Annual Report on Zoonoses in Denmark 2010

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Annual Report on Zoonoses in Denmark 2010

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Introduction

In 2010, the *Salmonella* source account estimated that almost half of the human salmonellosis cases were acquired abroad, which is an increase compared to previous years. For the domestic acquired cases, Danish produced pork was estimated to be the most important source followed by imported pork. The contribution of these two sources increased markedly compared to 2009; for Danish produced pork this was mainly due to one large pork-related outbreak. In contrast, the number of human cases attributed to table eggs was the lowest ever estimated. Despite the increase in the estimated number of cases attributed to imported pork, the total contribution of imported foods was similar to last year, as the remaining imported foods (broilers, turkey and beef) were estimated to be responsible for a lower number of cases in 2010.

The number of human *Salmonella* Enteritidis cases was the lowest number recorded in 10 years and at the same time, the relative number of cases related to travelling abroad increased to 76%. There were two outbreaks due to *S.* Enteritidis; both were related to travelling.

An increase in the number of human *Campylobacter* cases was observed throughout the year compared to 2009. In total, 20% more cases was reported in 2010. Two water-related outbreaks caused by contaminated drinking water and contaminated sea water, respectively, explain a small proportion of the increase.

Norovirus, fruit and vegetables

Norovirus was by far the single most frequent pathogen reported in foodborne outbreaks in 2010 accounting for 47 of 77 outbreaks and around a half of all outbreak-related cases; in 20 of the norovirus outbreaks there were double infections with ETEC. A large number of these outbreaks was associated with imported fruit and vegetables, e.g. a series of 20 outbreaks was associated with the same batch of Lollo Bionda lettuce from France.

For many years, norovirus has been the most common pathogen involved in foodborne outbreaks and there has been a growing need for development and implementation of methods for analysis of viruses in food products. In 2010, routine analysis of viruses in oysters and mussels were initiated at the National Food Institute, Technical University of Denmark, and the methods are currently being expanded to cover raspberries and green lettuce. The development of virus detection methods are a very important tool when investigating foodborne outbreaks.

The importance of taking fruits and vegetables into account when assessing the risk of food products to human illness is supported by the increasing number of outbreaks related to contaminated fruit (berries) and vegetables during the last six years. Until now, all norovirus outbreaks from fruit and vegetables have been related to imported products. Due to the increased concern, the Danish Veterinary and Food Adminstration conduvted a survey of pathogens in Danish and imported vegetables and fresh herbs and results support the importance of vegetables and fresh herbs as sources for human infections.

Lightly fermented sausages

Fermented sausages are normally considered a safe food product, however, several outbreaks with e.g. VTEC and *Salmonella* related to this type of food product has been reported. During recent years, the production of some of these food products has changed towards more lightly preserved products by reducing salt and fat content and increasing the water activity. This may reduce the safety, so the margin allowed for errors during the production is reduced. Results from a project investigating the survival of pathogens (*Listeria*, *Salmonella* and *E. coli*) in lightly preserved fermented sausages are presented.

The annual Report on Zoonoses presents a summary of the trends and sources of zoonotic infections in humans and animals, as well as the occurrence of zoonotic agents in food and feeding stuffs in Denmark in 2010. Greenland and the Faroe Islands are not represented. The report is based on data collected according to the Zoonoses Directive 2003/99/EC, supplemented by data obtained from national surveillance and control programmes as well as data from relevant research projects. Corrections to the data may occur after publication resulting in minor changes in the presentation of historical data in the following years report. The report is also available at www.food.dtu.dk.

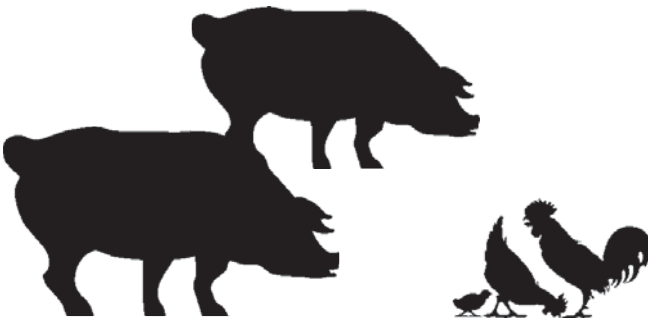
Broiler and table egg production

For the table egg production line, no *Salmonella* positive adult breeding flocks have been reported for many years, whereas for the broiler production line, positive breeding flocks have been reported yearly. In the EU, a 1% permanent target is set in the Regulation (EC) No 200/2010 for *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Infantis and *S.* Virchow for all adult breeding flocks. In 2010, 2.4% of the adult breeding flocks was positive with one of the five serovars, which is an increase compared to previous years.

In table egg layer flocks, the number of *Salmonella* positive flocks has been low for many years and 1.8% of the flocks was positive in 2010. The EU target of 2% set out in the Regulation (EC) No 1168/2006 for *S.* Typhimurium and *S.* Enteritidis in adult table egg layer flocks had to be reached by December 31st 2010. Denmark has been below this target for many years and the flock prevalence of *S.* Typhimurium and *S.* Enteritidis was 1.1% in 2010.

In the broiler production, the number of *Salmonella* positive flocks has been low for more than 10 years and 1.1% of the flocks slaughtered in Denmark was positive in 2010. The 1% EU target set out in the Regulation (EC) No 646/2007 for *S.* Typhimurium and *S.* Enteritidis in broiler flocks must be reached by December 31st 2011. In 2010, the flock prevalence of *S.* Typhimurium and *S.* Enteritidis was 0.3% in Danish broiler flocks.

From January 1st 2010, surveillance of *Campylobacter* in broilers became mandatory in Denmark and the producers are now obliged to sample the flocks at the farm. The result has to be available prior to slaughter as the results are used as a sorting tool for allocating positive flocks to frozen products. In 2010, 16.5% of the flocks was positive for *Campylobacter*. Results from this sampling are not directly comparable with results from the voluntary sampling at the slaughterhouse in previous years.



Turkey production

In 2010, EU Member States were obliged to report findings of *Salmonella* in turkey flocks according to a harmonised minimum surveillance programme for the first time. Since 2004, when the only major turkey slaughterhouse closed down in Denmark, less than 25 flocks have been slaughtered per year. The majority of flocks are exported as live animals to be slaughtered abroad. For many years, no or very little *Salmonella* has been found. In 2010, only one flock of 24 birds was positive. The 1% EU target set out in the Regulation (EC) No 584/2008 for *S.* Typhimurium and *S.* Enteritidis in turkey flocks must be reached by all Member States by December 31st 2012. In 2010, the prevalence of *S.* Typhimurium and *S.* Enteritidis in monitored Danish turkey flocks was 0%.

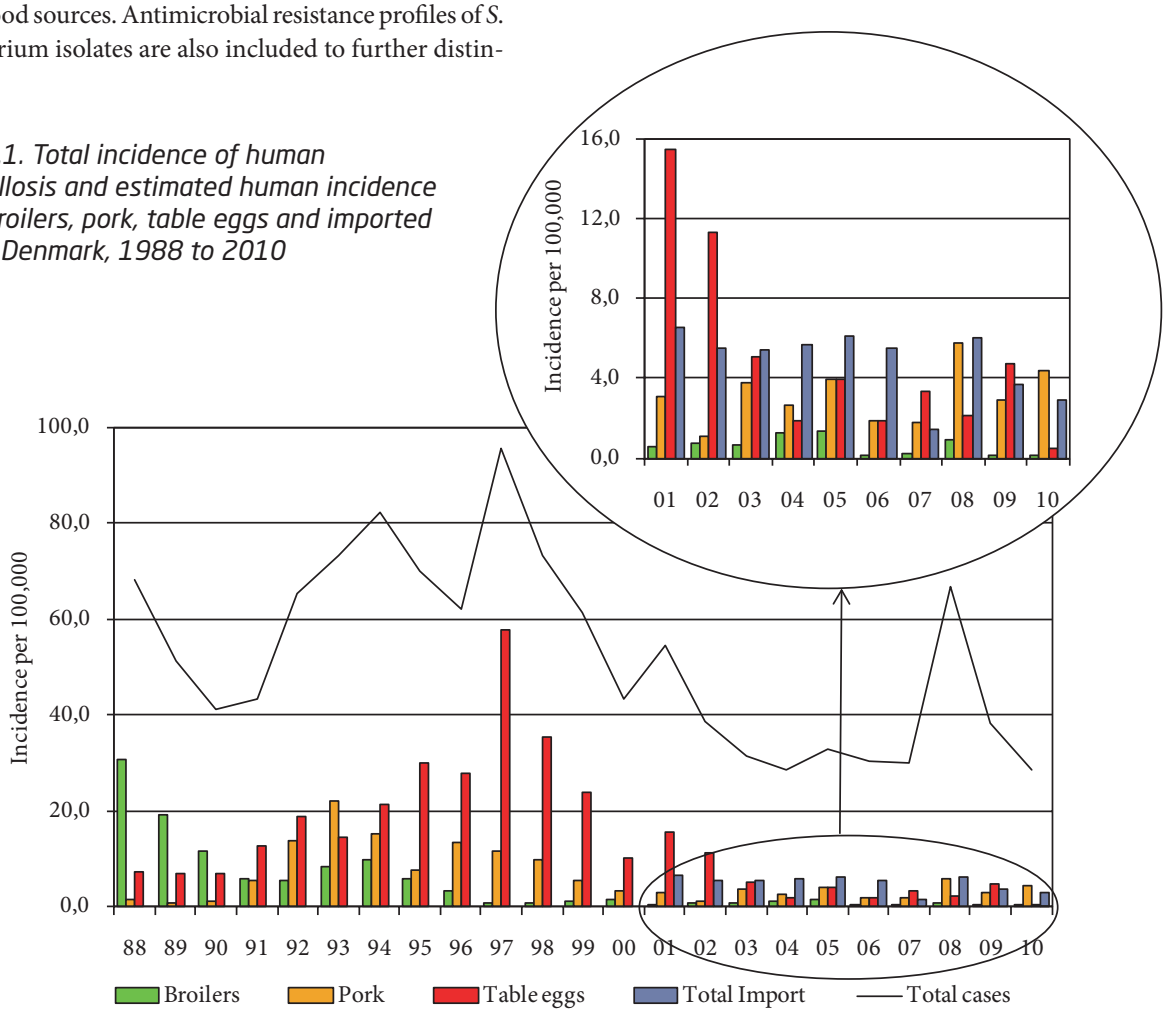
1. Trends and sources in human salmonellosis

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

Salmonella has been among the most important foodborne pathogens in Denmark in the last decades. The incidence of salmonellosis in the country reached a peak in the late 90's and has since then decreased sharply, reaching figures lower than the European average (1). Identifying the causative food sources and prioritizing interventions has been crucial to reduce the burden of foodborne salmonellosis in the population. To assist these risk management strategies, the Danish Zoonosis Centre, National Food Institute routinely applies a 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. In 2010, the European Food Safety Authority publiched an opinion on "*Salmonella* Typhimurium like" strains (2), and based on this it was decided to add these strains to *S.* Typhimurium in the source account model.

