

# Annual Report on Zoonoses in Denmark 2008



## **Annual Report on Zoonoses in Denmark 2008**

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## Introduction

Annual Report on Zoonoses presents a summary of the trends and sources of zoonotic infections in humans and animals and the occurrence of zoonotic agents in food and feeding stuffs in Denmark in 2008. Greenland and the Faeroe Islands are not covered by the report. The report is based on data from the national surveillance and control programmes including data collected according to Zoonoses Directive 2003/99/EC, supplemented by data from relevant research projects. The report is also available at www.food.dtu.dk.

A general description of the surveillance and monitoring of zoonoses is an important part of this report. All surveillance programmes and results are presented in appendix tables, and selected results are also presented in a narrative form.

This years report contains focused chapters on the typing methods used for surveillance and outbreak detection and on Verocytotoxin-producing *Escherichia coli* in Denmark. In addition, the 2008 *Salmonella* source account and descriptions of the most interesting outbreaks are presented in separate chapters. Please note that corrections to the data may occur after publication, resulting in minor changes in the presentation of historical data in the following years reports.

## Profile of the year

In 2008, there were several *Salmonella* Typhimurium outbreaks and, in particular, one very large *S*. Typhimurium phage type U292 outbreak with 1,224 registered cases in 2008 was the subject of an intensive investigation, but the source of the outbreak remained unknown. The outbreak is the largest known *Salmonella* outbreak in Denmark to date. Other relatively large outbreaks caused by *S*. Typhimurium strains belonging to the phage types DT135, DT120 and U288 also occurred.

The number of human *Salmonella* cases estimated to be due to travelling was at the same level as in 2007 (the first year with active surveillance for infections acquired abroad), however the number of cases attributed to Danish pork increased three-fold mainly due to the large outbreaks.

Infections with verocytotoxin-producing *E. coli* are of particular concern because of their relative seriousness. Approximately half of the country is covered by molecular diagnostics; at 2.9 cases per 100.000 population, the incidence was at the same level as in recent years. A case-control study of risk factors for sporadic infections indicated strawberries and contaminated public water supply as risk factors.

# 1. Trends and sources in human salmonellosis

By Sara Monteiro Pires (smpi@food.dtu.dk) and Tine Hald

#### 1.1 Salmonella source account 2008

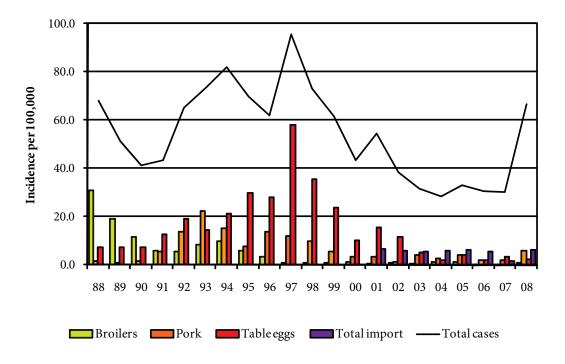
The Danish Zoonosis Centre routinely applies a so-called "source attribution" model to estimate the contribution of the major animal-food sources to human infections of *Salmonella*. The principle of the method is to compare the number of human cases caused by different Salmonella sero- and phage types with the distribution of the same subtypes isolated from the various animal-food sources. Antimicrobial resistance profiles of S. Typhimurium isolates are also included to further distinguish between similar phage types found in animals, food and humans. Since the model was first implemented in 1995, it has evolved from being purely deterministic to becoming a stochastic model, built under a Bayesian framework. In 2008, a new methodological development was introduced in the model (1), which applies data from multiple years thereby improving the robustness and accuracy of the results without compromising their comparability with estimates from previous

years. The proportion of cases that can be attributable to the major food sources is presented in Figure 1.1.

The incidence of human salmonellosis in 2008 was 66.8 cases per 100,000 inhabitants (11.7 for *S*. Enteritidis and 36.6 for *S*. Typhimurium) (appendix B, Table A2). The estimated mean incidence per 100,000 inhabitants attributed to the various food-animal sources was 28.9 for cases related to outbreaks, 15.6 for cases related to travel, 5.8 for pork, 3.5 for imported chicken, 2.1 for table eggs, 0.9 for broilers, 0.7 for ducks, 0.7 for imported pork, 0.5 for beef, 0.2 for imported beef and 8.8 for cases related to unknown sources.

In 2008, 1.572 human salmonellosis cases (43.0% of all cases) were associated with outbreaks. The number of outbreak-related cases that could not be linked to any source constituted 39.6% of all cases (Figure 1.2). This represents a substantial increase when compared to previous years, and is explained by the occurrence of one large long-lasting outbreak caused by *S.* Typhimurium U292 and also several smaller outbreaks (appendix A, Table A1). See Chapter 2 for further information on the outbreaks.

Figure 1.1. Total incidence of human salmonellosis and estimated human incidence due to broilers, pork, table eggs and imported foods in Denmark, 1988 to 2008



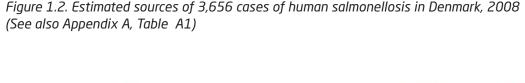
In total, 23.3% of all reported cases were estimated to be acquired abroad; among the 2,084 reported sporadic cases (excluding outbreak related cases), it was estimated that 41.0% of the cases were acquired abroad. In 2008, Statens Serum Institut attempted to interview all patients with diagnosed *Salmonella* infections and no existing travel information. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease onset. These data was complemented with information from general practitioners' reports. Travel information was available from a total of 86% of the reported cases. Among all reported cases of salmonellosis, 853 were estimated to be acquired abroad.

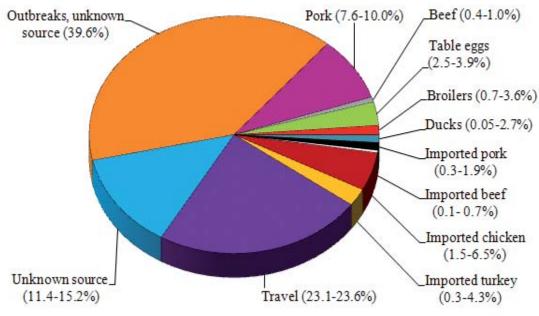
Pork was estimated to be the most important source of salmonellosis in 2008 (8.8%), followed by imported chicken (5.2%) and table eggs (3.2%) (appendix A, Table A1). The estimated number of cases attributed to the consumption of pork increased three-fold compared to 2007. This increase is partly explained by the occurrence of an unusual number of pork-related outbreaks in 2008. Likewise, the contribution of imported chicken to human salmonellosis markedly increased in 2008, and the number of cases attributed to this source was three times higher than in 2007. The relative contribution of table eggs to salmonellosis in humans decreased with 35.9% from 181 cases in 2007 to 116 in 2008.

In 2008, 547 *Salmonella* cases were attributed to domestic products, which is an increase compared to 2007 where 312 of the cases were attributed to domestic products. The increase is mainly due to the increase in the number of cases attributed to pork.

In total, 329 of all cases could be attributed to imported foods in 2008. The number of cases associated with imported foods almost tripled from 114 cases in 2007 and is at the same level as in 2006 (Appendix A, Table A1). The increase is mainly due to an increase in the number of cases atributed to imported chicken and to a lesser extent imported turkey. Despite of the increase in total number of cases due to imported foods, the relative proportion of cases attributed to imported foods decreased in 2007 and 2008 to half the level compared to 2006. This is partly explained by the improved travel information obtained in the last two years and, in 2008, by the many cases associated with Salmonella outbreaks. There is a considerable overlap between Salmonella subtypes isolated from patients that acquired the infection abroad and from imported foods. The improved travel information allowed for the more accurate identification of international travel as the source of infection.

The proportion of sporadic cases with unknown source of origin decreased from 23.4% in 2007 to 13.1% in 2008. This decrease is explained by the overall weight of out-





break-related cases in the total number of reported cases of salmonellosis. When analysing sporadic cases only, 23.0% of *Salmonella* cases could not be attributed to any known source, similar to 2007. These cases may be caused by foods not included in the national surveillance (e.g. imported or domestically produced fruits and vegetables), or by nonfood sources of infection.

Of the 638 S. Enteritidis cases, 60.8% was estimated to be related to international travel and only 8 cases (1.3%) were associated with outbreaks. Among the 2,002 S. Typhimurium cases, 74.0% was part of outbreaks and only 6.5% was estimated to be acquired abroad.

The majority of *S*. Typhimurium cases (75.0%) attributable to domestic products was caused by types susceptible to all antimicrobials, whereas 21.0% was caused by types resistant to one to three antimicrobial drugs, and 3.9% was caused by types resistant to four or more antimicrobial drugs (multi-resistant). In contrast, 38.8% of infections attributed to imported foods was caused by resistant types and 24.4% by multi-resistant types. The same trend was

observed in cases acquired abroad, where the majority of cases was caused by resistant and multi-resistant types (35.8% and 25.5%, respectively). Additionally, 11.9% of travel-related cases was caused by *S*. Typhimurium isolates resistant to quinolones. This resistance profile was not found in any of the Danish isolates.

The number of human cases of salmonellosis reported in 2008 reached a level not observed since the late 90's. This increase was mainly due to the unusual high number of outbreak-related cases, where most cases could not be attributed to any source. However, the number of cases attributable to food sources like Danish pork and imported poultry products also increased substantially in 2008.

#### References

(1) Pires SM, Hald T (2009). Assessing the differences in public-health impact of *Salmonella* subtypes using a Bayesian microbial subtyping approach for source attribution. Foodborne Pathogens and Diseases, 7(2).

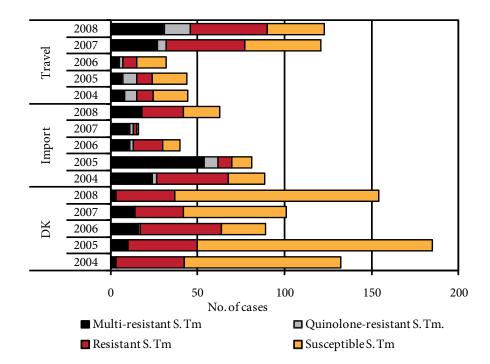


Figure 1.3. Sources of antimicrobial resistant S. Typhimurium infections in humans, 2004-2008







## 2. Outbreaks of special interest

#### By Steen Ethelberg (set@ssi.dk)

In Denmark, foodborne outbreaks are investigated by a number of different institutions, depending on the nature of the outbreak. Large, cross-regional foodborne outbreaks are typically investigated by Statens Serum Institut, the National Food Institute, Technical University of Denmark and the Danish Veterinary and Food Administration, whereas local outbreaks are handled by the Regional Veterinary and Food Control Authority in collaboration with the medical officer.

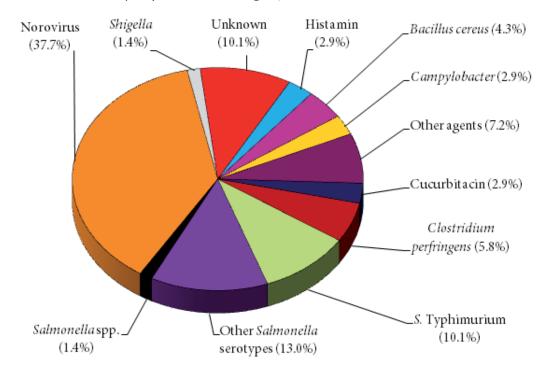
Outbreaks are reported in the Food- and waterborne Outbreaks Database (FUD) and all verified outbreaks in 2008 are presented in Appendix B, Table A4. Household outbreaks are not included in the table. The relative distribution of the outbreaks due to the different pathogens is presented in Figure 2.1. The reporting and outbreak investigation systems are described in further detail in Chapter 6.2. Some of the more notable outbreaks are outlined below.

2008 was an unusual year because of a very large outbreak of *Salmonella* Typhimurium. A total of 1,224 cases of *S.* Typhimurium U292 belonging to the same MLVA

cluster (FUD no. 788) were registered in what was the largest known *Salmonella* outbreak in Denmark to date (1-4). The outbreak was detected April 1<sup>st</sup> and during the summer 30 to 60 new cases appeared every week, gradually decreasing over the autumn and winter. This outbreak has been the subject of a very large and intensive investigation, among others including measures such as:

- A large number of trawling questionnaires
- Case-control and cohort analyses
- Investigations of a number of slaughter houses and food production facilities
- Comparative molecular subtyping of relevant isolates from many different sources
- Structured microbiological analyses of food samples from patients homes
- Investigation of shopping records obtained from supermarket computers
- Epi and trace-back analyses of embedded outbreaks where several persons have been ill when participating in the same event
- Risk ranking of slaughterhouses, shops food items and animal herds.

Figure 2.1. Aetiology of foodborne disease outbreaks reported with a causative agent in the Food- and waterborne Outbreak Database (FUD), 2008. Percentage of total outbreaks indicated in brackets



As of November 2009, the source of this outbreak is still not known. Only very few cases with the outbreak strain have been detected outside of Denmark and the main hypothesis remains that the outbreak is caused by a series of different foods and originates from a pig reservoir.

Two other relatively large outbreaks caused by *S*. Typhimurium strains belonging to the otherwise rare phage types DT135 (FUD no. 854) and DT3 (FUD no. 853) also occurred and also remain unsolved (2). Both outbreaks show similarities with the U292 outbreak and may share the same underlying cause.

Several other outbreaks with *S*. Typhimurium took place as well. One outbreak with phage type DT120 comprised 53 cases and occurred during June and July. This outbreak was solved as a spin-off of the U292 outbreak investigation when smoked ham sampled from the home of a case (the food samples were collected prior to MLVA typing) was found positive for the DT120 outbreak strain.

Two outbreaks with phage type U288 occurred; they were unrelated and belonged to different MLVA types. The first of these outbreaks was localised to central Jutland and comprised 37 cases (among which were two Norwegian tourists) in the spring of 2008 (FUD no. 793); it was traced back to a small group of shawarma restaurants. The second U288 outbreak occurred in the autumn and winter of 2008 and comprised 39 registered cases (FUD no. 855) most of whom lived on Zealand; four of the patients died. This outbreak was caused by pork in different forms; the outbreak strain was isolated from pork meat from different food producers that were supplied from the same slaughterhouse where the outbreak strain was also isolated. Part of the contaminated pork meat was sold to Sweden and gave rise to illness in both Sweden and Norway (5).

