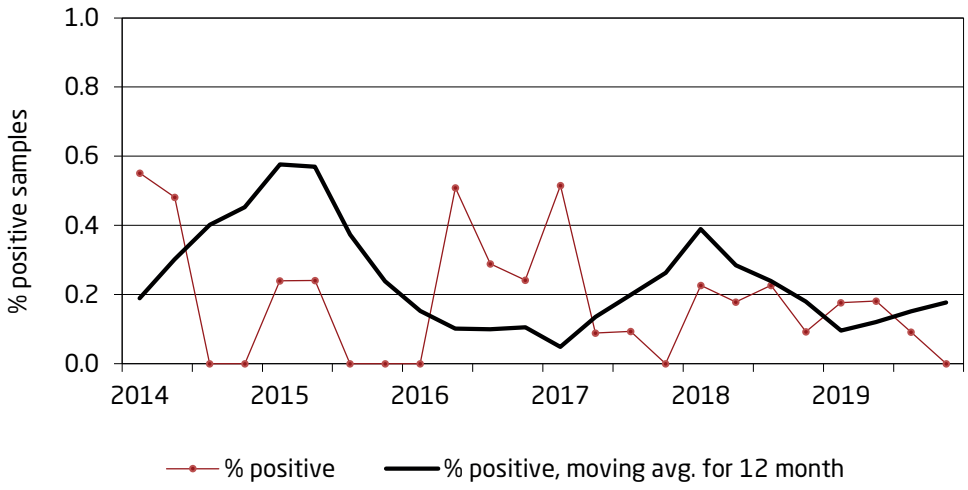
Table A12. Occurrence of zoonotic pathogens in cattle and beef in Denmark, 2019

	Animals/Samples		
Zoonotic pathogen	N	Pos	% pos
At farm			
Brucella spp.[a]	1,019	0	-
Mycobacterium bovis[b, c]	1,800	0	-
Coxiella burnetii	136[g]	3	-
At slaughterhouse			
Salmonella spp.[d,e](slaughtering >=7.500 cattle/year)	6,875	-	0.1
Salmonella spp.[d,e] (slaughtering 250 or more and 7.500 or less cattle/ year)	222	-	0
Mycobacterium spp.[b, f]	464,000	0	-
Echinococcusus granulosis/multilocularis[f]	464,000	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 intradermal 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) Swab samples from 4 designated areas after 12 hours chilling (4x100cm^2)
e) See Table A32 for description of the surveillance programme.
f) Slaughtered cattle were examined by the meat inspectors at slaughter.
g) Samples analysed using an ELISA method. Animals were tested by blood samples.
h) 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]*, 2014-2019*



a) For more information about the surveillance programme, see Table A33.

Source: Danish Veterinary and Food Administration

Table A13. Cattle herds in the Salmonella *Dublin surveillance programme[a], December 2019*

Salmonella Dublin level			Non-milk producing herds		Milk producing herds	
			N	%	N	%
Level 1		On the basis of milk samples	-	-	2,453	90.4
		On the basis of blood samples	12,684	97.4	-	-
	Total	Probably *S.* Dublin free	12,684	97.4	2,453	90.4
Level 2		Titer high in blood- or milk samples	146	1.1	207	7.6
		Contact with herds in level 2 or 3	140	1.1	26	1.0
		Other causes	51	0.4	20	0.7
Level 3		Salmonellosis, official supervision	7	0.1	7	0.3
	Total	Non *S.* Dublin free	344	2.7	260	9.6
Total number of herds			13,028		2,713	

a) See Table A32 for description of the surveillance programme.

Source: SEGES

Table A14. Salmonella *in three categories of meat and bone meal by-products not intended for human consumption[a], 2019*

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	435	7	455	3
2: By-product of this material may be used for feed for fur animals	-	-	3	0
3: By-products from healthy animals slaughtered in a slaughter-house. Products of these may be used for petfood[b] and for feed for fur animals	1,006	0	13	0
Total	1,441	7	471	3

a) Regulation (EC) No 1774 of 03/10/2002 as amended.
b) For cats and dogs. Only by-products from pigs are used in this pet food.