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 (3), which applies data from multiple years thereby improving the robustness and accurateness of the results without compromising their comparability with estimates from previous years. The proportion of cases that can be attributed to the major food sources is presented in Figure 1.1.

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 2010



Source: Danish Zoonosis Centre, National Food Institute

The incidence of human salmonellosis in 2010 was 28.7 cases per 100,000 inhabitants (7.0 for *S.* Enteritidis and 9.4 for *S.* Typhimurium including the "*Salmonella* Typhimurium like" strains) (appendix B, Table A2), which represents a substantial decrease when compared to 2008 and 2009 where several large outbreaks occurred.

In 2010, the most important source of salmonellosis in Denmark was estimated to be domestic pork (Figure 1.2). Domestic pork attributed with 15.1% of all *Salmonella* laboratory-confirmed cases followed by imported pork, which was estimated to contribute with 5.4% of reported cases (appendix A, Table A1). In addition, 20 outbreak-related *Salmonella* cases (1.3%) were caused by an imported mixed product containing pork and deer, which have been attributed to outbreaks with mixed or unknown source. The relative contribution of domestic and imported pork increased markedly compared to the previous years (more than two-fold for both sources). For domestic pork, the increase is mainly explained by a high number of cases related to a single pork-associated outbreak in 2010 (172 cases). In contrast, the relative importance of table eggs for salmonellosis decreased from 12.3% in 2009 to 1.8% in 2010. The large number of cases caused by table eggs in 2009 was due to two large egg-related outbreaks; the number of sporadic cases attributed to table eggs in 2010 was similar to estimates from 2009. The relative proportion of cases attributed to imported broilers and turkeys was 0.2% and 1.0%, respectively, which represents a decrease compared to the previous year. Cases attributed to imported beef decreased in 2010 as well, from 3% to 2%; all remaining animal-food sources were estimated to contribute with less than 1% of reported human cases. The total relative contribution of imported foods was similar to the previous year, despite the increase in the proportion of cases attributed to imported pork as the remaining imported foods were estimated to be responsible for a lower number of cases.

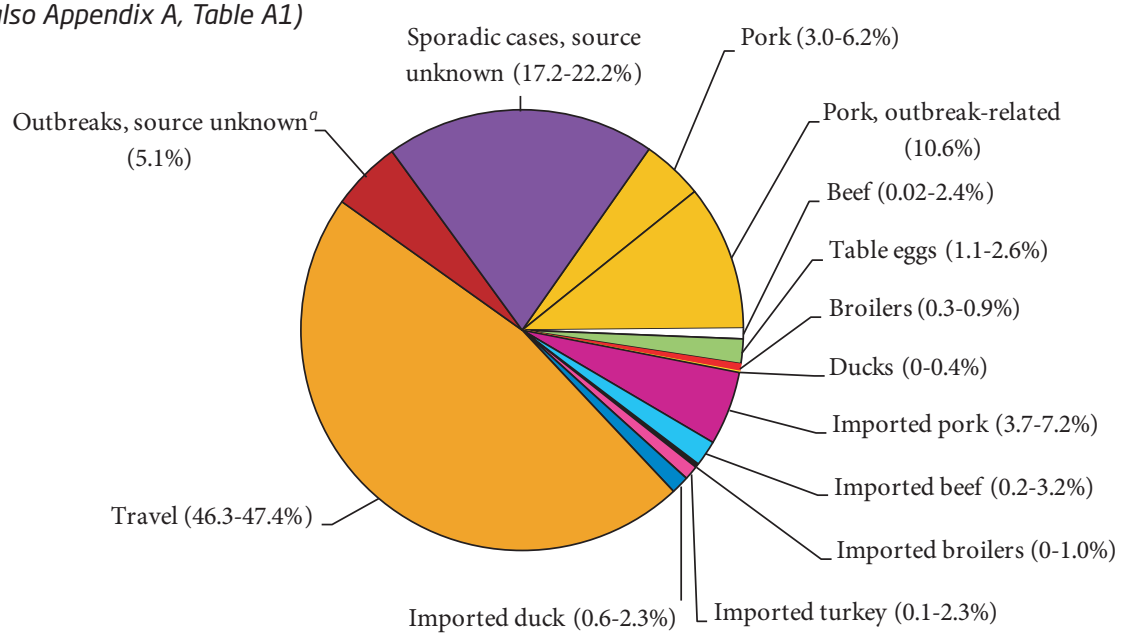
Nearly half (47%, 749 cases) of all *Salmonella* cases were estimated to be acquired abroad, of which 568 cases actually reported to have travelled within seven days prior to onset of symptoms. This represents a 14% increase in the estimated number of travel-related cases when compared to 2009.

Around 20% of reported sporadic *Salmonella* infections could not be associated with any of the included food sources. These cases may be caused by foods not included in the national surveillance (e.g. imported or domestic produced fruits and vegetables), or by non-food sources of infection such as direct contact with pet animals.

Of the 388 reported *S.* Enteritidis cases, 72.2% was estimated to be related to international travel and 5.2% to be associated with outbreaks related to international travel. There were no *S.* Enteritidis outbreaks related to Danish produced products in 2010. The estimated proportion of travel-related *S.* Enteritidis cases increased when compared to 2009, mainly due to two large domestic outbreaks in 2009 that increased the number of domestic cases compared to other years.

A total of 642 *S.* Typhimurium cases was reported in 2010 (including the 121 cases of "*S.* Typhimurium like strains"), of which 20.7% was estimated to be related to internatio-

Figure 1.2. Estimated sources of 1,598 cases of human salmonellosis in Denmark, 2010 (See also Appendix A, Table A1)



a) Includes mixed and unknown sources.
Source: Danish Zoonosis Centre, National Food Institute

nal travel and 34.4% associated with domestic outbreaks. The estimated number of *S.* Typhimurium cases acquired abroad increased compared to previous years when large domestic outbreaks influenced the relative distribution significantly. From the 59 *S.* Typhimurium cases attributed to domestic products, 77.0% was caused by types susceptible to all antimicrobials, 21.2 % by types resistant to one to three antimicrobial drugs, and 1.7% by types resistant to four or more antimicrobial drugs (multi-resistant); no cases caused by isolates resistant to quinolones were attributed to domestic foods (Figure 1.3). In contrast, the majority of *S.* Typhimurium infections attributed to imported food products (81 cases out of 642 cases) was caused by resistant (55.3%) or multi-resistant (19.3%) types. From the 133 S. Typhimurium cases acquired abroad, 42.2 % was caused by resistant types, 14.3 % by multi-resistant types, 11.7 % by types resistant to quinolones, and 27.7 % by types susceptible to all tested antimicrobials. These figures are similar to the estimates obtained for 2009 and reflect different levels of antimicrobial resistance in *Salmonella* isolates in Danish and imported food products.

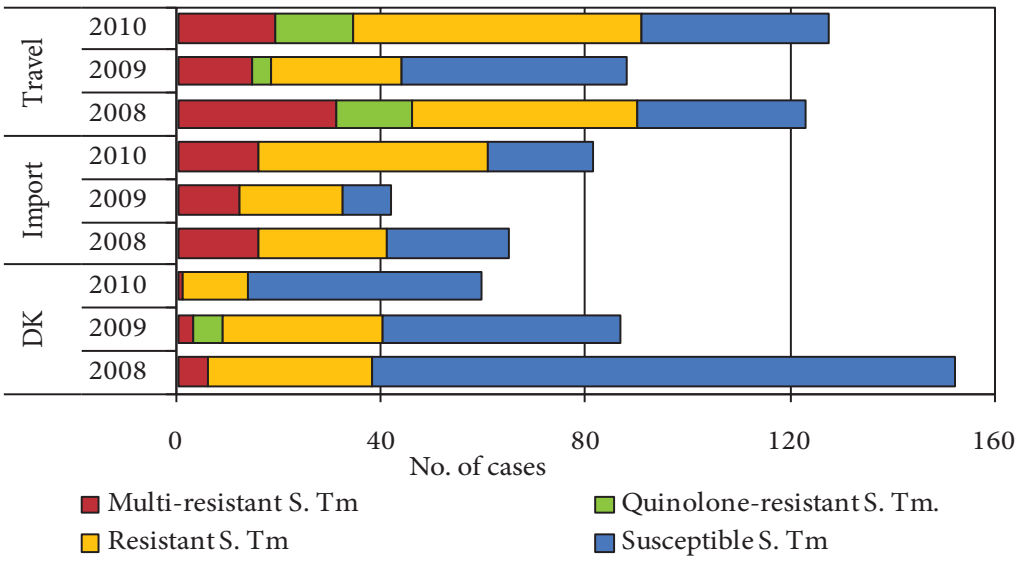
References

(1) The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2009 (2011). EFSA Journal 9(3):2090.

(2) Panel on Biological Hazards (BIOHAZ) (2010). Scientific Opinion on monitoring and assessment of the public health risk of "*Salmonella* Typhimurium-like" strains. EFSA Journal 8(10):1826.

(3) Pires SM and T Hald (2010). 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. Estimated sources of antimicrobial resistant[a] S. Typhimurium infections in humans, 2008-2010



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

Where do we acquire *Salmonella* infections?

In 2010, as in 2009, Statens Serum Institut attempted to interview all registered *Salmonella* cases where no travel information was reported by the general practitioner. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease onset. This information was complemented with information from general practitioners' reports and travel information was obtained from a total of 81% of the *Salmonella* cases in 2010. Among the cases with known travel history, 76% of the *S.* Enteritidis cases, 15% of the *S.* Typhimurium cases and 47% of cases with other serotypes were infected abroad. The group of other serotypes comprises considerable variation in terms of serotypes (Table 1.1).