Finally, a comparable outbreak with phage type U312 took place in the winter 2008/09 (FUD no. 863). In total, it comprised 42 cases (of which some occurred in 2009) and this outbreak was also caused by different forms of pork meat that originated from a specific slaughterhouse (6).

Norovirus is not a zoonosis, but it should be mentioned that, as in previous years, norovirus was the most frequent disease agent in the registered outbreaks. Of the 69 foodborne outbreaks in 2008, norovirus accounted for 26 with an average of 30 patients. These outbreaks were generally a result of contamination events associated with workplace lunch buffets, restaurants and private parties. Several of these outbreaks followed gastrointestinal symptoms in persons preparing the food.

#### References

- (1) Ethelberg S, Wingstrand A, Jensen T, Sørensen G, Müller L, Lisby M, Nielsen EM, Mølbak K (2008). Large outbreaks of *Salmonella* Typhimurium infection in Denmark in 2008. Euro Surveill; 13(44).
- (2) Ethelberg S, Wingstrand A, Jensen T, Sørensen G, Müller L, Nielsen EM, Mølbak K (2008). Large ongoing outbreak of infection with *Salmonella* Typhimurium U292 in Denmark, February-July 2008. Euro Surveill 13(28).
  - (3) Statens Serum Institut (2008): Epi-news, week 27-33.
  - (4) Statens Serum Institut (2008): Epi-news, week 49.
- (5) Bruun T, Sørensen G, Forshell LP, Jensen T, Nygard K, Kapperud G, Lindstedt BA, Berglund T, Wingstrand A, Petersen RF, Müller L, Kjelsø C, Ivarsson S, Hjertqvist M, Löfdahl S, Ethelberg S (2009). An outbreak of *Salmonella* Typhimurium infections in Denmark, Norway and Sweden, 2008. Euro Surveill; 14(10).
  - (6) Statens Serum Institut (2009): Epi-news, week 9.



# 3. Typing methods in the surveillance of foodborne pathogens

## By Eva Møller Nielsen (emn@ssi.dk), Gitte Sørensen, Dorte Lau Baggesen and Karl Pedersen

Characterisation – or typing – of bacterial isolates is an important tool for surveillance, source finding, outbreak detection, outbreak investigations, and source attribution. The methods used range from conventional phenotypic methods, such as serotyping and phage typing, to high-discriminatory molecular methods. The development of new, faster and cheaper molecular methods in recent years has made it possible to use these methods as an integrated part of surveillance of foodborne infections in human patients as well as in the food chain. Here, we briefly describe how the methods are used in the surveillance of foodborne pathogens in Denmark and how this has developed in recent years.

### 3.1 Surveillance of human infections

For some of the gastrointestinal infections, the bacterial isolates undergo a routine characterisation in the primary laboratory - typically at a regional clinical microbiological laboratory - where the pathogen is cultured from stool samples. For example, the two most prevalent Salmonella serotypes (S. Enteritidis and S. Typhimurium) are usually determined at this stage. For others, no characterisation is made beyond species or even genus level, e.g. thermophilic Campylobacter are designated C. coli/jejuni without species determination or any further typing. All isolates of Salmonella, verocytotoxin-producing E. coli (VTEC), and Listeria are sent to Statens Serum Institut for further characterisation (Table 3.1). Isolates of S. Typhimurium and S. Enteritidis are sent to the National Food Institute, Techical University of Denmark for phage typing. Campylobacter isolates from three selected geographic areas, covering approx. 25% of the country, are submitted to Statens Serum Institut.

## 3.2 Surveillance of food and animal isolates

Salmonella surveillance programmes are active on both production animals and food, although with different intensity. The most thorough surveillance is carried out in the poultry sector, where the Danish control programme for surveillance and eradication of Salmonella was launched in 1996. The programme has been modified several times over the years and comprehensive surveillance is still taking place at all production stages, i.e. breeding and

parent flocks, table egg layers and broilers. Every flock is tested for *Salmonella* by culture (breeding and parent flocks, layers and broilers) and serology (layers). See appendix D, tables A32-A34 for detailed information on surveillance programmes. All poultry isolates positive for *Salmonella* must be submitted to the National Veterinary Institute, Technical University of Denmark for serotyping. Isolates of *S.* Typhimurium and *S.* Enteritidis are then forwarded to the National Food Institute for phage typing.

Only very few flocks of duck or turkey are slaughtered in Denmark, as most are exported as live animals. Isolates collected before slaughter are analysed at private laboratories and positive isolates are sent to the National Veterinary Institute.

In pigs, there is a serological *Salmonella* surveillance programme in slaughter pig herds based on meat juice samples collected at the slaughterhouse, and in breeder and multiplier herds based on monthly blood samples from all herds. See appendix D, Table A37 for detailed information on the surveillance programmes. A high *Salmonella* index results in compulsory bacteriological examination of faecal samples from the pens. Isolates from these samples are sent for typing to the National Food Institute. A high *Salmonella* index in the slaughter pig herds will also result in compulsory collection of pen faecal samples in sow herds that have delivered pigs to the slaughter pig herd. This is done to clarify distribution and type of infection in the herd and also to clarify possible transmission from sow herds to slaughterpig herds.

In cattle, *Salmonella* surveillance is active in the primary production on the basis of serology specifically directed against *S*. Dublin. See appendix D, Table A36 for detailed information on the surveillance programme. In the dairy sector, serology is based on tank milk samples, and in beef cattle, serology is based on blood samples.

Clinical outbreaks of salmonellosis occasionally occur in pig and cattle herds, most often caused by *S*. Dublin (cattle) or *S*. Typhimurium, and isolates from such outbreaks are submitted to the National Food Institute for serotyping, phage typing (*S*. Typhimurium and *S*. Enteritidis) and antimicrobial susceptibility testing.

In food, there is surveillance for *Salmonella* in meat, in particular on pig and cattle carcasses at the slaughterhouses, however, other food products, both domestic and imported foods, are also examined. Isolates of *Salmonella* must be submitted to the National Food Institute for typing.

Table 3.1. Methods used in the surveillance of foodborne pathogens in Denmark

Dath a sa:	Mathada		Isolates				
Pathogen	Methods	Human	Food	Animal			
Salmonella	enterica						
	Serotype	All	All	All			
	Phage type	<ul><li>S. Typhimurium and</li><li>S. Enteritidis</li></ul>	<ul><li>S. Typhimurium and</li><li>S. Enteritidis</li></ul>	S. Typhimurium and S. Enteritidis			
	Antimicrobial resistance	<ul><li>S. Typhimurium, 50% of</li><li>S. Enteritidis, approx.</li><li>90% of other serotypes</li></ul>	S. Typhimurium and occasionally other serotypes	S. Typhimurium and occasionally other serotypes			
	MLVA	S. Typhimurium	S. Typhimurium (outbreak investigations)	S. Typhimurium (outbreak investigations)			
	PFGE	Outbreak investigations	Outbreak investigations	Outbreak investigations			
Campyloba	cter coli /jejuni						
	Antimicrobial resistance	Isolates from 3 districts for DANMAP surveillance	Only for DANMAP surveillance purposes	Only for DANMAP surveillance purposes			
	PFGE	Outbreak investigation	None	None			
	MLST	Outbreaks investigaions, research	None	None			
VTEC							
	Serotype	All	All	All			
	Virulence profile	All	All	All			
	PFGE	All	None	None			
Listeria							
	Serogroup	All	None	None			
	PFGE	All	None	None			
Yersinia en	terocolitica						
	O-group	Isolates from 1 district	None	None			

Source: Statens Serum Institut and Danish Zoonosis Laboratory, National Food Institute, Technical University of Denmark

Salmonella isolates submitted to or cultured by the National Food Institute are serotyped on a routine basis. In the case of S. Typhimurium or S. Enteritidis findings, isolates are phage typed, and S. Typhimurium isolates are also subjected to antimicrobial susceptibility testing. Further molecular typing is only carried out as a part of outbreak investigations and is coordinated with Statens Serum Institut.

In addition to *Salmonella*, there is surveillance of *Campylobacter* in broiler flocks at slaughter and on random samples of domestic and imported poultry meat. Samples are analysed at authorised private laboratories or by the National Veterinary Institute.

There is a very limited surveillance of VTEC on samples collected from fresh meat at cattle slaughterhouses, approximately 200 samples per year. The samples are analysed at the National Food Institute. Isolates – if any – are subjected to serotyping and toxin typing. No other routine typing is performed on bacterial pathogens of food or veterinary origin (Table 3.1).

## 3.3 Outbreak detection and investigation

Molecular typing of all S. Typhimurium, VTEC and Listeria isolates from human infections is carried out as routine investigations and used for outbreak detection. For the majority of investigated outbreaks with these pathogens, the detection of a cluster of isolates by the use of molecular methods is the first indication that triggers further outbreak investigations. Since mid-2004, both phage typing (1) and the high-discriminatory typing method MLVA (Multiple Locus VNTR Analysis) (2) have been used as routine typing of all S. Typhimurium isolates from humans. Prior to 2004, S. Typhimurium isolates were routinely phage typed and from 2003 to 2006 PFGE (Pulsed-Field Gel Electrophoresis) typed as well (3). However, the discrimination obtained by MLVA is significantly higher than by PFGE and the introduction of the MLVA method has lead to an increase in the number of detected and solved S. Typhimurium outbreaks in recent years (4).

The first indication of an outbreak caused by more rare serotypes of *Salmonella* or by *Yersinia*, *Shigella* and *Campylobacter* etc. is an increase in the total number of infections with one of the pathogens. In order to confirm the outbreak and define the outbreak related cases, molecular typing will typically be performed on isolates from human cases diagnosed during a specific time period (Table 3.1). See Chapter 6.1 for more detailed information on the reporting system.

Although all human infections caused by possible foodborne pathogens are centrally registered, the isolates of other organisms than *Salmonella* and VTEC are not always available for typing as they might be discarded after species confirmation at the regional laboratories.

When a cluster of human infections with a specific pathogen is detected, the National Food Institute will search their database for relevant animal or food isolates that may come from a possible source. If the pathogen has been registered in any animal or food sample, a hypothesis of the typical reservoir of the specific pathogen may become clear and isolates will undergo further typing to confirm a possible match to the human type. For *Salmonella* outbreaks, this search is typically based on the phage type and resistance profile. Isolates matching at this level are MLVA typed and/or PFGE typed and compared to the outbreak type.

## 3.4 National and international cooperation

The typing methods used for the central surveillance and outbreak investigations taking place at Statens Serum Institut, the National Food Institute and the National Veterinary Institute are fully standardised and comparable between the laboratories. For *Salmonella*, the shared database with molecular typing data is an important tool in the surveillance.

The phenotypic and molecular typing methods are internationally recognised methods and most of them are standard methods with international external quality assurance programmes, e.g. serotyping of *Salmonella* and VTEC, phage typing of *Salmonella*, antimicrobial resistance profiling of *Salmonella* and *Campylobacter*. The molecular typing methods are also international methods, however, not standardised to the same level as the other methods. For PFGE typing, the PulseNet US protocols are used (5). The method for MLVA typing of *S*. Typhimurium is used in several other European countries. This method needs further standardisation for the results to be fully comparable between laboratories and this work is ongoing (6). The Danish laboratories are actively involved in the international development and cooperation on typing methods.

If an outbreak is suspected to have an international aspect, international alerts or inquiries are sent out via PulseNet and the European Centre for Disease Prevention and Control. Further, in relation to identification of a possible animal reservoir or food source, inquiries are sent out in the network of Community Reference Laboratories (CRLs) and National Reference Laboratories (NRLs). In order to be able to extrapolate results between countries, it is important that the molecular typing data are comparable between laboratories and countries.

#### References

- (1) Anderson ES, Ward LR, Saxe MJ, de Sa JD. (1977). Bacteriophage-typing designations of *Salmonella* Typhimurium. J. Hyg. (Lond); 78:297-300.
- (2) Lindstedt BA, Vardund T, Aas L, Kapperud G. (2004). Multiple-locus variable-number tandem-repeats analysis of *Salmonella* enterica subsp. enterica serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. J Microbiol Methods; 59:163-172.
- (3) Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. (2006). Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis; 3:59-67.
- (4) Torpdahl M, Sørensen G, Lindstedt BA, Nielsen EM. (2007). Tandem repeat analysis for surveillance of human *Salmonella* Typhimurium infections. Emerg Infect Dis; 13:388-395.
- (5) Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, Rolando S, Gutierrez EP, Binsztein N. (2006). Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. Foodborne Pathog Dis; 3:36-50.
- (6) Larsson JT, Torpdahl M, Petersen RF, Sørensen G, Lindstedt BA, Nielsen EM. (2009). Development of a new nomenclature for Salmonella Typhimurium multilocus variable number of tandem repeats analysis (MLVA). Euro Surveill;14(15).



# 4. VTEC - status and human risk factors

## By Lone Jannok Porsbo (ljpo@food.dtu.dk), Jeppe Boel and Flemming Scheutz

Escherichia coli are part of the normal micro flora of the gastrointestinal tract of mammals and birds, but certain groups of E. coli have been associated with gastrointestinal diseases in both humans and animals. Verocytotoxinproducing E. coli (VTEC) is a group of E. coli that is characterized by the ability to produce verocytotoxins (VT). Human pathogenic VTEC usually harbour additional virulence factors that are important for the development of disease and a large number of serogroups of E. coli have been recognized as verocytotoxin producers. The two main types of VTs are encoded by the genes vtx1 and vtx2, with vtx2 being the most virulent type. Another important virulence gene found in VTEC is the eae gene, coding for the production of intimin. Most human VTEC infections are associated with a limited number of O:H serotypes. Of these, VT2-producing E. coli O157:H7 and O157:H (VTEC O157) are the most frequently reported types associated with human disease (1).

The majority of reported human VTEC infections are sporadic cases and are associated with watery or bloody diarrhoea (haemorrhagic colitis), which is often accompanied by abdominal cramps, usually without fever. The registered cases are mainly children less than 5 years of age. VTEC infections can result in haemolytic uraemic syndrome (HUS), which is characterized by acute renal failure, anaemia and lowered platelet counts. HUS develops in up to 10% of patients infected with

VTEC O157 and is the leading cause of acute renal failure in young children. VTEC infection is lethal in  $2-7\,\%$  of the cases (2). In Denmark, VTEC infections, including HUS, has been notifiable since 2000. From 2000 – 2008, 38 cases developed HUS corresponding to 3.1% of all patients infected with VTEC.