Source: Daka Denmark A/S

Table A15. Feed business operators own sampling of Salmonella *in compound feeds, feed processing and feed material (batch-based data), 2017-2019*

	2019		2018		2017	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control):						
Ordinary inspections - clean zone[a]	7,531	4[d]	8,018	6	7,263	7
Ordinary inspections - unclean zone[a]	1,257	25[e]	1,231	24	1,130	26
Compound feed, farm animals	1,918	1[f]	1,534	2	657	0
Feed materials, farm animals[b]	2,432	31[g]	1,734	18	1,445	22
Transport vehicles, clean zone/hygiene samples[c]	1,121	1[h]	1,141	1	1,216	0
Transport vehicles, unclean zone/hygiene samples[c]	346	3[i]	165	7	123	4

Note: Data are from one feed and grain trade organisation only, representing a proportion of feed at the Danish market.
a) Presence of *Salmonella* in compound feed is indirectly monitored by environmental samples collected during feed processing.
b) Predominantly products of soy (e.g. soybean meal) but also products of rape (e.g. rapeseed cake) and sunflower (e.g. sunflower meal).
c) Samples from transport vehicles (hygiene samples) prior to loading of feed compounds.
d) *S.* Falkensee.
e) *S.* Senftenberg, *S.* Rissen, *S.* 23:-:-, *S.* Putten, *S.* Falkensee, *S.* Kedougo, *S.* Mbandaka.
f) *S.* Falkensee.
g) *S.* Anatum, *S.* Coeln, *Salmonella* spp., *S.* Havana, *S.* Infantis, *S.* Mbandaka, *S.* Quakam, *S.* Rissen, *S.* Senftenberg, *S.* Soerenga, *S.* Yoruba, *S.* Typhimurium, *S.* Agona.
h) *S.* Typhimurium.
i) *S.* 23:-:-, *S.* 23:d:-, *Salmonella* spp..

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

Table A16. Control of Salmonella *in feed processing and feed material (batch-based data), 2017-2019*

	2019		2018		2017	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control)[a]:						
Ordinary inspections[b]	289	0	195	0	277	8
Feed materials, farm animals[c]	61	0	62	1	62	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.

Source: Danish Veterinary and Food Administration

Table A17. Listeria monocytogenes *in Danish produced ready-to-eat (RTE) foods[a], 2019*

		Samples analysed by a qualitative method[b]		Samples analysed by a quantitative method	
		Batches		Batches	
Food category	Sampling place	N	Positive	N	Positive[c]
Cheese, RTE[d]	Retail	9	0	-	-
Crustaceans, intended to be cooked	Retail	1	0	-	-
Crusteaceans, RTE[d]	Retail	2	0	-	-
	Processing plant	-	-	21	0
Dairy products (excluding cheeses), RTE[d]	Retail	1	0	-	-
Egg and egg products, RTE[d]	Retail	16	0	-	-
Fish and fishery products, intended to be cooked	Retail	8	0	-	-
	Processing plant	6	2	2	0
Fish and fishery products, RTE[d]	Retail	19	1	3	0
	Processing plant	34	1	17	0
Fruit and products made from fruit, RTE[d]	Retail	8	0	-	-
Infant formula and foodstuffs for special nutritional uses, RTE[d]	Processing plant	4	0	-	-
Products made from beef, RTE[d]	Retail	32	0	-	-
	Processing plant	4	0	8	0
Products made from beef, intended to be eaten raw	Retail	4	0	-	-
	Processing plant	2	0	2	0
Products made from mixed meat, RTE[d]	Retail	18	0	-	-
	Processing plant	1	0	13	0
Products made from pork, intended to be cooked	Retail	1	0	-	-
	Processing plant	-	-	5	0
Products made from pork, RTE[d]	Retail	84	1	1	0
	Processing plant	23	0	33	0
Products made from poultry, intended to be cooked	Retail	17	0	-	-
	Processing plant	-	-	1	0
Products made from poultry, RTE[d]	Processing plant	2	0	3	0
Other proccessed food products and prepared dishes, RTE[d]	Retail	96	3	3	1
Vegetables, RTE[d]	Retail	45	0	-	-
Total		437	8	112	1

a) Samples are collected by the local food control offices according to EU Regulation (EC) No 2073/2005.
b) *Listeria monocytogenes* present in a 25 g sample of the product.
c) Levels > 10 cfu/g.
d) Ready-to-eat.