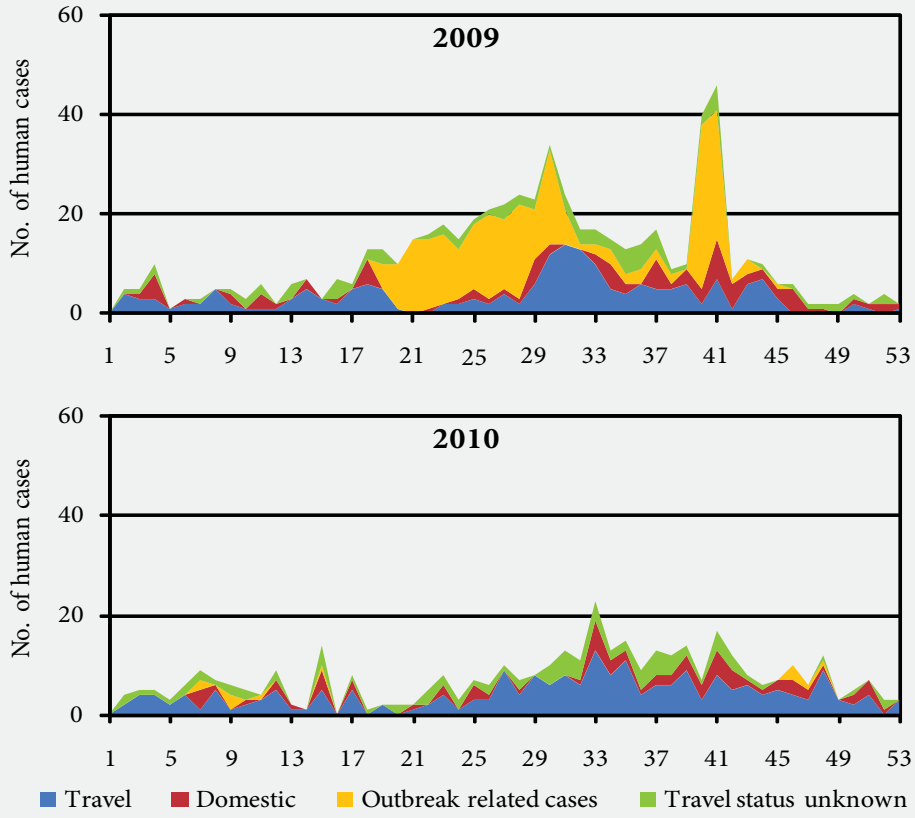
In 2010, the distribution pattern of travel-related and domestically acquired *Salmonella* infections was comparable to that of 2009 for most serotypes. However, for *S.* Enteritidis the percentage of cases acquired abroad increased from 46% in 2009 to 76% in 2010 (Figure 1.4). This shift is caused by the dramatic decrease in domestically acquired *S.* Enteritidis cases which was the lowest in 25 years. In 2009, the majority of *S.* Enteritidis cases was due to two large domestic outbreaks; the number of sporadic cases had already decreased in 2009. Most of the travel-related *Salmonella* infections in 2010 was acquired in Egypt, Thailand and Turkey.

Table 1.1. Top 10 Salmonella *serotypes in humans and place of infection, 2009-2010*

2009	Number of patients (%)	% patients infected[a] Abroad	Domestically	**2010**	Number of patients (%)	% patients infected[a] Abroad	Domestically
S. Typhimurium	767 (36)	10.6	89.4	S. Typhimurium	521 (33)	14.8	85.2
S. Enteritidis	600 (28)	45.7	54.3	S. Enteritidis	388 (24)	76.4	23.6
S . O:4,5,12;H:i:-	77 (4)	41.9	58.1	S. O:4,5,12;H:i:-	96 (6)	33.3	66.7
S. Dublin	46 (2)	6.9	93.1	S. Dublin	49 (3)	11.1	88.9
S. Newport	42 (2)	45.5	54.5	S. Infantis	38 (2)	37.1	62.9
S. Virchow	36 (2)	79.3	20.7	S. Newport	33 (2)	68.0	32.0
S. Agona	27 (1)	13.3	86.7	S. Virchow	32 (2)	76.9	23.1
S. Infantis	25 (1)	38.1	61.9	S. Stanley	30 (2)	73.7	26.3
S. Saintpaul	23 (1)	23.5	76.5	S. O:4,12;H:i:-	25 (2)	38.9	61.1
S. Muenchen	20 (1)	8.3	91.7	S. Java	22 (1)	47.4	52.6
Other serotypes	466 (22)	42.3	57.7	Other serotypes	364 (23)	62.9	37.1
Total	2,129 (100)	31.1	68.9	Total	1,598 (100)	45.2	54.8

a) Patients with unknown travel information (22.4% of all patients in 2010 and 26.4% of all patients in 2009) were excluded from the percent calculations.
Source: Statens Serum Institut

Figure 1.4. Weekly distribution of S. Enteritidis *cases, 2009-2010*



Source: Statens Serum Institut

2. Outbreaks of special interest

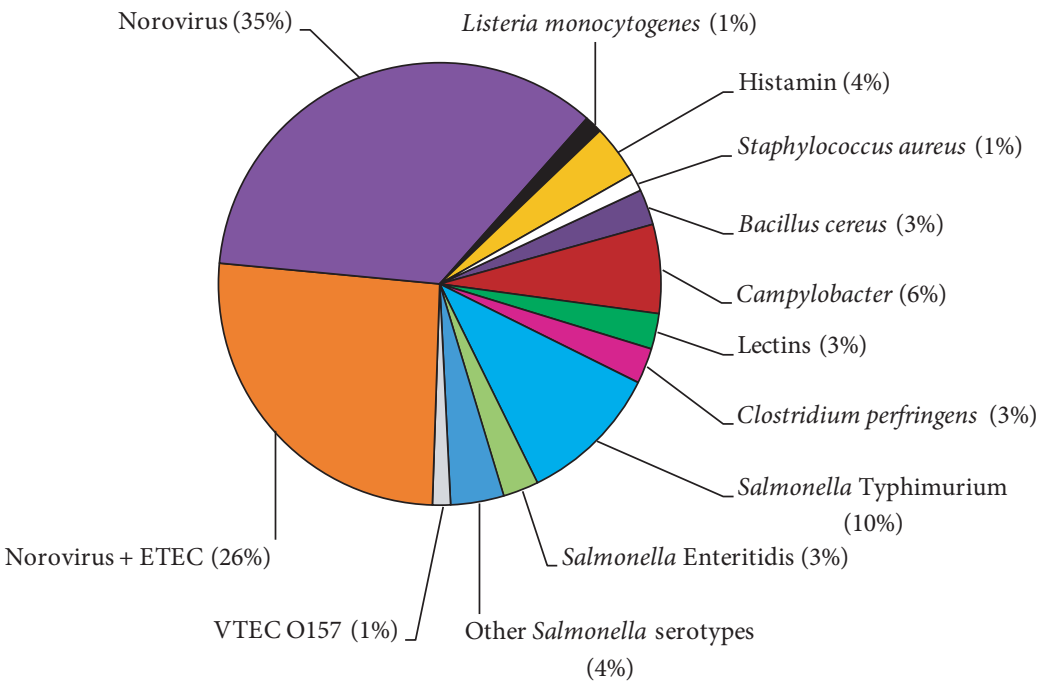
By Steen Ethelberg (set@ssi.dk)

In Denmark, foodborne outbreaks are investigated by a number of different institutions, depending on the nature of the outbreak. Local foodborne outbreaks are primarily handled by the Regional Veterinary and Food Control Authority. 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. These three institutions have a formalised cooperation involving mutual written agreements and weekly outbreak-response coordination meetings. The reporting and outbreak investigation systems are described in further detail in Chapter 7.2.

Outbreaks are reported in the Food- and waterborne Outbreaks Database (FUD). Outbreaks that occurred in 2010 are presented in appendix B, Table A3. Figure 2.1 shows the relative distribution of these outbreaks by the different pathogens that caused them. Household outbreaks and clusters that could not be verified as common source outbreaks are not included. Some of the more notable outbreaks are outlined below.

As in previous years, norovirus was the single most frequent disease agent in the registered outbreaks (appendix B, Table A3). Of the 77 reported foodborne outbreaks in 2010, norovirus accounted for 47 with a total of 1,266 registered cases. These outbreaks were often a result of contamination events associated with workplace lunch buffets, restaurants or private parties and as in previous years, many of these outbreaks followed gastrointestinal symptoms in persons preparing the food. Further, in 2010 a large number of norovirus outbreaks occurred with imported food products or food products produced in other EU countries. These were oysters mainly from France (1), raspberries from Serbia and two types of lettuce from Germany and France, respectively. Norovirus was detected in the foods by PCR methods (see Chapter 3 for more information on detecting norovirus in food). Romaine lettuce grown in Germany caused an outbreak in Southern Jutland (FUD

Figure 2.1. Aetiology of the 77 foodborne disease outbreaks reported with a causative agent in the Food- and waterborne Outbreak Database (FUD), 2010. Percentage of total outbreaks indicated in brackets



Source: Statens Serum Institut

no. 1008) and Lollo Bionda lettuce grown in France caused a series of outbreaks over a few days with norovirus and enterotoxigenic *E. coli* (2). In total, there were 20 registered outbreaks (FUD no. 952-953, 955-961, 963-964, 968, 970-972, 981-982, 984-986) and one household outbreak (FUD 983) associated with this one batch of Lollo Bionda lettuce. The lettuce was primarily used in sandwiches prepared by catering companies and this was part of the reason for the many registered outbreaks; a total of 405 cases were registered in the outbreaks. This one incident accounts for about a fourth of all registered outbreaks in 2010 and explains the increase in the number of reported outbreaks relative to the previous year.