VTEC (including VTEC O157) has been isolated from many different animal species. The gastrointestinal tract of healthy ruminants is the primary reservoir for VTEC, and foods of bovine origin are frequently reported as a source of human VTEC infections. The infectious dose is low and human infections can be acquired through the consumption of contaminated food or water, by direct transmission from person to person or from direct contact with infected animals. The importance of many of the VTEC types that can be isolated from animals and foodstuffs for infections in humans is not yet clear.

#### 4.1 Human cases in 2008

In 2008, there were 161 reported VTEC cases; an incidence of 2.9 per 100,000 inhabitants. VTEC cultures were obtained from 158 cases (some were diagnosed by PCR only, some were notifications from one of the district hospitals), of which 9% were caused by O157. The total distribution of VTEC O-groups, resulting in five or more episodes, is presented in appendix B, Table A3.

The number of registered infections was unchanged from 2007 to 2008. This follows several years of increase in the number of infections from the beginning of surveillance in 1997 to 2007 (appendix B, Table A2). The increase



10 8 Prevalence (%) 6 4 2 0 1999 2000 2001 2004 2008 2002 2003 2005 2006 2007

Year

Figure 4.1. VTEC 0157-prevalence in cattle, 1999-2008

Source: National Food institute, Technical University of Denmark

is primarily assumed to reflect improved diagnostics and increased awareness. However, Denmark does not have a centrally coordinated standard testing method for VTEC and the incidence through the past 10 years has been 3 to 10 times higher in laboratory uptake areas using a diagnostic approach involving molecular detection methods (appendix B, Figure A5). A rough estimate indicates that a little more than 50% of the Danish population was covered by molecular detection methods in 2008. In 2008, the age group specific incidence in areas using molecular methods was estimated to be 19.0 per 100,000 inhabitants in children less than 5 years and 3.9 per 100,000 inhabitants in the group with cases aged 5 years or more compared to 3.7 and 0.7, respectively, in areas using other methods.

There were four cases of VTEC related HUS in 2008. Unusually, all four cases were associated with non-O157 serotypes; O145:H- (2 boys aged 3 and 5 years), O111:H-(1 girl aged 2 years) and O121:H19 (1 girl aged 1 year). All cases were positive for the *eae* gene and *vtx2*. The O111:H- strain was also positive for *vtx1*. In 2008, all VTEC isolates were real-time sub-typed using PFGE at Statens Serum Institut.

Foodborne outbreaks caused by VTEC are rare in Denmark and in 2008, no general outbreaks occurred. In 2007, organic salami made from beef contaminated with VTEC O26:H11 caused a six week long outbreak (see also Annual Report 2007). During this period 20 cases, mainly small children (1 – 3 years of age) were laboratory confirmed. The symptoms were in general mild and one case of haemorrhagic colitis occurred. The epi-type was detected from the batch of imported frozen beef used to produce the salami. For the first time in Denmark, data from credit cards were used together with a case-control study in the epidemiological investigation (3). In 2004, an outbreak with VTEC O157 was epidemiological connected

to drinking pasteurized milk from a certain dairy (see also Annual Report 2004) (4).

#### 4.2 VTEC in cattle

The National Food Institute, Technical University of Denmark has monitored the occurrence of VTEC O157 in cattle since June 1997 through yearly examinations of approximately 200 fecal samples from slaughter calves. The samples are collected at the slaughterhouses as part of the DANMAP programme. The samples (25 g) are analysed by overnight enrichment followed by immunomagnetic separation and seeding on to sorbitol MacConkey agar supplemented with cefixime and potassium tellurite. Isolates of *E. coli* O157 are analysed for genes encoding verocytotoxins by PCR analysis.

In 2008, VTEC O157 was detected in 7.2% (16/222) of the analysed samples. This prevalence is in line with the findings in previous years, where the observed prevalence has ranged from 2.8% to 10.3% with an average prevalence of 5.7% (Figure 4.1).

## 4.3 VTEC in minced beef - 2008 investigation

In 2008, the Danish Veterinary and Food Administration conducted a VTEC survey of 263 samples of minced meat including 32 veal and 231 beef samples. The samples were collected at the retail level and the analyses were performed by the Regional Food Laboratory (further described in Chapter 6.4). The samples were enriched by overnight incubation. DNA was purified from the enriched culture and analysed for genes encoding verocytotoxin (*vtx1* and *vtx2*) and an *E. coli* serogroup O157 specific gene by real-time PCR analysis. The samples were regarded as real-time VTEC/O157 positive if they exhibited a Ct value of <30.

Attempts were made to isolate VTEC from 21 samples that exhibited Ct values <25 in the vtx real-time PCR assay. The primary BPW enrichment broth was seeded on tryptone bile x-glucuronide (TBX) agar. Approximately 10  $\beta$ -glucuronidase positive colonies per sample were examined for VT encoding genes by real-time PCR.

Enrichment broths from samples that were O157 real-time PCR positive were further examined as specified in the ISO 16654:2001 standard. Isolates of *E. coli* O157 were analyzed for verocytotoxin-encoding genes by *vtx* real-time PCR.

Thirty-three of the 263 samples (12.5%) were positive by the *vtx* real-time PCR assays; 17 samples were *vtx1* positive and 28 samples were *vtx2* positive. VTEC was isolated from four samples. Four samples were O157 real-time PCR positive and three of these samples were also positive in the *vtx* real-time PCR assay. VTEC O157 were isolated from two (0.8%) of the investigated samples.

## 4.4 Risk factors for sporadic VTEC infections in Denmark

In 2003–2005, Statens Serum Institute and the National Food Institute conducted a case-control study, which was the first systematic investigation of risk factors for sporadic VTEC infections in Denmark. The study was a matched case-control study with cases and controls matched on sex, age and municipality. Information on medical history, travel history, food exposures and contact to animals were collected through telephone interviews.

Cases and controls were excluded:

- If they had been travelling outside Denmark in two weeks prior to the first symptoms of infection
- If they had been part of an outbreak
- If the interview resulted in insufficient information to fulfill the criteria to be included in the study.

Before excluding travel associated cases, matched univariate analysis was conducted and foreign travel was found to be a significant risk factor for VTEC-infection, with Egypt and Turkey as the most frequently visited countries.

The final analysis contained 57 cases and 118 matched controls. Half of the cases were females (52.6%) and the median age was 4 years (age range 0–77 years). The preliminary multivariate analysis pointed out two exposures significantly associated with VTEC infection: Eating strawberries or having experienced irregularities with the domestic water supply, i.e. bad smell or soil in the water. The two risk factors were independent of season, and the domestic water supply was in all cases stated to be public water supply. The largest subgroup of cases was 27 children 0 – 3 years of age. Matched multivariate analysis was conducted separately for this group of cases and two risk factors were found: Drinking unpasteurized milk and having had contact to ruminants.

Clinical symptoms, complications and hospitalizations were described for 73 cases interviewed in connection with the study. In total, 26.0% of cases was hospitalized in 3.5 days on average; 8.2% of cases developed HUS and 35.6% haemorrhagic colitis. The most frequent symptom was diarrhoea (98.6%) and abdominal pain (64.4%). VTEC O157 was the most frequent serotype (26%); all O157 samples were *vtx2* and *eae* positive; *vtx1* was only seen in connection with *vtx2*. VTEC O157 was responsible for 66.7% of the HUS incidence and 57.9% of the incidence with haemorrhagic colitis. The most common serotypes besides O157 were O103, O26 and O145.

#### References

- (1) Duffy G (2006). Emerging pathogenic E. coli. In: Motarjemi Y, Adams M, red. Emerging Foodborne Pathogens. Woodhead Publishing Ltd. 10: 253-281.
- (2) Nelson S, Clarke RC, Woods LM (1998). Verocytotoxin-producing *Escherichia coli* (VTEC) infections. In: Palmer SR, Lord Soulsby, Simpson DIH red. Zoonoses. Oxford Medical Publications, p. 89-104.
- (3) Ethelberg S, Smith B, Torpdahl M, Lisby M, Boel J et al (2009). Outbreak of Non-O157 Shiga Toxin-Producing *Escherichia coli* Infection from consumtion of Beef Sausage. CID 48(15 April), Brief Report.
- (4) Jensen C, Ethelberg S, Gervelmeyer A, Nielsen EM, Olsen KEP, Mølbak K, and the outbreak investigation team (2006). First general outbreak of Verocytotoxin-producing *Escherichia coli* O157 in Denmark. Euro Surveill 11(2).

## 5. EU related topics

## 5.1 Trichinella special status

In July 2007, the European Commission and the other Member States assigned Denmark status as a region where the risk of *Trichinella* in domestic swine is officially recognised as negligible (EU Regulation (EC) No 2075/2005).

As a result of this status the future monitoring programme for *Trichinella* can be risk based. Slaughter pigs reared under controlled housing conditions in integrated production systems do not have to be tested for *Trichinella* any more. All other categories of pigs and other species (domestic or game) which can become infected with *Trichinella* will be examined in accordance with the methods laid down in Regulation (EC) No 2075/2005. Further, pork exported to third market countries will be tested for *Trichinella* unless the importing country accepts the new monitoring programme.

In order to fulfil the requirements set out by the Regulation, a monitoring programme for *Trichinella* in wildlife must be in place. This programme was initiated in 2008. In total, 300 foxes and 50 other carnivores will be examined annually. In 2008, *Trichinella pseudospiralis* was found in two wild mink on the island of Bornholm. Due to that, a large number of mink and crows were examined on Bornholm. There were no other findings. A report on results of the Danish *Trichinella* testing and monitoring for 2008 has been forwarded to the Commission.

# 5.2 Control of *Salmonella* in animal populations - EU regulations and studies

#### **EU Baseline studies**

Based on the Zoonosis Directive 2009/99/EC and Regulation (EC) 2160/2003 the Commission has initiated EUstudies – the Baseline Studies - of the *Salmonella* prevalence in laying hens, broilers, broiler carcasses, breeding pigs, slaughter pigs and turkeys, of the *Campylobacter* prevalence in broilers and broiler carcasses and of Methicillinresistant *Staphylococcus aureus* (MRSA) in breeding pigs. The objectives of the studies are to generate comparable prevalence data from all Member States with the purpose of setting common EU targets for the reduction of the pathogen in question.

In 2008, baseline studies on *Salmonella* and *Campylo-bacter* prevalence in broilers and broiler carcasses and of *Salmonella* and MRSA in breeding pigs was carried out and the results are still being analysed.

#### **EU** harmonised surveillance programmes

2008 was the first year Member States was obliged to report the prevalence in laying hen flocks based on a harmonised surveillance programme according to Regulation (EC) 2160/2003. Due to the large variation between Member States in prevalence of laying hen flocks reported in the baseline study finalised in 2005 (See Annual Report 2006 for overview of Danish results), the Commission decided to set a Member State reduction target depending on the prevalence in the preceding year until the prevalence falls below 2.0% (Regulation (EC) No 1168/2006). This target has to be reached by December 31st 2010. Denmark has had intesive *Salmonella* surveillance programmes for many years and the target of 2.0% has already been reached.

### Survey on prion protein genotypes in sheep

Denmark has a population of approximately 200,000 sheep and lambs. In the sheep population, some animals have a genotype resistant to classical scrapie. Although less conclusive, evidence also suggests that the same genotype is resistant to BSE. The pathogenic prion load in these resistant sheep is much lower than in non-resistant sheep. Therefore, the resistant sheep will pose a much lower public health risk, compared to that of non-resistant sheep. In 2008, like previous years, a study was conducted to determine the prion protein genotypes from a sample of ovine animals according to Regulation (EC) No 999/2001 (as amended). The study included 100 randomly selected animals. Results showed that 29.4% of sheep had the resistant prion protein genotype ARR/ARR which is an increase compared to last year where 16.0% of the sheep had the resistant prion protein genotype (Appendix C, Table A26).

# 6. Surveillance and monitoring

#### 6.1 Surveillance of human disease

Presented in this report is the occurrence of zoonotic enteric pathogens in Denmark:

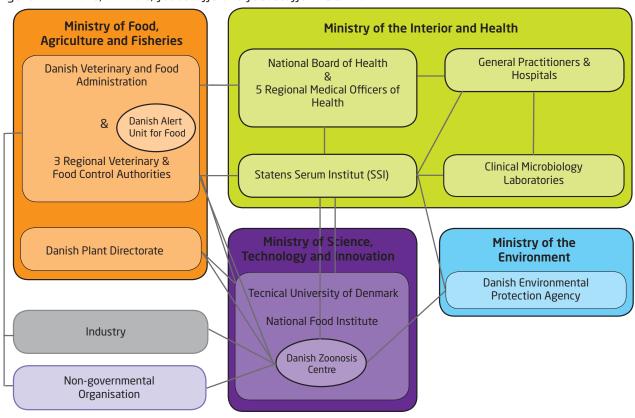
- Notifiable through the laboratory surveillance system: Salmonella, Campylobacter, Yersinia, Verocytotoxin-producing E. coli (VTEC) and Listeria
- Individually notifiable zoonotic pathogens: *Chlamydia psittacci* (ornithosis), *Leptospira, Mycobacterium*, Bovine Spongieform Encephalopathy (BSE) prions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

An overview of these notifiable and non-notifiable human diseases is provided in appendix D, Table A30.

In Denmark, the physicians report individually notifiable zoonotic diseases to the medical officers and the De-

partment of Epidemiology at Statens Serum Institut (Figure 6.1). Positive cases diagnosed by a clinical microbiological laboratory are reported through the laboratory surveillance system to the Unit of Gastrointestinal Infections at Statens Serum Institut. Physicians send specimens from suspect cases to one of 15 clinical microbiology laboratories depending on county of residence of the requesting physician. The laboratories must report positive results to Statens Serum Institut within one week. Furthermore, all Salmonella and VTEC isolates are sent to the reference laboratory at Statens Serum Institut for further sero- and genotyping. The Salmonella positive isolates are sent to the National Food Institute, Technical University of Denmark for phage typing. See chapter 3 for more detailed information on typing methods. The results are recorded in the Register of Enteric Pathogens maintained by Statens Serum Institut. Positive cases are reported as episodes, i.e. each patient-infectious agent combination is only recorded

Figure 6.1. Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feedstuffs in Denmark



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 B, Table A2
- Regional distribution of human cases of salmonellosis is presented in appendix B, Figures A1-A2
- Regional distribution of human cases of campylobacteriosis is presented in appendix B, Figure A3
- Regional distribution of human cases of yersiniosis is presented in appendix B, Figure A4
- Regional distribution of human cases due to VTEC is presented in appendix B, Figure A5.