Source: Danish Veterinary and Food Administration

Table A18. Histamine in batches of Danish and non-Danish fish products[a], 2019

	Danish		Non-Danish[b]	
Food category	N	Positive	N	Positive
Canned herring	3	0	-	-
Canned mackerel	3	0	2	0
Canned tuna	-	-	45	0
Fresh garfish	2	0	-	-
Fresh herring	2	0	1	0
Frozen fish, unspecific	-	-	1	0
Frozen mackerel	2	0	13	0
Frozen sardines	-	-	1	0
Smoked mackerel	-	-	1	0
Total	12	0	64	0

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

b) Samples from Chile, China, Colombia, Ecuador, Ghana, Greenland, Indonesia, Ireland, Mauritius, Phillippines, Poland, Seychelles, Spain, Thailand, United Kingdom and Vietnam.

Source: Danish Veterinary and Food Administration

Table A19. Salmonella *in Danish and non-Danish produced food items*[a], 2019

		Danish		Non-Danish[b]	
Food category	Sampling place	N	Positive	N	Positive
Crustaceans, intended to be cooked	At border inspection	-	-	11	0
Molluscan shellfish, intended to be cooked	At processing	-	-	25	0
Products made from beef, intended to be cooked	At border inspection	-	-	10	0
	At processing	162	0	-	-
	At retail	5	0	-	-
Products made from duck, intended to be cooked	At border inspection	-	-	5	0
Products made from pork, intended to be cooked	At border inspection	-	-	5	0
	At processing	424	8	-	-
Products made from poultry, intended to be cooked	At border inspection	-	-	15	5
	At processing	211	0	-	-
Crusteaceans, RTE[c]	At border inspection	-	-	40	0
	At processing	-	-	100	0
Molluscan shellfish, RTE[c]	At border inspection	-	-	15	0
Products made from beef, RTE[c]	At processing	30	0	-	-
	At retail	5	0	-	-
Products made from pork, RTE[c]	At processing	124	0	-	-
	At retail	5	0	-	-
Products made from poultry, RTE[c]	At border inspection	-	-	5	0
Infant formula, dried	At processing	90	0	-	-
Seeds, dried	At border inspection	-	-	25	0
Other Food	At border inspection	-	-	15	0
Total		1,056	8	271	5

a) Samples are collected by the local food control offices according to EU Regulation (EC) No 2073/2005.
b) Samples from Argentina, Belgium, Brazil, Canada, Chile, China, Greenland, India, Ireland, Netherland, Poland, Thailand and Vietnam.
c) Ready-to-eat.

Source: Danish Veterinary and Food Administration

Table A20. Occurrence of zoonotic pathogens in pets and zoo animals in Denmark[a], 2019

	Pet animals						Zoo animals			
	Dogs		Cats		Others		Mammals & reptiles		Birds	
Zoonotic pathogen	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
Chlamydia psittaci	-	-	-	-	1,079[b]	57[c]	-	-	-	-
Echinococcusus spp.	1	0	-	-	-	-	-	-	-	-
Lyssavirus (classical)	1	0	2	0	2[d]	0	-	-	-	-
European Bat *Lyssavirus*	1	0	2	0	2[d]	0	-	-	-	-

a) All samples are analysed based on suspicion of disease, and does not reflect the country prevalence.
b) Psittacidae (6), pigeon (1073)
c) Psittacidae (3), pigeon (54)
d) Sheep (1), cattle (1).

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

Table A21. Occurrence of zoonotic pathogens in wild and farmed wildlife in Denmark[a], 2019

	Farmed wildlife						Wildlife			
	Wild boar		Mink and chinchillas		Birds		Mammals		Birds	
Zoonotic pathogen	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
Echinococcus multilocularis	-	-	-	-	-	-	33[c]	0[d]	-	-
Lyssavirus (classical)	-	-	-	-	-	-	13[e]	0	-	-
European Bat *Lyssavirus*	-	-	-	-	-	-	13[e]	0	-	-
West Nile virus	-	-	-	-	400[f]	0	-	-	410[g]	13[h]

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 ot EU regulation (EC) No 2075/2005 came into force stating that slaughter pigs, sows and boars kept under "controlled housing conditions" in Denmark are extempted testing for *Trichinella*. Free range pigs, horses and wild game and other species susceptible to *Trichinella* must be tested.
c) Fox.
d) 1 sample could be positive, but needs retesting (because of COVID-19 this has not been possible jet).
e) Bat (11), fox (2).
f) Mallards (140), pheasants (160), poultry (100).
g) Migratory birds (322), dead wild birds (88).
h) Lesser whitethroat (2), whitethroat (2), willow warbler (6), redstart (2), tree pipit (1).