One large outbreak occurred caused by *S.* Typhimurium U 323 of a specific MLVA type (FUD no. 979). The source of the outbreak was different types of pork products that were traced back to a specific slaughterhouse. The outbreak strain with matching MLVA type was found in the slaughterhouse on several occasions. Among the positive products was a spreadable pork sausage, which in a case-control investigation was shown to have caused a number of illnesses towards the end of the outbreak period. Despite good understanding of the source of the outbreak, it proved difficult to thoroughly clean the slaughterhouse and also to obtain valid information concerning distribution of meat from later stages of the production chain (cutting plant), and the outbreak ended up comprising a total of 172 registered cases over a period of six months (3).

Another *S.* Typhimurium DT 120/DT 7 outbreak was detected as a clustering of a particular MLVA type in patients from the national surveillance system (FUD 996). Trawling interviews lead to the hypothesis of a particular sliced salami containing meat from pigs and deer. This was confirmed in a subsequent case-control investigation. The sausage was produced in Germany for a Danish supermarket chain, but the suspected batch of sausages was sold out before microbiological evidence could be obtained (4).

A large waterborne *Campylobacter* outbreak took place in May among approximately 20,000 recipients of water from the municipal waterworks in the city of Køge south of Copenhagen (FUD no. 1001). A total of 61 cases of *Campylobacter jejuni* was laboratory confirmed and the majority of isolates found to belong to the same clone based on flaA-typing. In a questionnaire study performed among the inhabitants, a little more than 1,500 inhabitants could be included in the analysis and of these some 400 were cases. This study showed a dose-response relationship between intake of tap water and the risk of becoming ill. A boiling order was in place during the investigation. A very thorough technical investigation into the possible causes of the contamination was conducted, however no likely explanation for the cause was found.

A *Listeria* outbreak (FUD no. 1035) took place in the autumn. It comprised nine cases of which five were pregnant women. The MLVA/PFGE pattern of the strain was among the most common in Denmark, but the outbreak investigation was initiated as a result of the unusually high number of pregnant cases. Based on case interviews, a hypothesis of smoked salmon was formed.

Finally, an unusual outbreak occurred in August when a number of participants in a Triathlon competition fell ill after competing in contaminated sea water outside of Copenhagen (FUD no. 1015). The swimming leg of the competition was held on the morning following an unusually powerful rainfall that flooded the Copenhagen sewer system and lead to a sudden, transient microbial pollution of coastal waters. In a questionnaire investigation conducted among all participants (of which about half were foreign), close to 800 (about 60%) answered the questionnaire and of these 55% indicated to have had symptoms of acute gastroenteritis. There was an association between illness and the amount of sea water that the participants indicated to have accidentally swallowed. Some participants had stool samples examined after the competition and results thereof indicated an outbreak of mixed etiology including *Campylobacter* and enterotoxigenic *E. coli* (3).

References

(1) Westrell T, V Dusch, S Ethelberg et al (2010). Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. Euro Surveill 25;15(12).

(2) Ethelberg S, M Lisby, B Bottiger et al (2010). Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. Euro Surveill 11;15(6).

(3) Statens Serum Institut (2010): EPI-NEWS, week 42-43.

(4) Statens Serum Institut (2010): EPI-NEWS, week 25.

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3. Foodborne virus as a cause of disease - detection and future control perspectives

By Anna Charlotte Schultz (acsc@food.dtu.dk) and Blenda Böttiger

Human noroviruses (NoVs) cause gastroenteritidis and are transmitted through the faecal-oral route. Infected persons may excrete up to 10^8 viral particles/ml faeces and vomit (1), and with infective dose (ID_{50}) as low as 18 viral particles (2) they are highly contagious and spread rapidly through person-to-person contact and airborne droplets.

NoVs are extremely resistant towards environmental stress which allows them to survive outside their host and spread via the environment. In addition, infection by NoVs provides only short-time immunity. Combined, these characters explains why NoVs worldwide have been reported to cause up to 95% of viral gastroenteritis outbreaks in humans of all age groups (3, 4) and repeatedly are implicated in large-scale disease outbreaks (1, 3, 4). Finally, NoVs have been identified as the most frequent cause of foodborne disease outbreaks in most Western countries such as in the United States (5) and EU (6). In Denmark, they have accounted for 36-61% of total foodborne outbreaks during the years 2007-2010 (Annual Report 2007-2010). Besides NoVs, other enteric viruses (e.g. sapoviruses, astroviruses and rotaviruses) and hepatic viruses (e.g. hepatitis A virus) can be foodborne.

NoVs comprise a genus in the family Caliciviridae and are divided into five genogroups (G I – G V) with a great diversity of genomic sequences. Human NoVs constitute 25 genotypes and many more subtypes that belong to G I, G II or G IV (7). The majority of animal strains have been identified within G III (bovine) and G V (murine), but porcine and lion strains also cluster within G II and G IV, respectively. Despite fractional sequence similarities between some human and animal NoVs, no zoonotic transmission has yet been identified (3).

Since NoVs cannot be grown in cell culture (8), detection of genomic RNA by reverse transcriptase-realtime PCR (RT-qPCR) has become the method of choice for laboratory diagnosis (9-12) and strains can be genotyped by conventional RT-PCR targeting the polymerase (13) or capsid (14) region.

3.1 Human cases

There is no reporting system for NoV infections or for gastroenteritis outbreaks in Denmark, except if it is a suspected foodborne outbreak. As diagnostics of NoV infections today is carried out in several microbiological laboratories, there is no concise picture of the NoV seasonality and epidemiology in Denmark. However, restricted NoV surveillance has been performed during the last couple of years by genotyping a selection of NoV positive samples. Characterisation of positive NoV findings by genotyping is also an important tool in the investigation of outbreaks. The genotyping results can link separate outbreaks to each other and can confirm or oppose a suspicion of a common source of infection.

3.2 Foodborne outbreaks

Transmission of NoVs occur through the faecal-oral route either directly from person-to-person by ingestion of aerosolised vomit, or by indirect exposure via contaminated environmental surfaces, food and water. Most often, foodborne transmission occurs by contamination from food handlers with gastrointestinal symptoms. However, contamination earlier in the food production chain with human waste or polluted irrigation water has been demonstrated frequently as well. In Denmark, NoV outbreaks due to e.g. contaminated oysters, raspberries, lettuce as well as drinking water has occurred (Annual report 2005-2010).

3.3 Virus detection in food

Despite that a large part of foodborne outbreaks around the world are estimated to be caused by enteric viruses (e.g. norovirus), the official control programmes of food and water does not include routine monitoring of these pathogens in any part of the world. The reason is the absence of standardized analytical methods.

The major problems for detection of NoVs in food samples are:

- The presumable low levels of virus contamination
- The variability in virus or nucleic acid extraction
- The presence of interfering substances that inhibit molecular detection
- The genetic variability of NoVs.

However, with the growing acknowledgement of the risk of virus transmission to food, efforts to develop methods for virus detection have resulted in significant progress during the past 15 years.

3.3.1 Efforts at EU level

A two-part (quantitative and qualitative) standard has recently been drafted by a technical working group within European Committee on Standardisation (CEN/TC 275/WG6/TAG4). After an upcoming formal study of validation this standard has the potential to be incorporated into EU legislation as a reference method (15).

3.3.2 Implementation of methods in Denmark

At the National Food Institute, Technical University of Denmark routine analysis of viruses in oysters and mussels were initiated in the beginning of 2010 and are currently being expanded primarily to cover raspberries and green lettuce. Although only for research purposes, the quantitative levels of detected viruses are being estimated in order to accumulate data on viral loads in the field and from samples related to outbreaks. It is the aim that such data can help the risk assessors in the estimation of the human health risk when consuming foods contaminated in different levels.

The majority of foodborne outbreaks caused by infected food handlers can often be solved by epidemiological data obtained from questionnaires combined with the linkage of the specific type of virus by clinical examinations of patient samples and food handlers with gastroenteritidis.

For outbreaks thought to be caused by foods contaminated during production and distributed in large consignments, analysis of relevant food samples has proved useful in the aim to complete the argumentation for withdrawal of the suspected batch. Therefore, the development of methods for virus detection in foods at the National Food Institute has focused primarily on vehicles likely to be contaminated during production such as oysters, raspberries and lettuce.

During the past 18 months, samples from 12 different batches of food implicated in 38 Danish NoV outbreaks has been analysed (Table 3.1). Except for one batch, NoV GI, GII or both were detected in samples from all 12 batches. As the characterisation of the detected NoVs in the food samples are so far only at genogroup level, opposed to the genotype characterisation in patient samples, a positive virus detection in food samples may not prove a direct link between the suspected food in question and the patients. However, it proves the food to be contaminated with NoV, and in outbreaks where the data obtained from the epidemiological and clinical findings are insufficient, it can support the arguments needed by the authorities to withdraw the batch in question from the market.

Table 3.1. Food consignments contaminated during production and implicated in viral outbreaks in Denmark, September 2009-March 2011

Origin of batch	No. of outbreaks	Outbreak year/ month	Setting	No. of cases	Virus detected in humans[a]	Virus detected in outbreak batch[b]	FUD no.[c]
Live oysters							
France (Creuse Normandie, Isigny, Utah)	1	2010/02	Parties	27	NoV G I.7, NoV G II.2, II.12	NoV G I, NoV G II	973
France (Oleron)	1	2010/03	Vine bar	2	No samples	NoV G I, NoV G II	-
France (Marennes d'Oleron)	2	2011/01	Private dinners	11	No samples	NoV G I, NoV G II	1048
Fresh lettuce							
France	20[d]	2010/01	Cantines, take aways etc.	405	NoV G I.6, I.13, NoV G II.4, II.c, II.14,SaV, AsV	NoV G II	952-953 955-961 and more
Germany	1	2010/08	Catering	14	NoV G I.3 / I.14	NoV G I, NoV G II	1008
Frozen raspberries							
Serbia	1	2009/09	Private party	6	NoV G II.8	NoV G I, NoV G II	936
China	1	2010/01	Private party	10	NoV	NoV G II	-
Serbia	1	2010/10	Company canteen	30	NoV G I.b / I.6	NoV G II	1020
Serbia	1	2010/11	Conference centre	60	NoV G I.b / I.6	Not detected	1030
Serbia	2	2011/01	Employee at hospital canteen/ company canteen	127	NoV G I.b / I.6	NoV G I, NoV G II	1051, 1057
Serbia	2	2011/01	Café/company canteen	7	NoV G I.b / I.6	NoV G I, NoV G II	1058, 1059
China	1	2011/01	Private party	8	NoV G I.4	NoV G II	1049

a) NoV=Noroviruses, SaV=Sapoviruses, AsV=Astroviruses.
b) Food samples were only analysed for norovirus.
c) Outbreaks reported in the Food- and waterborne Outbreaks Database (FUD). For further information on FUD, see Chapter 2 and appendix B, Table A3.
d) In addition, one household outbreak (FUD 983). For further information, see appendix B, Table A3.
Source: National Food Institute.