Further, additional information on human infections are presented as follows:

- The *Salmonella* sero- and phage type distributions are presented in appendix C, Tables A5-A7
- VTEC O-group distribution in humans is presented in appendix B, Table A3.

## 6.2 Outbreaks of zoonotic gastrointestinal infections

In Denmark, local foodborne outbreaks are typically investigated by the Regional Veterinary and Food Control Authority in collaboration with the medical officer; often with the participation of the regional clinical microbiology laboratory. Larger outbreaks involving more than one region are typically investigated by Statens Serum Institut, the National Food Institute and the Danish Veterinary and Food Administration. These institutions may also aid in the investigation of local outbreaks. Representatives from these institutions meet regularly to discuss surveillance results, compare the reported occurrence of zoonotic agents in animals, food and feedstuffs with that in humans, and review major outbreaks. The formal responsibility of investigating food- or waterborne outbreaks is currently divided between three ministries based on the outbreak source: the Ministry for the Interior and Health for infectious diseases; the Ministry of Food, Agriculture and Fisheries for food and animal related diseases; and the Ministry of the Environment (along with the municipalities) for water related diseases.

Outbreaks may be detected in various ways. Individuals who experience illness related to food intake in settings such as restaurants or work place cantinas may report these incidents directly to the Regional Veterinary and Food Control Authorities. Physicians are obligated to report all suspected water- and foodborne infections to the regional medical officer, who then reports to Statens Serum Institut. Clusters of cases may be noted in the laboratory or identified at Statens Serum Institut through the laboratory surveillance system of gastrointestinal bacterial infections or through subtyping of bacterial isolates from patients.

A list of verified outbreaks (not including household outbreaks) reported to the Food- and waterborne Outbreaks Database (FUD) are presented in Appendix B, Table A4 and some of the more notable outbreaks are outlined in Chapter 2.

## 6.3 Surveillance and monitoring of animals and animal products

Surveillance and monitoring programmes for poultry, pigs and cattle are presented in Appendix D, Tables A32-A37. Sample analysis is performed at authorised private laboratories, the Regional Veterinary and Food Control Authorities, the National Food Institute or the National Veterinary Institute, Technical University of Denmark. Isolates positive with *Salmonella* are forwarded to the National Food Institute for subtyping (sero-, phage and genotyping as well as antimicrobial susceptibility testing). See chapter 3 for more detailed information on typing methods.

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

- Results from the table-egg production are presented in appendix C, Tables A5-A9 and Figures A8-A9
- Results from the broiler production are presented in appendix C, Tables A5, A7 and A10
- Results from the duck and turkey produtions are presented in appendix C, Table A14
- Results from the pig production are presented in appendix C, Tables A5-A7, A15 and Figures A6-A8
- Results from the cattle production are presented in appendix C, Tables A5, A7, A16-17 and Figure A9
- Results from the feeding stuff production are presented in appendix C, Tables A21
- Results from the rendering plants are presented in appendix C, Table A22
- Results from pets, zoo animals and wild life are presented in appendix C, Table A23.

Salmonella sero- and phage type distribution in cattle and pig herds investigated due to clinical disease (not necessarily salmonellosis) and found positive for Salmonella are presented in appendix C, Table A18. Cattle herds with confirmed infections of multiresistant S. Typhimurium DT104 (MR DT104) or herds that have been in contact with herds infected with MR DT104 are placed under official veterinary supervision. Cattle herds with confirmed infection of S. Dublin are subject to hygienic slaughter.

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

- Results from the poultry production are presented in appendix C, Tables A11 and A13
- Results from pig and cattle herds are presented in appendix C, Tables A19
- Results from pets, zoo animals and wild life are presented in appendix C, Table A23.

## Changes to the Salmonella surveillance programme for broiler flocks

In 2008, the *Salmonella* surveillance programme for broiler flocks was changed; one additional AM-testing of broiler flocks was introduced 7-10 days prior to slaughter in addition to the samples already collected 2-3 weeks before slaughter. The results of the tests must be available before slaughter. All flocks are sampled, also flocks intended for export before slaughter.

From mid 2008, all AM-positive flocks were slaughtered at one slaughterhouse in Denmark and special hygienic precautions are in action at slaughter; all positive flocks are heat treated and 300 samples are collected from the flock thereafter.

Surveillance of *Salmonella* in broiler flocks is carried out according to Order no 1261 of 15/12/2008, and further information on the surveillance programme are given in appendix D, table A33.

Pig and cattle carcasses are screened for *Mycobacterium* and *Echinococcus* during meat inspection at the slaughterhouse. Although Denmark is assigned as a region where the risk of *Trichinella* in domestic swine is negligible (see paragraph 5.1), all slaughter pigs slaughtered at export approved slaughterhouses are still examined for *Trichinella* as well as all horses slaughtered for human consumption and all wild boars. In addition, boars and bulls are tested for *Brucella* and bulls are tested for *Mycobacterium* at semen collection centres. All positive results for notifiable infectious diseases are reported to the Danish Veterinary and Food Administration. Results are presented in appendix C, Table A15-A16.

Results from the surveillance for Bovine Spongiform Encephalopathy (BSE) in cattle, Transmissible Spongiform Encephalopathy (TSE) in sheep/goats and Chronic Wasting Disease (CWD) in deer are presented in appendix C, Tables A24-A25 and A27.

Results from the monitoring of *Coxiella brunetii* (Q fever) in cattle are presented in appendix C, Table A14.

Appendix D, Table A31 gives an overview of notifiable and non-notifiable zoonoses presented in this report along with the relevant legislation.

## 6.4 Official testing of zoonotic pathogens in foodstuffs

In Denmark, control of pathogens in foodstuffs is coordinated both at the regional and at the central level of administration. Each Regional Veterinary and Food Control Authorities is responsible for the control carried out within its own region, and the Danish Veterinary and Food Administration is responsible for the regulation, control strategy and the surveillance at the national level.

The main purpose of the regional microbiological control system is to verify that the own-check programmes implemented at food establishments are functioning effectively and to verify the compliance with the microbiological criteria laid down in the legislation.

Regional microbiological control is carried out as follows:

- Targeted survey sampling primarily at the retail level.
   These surveys are focused on collecting samples from high risk products, specific types of production processes or specific types of food establishments
- Other types of sampling at the food wholesale and retail level include:
  - \* Sampling based on suspicion to support findings from inspection of food establishments
  - \* Sampling at the wholesale level to verify compliance with microbiological criteria in the legislation
  - \* Sampling in relation to the investigation of foodborne outbreaks
  - \* Sampling in response to consumer complaints.

Centrally co-ordinated control is carried out as national projects or surveys. The purposes of these projects are to:

- Verify compliance with microbiological criteria laid down in the legislation
- Discover emerging problems with microbiological contaminants
- Generate data for the preparation of risk profiles and risk assessments to support microbial risk management
- Monitor the effect of established risk management procedures in order to evaluate if these provide the desired results or if they need to be reconsidered.

Appendix C, Table A28 provides information on the centrally co-ordinated projects conducted in 2008. Information on the following projects is presented:

- The intensified control of Salmonella and Campylobacter in Danish and imported meat are presented in Appendix C, Table A20
- The findings of *Campylobacter* in non-heat treated meat cuts from broilers are presented in Appendix C, Tables A11 and A12
- Findings of *Listeria monocytogenes* in ready-to-eat products are presented in Appendix C, Table A29.

For further information consult the webpage of the Danish Veterinary and Food Administration, www.fvst. dk (in Danish).



For information on antimicrobial resistance in zoonotic bacteria please refer to the annual report "DANMAP - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark". The 2008 DANMAP report is available from www.danmap.org or may be ordered from the Danish Zoonosis Centre (vibb@food.dtu.dk).

# Appendix A

## Trends and sources in human salmonellosis

Table A1. Estimated no. of reported human cases and percentage of cases per major food source, travel or outbreaks, 2006-2008

	2008		2007		2006	
Source	Estimated no. of reported cases (95% credibility interval <sup>b</sup> )	Percentage of reported cases	Estimated no. of reported cases (95% credibility interval)	Percentage of reported cases	Estimated no. of reported cases (95% credibility interval)	Percentage of reported cases
Pork	320 (277-367)	8.8	107 (59-159)	6.5	101 (77-129)	6.1
Beef	26 (16-36)	0.7	12 (2-27)	0.8	23 (12-33)	1.4
Table eggs	116 (91-143)	3.2	181 (147-217)	11.0	103 (81-124)	6.2
Broilers	47 (25-133)	1.3	12 (2-30)	0.8	8 (3-16)	0.5
Ducks Imported pork Imported beef	38 (2-99) 39 (12-70) 12 (3-25)	1.0 1.1 0.3	21 (4-46) 20 (10-29)	1.3 1.2	12 (3-23) 26 (12-43) 22 (12-34)	0.7 1.6 1.3
Imported chicken	191 (120-250)	5.2	61 (34-87)	3.7	152 (123-184)	9.2
Imported turkey	87 (8-151)	2.4	12 (2-29)	0.7	87(67-108)	5.2
Imported duck	-	-	-	-	11 (5-20)	0.7
Travels <sup>1</sup>	853 (843-864)	23.3	762 (731-794)	46.3	410	24.7
Unknown source	480 (413-547)	13.1	386 (329-441)	23.4	605 (556-653)	36.5
Outbreaks, unknown source	1,447	39.6	73	4.4	98	5.9
TOTAL	3,656		1,647		1,658	

<sup>1)</sup> The estimate of travel related cases should be interpreted carefully, since availability of travel history data was incomplete for 2006

<sup>2)</sup> The model is based on a Bayesian framework which gives 95% credibility intervals.

# Appendix B

## Human disease and outbreak data

Table A2. Zoonoses in humans, number of laboratory-confirmed cases, 1999-2008

	Incidence per 100,000		R	ises			
Zoonotic pathogen	2008	2008	2007	2006	2005	2004	1999
Bacteria							
Brucella abortus/melitensis <sup>a,c</sup>	-	8	20	9	15	4	-
Campylobacter coli/jejuni <sup>b</sup>	63.1	3,454	3,868	3,242	3,671	3,724	4,164
Chlamydia psittaci <sup>b</sup>	0.1	6	11	7	22	8	-
Leptospira spp. <sup>b</sup>	0.2	13	10	15	24	33	23
Listeria monocytogenes <sup>b</sup>	0.9	51	58	56	46	41	44
Mycobacterium bovis <sup>b</sup>	0.01	1	1	3	0	2	2
Salmonella <sup>b</sup>	66.8	3,656	1,647	1,658	1,775	1,538	3,268
S. Enteritidis <sup>b</sup>	11.7	638	566	562	642	546	2,025
S. Typhimurium <sup>b</sup>	36.6	2,002	343	411	565	467	584
Other serotypes <sup>b</sup>	18.6	1,016	740	687	568	525	659
VTEC total <sup>b</sup>	2.9	161	161	146	154	168	51
O157	0.3	15	25	19	25	47	10
other or non-typeable	2.6	143	136	127	129	121	41
Yersinia enterocolitica <sup>b</sup>	6.0	330	270	215	241	228	339
Parasites	_						
Cryptosporidium spp. <sup>a,c</sup>	-	92	49	-	-	-	-
E. multilocularis <sup>a,e</sup>	-	0	3	-	-	-	-
E. granulosus <sup>a,e</sup>	-	5	9	-	-	-	-
Toxoplasma gondii <sup>a,f</sup>	-	-	-	14	9	8	-
Trichinella spp. <sup>a,c,e</sup>	-	0	1	-	-	-	
Viruses							
Lyssavirus <sup>b</sup>	-	0	0	0	0	0	0

a) Not notifiable hence the incidence cannot be calculated.

b) Notifiable.

c) Data presented are from one laboratory (Statens Serum Institute) only, representing a proportion of the Danish population (approximately 1/3 in 2008). The proportion of the population represented varies from year to year, thus results from different years are not comparable. Testing for these pathogens is carried out only if specifically requested on the submission form.

e) The cases were imported.

f) The nation-wide neonatal screening for congenital toxoplasmosis stopped in 2007.