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

Table A22. The Bovine Spongiform Encephalopathy (BSE) surveillance programme[a] for cattle, 2019

Type of surveillance	N[b]	Positive
Active surveillance		
Slaughtered animals	-	-
Risk categories:		
Emergency slaugthers	1,705	0
Slaughterhouse antemortem inspection revealed suspicion or signs of disease	-	-
Fallen stock	22,872	0
Animals from herds under restriction	-	-
Passive surveillance		
Animals suspected of having clinical BSE	1	-
Total	24,578	0

a) According to the EU Regulation (EC) 999/2001 as amended, Commission Decision 2009/719/EC as amended and Danish Order no. 1442 of 11/12/2019 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: Danish Veterinary and Food Administration, data extraction from the EFSA database, May 2020

Table A23. The Transmissible Spongiform Encephalopathy (TSE) surveillance programme[a] for sheep and goats, 2019

Type of surveillance	N[b]	Positive
Active surveillance		
Slaugthered for human consumption	-	-
Not slaugthered for human comsumption	627	0
Fallen stock (>18 months)	-	-
Animals from herds under restriction	-	-
Passive surveillance		
Animals suspected of having clinical TSE	1	0
Total	628	0

a) According to the EU Regulation (EC) 999/2001 as amended, Commission Decision 2009/719/EC as amended and Danish Order no. 1491 of 12/12/2019 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: Danish Veterinary and Food Administration, data extraction from the EFSA database, May 2020

Table A24. Centrally coordinated studies conducted in 2019

Title of project	No. of planned samples	Pathogen surveyed	Further information
Norovirus in samples of oysters from Danish production sites	20	Norovirus, *E.coli*	To be published by Cefas
BU microbiology - slaughterhouses	50	Various	Not published
Campylobacter in minced beef-surveillance	450	*Campylobacter* spp.	To be published[a]
Campylobacter spp. in fresh, chilled Danish broiler meat at slaughterhouses (conventional)	1,250	*Campylobacter* spp.	Appendix Table 10
Campylobacter spp. in fresh, chilled Danish and imported broiler meat	1,000	*Campylobacter* spp.	To be published[a]
DANMAP - antibiotic resistance in poultry, pork and cattle	165	*E. coli, Campylobacter* spp., *Salmonella* spp., ESBL, AmpC, carbapenemase-producing *E. coli*	To be published in the 2019 DANMAP report
DANMAP and EU surveillance of antibiotic resistance in broiler, pork and cattle meat at retail (caecum samples)	660	*E. coli, Campylobacter* spp., ESBL, AmpC, carbapenemase-producing *E. coli*	To be published in the 2019 DANMAP report
EU surveillance of antibiotic resistance in retail	660	ESBL, AmpC, carbapenemase-producing *E. coli*	To be published[a]
Export - USA environmental samples	100	*Listeria monocytogens*	Not published
Export- USA swab	468	*Salmonella*	Not published
Import - intensified control of Brazilian beef and poultry meat	5	*Salmonella, Listeria monocytogenes*	Appendix Table 19 and 21
Import - microbiologic control of fish, fish products and bivale molluscan shellfish from 3rd.countries	140	*Listeria monoctogenes, Salmonella*	Appendix Table 19 and 21
Import - microbiologic control of some fishproducts - Greenland	10	*Listeria monoctogenes, Salmonella*	Appendix Table 19 and 21
Import - microbiological control of food of animal origin, excluding fish	25	*Listeria monoctogenes, Salmonella*	Appendix Table 19 and 21
Import - special control microbiology - not animal (Reg. 669/2009)	100	Various	To be published[a]
Listeria monocytogenes, Salmonella spp., *Escherichia coli* and *Staphylococci* in fish producs from Greenland	100	*Listeria monocytogenes, Salmonella spp., Escherichia coli, staphylococci*	To be published[a]
Listeria in ingredients for cold meals	500	*Listeria monocytogenes*	To be published[a]
Listeria in ingredients for hot meals	500	*Listeria monocytogenes*	To be published[a]
Listeria - WGS of isolates from official samples and follow-up on outbreaks	120	*Listeria monocytogenes*	To be published[a]
Microbiologic classification of mussel production areas in Denmark	60	*Salmonella* spp., *Escherichia coli*	To be published[a]
Part 2: Prepared meat - wholesale	450	According to Reg. 2073/2005	To be published[a]
Part 3: Ready-to-eat meat products - wholesale	450	According to Reg. 2073/2005	To be published[a]
Part 6: Fish and fish products - wholesale	250	According to Reg. 2073/2005	To be published[a]