3.4 Discussion

Implementation of virus testing into formalised use for official control monitoring and surveillance is a complicated task as several knowledge gaps needs to be addressed prior to such decisions. Given the nature of PCR detection of pathogens, a major result interpretation issue is that it is not clear whether presence of virus genome correlates with presence of infectious virus and thus human health risk. In addition, information on how viruses are distributed, e.g. within a harvesting area of oysters or a farm of raspberries, is needed to support the development of appropriate sampling plans to be used in field and consignment studies. Using these new quantitative methods in a systematic approach to surveillance of virus in relevant food production chains will considerably assist management and interpretation of outbreak related incidents.

References

(1) Koopmans M and E Duizer (2004). Foodborne viruses: an emerging problem. Int J Food Microbiol 90:23-41.

(2) Teunis PF, C L Moe, P Liu, E Miller, L Lindesmith, RS Baric, PJ Le and RL Calderon (2008). Norwalk virus: How infectious is it? J Med Virol 80:1468-1476.

(3) Glass RI, UD Parashar and MK Estes (2009). Norovirus gastroenteritis. New Engl J Med 361:1776-1785.

(4) Green KY (2007). Caliciviridae: The Noroviruses, p. 949-979. In Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B and Straus SE (ed.), Fields Virology, 5th edition. Lippincott Williams and Wilkins, Philadelphia, PA.

(5) CDC&P (2009). Surveillance for foodborne disease outbreaks - United States, 2006. MMWR. 58:609-615.

(6) The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks 2009 (2011). EFSA Journal 9(3):2090.

(7) Atmar RL (2010). Noroviruses: State of the Art. Food Environ Virol 2:117–126.

(8) Duizer E, KJ Schwab, FH Neill, RL Atmar, MP Koopmans and MK Estes (2004). Laboratory efforts to cultivate noroviruses. J Gen Virol 85:79-87.

(9) Kageyama T, S Kojima, M Shinohara, K Uchida, S Fukushi, FB Hoshino, N Takeda and K Katayama (2003). Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol 41:1548-1557.

(10) Loisy F, RL Atmar, P Guillon, P Le Cann, M Pommepuy and FS Le Guyader (2005). Real-time RT-PCR for norovirus screening in shellfish. J Virol Methods 123:1-7.

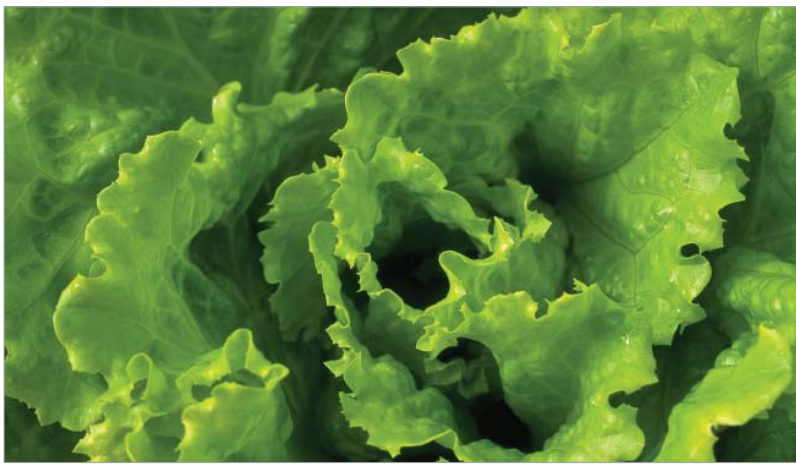
(11) da Silva A K, JC Le Saux, S Parnaudeau, M Pommepuy, M Elimelech and FS Le Guyader (2007). Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviors of genogroups I and II. Appl Environ Microbiol 73:7891-7897.

(12) Svraka S, E Duizer, H Vennema, E de Bruin, B van der Veer, B Dorresteijn and M Koopmans (2007). Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. J Clin Microbiol 45:1389-1394.

(13) Vennema H, E de Bruin and M Koopmans (2002). Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction. J Clin Virol 25:233-235.

(14) Atmar RL and MK Estes (2006). The epidemiologic and clinical importance of norovirus infection. Gastroen Clin N Am 35:275-290.

(15) Lees D and CEN WG6 TAG4 (2010). International standardisation of a method for detection of human pathogenic viruses in molluscan shellfish. Food Environ Virol 2:146-155.



4. Pathogens in fruit and vegetables

By Luise Müller (lum@ssi.dk), Anna Irene Vedel Sørensen and Annette Perge

In recent years, fresh fruit and vegetables have increasingly been recognized as a source of foodborne outbreaks. Several conditions may explain this including increasing consumption of fruit and vegetables, changes in processing and distribution patterns and growing awareness among public health personal of this transmission route (1). Worldwide, a number of large outbreaks caused by fruit and vegetables have occurred within the last few years; well described examples include a large multistate *Salmonella* Saintpaul outbreak in the USA involving 1,500 cases of which 21% were hospitalized and two died (2). The results of the outbreak investigation indicated jalapeño peppers as the major vehicle for transmission. In Sweden, a large outbreak of verocytotoxin-producing *Escherichia coli* (VTEC) O157 was associated with locally produced lettuce (3). In total, 135 cases were identified, 11 of these developed hemolytic uremic syndrome (HUS). The global distribution of fruits and vegetables has also led to international outbreaks, e.g. *S.* Thompson in rucola lettuce in 2004 with cases in Norway, Sweden and England (4).

Increasing focus on a healthy lifestyle among Danish consumers has increased the demand for availability of all types of fruit, vegetables and fresh herbs all year around. Therefore, fruit and vegetables from all over the world have become available on the Danish market. Many of these types of products are usually eaten raw (e.g. lettuce and other leafy vegetables) or added to dishes after heat treatment (e.g. fresh herbs) increasing the potential for pathogenic microorganisms present in these products to cause human disease.

4.1. Outbreaks related to fruit and vegetables in Denmark

In Denmark, a number of foodborne outbreaks have been linked to the consumption of fresh fruit and vegetables from 2005-2010 (Table 4.1). The largest outbreaks were due to norovirus in frozen raspberries from Poland with a series of sub-outbreaks in 2005 (5) with 1,010 cases and frozen raspberries from Serbia in 2010-11 with 237 cases. The 2005 outbreak caused the largest recorded number of human cases attributable to a single known vehicle in Denmark. Other outbreaks were caused by fresh basil from Israel (7), baby corn from Thailand (8), sugar peas from Kenya (9), Lollo Bionda lettuce from France (6) and Romaine lettuce from Germany (Table 4.1). The two latter outbreaks, which took place in 2010, are described in more details in chapter 2.

On several occasions, Danish cases could be linked to cases in other countries, as was seen in the baby corn outbreak where 12 cases were reported from Australia (8) and a *Salmonella* Weltevreden outbreak where contaminated alfalfa seeds had been sold in Norway and Finland as well (10). Furthermore, Danish cases of *S.* Java from baby spinach (11) and *S.* Stanley in alfalfa sprouts (12) were suspected part of international outbreaks.

The outbreaks related to fruit and vegetables in Denmark show a variety of different pathogens involving both virus and bacteria as well as many different sources; from berries, herbs and lettuce to more tropical vegetables such as sugar snaps and baby corn. Fruit and vegetables are gradually being recognised as possible vehicles for foodborne outbreaks and the identified outbreaks only constitute a part of the full picture as outbreaks and sporadic cases where the source is not known may to some extent be associated with fruits and vegetables.

4.2. Project on control of pathogens in vegetables and fresh herbs 2009-10

Due to the increased concern about possible pathogens in fruit and vegetables, the Danish Veterinary and Food Administration initiated a survey to investigate the presence of pathogens in Danish and imported ready-to-eat vegetables and fresh herbs on the Danish market.

From May 2009 to September 2010, 334 batches of vegetables and fresh herbs were sampled by the Regional Veterinary and Food Control Authorities. The sampling occurred at the point of entry for imported products and at wholesalers for Danish products. Five samples of at least 100 g were obtained from each batch and analysed for *Salmonella*, *Campylobacter* and *Escherichia coli* using the regional laboratories standard methods. Sampling included baby corn, sugar peas (e.g. sugar peas, sugar snaps, mange tout), sprouted seeds (e.g. bean sprouts, alfalfa sprouts, chick pea sprouts), leafy vegetables (e.g. baby spinach, rocket lettuce, iceberg lettuce), and fresh herbs (e.g. basil, coriander, mint, parsley, chives). Frozen vegetables, pre-cut vegetables and dried herbs were not sampled.

A batch was defined as positive for *Campylobacter* or *Salmonella* if the pathogen was isolated from at least one of five samples. Batches were examined for the presence

Table 4.1. Outbreaks due to fruit and vegetables in Denmark, 2005-2010

Year	Pathogen	Number of patients	Setting	Source	Country of origin	Ref.	FUD no.[f]
2005	Norovirus	1,010	5 sub-outbreaks in different institutions	Frozen raspberries	Poland	5	-
2005	Norovirus GGII.7	34	Company outbreak	Frozen raspberries	Poland	-	462
2006	ETEC/*Salmonella* Anatum	250	High school party	Fresh basil	Israel	7	661
2007	*Shigella Sonnei*	200[a]	Company outbreak/ international and sporadic	Baby corn	Thailand	8	726
2007	*Salmonella* Weltevreden	19[b,c]	International and sporadic	Alfalfa sprouts	Italy -the seeds	10	743
2007	Norovirus GGII.7	9	Private party	Frozen raspberries	China	-	708
2008	*Cl. perfringens*	19	Restaurant	Chick peas/Humus	-	-	831
2009	*Shigella Sonnei*	10[b]	Sporadic	Sugar peas	Kenya	9	888
2009	Norovirus GGI.8	6	Private party	Frozen raspberries	Serbia	-	936
2010	Norovirus GGII	10	Private party	Frozen raspberries	China	-	-
2010	ETEC/Norovirus	405[d]	20[e] sub-outbreaks from catering companies	Lollo Bionda lettuce	France	6	-
2010-2011	Norovirus GGI.6/I.b	237	6 sub-outbreaks	Frozen raspberries	Serbia	-	-
2010	Norovirus GGI.3	14	Catering and sporadic	Romaine lettuce	Germany	-	1008

a) Additional 12 cases in Australia.
b) Laboratory confirmed cases.
c) Additional 19 cases in Norway and 7 in Finland.
d) Additional 2 outbreaks in Norway.
e) Additional 1 household outbreak.
f) Outbreaks reported in the Food- and waterborne Outbreaks Database (FUD). For further information on FUD, see Chapter 2
Source: National Food Institute and Statens Serum Institut

of *E. coli* as an indicator of faecal contamination, and batches were only included as positive when levels of *E. coli* exceeded 100 cfu/g in one or more of five samples. *Campylobacter, Salmonella* or samples containing more than 100 cfu/g *E. coli* were isolated from 1.5%, 1.8% and 6.0% of the tested batches, respectively (Table 4.2).