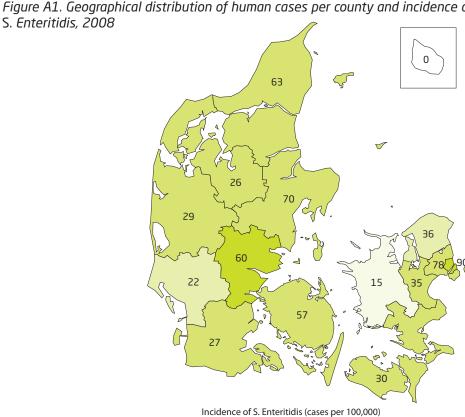


Figure A1. Geographical distribution of human cases per county and incidence of human infections with

Source: Statens Serum Institut

Figure A2. Geographical distribution of human cases per county and incidence of human infections with S. Typhimurium, 2008

0-<5 5-<10 10-<15 15-<20

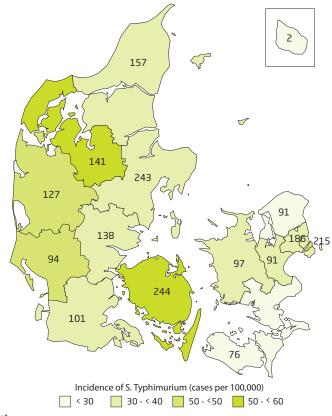
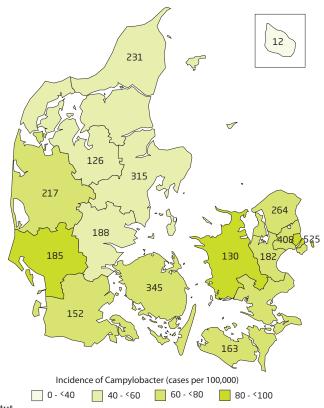


Figure A3. Geographical distribution of human cases per county and incidence of human infections with Campylobacter, 2008



Source: Statens Serum Institut

Figure A4. Geographical distribution of human cases per county and incidence of human infections with Yersinia, 2008

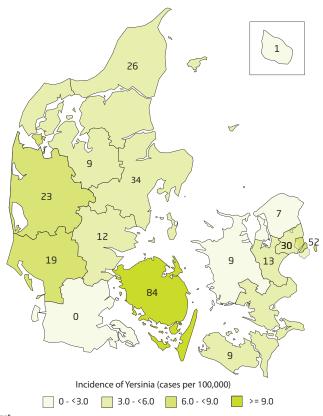
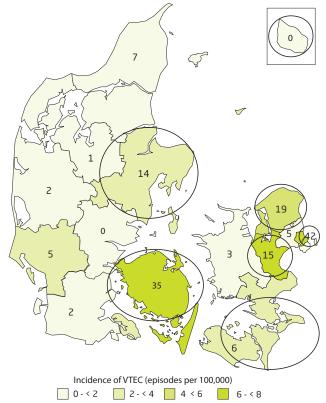


Figure A5. Geographical distribution of human cases per county and incidence of human infections with VTEC, 2008. The circled counties offer testing by molecular detection



Source: Statens Serum Institut

Table A3. VTEC O-group distribution in humans, 2008

Oa	Number of
O-group <sup>a</sup>	episodes
O103	19
O157	15
O146	12
O91	11
O145	11
O111	9
O117	8
O128ab	7
O26	6
O121	5
O- rough	6
Other O-groups or not-typed	49
TOTAL	158

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

Table A4. Foodborne disease outbreaks<sup>a</sup> reported in the Food- and waterborne Outbreak Database

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Suspected source	FUD no.
Cucurbitacin	10		Hotel	Composite meal	811
Cucurbitacin	4		Farm	Fresh vegetables	835
Histamin	2		Shop	Fish	834
Histamin	2		Restaurant/catering	Fish	805
Norovirus	11	2	Other	Unknown	814
Norovirus	7	0	Restaurant/catering	Unknown	791
Norovirus	36		Canteen	Unknown	819
Norovirus	24		Private party	Buffet meals	830
Norovirus	22		Hospital	Unknown	787
Norovirus	114	6	Canteen	Buffet meals	850
Norovirus	16		Shop	Composite meal	849
Norovirus	91	3	Restaurant/catering	Buffet meals	847
Norovirus	18		Restaurant/catering	Buffet meals	846
Norovirus	3		Restaurant/catering	Composite meal	842
Norovirus	20	5	Institution	Buffet meals	841
Norovirus	24		Canteen	Composite meal	839
Norovirus	30		Restaurant/catering	Buffet meals	838
Norovirus	6		Canteen	Buffet meals	83
Norovirus	27		Canteen	Buffet meals	83
Norovirus	66		Restaurant/catering	Buffet meals	829
Norovirus	23	2	Restaurant/catering	Buffet meals	803
Norovirus	27	6	Restaurant/catering	Buffet meals	80
Norovirus	28	4	Hotel	Buffet meals	800
Norovirus	32	6	Restaurant/catering	Composite meal	780
Norovirus	16		Shop	Composite meal	779
Norovirus	5	2	Restaurant/catering	Composite meal	800
Norovirus	68		Canteen	Unknown	883
Norovirus	13		Hotel	Unknown	882
Norovirus	22		Hotel	Unknown	879
Norovirus	32	12	Restaurant/catering	Unknown	867
Calcivirus (not norovirus)	15	4	Canteen	Buffet meals	799
Virus	15		Institution	Unknown	820
Virus	11		Restaurant/catering	Unknown	780
Staphylococcus aureus	42		Other	Composite meal	88
Shigella flexneri	17	4	Private party	Unknown	79.
Salmonella	43		Private party	Unknown	809
S . Agona	4	4	Canteen	Composite meal	810
S . Agona		10	General outbreak	Unknown	859
S . Enteritidis	40	8	Private party	Unknown	87.
S. Enteritidis			Tourists from Greece	Unknown	86
S . Derby		10	General outbreak	Pork	87.
S. Derby		22	General outbreak	Unknown	874
S . Kottbus	•	5	General outbreak	Unknown	78

Continued...

Table A4. Foodborne disease outbreaks<sup>a</sup> reported in the Food- and waterborne Outbreak Database (FUD), 2008 (Continued from page 29)

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Suspected source	FUD no.
S. Typhimurium U312 <sup>b</sup>		24	Slaughterhouse	Pork	863
S. Typhimurium U288		39	Food producer	Pork	855
S. Typhimurium DT 135 <sup>b</sup>		109	General outbreak	Unknown	854
S. Typhimurium DT 3 <sup>b</sup>		61	General outbreak	Unknown	853
S. Typhimurium DT 120		53	Shop	Pork (ham)	852
S. Typhimurium U288		37	Restaurant/catering	Shawarma	793
S. Typhimurium U292 <sup>b</sup>		1,224	General outbreak	Unknown	788
S . Stanley		5	General outbreak	Fresh vegetables	860
S. Chester	9	8	General outbreak	Unknown	796
Clostridium perfringens	58		Canteen	Buffet meals	812
Clostridium perfringens	19	1	Restaurant/catering	Fresh vegetables	831
Clostridium perfringens	35		Private party	Composite meal	823
Clostridium perfringens	5		Restaurant/catering	Other foods	802
Campylobacter	5	1	Restaurant/catering	Chicken	845
Campylobacter	28	11	Tourists from India	Chicken	781
Bacillus cereus	23		Canteen	Composite meal	840
Bacillus cereus	4		Restaurant/catering	Composite meal	825
Bacillus cereus	10		Restaurant/catering	Other meat	778
Listeria monocytogenes	3	2	Other	Other meat	878
Unknown	26		Institution	Composite meal	818
Unknown	6		Restaurant/catering	Unknown	807
Unknown	16		Hotel	Unknown	827
Unknown	15	0	Hotel	Buffet meals	797
Unknown	2		Canteen	Composite meal	792
Unknown	2	0	Restaurant/catering	Composite meal	777
Unknown	22		Private party	Unknown	783
Total	1,274	1,690			

a) In addition, 14 confirmed household outbreaks were registered. These were caused by Campylobacter (3 outbreaks), S. Infantis

 $<sup>(1),</sup> Norovirus\ (2), {\it Clostridium\ perfringens}\ (1), Cucurbitacin\ (squash\ 1), Histamin\ (1)\ and\ unknown\ pathogens\ (5).$ 

b) The outbreak continued in 2009, only cases from 2008 are reported here.

## Appendix C

## Monitoring and surveillance data

Table A5. Serotype distribution (%) of Salmonella from humans, animals, carcasses at slaughterhouse and imported meat, 2008

imported meat, i	Pig			Cattle		Layer	Broiler	Duck		Impor	ted meat	•
	Human	herds <sup>a</sup>	$Pork^{b}$	herds <sup>c</sup>	Beef <sup>b</sup>	flocks d	flocks <sup>e</sup>	flocks <sup>e</sup>	Pork	Beef	Chicken	Turkey
Serotype	N=3,656	N=554	N=199	N=33	N=11	N=4	N=43	N=76	N=61	N=6	N=110	N=66
Typhimurium	54.8	70.4	44.2	60.6	9.1	25.0	25.6	3.9	54.1	16.7	7.3	24.2
Enteritidis	17.5	0	0.5	0	0	25.0	0	1.3	0	0	10.0	1.5
Agona	1.9	0.4	1.0	0	0	0	0	0	0	0	8.2	0
Newport	1.6	0	0	0	0	50.0	0	1.3	0	0	0	6.1
O:4,5,12; H:i:-	1.6	0	0	0	0	0	0	0	0	0	0	0
Derby	1.5	19.3	27.6	0	0	0	4.7	0	16.4	0	2.7	6.1
Stanley	1.2	0	0	0	0	0	0	0	0	0	0	0
Java	1.1	0	0	0	0	0	0	0	0	0	0	0
Infantis	1.0	3.4	6.5	0	0	0	9.3	0	3.3	0	5.5	0
Saintpaul	1.0	0	0	0	0	0	0	0	0	0	1.8	36.4
Others	16.8	6.5	20.1	39.4	90.9	0	60.5	93.4	26.2	83.3	64.5	25.8
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100

- a) Isolates obtained from sampling of herds placed in level 2 and 3 (See Table A37 for detailes on the surveillance programme).
- b) Swab samples of pork and beef carcasses from the surveillance programme at slaughterhouses.
- c) Cattle herds examined based on clinical indication. The data are not representative for the Danish cattle population.
- d) Represenative samples from the surveillance programme in prodution flocks.
- e) Representative faecal or sock samples from the mandatory AM inspection prior to slaughter.
- f) Case-by-case monitoring of imported meat and meat products.

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

Table A6. Phage type distribution (%) of S. Enteritidis<sup>a</sup> from humans, animals and imported meat, 2008

				Importe	ed meat <sup>f</sup>
	Human	$Pork^b$	Layer flocks <sup>d</sup>	Chicken	Turkey
	n=638	n=1	n=1	n=11	n=1
PT 8	24.1	100	100	0	0
PT 21	16.5	0	0	0	0
PT 14B	12.9	0	0	0	0
PT 1	10.8	0	0	18.2	0
PT 4	8.3	0	0	54.5	100
PT RDNC	7.1	0	0	9.1	0
PT 6	3.1	0	0	9.1	0
PT 6A	2.8	0	0	0	0
PT 1B	2.0	0	0	0	0
PT 13A	1.9	0	0	0	0
Others	10.5	0	0	9.1	0
Total	100	100	100	100	100

a) The total number of samples may differ between phage type and serotype tabels (Tables A5-A7), since isolates of one serotype may contain more than one phage type.

Additionally, one isolate from a turkey flock and one isolate from a duck flock were positive with *S*. Enteritidis.

Source: Danish Veterinary and Food Administration, Statens Serum Institute and National Food Institute, Technical university of Denmark

b, d and f): See Table A5.

Table A7. Phagetype distribution (%) of S. Typhimurium<sup>9</sup> from humans, animals and imported meat, 2008

		Pig		Cattle		Layers	Broiler	Duck		Impor	ted meat <sup>f</sup>	
	Human	herds <sup>a</sup>	Pork <sup>b</sup>	herds <sup>c</sup>	Beef <sup>b</sup>	flocks <sup>d</sup>	flocks <sup>e</sup>	flocks <sup>e</sup>	Pork	Beef	Chicker	n Turkey
Phagetype	n=2002	n=390	n=95	n=20	n=1	n=1	n=11	n=3	n=33	n=1	n=8	n=16
U292	51.0	0	0	15.0	0	0	18.2	0	0	0	0	0
DT 120	6.0	20.0	18.9	0	0	0	9.1	0	12.1	100	0	12.5
DT RDNC	6.0	7.9	4.2	0	0	0	0	0	3.03	0	50.0	6.3
DT 135	4.9	1.5	2.1	0	0	0	0	0	0	0	0	0
U288	3.0	2.1	1.1	0	0	0	0	0	0	0	0	0
DT 3	2.7	0	0	0	0	0	0	0	0	0	0	0
DT 193	2.5	6.7	2.1	0	0	0	0	0	24.2	0	25.0	31.3
DT 12	1.8	14.6	15.8	5.0	0	0	27.3	0	0	0	0	0
U302	1.7	2.6	8.4	0	0	0	0	0	0	0	0	0
DT 104+104B	1.7	12.1	8.4	25.0	0	0	0	0	21.2	0	12.5	37.5
Others	18.6	32.6	38.9	55.0	100	100	45.5	100	39.4	0	12.5	12.5
Total	100	100	100	100	100	100	100	100	100	100	100	100

a-f) See Table A5.

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

Table A8. Occurrence of Salmonella in the table egg production, 2000-2008

	Rearii	Rearing period		Production period		ng flocks	Laye	r flocks
	(pare	nt flocks)	(pare	nt flocks)				
	N	Positive	N	Positive	N	Positive	N	Positive
2000	15	0	29	0	374	8	688	32
2001	14	0	22	0	339	4	607	35
2002	15	0	22	0	330	9	619	15
2003	24	0	15	0	367	4	611	10
2004	9	$2^{\mathrm{b}}$	9	0	368	1	641	5
2005	16	0	9	0	255	6	655	7
2006	17	0	11	0	289	2	565	2
2007	11	0	12	0	326	0	510	5
2008	10	0	6	0	258	$1^{c}$	508	$4^{d}$

a) See Tables A32 and A34 for describtion of the surveillance programmes.

Source: Danish Veterinary and Food Administration

g) The total number of samples may differ between phage type and serotype tabels (Tables A5-A7), since isolates of one serotype may contain more than one phage type.

b) Two positive flocks in the same holding; the second flock was registered approx. six weeks after the first flock.

c) S. Typhimurium DT 7.

d) One battery flock positive with *S.* Enteriditis PT 8, one organic flock positive with *S.* Typhimurium DT 41 and two free range flocks positive with *S.* Newport (same holding and same house found twice in 2008).

Table A9. Occurrence of Salmonella in the table egg layer flocks according to type of production, 2000-2008

	Dee	p litter	Free	e range	Or	ganic	Battery	
	N	Positive	N	Positive	N	Positive	N	Positive
2000	86	0	48	5	111	9	79	16
2001	122	2	46	16	137	3	129	14
2002	123	1	49	4	130	4	127	7
2003	191	2	71	2	173	1	167	9
2004	214	0	72	2	175	1	177	2
2005	217	3	70	0	178	0	175	4
2006	185	0	62	0	164	2	148	0
2007	155	2	56	0	146	2	146	1
2008	151	0	61	$2^{a}$	145	$1^{\mathrm{b}}$	135	1 <sup>c</sup>

a) Two flocks positive with S. Newport - same holding and same house found twice in 2008.