Continued on the next page

Table A24. Centrally coordinated studies conducted in 2019 (Continued from previous page)

Title of project	No. of planned samples	Pathogen surveyed	Further information
Part 9: *Listeria monocytogenes* in ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes	240	*Listeria monocytogenes*	To be published[a]
Salmonella in imported pork and beef and in duck meat	700	*Salmonella* spp.	To be published[a]
Salmonella and resistance in pig/pork - surveillance	470	*Salmonella* spp.	To be published[a]
Salmonella and STEC in minced beef	450	*Salmonella* spp., STEC	To be published[a]
Salmonella in feed materials from feed companies	60	*Salmonella* spp.	Appendix Table 16
Salmonella in intratraded shell eggs retail	25	*Salmonella* spp.	To be published[a]
Salmonella in intratraded shell eggs wholesale	25	*Salmonella* spp.	To be published[a]
Salmonella process samples from feed companies	280	*Salmonella* spp.	Appendix Table 16
Salmonella spp. and *Escherichia coli* in raw frozen scallops from Greenland	25	*Salmonella* spp., *Escherichia coli*	To be published[a]

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

Source: Danish Veterinary and Food Administration

Table A25. Status on targets for Campylobacter *and* Salmonella, 2019

National Action Plans	Target	Status
Campylobacter in broilers 2018-2021		
Flocks at farm	Maintaining low prevalence in flocks of 17.3%	The prevalence in flocks in 2019 was 22.7%
Fresh meat at slaughterhouse	Reduction of the relative human risk (RR) by 50% compared to the level in 2013[a]	A reduction in relative risk of 6% was obtained in 2019 compared to 2013
Salmonella in poultry[b]		
Laying hen flocks of *Gallus gallus*	Initially eradication, later a reduction strategy in the table egg production	8 positive flocks (Table A5-A6) 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.	0 positive batch (Table A7) Positive batches are heat treated
Salmonella in pigs 2014-2017		
Carcasses at slaughterhouse	Max. 1% *Salmonella* at carcass level	1.2% (Table A11)
Salmonella Dublin in cattle 2017-2020		
Herds at farm	Eradication of *S.* Dublin in all herds, i.e. all herds in level 1[c]	9.6% of milk-producing herds and 2.7% of non-milk producing herds are in level 2 or 3 (January 1, 2020) (Table A13)
EU Regulations		
Regulation (EC) No. 1190/2012		
Breeding and fattening turkey flocks	Max. 1% positive for *S.* Enteritidis and *S.* Typhimurium[d]	No fattening flocks positive with target serovars (N=85) (Table A8)
Regulation (EC) No. 200/2010		
Breeding flocks of *Gallus gallus*	Max. 1% adult flocks positive for *S.* Typhimurium[d], *S.* Enteritidis, *S.* Hadar, *S.* Infantis and *S.* Virchow	0.7% (1 flock)[e] (Table A5 and A7)
Regulation (EC) No. 1168/2006		
Laying hen flocks of *Gallus gallus*	MS specific targets, for Denmark: Max. 2% adult flocks positive for *S.* Typhimurium[d] and *S.* Enteritidis	0.7% (3 flocks) positive with target serovars (Table A5)
Regulation (EC) No. 646/2007		
Broiler flocks of *Gallus gallus*	Max. 1% positive *S.* Typhimurium[d] and *S.* Enteritidis	0.2% (9 flocks) positive with target serovars (Table A7)

a) 2013 is agreed as the baseline since 2012 data are not compareable with data from 2013 and onwards due to a nessessary improvent in the data collection.
b) Supplementary to EU-regulations.
c) See Table A32 for explanation of the herd levels.
d) Including the monophasic variant of *S.* Typhimurium (*S.* 1,4,[5],12:i:-).
e) One flock positive for *S.* Hadar