Campylobacter was detected in three (2.9%) of 104 tested batches of leafy greens (lettuce, rocket lettuce, red mangold lettuce) and two (1.6%) of 125 tested batches of fresh herbs (parsley and spring onions). *Salmonella* was detected in one of 20 tested batches of baby corn and in five (4.0%) of the 125 tested batches of fresh herbs (one batch of estragon and four batches of basil). The isolated *Salmonella* serovars were *S.* Weltevreden, *S.* Stanley, *S.* Aberdeen, *S.* Rubislaw and *S.* Chicago. All batches of herbs positive for *Salmonella* had one or more samples where the level of *E. coli* exceeded 100 cfu/g as well. More than 100 cfu/g indicator *E. coli* were detected in a wide range of products including sprouts (three of 38 tested batches) and baby corn (five of 20 tested batches). *Salmonella* and *E. coli* were found in five (10.4%) and seven (14.6%) of 48 tested batches of herbs imported from third countries, respectively.

3. Discussion

The number of outbreaks caused by fruits and vegetables in recent years and the results of the presented survey demonstrate the relevance of taking fruit, herbs and vegetables into account as sources of foodborne illness. Timely and detailed outbreak investigations and thorough trace-back investigations are important to identify likely sources of contamination in the food production chain. Furthermore, molecular subtyping has become an important tool in outbreak investigations, especially when linking cases and products in different countries (4).

The contamination of fruit and vegetables is most likely to occur in the field during the initial processing or during the final preparation in the kitchen (1) and might reflect problems in the production environment; e.g. use of contaminated irrigation water, use of manure as fertilizer, use of dirty equipment (13) and/or the fact that the surface of some vegetables might be difficult to clean properly once it has been contaminated from the surrounding environment. Prevention of contamination of fruit and vegetables in the field and during the following processing and/or packaging steps is essential as fruit and most vegetables are considered to be ready-to-eat products.

Table 4.2. The occurence of Campylobacter, Salmonella *and* E. coli *in batches of fresh herbs and greens on the Danish market in 2009-2010 by country of origin*

Product type	Country of origin	N	*Campylobacter*		*Salmonella*		*E. coli* (>100 cfu/g)	
			Pos	% Pos	Pos	% Pos	Pos	% Pos
Baby corn	Third countries	20	0	-	1	5.0	5	25.0
Sugar peas	Denmark	1	0	-	0	-	0	-
	Other EU MSs	1	0	-	0	-	0	-
	Third countries	42	0	-	0	-	1	2.4
	Unknown	2	0	-	0	-	0	-
Sprouts	Denmark	30	0	-	0	-	3	10.0
	Other EU MSs	2	0	-	0	-	0	-
	Third countries	0	-	-	-	-	-	-
	Unknown	6	0	-	0	-	0	-
Leafy greens	Denmark	33	1	3.0	0	-	1	3.0
	Other EU MSs	59	2	3.4	0	-	0	-
	Third countries	3	0	-	0	-	0	-
	Unknown	10	0	-	0	-	0	-
Fresh herbs	Denmark	36	1	2.8	0	-	3	8.3
	Other EU MSs	39	1	2.6	0	-	0	-
	Third countries	48	0	-	5	10.4	7	14.6
	Unknown	2	0	-	0	-	0	-
In total		334	5	1.5	6	1.8	20	6.0

Source: National Food Institute and Danish Veterinary and Food Administration

Some products on the Danish market are almost exclusively imported from one country, e.g. baby corn whereas other products such as fresh herbs are available from a wide range of countries. Production practices and sanitary conditions such as access to clean water might vary between and within the countries, leading to differences in the observed prevalence within a product type. Isolation of pathogenic bacteria or virus from fruits and vegetables produced all over the world including the EU show that this is a global problem.

Internationally, in FAO/WHO and Codex Alimentarius as well as the EU awareness about these problems has been increasing (14). Examples are the implementation of Regulation (EC) No 2073/2005 defining among other things the Microbiological Criteria for the presence of *Salmonella* in sprouts as well as *Salmonella* and *E. coli* in pre-cut ready-to-eat fruits, vegetables and unpasteurized juices; and latest with the requirement for official testing of fresh herbs of coriander, basil and mint from Thailand for *Salmonella* according to the latest amendment of Regulation (EC) No 669/2009 on "Intensified control of certain non-animal products".

The risk of foodborne outbreaks caused by contaminated fruit and vegetables might be reduced by encouraging importers of these products to require certain hygiene standards from their suppliers. The Danish Veterinary and Food Administration will prepare guidance to importers of fresh fruit and vegetables as to how they can ensure the marketed products are free from pathogens; including requirements for hygienic conditions at the site of produc-tion. This information will be available on the website of the Danish Veterinary and Food Administration.

Regarding prevention of foodborne disease at the consumer level or commercial settings such as institutions and restaurants, the Danish Veterinary and Food Administration recommend washing fruits and vegetables before consumption. Where possible, it is recommended to blanch products which often have been linked to human disease and are not intended to be eaten raw in the country of origin (e.g. sugar peas and baby corn). Furthermore, the norovirus outbreaks caused by imported frozen raspberries, has led to recommendations on boiling frozen raspberries before consumption; for kitchens catering for immuno-compromised individuals the recommendation covers all types of frozen berries.

References

(1) Lynch MF, RV Tauxe, CW Hedberg (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137(3):307-15.

(2) Barton Behravesh C, RK Mody, J Jungk, L Gaul, JT Redd, S Chen, S Cosgrove, E Hedican, D Sweat, L Chávez-Hauser, SL Snow, H Hanson, TA Nguyen, SV Sodha, AL Boore, E Russo, M Mikoleit, L Theobald, P Gerner-Smidt, RM Hoekstra, FJ Angulo, DL Swerdlow, RV Tauxe, PM Griffin, IT Williams (*Salmonella* Saintpaul Outbreak Investigation Team) (2011). 2008 outbreak of *Salmonella* Saintpaul infections associated with raw produce. N Engl J Med 10;364(10):918-27.

(3) Söderström A, P Österberg, A Lindqvist, B Jönsson, A Lindberg, S Blide Ulander, C Welinder-Olsson, S Löfdahl, B Kaijser, B De Jong, S Kühlmann-Berenzon, S Boqvist, E Eriksson, E Szanto, S Andersson, G Allestam, I Hedenström, L Ledet Muller, Y Andersson (2008). A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce. Foodborne Pathog Dis 5(3):339-49.

(4) Nygård K, J Lassen, L Vold, Y Andersson, I Fisher, S Löfdahl, J Threlfall, I Luzzi, T Peters, M Hampton, M Torpdahl, G Kapperud, P Aavitsland (2008). Outbreak of *Salmonella* Thompson infections linked to imported rucola lettuce. Foodborne Pathog Dis 5(2):165-73.

(5) Falkenhorst G, L Krusell, M Lisby, SB Madsen, B Böttiger, K Mølbak (2005). Imported frozen raspberries cause a series of norovirus outbreaks in Denmark, 2005. Euro Surveill 10(38).

(6) Ethelberg S, M Lisby, B Böttiger, AC Schultz, A Villif, T Jensen, KE Olsen, F Scheutz, C Kjelsø, L Müller (2010). Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. Euro Surveill 15(6).

(7) Pakalniskiene J, G Falkenhorst, M Lisby, SB Madsen, KE Olsen, EM Nielsen, A Mygh, J Boel, K Mølbak (2006). A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, November 2006. Epidemiol Infect 137(3):396-401.

(8) Lewis HC, S Ethelberg, KE Olsen, EM Nielsen, M Lisby, SB Madsen, J Boel, R Stafford, M Kirk, HV Smith, S Tikumrum, A Wisetrojana, A Bangtrakulnonth, J Vithayarungruangsri, P Siriarayaporn, K Ungchusak, J Bishop, K Mølbak (2009). Outbreaks of *Shigella sonnei* infections in Denmark and Australia linked to consumption of imported raw baby corn. Epidemiol Infect 137(3):326-34.

(9) Müller L, T Jensen, RF Petersen, K Mølbak, S Ethelberg (2009). Imported fresh sugar peas as suspected source of an outbreak of *Shigella sonnei* in Denmark, April – May 2009. Euro Surveill 14(24).

(10) Emberland KE, S Ethelberg, M Kuusi, L Vold, L Jensvoll, BA Lindstedt, K Nygard, C Kjelsø, M Torpdahl, G Sørensen, T Jensen, S Lukinmaa, T Niskanen, G Kapperud (2007). Outbreak of *Salmonella* Weltevreden infections in Norway, Denmark and Finland associated with alfalfa sprouts, July-October 2007. Euro Surveill 29;12(11).

(11) Denny J, J Threlfall, J Takkinen, S Löfdahl, T Westrell, C Varela, B Adak, N Boxall, S Ethelberg, M Torpdahl, M Straetemans, W van Pelt (2007). Multinational *Salmonella* Paratyphi B variant Java (*Salmonella* Java) outbreak, August – December 2007. Euro Surveill 12(51).