Source: Danish Veterinary and Food Administration

Table A10. Occurrence of Salmonella in the broiler production, 2000-2008

	Rearing period (parent flocks)		Production period (parent flocks)		Broiler flocks		Slaughterhouse (flocks/batches)	
	N	Positive	N	Positive	N	Positive	N	Positive
2000	222	3	345	3	4,500	95	4,543	131
2001	243	0	325	7	4,571	76	1,695 <sup>a</sup>	69
2002	241	2	330	2	4,443	68	1,667	92
2003	265	2	182 <sup>c</sup>	4	4,414	77	1,552	77
2004	275	1	155 <sup>c</sup>	6	4,246	64	1,472	24
2005	214	0	185 <sup>c</sup>	0	4,034	87	1,174	27
2006	190	0	282	5	3,621	71	775 <sup>b</sup>	17
2007	152	0	258	3	3,736	60	828	10
2008	146	0	293	$2^{d}$	3,717	43	518 <sup>e</sup>	3

a) PM sampling at the slaughterhouse were changed from pooled neck skin samples of flocks to chicken cuts sampling of batches.

b) One flock positive with S. Typhimurium DT 41.

c) One flock positive with S. Enteritidis PT 8.

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

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

d) One flock positive with S. Typhimurium DT 41, one with S. Typhimurium DT 120.

e) From 2008, all AM positive flocks are heat treated at slaughter. Sampling is now carried out as verification of the AM results of the negative flocks. See Tables A32 and A33 for describtion of the surveillance programmes. Source: Danish Agriculture and Food Council and Danish Veterinary and Food Administration

Table A11. Occurrence of Campylobacter in broiler flocks and in fresh meat at slaughter, 2000-2008

Year	Broiler flocks <sup>a</sup>		Chilled broiler m	eat <sup>b</sup>
	N	% pos	N	% pos
2000	6,146	37.6	-	-
2001	6,054	41.8	-	-
2002	6,208	42.6	-	-
2003	5,373	34.2	-	-
2004	5,157	27.0	1,603	17.8
2005	4,952	30.4	1,689	12.3
2006	4,522	30.8	959	7.9
2007	4,527	26.8	439	8.2
2008	4,912	25.9	484 <sup>c</sup>	14.7°

a) Flocks investigated by cloacal swabs collected at slaughter, samples are pooled and analysed as one sample using PCR.

Source: Danish Veterinary and Food Administration, Danish Agriculture and Food Council and National Veterinary Institute, Tecnical University of Denmark

Table A12. Occurrence of Campylobacter in non-heat treated broiler meat at retail<sup>a,b</sup>, 2001-2008

Chilled broiler meat (samples)				Frozen broiler meat (samples)				
Year	Denmark		Import		Denmark		Import	
	N	% pos	N	% pos	N	% pos	N	% pos
		(adjusted)		(adjusted)		(adjusted)		(adjusted)
2001-200	2 762	36.7	190	55.0	485	15.6	215	34.5
2002-200	3 403	40.8	139	78.5	324	18.3	167	24.9
2003-200	4 334	27.2	170	65.7	566	10.9	272	19.6
2004-200	5 517	31.1	299	73.2	937	12.2	391	25.9
2005-200	6 401	29.8	854	56.3	1,087	13.5	698	31.3
2006-200	7 363	31.0	1,128	51.1	897	19.0	812	33.9
2007-200	8 1,059	32.9	1,067	53.9	655	29.6	577	44.4

a) Centrally co-ordinated studies, retail samples (see section 6.4 for describtion). 2000-2002: detection limit <0.4 cfu/g; 2003-2008: detection limit <0.1 cfu/g.

Table A13. Relative distribution of Campylobacter species (%) in broilers before slaughter<sup>a</sup>, 2002-2008

Year	N	C. jejuni	C. upsaliensis	C. coli	NT/other
		% pos	% pos	% pos	% pos
2002	178	93.3	0	6.7	0
2003	113	92.9	0	6.2	$0.9^{\mathrm{b}}$
2004	101	94.1	0	5.9	0
2005	109	90.8	2.8	0	6.4
2006	113	92.0	0.9	7.1	0
2007	111	91.9	5.4	0.9	1.8
2008	100	90.5	2.8	0.0	6.6

a) Positive isolates collected as part of the DANMAP programme was examined using conventional microbiological methods.

Source: National Veterinary Institute, Technical University of Denmark

b) Centrally co-ordinated studies, slaughterhouse samples (see section 6.4 for describtion). Detection limit <10 cfu/g.

c) Data are not compareable to previous years as they only represent the last two quarters of the year, which is the high prevalent period.

b) The prevalence is calculated as a mean of quarterly prevalences based on the sum of data from the two years specified. Source: National Food Institute, Technical University of Denmark

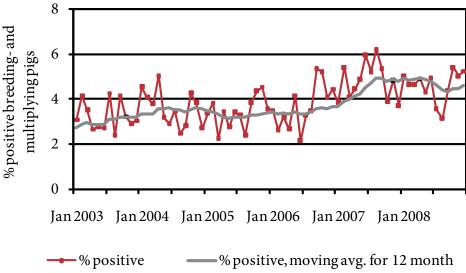
Table A14. Occurrence of Salmonella in turkey and duck flocks<sup>a,b</sup>, 2006-2008

	Duck flocks	Turkey flocks		
Year	N	% pos	N	% pos
2006	255	81.2	32	0
2007 <sup>c</sup>	n.a.	n.a.	n.a.	n.a.
2008	61	70.5	69	1.4

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

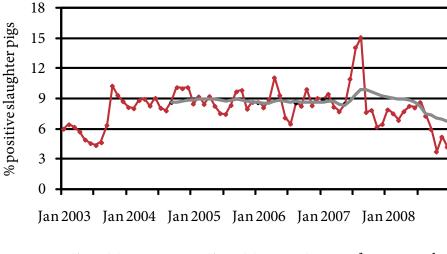
Source: Danish Agriculture and Food Council

Figure A6. Serological surveillance of Salmonella in breeding and multiplying pigs based on monthly testing of blood samples, 2003-2008. For more information about the surveillance programme, see table A37



Source: Danish Agriculture and Food Council

Figure A7. Serological surveillance of Salmonella in slaughterpigs, 2003-2008. Percentage of seropositive meat juice samples (first sample per herd per month). The abrupt increase in 2003 was attributed, in part, to analytical-technical adjustments. The peak in late summer 2007 and the very low level during 2008 were due to technical problems in the laboratory. For more information about the surveillance programme, see table A37



Source: Danish Agriculture and Food Council

——% positive, moving avg. for 12 month

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b) The two major turkey and duck slaughterhouses in Denmark closed down in 2004 and 2007, respectively.

Therefore, most commercially raised duck and turkey flocks are transported abroad for slaughter.

c) Not available.

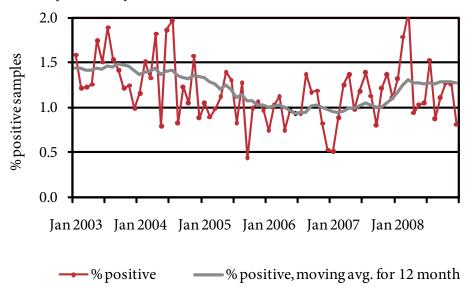
Table A15. Occurrence of zoonotic pathogens in pigs and pork in Denmark, 2008

	Primary production			Slaughterhouse (slaughtering >50 pigs per month)		Slaughterhouse (slaughtering 50 or less pigs per month)	
	Herds	Herds/ Animals	Animals/ samples	Samples		Samples	
Zoonotic pathogen	N	Positive	N	N	% pos	N	% pos
Salmonella <sup>a</sup>	9,445 <sup>b</sup>	185 <sup>b</sup>	-	27,045 <sup>c</sup>	1.3 <sup>d</sup>	144 <sup>c</sup>	2.0 <sup>d</sup>
Brucella abortus <sup>e</sup>	-	0	24,796	-	-	-	-
Mycobacterium bovis <sup>f</sup>	-	0	18,582,288	-	-	-	-
Echinococcus <sup>f</sup>							
granulosis/multilocularis	-	0	18,582,288	-	-	-	-
Leptospira <sup>g</sup>	26	0	69	-	-	-	-
Trichinella spp.h	-	0	18,582,288	-	-	-	-

- a) See Table A37 for describtion of the surveillance programme.
- b) Data are from December 2008. Slaughterpig herds monitored using serological testing of meatjuice samples collected at slaughter. Herds belonging to level 2 and 3 were defined as *Salmonella* positive.
- c) Swabs from three areas of the half-carcass were collected at the slaughterhouse after min. 12 h chilling. Sample size is 3x100 cm². Samples from 5 animals were pooled, except at slaughterhouses where 50 pigs or less were slaughtered per month, in which case samples were analysed individually.
- d) 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.
- e) Including samples from boars (examined at pre-entry, every 18 month, and prior to release from semen collection centres)
- (15,739 samples), samples collected in connection with export (8,790 samples), import (124 samples) or fertility problems (143 samples). 5-8 ml blood samples were analysed using either the SAT, RBT or CFT methods.
- f) Slaughtered pigs were examined by slaughterhouse meat inspectors.
- g) Sampling is based on suspicion of leptosporosis due to increased abortions or other reproductive problems in a herd. Samples are investigated using immunoflourescence techniques.
- h) Samples from all pigs slaughtered at export approved slaughterhouses were examined using the method described in Directive 2075/2005/EEC. In 2007, Denmark achieved official status as region with negligible risk of *Trichinella*, according to EU Regulation (EC) No 2075/2005.

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

Figure A8. Salmonella in pork, monitored at slaughterhouses, 2003-2008. Swab samples from three designated areas of chilled half-carcasses



Source: Danish Veterinary and Food Administration

Table A16. Occurrence of zoonotic pathogens in cattle and beef in Denmark, 2008

	Primary p	Primary production			house ring >50 month)	(slaugh	Slaughterhouse (slaughtering 50 or less cattle per month)		
	Herds	Herds/ Animals	Animals/ Samples	Samples		Sample	S		
Zoonotic pathogen	N	Positive	N	N	% pos	N	% pos		
Salmonella <sup>a,b</sup>	-	-	-	7,915	0.2	205	0		
Brucella abortus <sup>c,d</sup>	-	0	2,994	-	-	-	-		
Mycobacterium bovis <sup>e,f</sup>	-	0	511,300	-	-	-	-		
Echinococcusus <sup>f</sup>									
granulosis/multilocularis	-	0	511,300	-	-	-	-		
Coxiella brunetii	-	26	229 <sup>g</sup>	-	-	-	-		
	$607^{\rm h}$	362	-	_	-	_	-		

- a) Swabs from three areas of the half-carcass were collected at the slaughterhouse after min. 12 h chilling. Sample size is 3x100 cm<sup>2</sup>. Samples from 5 animals were pooled, except at slaughterhouses where 50 cattle or less were slaughtered per month, in which case samples were analysed individually. See Table A36 for describtion of the surveillance programme.
- b) 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.
- c) Denmark has been declared officially brucelosis free since 1979. The last outbreak was recorded in 1962.
- d) Including samples from boars (examined at pre-entry, every 18 month, and prior to release from semen collection centres) (2.627 samples), samples collected in connection with export (225 samples) or fertility problems (142 samples). 5-8 ml blood samples were analysed using either the SAT, RBT, CFT or ELISA methods.
- e) Denmark has been declared officially tuberculosis free since 1980. The last case of TB in cattle was diagnosed in 1988.
- f) Slaughtered cattle were examined by the slaughterhouse meat inspectors.
- g) Serum samples taken for diagnostic testing and analysed using an ELISA method. An additional 25 samples from placenta was analysed using the FISH method, 4 samples were positive.
- h) Bulk tank milk samples taken for diagnostic testing and analysed using an ELISA method.

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

Figure A9. Salmonella in beef, monitored at slaughterhouses, 2003-2008. Swab samples taken from three designated areas of chilled half-carcasses

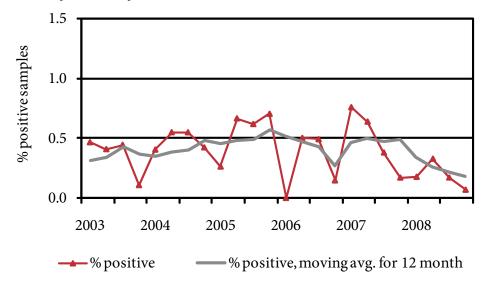


Table A17. Cattle herds assigned to level 1-3 according to the S. Dublin surveillance programme<sup>a</sup>, January 2009

Salmonella I	Duhlin level	Non	-milk	Milk producing		
Samonena 1	Jubini level	produci	ng herds	herds		
		N	% pos	N	% pos	
Level 1						
1a	On the basis of milk samples	1,084	6.8	3,753	84.9	
1b	On the basis of blood samples	12,576	78.6	25	0.6	
Total	Probably Salmonella Dublin free	13,660	85.4	3,778	85.4	
Level 2						
2	Titer high in blood- or milk samples	561	3.5	594	13.4	
2	Contact with herds in level 2 or 3	965	6.0	39	0.9	
2	'Non-Level 1' due to too few blood samples	9	0.1	5	0.1	
Total	Non Salmonella Dublin free	1,535	9.6	638	14.4	
Level 3						
Total	Salmonellosis, official supervision	2	<0.1	6	0.1	
Unknown	Too few blood samples	798	5.0	0	0	
TOTAL		15,995		4,422		

a) See Table A36 for describtion of the surveillance programme.

Table A18. Isolation of Salmonella from outbreaks of clinical disease in pig and cattle herds, 2008

Serotype and phage type	Pigs herds	Cattle herds
S. Dublin		13
S. Typhimurium	1	10
S. Typhimurium DT104	2	5
S. Typhimurium DT12		1
S. Typhimurium DT107	1	1
S. Typhimurium DT193	1	
S. Typhimurium U292		3
TOTAL	5	33

Table A19. Distribution of Campylobacter (%) in pig and cattle herds<sup>a</sup>, 2000-2008

	Pigs				Cattle			_
	N		% pos		N		% po	s
		C. coli	C. jejuni	other/unknown		C. coli	C. jejuni	other/unknown
2000	310	59.4	4.2	0.6	90	1.1	56.7	3.3
2001	238	68.5	2.9	5.5	76	6.6	53.9	11.8
2002	240	78.8	1.7	0.0	87	0	63.2	2.3
2003	259	-	-	93.4	88	-	-	63.6
2004	191	78.0	1.0	0.5	67	1.5	62.7	0
2005	185	83.2	2.2	0	73	0	42.5	0
2006	295	50.8	1.4	0	224	6.7	37.5	0
2007	261	76.6	1.9	0	132	3.0	67.4	0
2008 <sup>b</sup>	292	66.1	1.7	-	168	3.0	58.3	-

a) Samples were collected as part of the DANMAP programme. Caecal content was tested from one animal per herd.