Source: Danish Veterinary and Food Administration

Monitoring and surveillance programmes

Table A26. Overview of notifiable and non-notifiable human diseases presented in this report, 2019

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

a) Danish Order no. 277 of 14/04/2000. Cases must be notified to Statens Serum Institut.
b) The regional microbiological laboratories report confirmed cases.
c) The physician report individually notifiable infections.
d) The laboratories voluntarily report confirmed cases.

Source: Statens Serum Institut

Table A27. Overview of notifiable and non-notifiable animal diseases presented in this report, 2019

Patogen	Notifiable	EU legislation	Danish legislation
Bacteria			
Brucella spp.	1920[a]		
Cattle	OBF in 1979[b]	Decision 2003/467/EC	Order no 305 of 3/5/2000
Sheep and goats	ObmF in 1995[c]	Decision 2003/467/EC	Order no. 739 of 21/8/2001
Pigs	No cases since 1999	Directive 2003/99/EC	Order no. 575 of 29/5/2018
Campylobacter spp.	no	-	-
Chlamydophila psittaci	-	-	-
Birds and poultry	1920	-	Order no. 575 of 30/5/2017
Listeria monocytogenes	no	-	-
Leptospira spp. (only in production animals)	2003	-	Order no. 532 of 25/5/2018
Mycobacterium bovis/tuberculosis	1920[a]		
Cattle	OTF in 1980[d]	Decision 2003/467/EC	Order no. 1417 of 11/12/2007 (Order no. 1079 of 6/10/2014)
Coxiella burnetii	2005	-	Order no. 532 of 25/5/2018
Salmonella spp.	1993[e]		
Cattle		-	Order no. 1687 of 18/12/2018
Swine		-	Order no. 1426 of 30/11/2018
Eggs for consumption		-	Order no. 1422 of 30/11/2018
Hatching eggs		-	Order no. 1423 of 30/11/2018
Poultry for slaugther		-	Order no. 1273 of 30/11/2018
STEC	no	-	-
Yersinia enterocolitica	no	-	-
Parasites			
Cryptosporidium spp.	no	-	-
Echinococcus multilocularis	2004	Council Directive 64/433/EC	Order no. 532 of 25/5/2018
Echinococcus granulosus	1993	Council Directive 64/433/EC	Order no. 532 of 25/5/2018
Trichinella spp.	1920[a]	Regulation (EU) 2015/1375	Order no. 1714 of 15/12/2015
Viruses			
Lyssavirus (Rabies)	1920	-	Order no. 330 of 14/04/2011
Prions			
TSE			
Sheep and goats	yes	Regulation 999/2001/EC (as amended)	Order no. 1288 of 20/12/2011
BSE			
Cattle	yes[f]	Regulation 999/2001/EC (as amended)	Order no. 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 Commision Decision 2003/467/EC. No cases in since 1962.
c) Officially *Brucella melitensis* Free (ObmF) according to Council Directive 91/68/EC and Commision Decision 2003/467/EC. 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 neglible risk for BSE at World Organisation for Animal Health (OIE) general session in May 2011.

Source: Danish Veterinary and Food Administration

Table A28. Salmonella *surveillance programme for the rearing flocks and adult flocks of the grandparent and parent generation of the broiler and table egg production, 2019*

Time	Samples taken	Material	Material
Rearing flocks		*Grandparent generation*	*Parent generation*
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 (>1 m^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		*Grandparent generation*	*Parent generation*
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 Danish Order no. 1423 of 30/11/2018.
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 A29. Salmonella *and* Campylobacter *surveillance programme for the broiler flocks, 2019*