(12) Werner S, K Boman, I Einemo, M Erntell, B de Jong, A Lindqvist, M Löfdahl, S Löfdahl, A Meeuwisse, G Ohlen, M Olsson, U Stamer, E Sellström, Y Andersson (2007). Outbreak of *Salmonella* Stanley in Sweden associated with alfalfa sprouts, July-August 2007. Euro Surveill 12(42).

(13) Oliveira M, J Usall, I Viñas, M Anguera, F Gatius and M Abadias (2010). Microbiological quality of fresh lettuce from organic and conventional production. Food Microbiology 27:679-684.

(14) World Health Organization, Food and Agriculture Organization of the United Nations (2008). Microbiological hazards in fresh leafy vegetables and herbs. Microbiological Risk Assessment Series - 14.

5. Survival of pathogens in lightly preserved fermented sausages

By Jens Kirk Andersen (jkia@food.dtu.dk) and Søren Aabo

Production of lightly fermented sausages is known to be a delicate matter and in recent years it has been identified as a source of outbreaks on several occasions (1, 2, 3). Therefore, the microbiology of fermented sausages has been the subject for increased attention from the authorities.

In Denmark, an outbreak caused by verocytotoxin producing *Escherichia coli* (VTEC) O26:H11 in organic semi-dried fermented beef sausage occurred in 2007 (3). Epidemiological investigations identified a batch of 19,000 organic semi-dried fermented beef sausages as the likely source of infection. Subsequently, the outbreak strain was recovered from sausages and frozen beef used to produce the sausages. The sausages were found to be improperly fermented. The outbreak raised the question whether this type of sausage is intrinsically problematic with regard to VTEC.

Production of lightly preserved fermented sausages includes steps where temperature and time are set relatively high (about 15°C) to allow growth of the fermenting flora and drying of the sausage. This procedure develops taste and at the same time provides protection against growth and survival of unwanted microorganisms through a pH decline and competition. However, in some cases these conditions provide opportunity for potentially disease-causing organisms to grow. The safety of the production is therefore determined by the balance between sufficient growth of the desired microflora and keeping the unwanted flora below the threshold level.

In recent years, a healthy lifestyle has been increasingly important for many consumers. Some food business operators have therefore in order to meet the consumer demands changed the recipes of their products so they contain less fat and salt, which may result in an increased water activity. Such changes may have the possible consequence that the growth potential of pathogenic microorganisms increases.

On this background, the Danish Veterinary and Food Administration decided to investigate microbiological and biochemical factors of a substantial number of lightly preserved fermented sausages. The sausages were collected according to the sampling plan described in the EU regulation on Microbiological Criteria (Regulation (EC) No 2073/2005). In total, 130 Danish batches and 27 imported batches with a total of 696 single samples were analysed. The samples were investigated for presence of pathogenic microorganisms as well as microbial indicators of general hygiene. The samples were analysed for presence of *Salmonella* and VTEC (the latter only in batches containing beef) and quantitatively for *Listeria monocytogenes*. pH and water activity (a_W) was measured to allow inference on the possible correlation of these parameters to the results of microbiological analysis, in particular to the presence of pathogenic organisms.

L. monocytogenes was detected in four of the Danish batches sampled. In one batch, three of five samples were positive with 100 cfu/g in two samples and 50 cfu/g in one sample. Three other batches showed one of five samples positive with 10 cfu/g detected in two cases, and 20 cfu/g in one case. None of the imported batches were positive.

Salmonella was detected in one Danish and one imported batch from Germany. In the Danish batch, three of five samples were found positive with *S.* Indiana, and in the imported batch, one of five samples contained *S.* Ohio.

E. coli O157 was not detected in any of the batches.

The few findings of pathogenic microorganisms provide little background for an analysis on correlation of the pathogens detected and the measurements of pH and a_W. Measurement of a_W is a key parameter in the control of growth of microorganisms, but it is difficult for the laboratories to measure it; and for some of the batches, results of a_W were not reported. The sampled batches cover a wide range of products, which become very apparent when looking at the correlation between pH and a_W (Figure 5.1). All products are refrigerated after the end of drying and at this point *Salmonella* and VTEC will have halted growth, while possible growth of *L. monocytogenes* may still cause concern. *Listeria* may grow at:

- pH-levels above 4.4
- a_W-levels above 0.92
- Conditions with a combination of pH-levels above 5.0 and a_W above 0.94
- Refrigeration and pH above 5.0.

The results of pH and a_W (Figure 5.1) suggest that fermented sausages quite frequently provide growth potential for *L. monocytogenes*.

Salmonella and VTEC will also have growth potential during the early stages of the production when the temperature is elevated during fermentation and drying. It is evident that growth inhibition from these hurdles alone

is not sufficient to halt the growth in a large part of the sausages examined. Other factors may render the products safety as well, for example:

- The starter culture
- Organic acids
- Phenolic components from smoking
- Nitrite.

Using freely available modelling tools (ComBase Predictor and Pathogen Modelling Program) to perform predictive modelling of the different process steps, it appears that these lightly fermented products under some circumstances do allow growth of *L. monocytogenes* (and in fact also *Yersinia enterocolitica*) at different stages during the processing, even during the cold storage of the final product. For VTEC, the potential of growth was predicted during fermentation and until a point in the drying process. During cold storage the VTEC level - if present - would decline. However, caution in interpretation of the simplified model predictions should be taken; for example a simplified model based on pH and a_W predicted a substantial growth (about 6 log-units) of *L. monocytogenes*. However, it was evident that the prediction of growth of pathogens was vastly exaggerated (4).

In order to provide substantial knowledge on the safety of the sausage production, a research project (ConFood) aim at developing a web-based neural network model. This will give guidance to HACCP programmes for producers of fermented sausages with regard to the overall safety of their process conditions and recipes. Key factors as type of starter culture, salt in the water phase, pH, nitrite and fat content and carbohydrate source have been tested in a pilot plant sausage production. The neural network models are presently under development.

Fermented sausages are normally considered a safe food product. However, changes in the production towards more lightly preserved products by reducing salt and fat content and increasing the water content may reduce the safety, so the margin allowed for errors during the production, i.e. less efficient fermentation or prolonged drying, is reduced critically. The food business operators therefore need to be concerned not only about culinary demands from the consumers, but also of the inherent risk of creating a product that does not include sufficient steps during the production to protect against growth and survival of unwanted microorganisms.

References

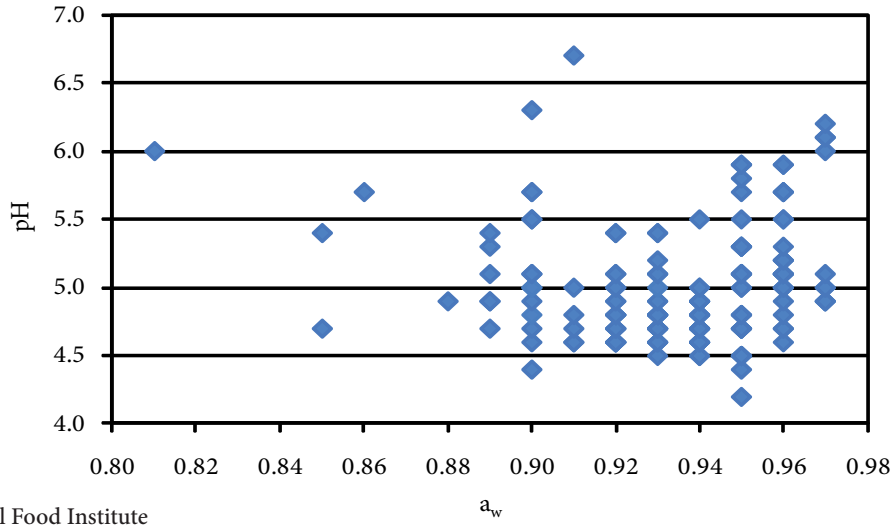
(1) Sartz L, B De Jong, M Hjertqvist, L Plym-Forsell, R Alsterlund, S Löfdahl, B Osterman, A Ståhl, E Eriksson, HB Hansson and D Karpman (2008). An outbreak of *E. coli* O:157:h7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. Epidemiol Infect 136, 370-380.

(2) Schimmer B (2006). Outbreak of haemolytic uraemic syndrome in Norway: update. Euro Surveill 11: 4.

(3) Ethelberg S, B Smith, M Torpdahl, M Lisby, J Boel, T Jensen and K Mølbak (2007). An outbreak of Verocytotoxin-producing *Escherichia coli* O26:H11 caused by beef sausage, Denmark 2007. Euro Surveill 12: 5.

(4) Hansen TB, COA Møller, JK Andersen (2009). Using ComBase Predictor and Pathogen Modeling Program as support tools in outbreak investigation: an example from Denmark. In: 6th International Conference Predictive modeling in Foods (IC PMF 2009), Washing DC, USA, Proceedings, pp. 263-266.

Figure 5.1. Plot of pH vs. water activity (a_w) measured in the fermented sausages investigated



Source: National Food Institute

6. EU related topics

6.1 Antimicrobial Resistance - initiatives

In 2010, the Commission Working Group on Antimicrobial Resistance was established and held its first meeting in May at which matters regarding ways and means of dealing with antimicrobial resistance were discussed. The Commission has requested the European Medicines Agency to lead a project aiming at collecting comparable data on the sales and use of antimicrobials in animals in the Member States, Norway and Switzerland. The Commission has also requested the European Food Safety Authority to provide a scientific opinion on resistance caused by bacteria producing extended spectrum cephalosporinases in food and food producing animals.

6.2 Control of zoonoses in animal populations

6.2.1 EU coordinated monitoring studies

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

In 2010, the Commission decided to finance a one year study on the prevalence of *Listeria monocytogenes* in certain ready-to-eat products. The study is carried out in 2010 and 2011. Samples include smoked fish, meat products as well as soft and semisoft cheeses collected at retail level in major cities. The aim of the study is to evaluate compliance with the Microbiological Criteria for *L. monocytogenes* laid down in Regulation (EC) No 2073/2005 for products marketed in EU. Additionally, the growth potential for *L. monocytogenes* in smoked fish will be evaluated.

6.2.2 EU harmonised surveillance programmes

In 2010, Member States were for the first time obliged to include breeding and fattening turkey flocks in the control and surveillance of *Salmonella* according to Regulation (EC) No 584/2008. The EU target of 1% for breeding and fattening turkey flocks positive with *S.* Typhimurium and *S.* Enteritidis is based on the results of the EU baseline study carried out in 2006-2007 and decided by the Commission in 2008 (See Annual Report 2008 for an overview of Danish results). These targets have to be reached by December 31st 2012. In Denmark, no turkey flocks of 24 flocks tested were positive with *S.* Typhimurium or *S.* Enteritidis in 2010 (appendix C, Table A13).