Source: National Food Institute, Tecnical University of Denmark

Table A20. Results from the intensified control of Salmonella and Campylobacter in fresh meat based on a case-by-case risk assessment, 2008

	_					
		No. of batches tested	No. of batches positive	No. of batches sanctioned	Mean prevalence in positive batches <sup>a,b</sup>	Mean relative human risk in positive batches <sup>a</sup>
Campylobac	ter					
Danish	Poultry	310	41	2	30.9%	2.6
Imported	Poultry	938	192	13	32.2%	2.8
Salmonella						_
Danish	Beef	318	9	9	10.3%	110.5
	Pork	310	38	13	17.3%	12.2
	Poultry	310	1	0	1.7%	0.04
Imported	Beef	137	3	3	36.1%	95.4
	Pork	490	53	15	6.9%	4.8
	Poultry	938	143	50	18.6%	2.3

a) Include positive batches where a risk assessments has been performed. Risk assessments of positive batches of marinated meat is not required, but conducted in most cases.

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

b) In 2008, samples were only tested for C. coli and C. jejuni.

b) The *Salmonella* prevalence in each batch is based on the proportion of positive pooled samples (12 pools per batch) and number of subsamples per pool.

Table A21. Control of Salmonella in compound feeds, feed processing and feed material, 2004-2008

	2008		2007	2007		2006		2005		2004	
	Sampl	es	Sampl	Samples		Samples		Samples		es	
	N	Positive	N	Positive	N	Positive	N	Positive	N	Positive	
Feed processing plants											
(process control) <sup>a</sup> :											
Ordinary inspections	1,085	$18^{d}$	976	17	1,589	31	1,885	29	2,008	30	
Additional inspections			-	-	174	13	175	15	156	21	
Feed materials, farm											
$animals^b$	174	12 <sup>e</sup>	71	3	336	16	1,119	72	1,127	49	
Transport vehicles,											
hygiene samples <sup>c</sup>	3	0	95	0	191	2	254	3	317	3	

a) The presence of *Salmonella* in compound feed is indirectly monitored by the environmental samples collected during feed processing. Companies are sampled one to four times per year.

Table A22. Three categories of meat and bone meal by-products not intended for human consumption, 2008

Category of		Own-check	samples	Product sa	mples
processing plant	:	N	Pos	N	Pos
1	By-products of this material cannot be used for				
	feeding purposes	-	-	5	0
2	By-product of this material may be used for feed				
	for fur animals	-	-	175	1
3	By-products from healthy animals slaughtered				
	in a slaughterhouse. Products of these may be				
	used for petfood and for feed for fur animals	1,085	11	2,389	66
	TOTAL	1,085	11	2,569	67

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

b) Sampling of feed materials used without further heattreatment (predominantly soy bean meal and rapeseed cake).

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

d) 1 S. Agona, 5 S. Infantis, 1 S. Isangi, 1 S. Kralingen, 1 S. Lexington 15,34, 2 S. Liverpool, 2 S. Mbandaka, 1 S. Montevideo, 1 S. Ruiru and 3 S. Senftenberg.

e) 3 S. Agona, 1 S. Cubana, 1 S. Infantis, 1 S. Kralingen, 1 S. Lexington, 1 S. Livingstone, 1 S. Mbandaka and 3 S. Senftenberg. Source: Danish Plant Directorate

Table A23. Occurrence of zoonotic pathogens in pets, zoo animals and wild life in Denmark<sup>a</sup>, 2008

	Pet animals				Zoo	Zoo animals			Wild	llife				
	Dogs	3	Cats		Othe	ers		nmal ptiles	Birds	S	Man	nmal	Birds	S
Zoonotic pathogen	N	pos	N	pos	N	pos	N	pos	N	pos	N	pos	N	pos
Salmonella	10	0	4	1	0	-	19	2 <sup>g</sup>	15	1 <sup>h</sup>	116	13 <sup>i</sup>	23	1 <sup>j</sup>
Campylobacter spp.	19	0	14	1	5	0	10	$3^{b}$	0	0	72	3 <sup>c</sup>	8	0
Brucella canis/abortus	31	0	0	-	0	-	75	0	0	-	2	0	0	-
Chlamydia psittaci	0	-	1	0	4	0	0	-	46	0	0	-	0	-
Cryptosporidium spp.	36	7	9	1	1	$1^{d}$	35	6 <sup>e</sup>	0	-	4	$4^{\mathrm{f}}$	0	-
Echinococcus spp.	0	-	0	-	0	-	0	-	0	-	4	0	0	-
Trichinella spp. <sup>k</sup>	0	-	0	-	0	-	4	0	0	-	281	$2^{l}$	50	0
European Bat Lyssavirus	0	-	0	-	0	-	0	-	0	-	16	0	0	-

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

Source: National Veterinary Institute, Technical University of Denmark

Table A24. The Bovine Spongiform Encephalopathy (BSE) surveillance programme for cattle, 2008

Type of surveillance	N	Positive
Active surveillance <sup>a,b</sup>		
Healthy slaughtered animals (>30 month)	190,824	0
Risk categories:		
Emergency slaugthers (>24 month)	1,612	0
Slaughterhouse ante-mortem inspection revealed suspicion	n or signs of	
disease (>24 month)	3	0
Fallen stock (>24 month)	40,720	0
Animals from herds under restriction	0	0
Passive surveillance		
Animals suspected of having clinical BSE	3	
TOTAL	233,162	0

a) Samples (brain stem material) are tested using a IDEXX technique or Prionics-Check PrioStrip.

b) 1 orangutan, 1 spider monkey, 1 barbary ape.

c) 1 roe deer, 1 fox, 1 mink.

d) 1 chinchilla.

e) 4 ring-tailed lemurs, 1 capybara, 1 golden lion tamarin.

f) 3 roe deer, 1 hedgehog.

g) 1 elephant, 1 lizzard.

h) 1 rainbow lorikeet.

i) 12 European hedgehogs, 1 badger.

j) 1 black-headed gull.

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

l) Two mink out of 142 was positive with Trichinella pseudospiralis.

b) Confirmatory testing is carried out using histopathology or immunohistochemistry. Further confirmation is performed at the Community TSE reference laboratory.

*Table A25. The* Transmissible Spongiform Encephalopathy (*TSE*) surveillance programme for sheep and goats, 2008

Type of Surveillance	$N^{a,b}$	Positive
Active surveillance		
Fallen stock (>18 mo.)	8,788	2
Healthy slaughtered animals (>18 mo.)	0	0
Animals from herds under restriction	0	0
Passive surveillance		
Animals suspected of having clinical TSE	0	0
TOTAL	8,788	2

a) Samples (brain stem material) are tested using a IDEXX technique.

Table A26. Distribution<sup>a</sup> (%) of prion protein genotype of sheep randomly selected, 2008

randoning Sciected, 2000					
	Canatyma	Sheep			
	Genotype	n=102			
NSP 1	ARR/ARR	29.4			
NSP 2	ARR/AHQ	2.0			
	ARR/ARQ	11.8			
	ARR/ARH/Q	2.0			
NSP 3 (ARQ/ARQ)	ARQ/ARQ	28.4			
NSP 3 (Other)	AHQ/AHQ	2.0			
	ARH/ARH	1.0			
	ARQ/ARH	2.9			
	ARQ/AHQ	8.8			
NSP4	ARR/VRQ	2.9			
NSP5	ARQ/VRQ	8.8			
Total		100			

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

Source: National Veterinary Institute, Technical University of Denmark

Table A27. The Cronic Wasting Disease (CWD) surveillance programme for deer, 2008

Type of Surveillance	$N^{a,b}$	Positive
Active surveillance		
Wild deer - road injured/hunted (>18 mo.)	45	0
Farmed deer - culled (>18 mo.)	35	0
TOTAL	80	0

a) Samples (brain stem material) are tested using a IDEXX technique.

b) Confirmatory testing is carried out using histopathology or immunohistochemistry. Further confirmation is performed at the Community TSE ferefence laboratory.

NSP 1: Genetically most resistant. NSP 2: Genetically resistant.

NSP 3: Genetically little resistance. NSP 4: Genetically susceptible.

NSP 5: Genetically highly susceptible.

b) Confirmatory testing is carried out using histopathology or immunohistochemistry. Further confirmation is performed at the Community TSE reference laboratory.

Table A28. Centrally coordinated studies conducted in 2008

Title of project	No. of	Agents analysis per sample	Futher
Title of project	samples	(regional laboratories)	information
Microbiological classification of the production areas for bivalve molluscs	150	E. coli, Salmonella, vira	Results are not presented
Antimicrobial resistance in Danish and imported meat (chicken, beef and pork)	1,000	Campylobacter, Salmonella, E. coli, Enterococcus	Results are not presented
Campylobacter in fresh, chilled Danish chicken meat	860	Campylobacter	Appendix C, Table A11
Campylobacter in fresh chilled and frozen Danish and imported chicken meat and frozen Danish chicken meat	1,800	Campylobacter	Appendix C, Table A12
Campylobacter in fresh chilled imported poultry meat (duck and turkey)	800	Campylobacter	Results are not presented
Intensified control for <i>Salmonella</i> and <i>Campylobacter</i> in fresh Danish meat	900 <sup>a</sup>	Salmonella, Campylobacter (quantitative)	Appendix C, Table A20
Intensified control for <i>Salmonella</i> and <i>Campylobacter</i> in fresh imported meat	1,500 <sup>a</sup>	Salmonella, Campylobacter (quantitative)	Appendix C, Table A20
Hygiene quality and shelf life of sliced meat production	100	Total aertobic bacterial count and lactic acid producing bacteria	Results are not presented
VTEC in minced beef	300	VTEC	Chapter 4
Salmonella dublin in Danish dairy herd	200	Salmonella	Results are not presented
Samples of different origin related to humane outbreak of <i>Salmonella</i> Typhimurium U292 (food stuffs, swabs, pen-faecal samples)	3,100	Salmonella	Results are not presented

a) Batches

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

Table A29. Listeria monocytogenes in ready-to-eat foods<sup>a</sup>, 2008

		Samples analysed by a qualitative method			Samples analysed by a quantitative method		
Food category	Sampling place	N	Positive <sup>b</sup>	N	Samples with < 10 cfu <sup>c</sup> /g	Samples between 10 and 100 cfu/g	Samples with >100 cfu/g
RTE products of	At processing	64	0	332	332	0	0
meat origin	At retail	127	3	644	640	2	2
Milk and dairy							
products, RTE	At processing	24	0	11	11	0	0
Cheese, RTE	At processing	26	0	8	8	0	0
Fishery products,	At processing	11	0	59	59	0	0
	At retail	4	0	24	24	0	0
Fruit and vegetables	At processing	5	0	82	79	3	0
	At retail	11	0	213	211	2	0
Other RTE products	At processing	12	0	85	85	0	0
	At retail	2	0	74	74	0	0

a) Samples are collected by the Regional Veterinary and Food Control Authorities according to European Regulation (EC) No 2073/2005

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

c) cfu: The number of colony forming units.

# Appendix D

### **Monitoring and surveillance programmes**

Table A30. Overview of notifiable and non-notifiable human diseases presented in this report, 2008

	Notifiable in humans	Notification route
Bacteria		
Brucella spp.	no	-
Campylobacter spp.	1979 <sup>a</sup>	Lab <sup>b</sup>
Chlamydophila psittaci	1980 <sup>a</sup>	Physician <sup>c</sup>
(Ornithosis)		
Listeria monocytogenes	1993 <sup>a</sup>	Physician
Leptospira spp.	1980 <sup>a</sup>	Physician
Mycobacterium bovis/	1905 <sup>a</sup>	Physician (and lab <sup>d</sup> )
tuberculosis		
Coxiella burnetii	no	-
Salmonella	1979 <sup>a</sup>	Lab
VTEC	$2000^{a}$	Physician and Lab
Yersinia enterocolitica	1979 <sup>a</sup>	Lab
Parasites		
Cryptosporidium spp.	no	-
Echinococcus multilocularis	no	-
Echinococcus granulosus	no	-
Toxoplasma gondii	no	-
Trichinella spp.	no	-
Viruses		
Lyssa virus (Rabies)	1964 <sup>a</sup>	Telephone and
		physician
Prions		
TSE	-	-
BSE/Creutzfeld Jacob	1997 <sup>a</sup>	Physician

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

Source: Statens Serum Institute and Danish Veterinary and Food Administration

b) The regional microbiological laboratories report confirmed cases.

c) The physician report individually notifiable infections.

d) The laboratories voluntarily report confirmed cases.

Table A31. Overview of notifiable and non-notifiable animal diseases presented in this report, 2008

2008	Notifiable in animals	EU legislation	Danish legislation
Bacteria			
Brucella spp.	1920 <sup>e</sup> , OBF in 1979 <sup>b</sup> , no cases since 1962.	Cattle - Decision 2004/320/EC	Order no 305 of 3/5 2000
	Never detected, ObmF in 1995 <sup>c</sup>	Sheep and goats - 2004/320/EC	Order no. 739 of 21/8 2001
	-	Pigs - Directive 2003/99/EC	Order no. 205 of 28/3 2009
Campylobacter spp.	no	-	-
Chlamydophila psittaci (Ornithosis)	yes	-	Birds and poultry - order no. 78 of 30/1 1997
Listeria monocytogenes	no	-	-
Leptospira spp. (only in production animals)	yes	-	Act no. 432 of 09/06/2004
Mycobacterium bovis/	1920 <sup>e</sup>	Cattle - Decision	Cattle - Order no. 1417 of
tuberculosis	OTF since 1980 <sup>d</sup>	2004/320/EC	11/12 2007
Coxiella burnetii	2005	-	Act no. 432 of 09/06/2004
Salmonella	1993 <sup>a</sup>	-	Cattle/swine - Order no. 112 of 24/02/2005
VTEC	no	-	-
Yersinia enterocolitica	no	-	-
Parasites			
Cryptosporidium spp.	no	-	-
Echinococcus multilocularis	2004	Council directive 64/433/EC	Act no. 432 of 09/06/2004
Echinococcus granulosus	1993	Council directive 64/433/EC	Act no. 432 of 09/06/2004
Toxoplasma gondii	no	-	-
Trichinella spp.	1920 <sup>e</sup>	Regulation 2075/2005/EC	Circular no. 9466 of 12/07/2006
Viruses			
Lyssa virus ( Rabies)	1920	-	Order no. 14 of 11/01/1999 and Order no. 914 of 15/12/1987
Prions			
TSE	yes	Sheep & goats - Regulation 999/2001/EC (as amended)	Order no. 930 ot 07/09/2006
BSE	yes	Cattle - Regulation 999/2001/EC (as	Order no. 800 of 13/07/2006

a) Only clinical cases notifiable.