Time	Samples taken	Material
Salmonella		
15 - 21 days before slaughter[a,b,c]	Per flock	5 pairs of boot swabs
7 - 10 days before slaughter[d,e]	Per flock	5 pairs of boot swabs
After slaughter[b,d,f]	Per batch	From slaughterhouses slaughtering 1,000 chickens or hens per day or more: 300 neck skin samples of 1 gram, pooled into subsamples of 60 gram from one batch per week. From slaughterhouses slaughtering less than 1,000 chickens or hens per day: 15 neck skin samples of approx. 10 gram pooled into 5 subsamples of 25 gram from one batch every fifth day of slaughter
Campylobacter		
After slaughter[b,d]	Per flock	12 cloacal swabs from 24 animals, analysed in one pool[g,h]
After slaughter[b,f]	Per batch	From slaughterhouses slaughtering 1,000,000 chickens or more per year: 15 neck skin samples of approx 10 gram, pooled into five subsamples of 25 gram from one batch per week. From slaughterhouses slaughtering less than 1,000,000 chickens per year and more than 10,000: 15 neck skin samples of approx. 10 gram pooled into 5 subsamples of 25 gram from one batch every tenth day of slaughter

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 Danish Order no. 1424 of 30/11/2018.
e) Samples are collected by a representative of the slaughterhouse, laboratorium 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 A30. Salmonella *surveillance programme for the pullet-rearing, table egg layer and barnyard/hobby flocks in the table egg production, 2019*

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 *S.* Enteritidis, *S.* Hadar, *S.* Infantis, *S.* Virchow or *S.* Typhimurium including the monophasic variant *S.* 1,4,[5],12: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)[g]
Barnyard and hobby flocks[e]		
Every 18 weeks[a,b,f]	Per flock	Egg samples (serology)

a) Sampling requirements set out by Danish Order no. 1422 of 30/11/2018.
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, who are informed about the fact that no *Salmonella* control was performed.
g) If samples are negative, sampling is repeated 14 days later.

Source: Danish Veterinary and Food Administration

Table A31. Salmonella *surveillance programme for the turkey flocks, 2019*

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) 1190/2012 and Danish Order no. 1424 of 30/11/2018.
b) Samples collected by the food business operator or the local food control offices.

Source: Danish Veterinary and Food Administration

Table A32. Salmonella *surveillance programme[a] for the cattle production, 2019*

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, analysed 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, analysed 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) Danish Order no. 1687 of 18/12/2018 as amended. In 2013 and 2014, the programme for eradication of *Salmonella* Dublin from the Danish cattle production was intensified. This implies compulsory eradication in Level 2 and 3 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: Danish Veterinary and Food Administration and SEGES

Table A33. Salmonella *surveillance programme[a] for the pig production, 2019*

Time	Samples taken	Purpose/Comment
Breeding and multiplier herds		
Every month	10 blood samples per epidemiological unit	Calculation of *Salmonella*-index based on the mean seroreaction from the last three months with more weight to the results from the more recent months (1:3:6)[b]
Max. twice per year	Herds with *Salmonella*-index 5 or above: Pen-faecal samples	Clarify distribution and type of infection in the herd[c]
Sow herds		
When purchaser of piglets is assigned to level 2 or 3, max. twice per year	Pen-faecal samples	Clarify distribution and type of infection in the herd, and possible transmission from sow herds to slaughter pig herds
Herds positive with *S.* Typhimurium, *S.* Infantis, *S.* Derby and *S.* Choleraesuis are considered positive for the following 5 years[d]	No samples are collected from the herd during the 5 year period when the herd is considered positive, unless the herd is proven negative	Reduce repeated sampling in positive herds infected with a persistent serotype
Slaughter pigs, herds		
At slaughter	Meat juice, 60-100 samples per herd per year. Herds in RBOV[e]: one meat juice sample per month	Calculation of slaughter pig index based on the mean proportion of positive samples from the last three months with most weight to the result from the most recent month (1:1:3)[f]. Assigning herds to level 1-3 and assigning herds to risk-based surveillance (RBOV)[e, f]
Slaughter pigs, animals		
At slaughter[g]	Caecum 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 Danish Order no. 539 of 03/06/2016, replaced by Danish Order no. 1426 of 30/11/2018.
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 variant *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 A24).

Source: Danish Veterinary and Food Administration

Table A34. Typing methods used in the surveillance of foodborne pathogens in Denmark, 2019

Methods	Human	Food	Animal
Salmonella enterica			
Serotyping	All isolates (mainly WGS)	All isolates (by WGS)	All isolates (by WGS)
Antimicrobial resistance testing	All *Salmonella* except *S.* Enteritidis	Almost all isolates	Isolates for DANMAP and EFSA
MLVA	In relation to International outbreak	None	None
WGS	All isolates	All isolates	All isolates
Campylobacter coli/jejuni			
Antimicrobial resistance testing	Isolates from 4 districts for DANMAP surveillance	Isolates for DANMAP and EFSA	Isolates for DANMAP and EFSA
WGS	Subset representing 10-15% of isolates	Few (isolates from chilled chicken meat)	None
STEC			
Serotyping	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
WGS	All isolates	All isolates	None
Listeria			
WGS	All isolates	Selected isolates (ST typing and outbreak investigations)	None
Yersinia Enterocolitica			
serotype	All pathogenig isolates sent to SSI	None	None
WGS	Outbreaks investigations, research	None	None

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

Population and slaughter data

Table A35. Human population, 2019

Age groups (years)	Males	Females	Total
0-4	157,512	148,737	306,249
5-14	332,462	315,896	648,358
15-24	371,928	355,465	727,393
25-44	735,893	715,703	1,451,596
45-64	768,169	765,708	1,533,877
65+	527,992	618,996	1,146,988
Total	2,893,956	2,920,505	5,814,461

Source: Statistics Denmark, 1 July 2019

Table A36. Number of establishments, livestock and animals slaughtered, 2019

	No. of establishments	Livestock (capacity)	Number slaughtered
Slaughter pigs	7,345	13,350,704	16,754,410
Cattle	16,101	1,505,474	464,000
Broilers	247	18,924,398	104,155,000
Layers (excl. barnyard)	173	4,767,399	-
Turkeys	29	327,588	200
Sheep & lambs	6,211	143,080	71,000
Goats	2,980	19,744	-
Horses	-	-	820

Source: Statistics Denmark and Danish Veterinary and Food Administration - the Central Husbandry Register, May 2020 and 1 July 2019

Table A37. Number of establishments, flocks and livestock capacity in the broiler production, 2019

	No. of establishments	No. of flocks	Livestock (capacity)
Rearing period (grandparent)	2	10	50,000
Adult period (grandparent)	3	10	82,500
Rearing period (parent)	21	98	770,810
Adult period (parent)	41	140	1,108,900
Hatcheries	5	-	-
Broilers	247	619	18,924,398

Source: Danish Veterinary and Food Administration, March 2020

Table A38. Number of establishments, flocks and livestock capacity in the table egg production, 2019

	No. of establishments	No. of flocks	Livestock (capacity)
Rearing period (grandparent)	2	2	47,500
Adult period (grandparent)	2	7	75,000
Rearing period (parent)	7	7	23,010
Adult period (parent)	8	9	43,556
Hatcheries	4	-	-
Pullet-rearing	42	67	1,095,289
Layers (excl. barnyard)	173	281	4,767,399

Source: Danish Veterinary and Food Administration, March 2020

List of Figures

List of Tables

Contributing institutions:

National Food Institute
Technical University of Denmark
Kemitorvet
Building 202
DK - 2800 Kgs. Lyngby
Tel: +45 3588 7000
E-mail: food@food.dtu.dk
www.food.dtu.dk

Statens Serum Institut
Artillerivej 5
DK - 2300 København S
Tel: +45 3268 3268
E-mail: serum@ssi.dk
www.ssi.dk

The Danish Veterinary and Food Administration
Stationsparken 31-33
DK - 2600 Glostrup
Tel: +45 7227 6900
E-mail: fvst@fvst.dk
www.fvst.dk

National Veterinary Institute
Technical University of Denmark
Kemitorvet
Building 202
DK - 2800 Kgs. Lyngby
Tel: +45 3588 6000
E-mail: vet@vet.dtu.dk
www.vet.dtu.dk

Danish Agriculture and Food Council
Axelborg, Axeltorv 3
DK - 1609 Copenhagen V
Tel: +45 3339 4000
E-mail: info@lf.dk
www.lf.dk

National Food Institute
Technical University of Denmark
Kemitorvet, Building 202
DK - 2800 Kgs. Lyngby

T: 35 88 70 00
F: 35 88 70 01
www.food.dtu.dk

ISSN: 1600-3837