Source: Statens Serum Institute and Danish Veterinary and Food Administration

b) OBF according to Council Directive 64/432/EEC as amended by Council Directive 97/12/EC and Commission Decisions 93/52/EEC, 2003/467/EC and 2004/320/EC.

c) ObmF according to Council Directive 91/68/EEC and Commission Decisions 93/52/EEC, 94/877/EEC, 2003/467/EC and 2004/320/EC.

d) OTF according to Council Directive 64/432/EEC as amended by Council Directive 97/12/EC and regulation (EC) 1226/2002, and Commission Decision 2003/467/EEC.

e) Clinical cases, observations during the meat inspection at the slaughterhouse, positive blood sampes or finding of agens are notifiable.

Table A32. Salmonella surveillance programme for the rearing breeding flocks and adult breeding flocks of the grandparent and parent generation of the broiler and table egg production, 2008

Rearing flocks		Grandparent generation	Parent generation
Time	Sample taking	Material	Material
Day-old <sup>a,b</sup>	Per delivery	5 transport crates from one delivery: crate liners (>1m² in total) or swab samples (>1m² in total). Analysed as one pool.	5 transport crates from one delivery: crate liners (>1m² in total) or swab samples (>1m² in total). Analysed as one pool.
1 <sup>st</sup> & 2 <sup>nd</sup> week <sup>b, f</sup>	Per unit <sup>c</sup>	-	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 gram.
4 <sup>th</sup> week <sup>a,b</sup>		5 pairs of boot swaps (analysed as two pooled samples), or 1 faeces sample consisting of 2x150 gram.	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 gram.
8 <sup>th</sup> week <sup>b,f</sup>	Per unit	2 pairs of boot swabs (analysed as one pooled sample), or 1 faeces sample of 60 gram.	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 gram.
2 weeks prior to moving <sup>a,d</sup>	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150 gram.	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 gram.
Production flocks		Grandparent generation	Parent generation
Time	Sample taking	Material	Material
Every two weeks <sup>b</sup> (Every 16th week <sup>d</sup> ) <sup>e</sup>	Per flock	Hatcher basket liners from 5 baskets (>1m² in total) or 10g of broken eggshells from each of 25 hatcher baskets (reduced to 25g sub-sample). Analysed as one pool.	Hatcher basket liners from 5 baskets (>1m² in total) or 10g of broken eggshells from each of 25 hatcher baskets (reduced to 25g sub-sample). Analysed as one pool.
After each hatch <sup>b</sup>	Per hatch	Wet dust samples. Up to 4 hatchers of the same flock can be pooled.	Wet dust samples. Up to 4 hatchers of the same flock can be pooled.
Every week <sup>b</sup>	Per unit	-	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60 gram.
0-4 weeks after moving, 8-0 weeks before	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150 gram.	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150 gram.
After positive findings d	Per unit	5 pairs of boot swabs (analysed as two pooled samples).	5 pairs of boot swabs (analysed as two pooled samples).

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

b) Samples collected by the food business operator.

c) A unit (house) may harbor part of a flock or more than one flock, depending on the size of the unit.

d) Samples collected by the Regional Veterinary and Food Control Authorities.

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

f) Order no 1259 of 15/12/2008.

Table A33. Salmonella surveillance programme° for the broiler flocks, 2008

roiler production		
Time	Samples taken	Material
15 - 21 days before slaughter, Ante mortem (AM) <sup>b,c</sup>	Per flock	5 pairs of boot swabs. Analysed individually.
7 - 10 days before slaughter, Ante mortem (AM) <sup>d</sup>	Per flock	5 pairs of boot swabs. Analysed individually.
After slaughter Post mortem (PM) <sup>b</sup>	Per batch	Sampling is depending on whether the slaughterhouse slaughters only AM-negative flocks or AM-negative as well as AM-positive flocks.

a) According to Order no 1261 of 15/12/2008.

Table A34. Salmonella surveillance programme for the pullet-rearing, layer and barnyard/hobby flocks in the table egg production, 2008

Pullet-rearing flocks		
Time	Sample taking	Material
Day-old <sup>a,d</sup>	Per delivery	5 transport crates from one delivery: Crate liner (> $1 \text{ m}^2$ in total) or swab samples (> $1 \text{ m}^2$ in total) (Analysed as one pooled sample).
4 weeks old <sup>b,d</sup>	Per flock	5 pairs of boot swabs. Analysed as two pooled samples. Cage birds: 5x60 samples of fresh droppings (1g).
2 weeks before moving <sup>a,c</sup>	Per flock	5 pairs of boot swabs. Analysed as two pooled samples. Cage birds: 5x60 samples of fresh droppings (1g). 60 blood samples (serology).
ayers (Production for certif	ied packing statio	ons)
Time	Sample taking	Material
24 weeks old <sup>a,c</sup>	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 gram. 250 ml (100 g) dust of
		1 pair of boot swabs. 60 eggs <sup>b</sup> (serology).
Every 9 weeks <sup>d,e</sup>	Per flock	
Every 9 weeks <sup>d,e</sup> Barnyard and hobby flocks	Per flock	<ul> <li>1 pair of boot swabs. 60 eggs<sup>b</sup> (serology).</li> <li>2 pairs of boot swabs. Analysed as one pooled sample. Cage birds: 60 samples of fresh drop-pings (1g). Analysed as one</li> </ul>
, 	Per flock Sample taking	1 pair of boot swabs. 60 eggs <sup>b</sup> (serology).  2 pairs of boot swabs. Analysed as one pooled sample. Cage birds: 60 samples of fresh drop-pings (1g). Analysed as one pool. 60 eggs <sup>b</sup> (serology).

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

b) Samples collected by the food business operator.

c) Once a year, the samples are collected by the Regional Veterinary and Food Control Administration.

d) Samples are collected by a representative of the slaughter house, laboratorium or the Regional Veterinary and Food Control

b) According to Order no 1260 of 15/12/2008.

c) Samples collected by the Regional Veterinary and Food Control Administration.

d) Samples collected by the food business operator.

e) According to Regulation (EC) 2160/2003 sample collection must be carried out every 15 weeks as a minimum. Source: Danish Veterinaty and Food Administration

Table A35. Salmonella surveillance programmes<sup>a</sup> for ducks and turkey flocks, 2008

Duck production		
Time	Samples taken	Material
Max. 21 days before slaughter, Ante	Per flock	2 pairs of boot swabs. Analysed
mortem (AM) <sup>b</sup>		individually.
Turkey production		
Time	Samples taken	Material
Max. 21 days before slaughter, Ante	Per flock	5 pairs of boot swabs. Analysed
mortem (AM) <sup>b</sup>		individually.

a) According to Order no 1261 of 15/12/2008.

Table A36. Salmonella Dublin surveillance programme<sup>a</sup> for the cattle herds and Salmonella surveillance programme at slaughter. 2008

surveillance programme at slaughte	er, 2008	
Milk producing herds		
No. of tests	Sample taken	Herd level
4 samples distributed over 13 months	Tank milk	1a
8 samples	Blood samples	1b
Non-milk producing herds		
No. of tests	Sample taken	Herd level
1 sample <sup>b</sup>	Blood samples	1b
If the owner wants a herd moved from	Blood samples	2 ->1b
level 2 to 1b <sup>c</sup>		
Beef carcasses at the slaughterhouse		
No. of samples	Sample taken	Sampling time and no. of animals
		slaughtered
5 samples daily pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3-100m <sup>2</sup> )	> 200 cattle slaughtered per day
5 samples per 200 slaughtered cattle	Swab samples from 3 designated	> 200 cattle slaughtered per
pooled into one analysis	areas after 12 hours chilling (3-	month,
	$100\mathrm{m}^2)$	≤ 200 cattle slaughtered per day
5 samples every 3 <sup>rd</sup> month pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3- $100 \mathrm{m}^2$ )	> 50 cattle slaughtered per month, < 200 cattle slaughtered per month
1 sample every 3 <sup>rd</sup> month	Swab samples from 3 designated areas after 12 hours chilling (3- $100\text{m}^2$ )	< 50 cattle slaughtered per month

a) Order no. 112 of 24/02/2005 as ammended

b) Samples collected by the food business operator.

b) If the herd has been tested for *S*. Dublin within the last 120 days or 8 samples have been tested within the last 12 months no samples are taken.

c) 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

Table A37. Salmonella surveillance programme for the pig production, 2008

Breeding- and multiplier herds		
Time	Sample taken	Purpose
Every month	10 blood samples per epidemiological unit	Calculation of Salmonella -index based on the mean from the last three months with most weight to the result from the more recent months (1:3:6)
Max. twice per year	Herds with <i>Salmonella</i> -index 5 or above: Pen-faecal samples <sup>a</sup>	Clarify distribution and type of infection in the herd
Sow-herds		
Time	Sample taken	Purpose
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 clarify possible transmission from sow herds to slaughter-pig herds
Slaughter-pig herds		
Time	Sample taken	Purpose
At slaughter	Meat juice, 60-100 samples per herd per year.  Herds in RBOV <sup>a</sup> : one meat juice sample per month	Calculation of slaughter-pig index based on the mean from the last three months with most weight to the result from the most recent month (1:1:3). Assigning herds to level 1-3 and assigning herds to risk based surveillance (RBOV) <sup>b</sup>
Herds assigned to level 2 or 3, max. twice per year	Pen-faecal samples <sup>c</sup>	Clarify distribution and type of infection in the herd
Pork carcasses at the slaughterhou	se	
No. of samples	Sample taken	Time and no. of animals slaughtered
5 samples daily, pooled into one analysis	Swab samples from 3 designated areas (3x100 cm <sup>2</sup> ) after min. 12 h chilling	> 200 pigs slaughtered per day
5 samples per 200 slaughtered pig, pooled into one analysis	Swab samples from 3 designated areas $(3x100 \text{ cm}^2)$ after min. 12 h chilling	> 200 pigs slaughtered per month, ≤ 200 pigs slaughtered per day
5 samples every 3 <sup>rd</sup> month, pooled into one analysis	Swab samples from 3 designated areas $(3x100 \text{ cm}^2)$ after min. 12 h chilling	> 50 pigs slaughtered per month, < 200 pigs slaughtered per month
1 sample every 3 <sup>rd</sup> month	Swab samples from 3 designated areas $(3x100 \text{ cm}^2)$ after min. 12 h chilling	< 50 pigs slaughtered per month

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

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

c) Producers are paid a reduced price per animal. Pigs from herds in Level 3 must be slaughtered under special hygienic precautions.

## Appendix E

#### Population and slaughter data

Human population, 2008

, ,			
Age group (years)	males	females	Total
0-4	166,580	159,026	325,606
5-14	350,693	333,618	684,311
15-24	326,145	311,915	638,060
25-44	758,646	744,561	1,503,207
45-64	738,401	733,165	1,471,566
> 65	372,201	480,840	853,041
Total	2,712,666	2,763,125	5,475,791

Source: Statistics Denmark

Number of herds/flocks, livestock and animals slaughtered, Dec 2008

	Herds/flocks	Livestock	Number
	Tierus/Hocks	(capacity)	slaughtered
Slaughterpigs	11,000	12,195,000	18,582,288
Cattle	32,000	1,600,000	511,300
Broilers	580	20,000,000	100,304,000
Layers (excl. barnyard)	215	2,900,000	_a
Turkeys	51	482,000	_a
Sheep & lambs	9,080	173,000	89,520
Goats	3,475	23,000	2,140
Horses	-	-	2,520

a) Animals are slaughtered abroad.

Source: The Central Husbandry Register, Statistics Denmark and Danish Veterinary and Food Administration

#### Number of farms in the broiler production, 2008

	No ofholdings	No. of	Livestock
	No. of holdings	houses/flocks	(capacity)
Rearing period (grand parent)	2	5	50,000
Production period (grand parei	4	18	50,000
Rearing period (parent)	16	92	130,000
Production period (parent)	46	154	720,000
Hatcheries	5	-	-
Broilers	243	580	n.a.ª

a) Not available.

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

Number of farms in the table egg production, 2008

	337	No. of	Livestock
	No. of holdings	houses/flocks	(capacity
Rearing period (parent)	3	4	21,000
Production period (parent)	5	5	33,000
Hatcheries	5		
Pullet-rearing	93	155	1,420,000
Layers (excl. Barnyard)	215	295	2,900,000

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

Distribution of import, export and production of fresh and frozen meat and the production of table eggs in Denmark, 2007-2008. Data is presented in tons

10113							
		$Pork^{a}$	Beefa	Broiler	Turkey	Duck	Table
				meat <sup>b</sup>	meat <sup>c</sup>	meat <sup>d</sup>	eggs <sup>e</sup>
Import	2007	40,201	80,287	30,390	8,423	3,845	-
	2008	83,057	81,427	32,480	8,264	4,494	-
Export	2007	1,263,169	61,374	105,741	1,692	454	-
	2008	1,386,849	66,690	109,725	2,345	772	-
Production	2007	1,447,894	134,374	168,354	34	2,956	66,800 <sup>g</sup>
	2008	1,602,648	149,744	157,543	49	37	67,900 <sup>g</sup>
$Consumption^f \\$	2007	224,925	153,287	93,003	6,765	6,347	-
	2008	298,857	164,481	80,298	5,968	3,722	-

- a) Salted, smoked, dried, cooked or preserved meat is not included.
- b) Cooked or preserved meat is not included. Natural-marinated chicken is included (product line 1602 3211).
- c) Cooked or preserved meat is not included.
- d) Salted, smoked or dried meat or mixed products of ducks, geese and guinea fowl are not included.
- e) Include fresh table eggs from battery, free-range, organic and deep litter production and table eggs for producers' own use and barnyard sale.
- $f)\ Consumption = Production + import export$
- g) Consumption of table eggs is assumed to be roughly the same as the production, since import and export of table eggs is minimal.

Source: Statistics Denmark

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