In breeding flocks of *Gallus gallus*, the target of 1% positive adult flocks had to be reached by the end of 2009 according to Regulation (EC) No 1003/2005. The target was set for *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Infantis and *S.* Virchow. This regulation has been replaced by Regulation (EC) No 200/2010 laying down a permanent target of maximum 1% adult flocks positive for *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Infantis and *S.* Virchow. The regulation does not differentiate between breeding flocks from the table egg and broiler production lines and in 2010, a total of 5 (2.4%) adult flocks were positive with one of the five serovars (appendix C, Table A8 and A10).

The EU baseline study on table egg laying flocks carried out in 2004 showed large differences in the prevalence between Member States. Therefore, Member States specific targets were set either as an annual 10-40% reduction of positive adult flocks dependant on the prevalence of adult flocks in the Member State the previous year or a maximum of 2% adult flocks positive (Regulation (EC) No 1168/2006). The target was set for *S.* Typhimurium and *S.* Enteritidis and had to be reached by December 31st 2010. For Denmark, the target is a maximum of 2% adult flocks positive for *S.* Typhimurium and *S.* Enteritidis. The prevalence in Denmark has been below 2% since 2004 . In 2010, 1.1% of the flocks was positive (appendix C, Table A8).

In broiler flocks of *Gallus gallus*, the target of maximum 1% flocks positive for *S.* Typhimurium and *S.* Enteritidis has to be reached by December 31st 2011 according to Regulation (EC) No 646/2007. Denmark has had intensive *Salmonella* control programmes for many years and the target of 1% has already been reached. In 2010, 0.3% of the broiler flocks was positive with *S.* Typhimurium and *S.* Enteritidis (appendix C, Table A10).

7. Surveillance and control programmes

The close collaboration between different national and regional authorities, the industry and non-governmental organizations is presented in Figure 7.1. According to the legislation, 41 infectious diseases are notifiable in Denmark. An overview of the notifiable and non-notifiable human and animal diseases presented in this report is provided in appendix D, Table A27 and Table A28, respectively, including the relevant legislation.

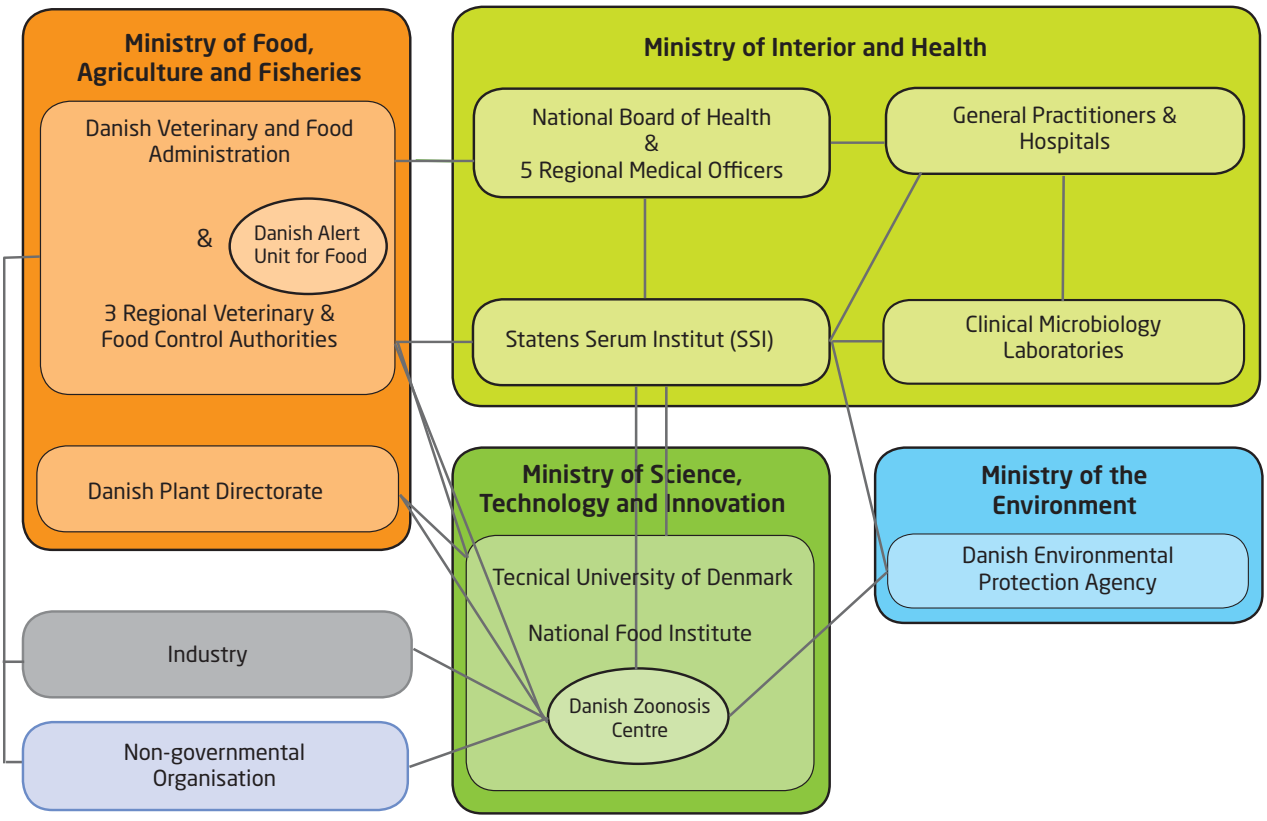
7.1 Surveillance of human disease

Information on human cases is reported to Statens Serum Institut through different channels depending on the disease:

- 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, Cryptosporidium, Echinococcus, Toxoplasma* and *Trichinella.*

In Denmark, the physicians report individually notifiable zoonotic diseases to the medical officers and the Department of Epidemiology at Statens Serum Institut. 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 the clinical microbiology laboratories depending on county of residence of the requesting

Figure 7.1. Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feedstuffs in Denmark



Source: Danish Zoonosis Centre, National Food Institute

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 appendix D, table 35 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 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
- VTEC O-group distribution in humans is presented in appendix B, Table A4.
- The *Salmonella* sero- and phage type distributions are presented in appendix C, Tables A5-A7.

7.2 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 investigate 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 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 Outbreak Database (FUD) are presented in appendix B, Table A3 and some of the more notable outbreaks from 2010 are outlined in Chapter 2.

7.3 Surveillance and control of animals and animal products

Salmonella surveillance and control programmes for poultry, pigs and cattle are presented in appendix D, Tables A29-A34. Sample analysis is performed at authorised private laboratories, the Regional Veterinary and Food Control Authorities, the National Food Institute or the National Veterinary Institute. *Salmonella* isolates are forwarded to the National Food Institute for serotyping, some isolates are also phage- and genotyped as well as tested for antimicrobial resistance. An overview of the methods used for subtyping is presented in appendix D, Table A35.

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

- Results from the table egg production are presented in appendix C, Tables A5-A9
- Results from the broiler production are presented in appendix C, Tables A5-A7 and A10
- Results from the duck and turkey productions are presented in appendix C, Table A13
- Results from the pig production are presented in appendix C, Tables A5-A6, A14 and Figures A1-A3
- Results from the cattle production are presented in appendix C, Tables A5-A6, A15-16 and Figure A4
- Results from the feeding stuff production are presented in appendix C, Tables A18-A19
- Results from the rendering plants are presented in appendix C, Table A20
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in appendix C, Table A21.

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

- Results from the poultry production are presented in appendix C, Tables A11-A12
- Results based on suspicion of diseases in pets, zoo animals and wild life are presented in appendix C, Table A21.

Pig and cattle carcasses are screened for *Mycobacterium* and *Echinococcus* during meat inspection at the slaughterhouse. Although Denmark is assigned as a region where the risk of *Trichinella* in domestic swine is negligible, all slaughter pigs slaughtered 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

Changes in the *Campylobacter* surveillance programme for broiler flocks

January 1st 2010, the Order no 1462/12/2009 came into force making for the first time surveillance for *Campylobacter* in broiler flocks mandatory. The producer is now obliged to sample the flocks at the farm using sock samples. The result has to be available for the slaughter house prior to slaughter as the *Campylobacter* status of the flock is used as a sorting tool for the allocation of positive flocks to frozen products.

The mandatory surveillance programme replaced an equivalent voluntary programme, which was part of the intervention strategy that has been in place since 2003. The strategy was described in detail in Annual Report 2003.

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 A14-A15.

Results from the surveillance for Bovine Spongiform Encephalopathy (BSE) in cattle, Transmissible Spongiform Encephalopathy (TSE) in sheep/goat are presented in appendix C, Tables A22-A24.

Results from the monitoring of *Coxiella burnetii* (Q fever) in cattle are presented in appendix C, Table A15.

Results based on suspicion of diseases with *Chlamydia psittacci, Cryptosporidium, Trichinella*, classical rabies and European Bat *Lyssavirus* in zoo animals and wild life are presented in appendix C, Table A21.

7.4 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 Authority 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 coordinated 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 need to be reconsidered.

Appendix C, Table A25 provides information on the centrally coordinated projects conducted in 2010. Results from the following projects are presented:

- Intensified control of *Salmonella* and *Campylobacter* in Danish and imported meat based on a case-by-case risk assessment (appendix C, Table A17)
- Findings of *Campylobacter* in non-heat treated meat cuts from broilers (appendix C, Tables A11 and A12)
- Findings of *Listeria monocytogenes* in ready-to-eat products (appendix C, Table A26)

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

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, 2008-2010

	2010		2009		2008	
Source	Estimated no. of reported cases (95% credibility interval[a])	Percentage of reported cases	Estimated no. of reported cases (95% credibility interval[a])	Percentage of reported cases	Estimated no. of reported cases (95% credibility interval[a])	Percentage of reported cases
Pork	242 (238-283)	15.1	162 (127-198)	7.6	320 (277-367)	8.8
Beef	12 (0-38)	0.7	4 (3-6)	0.2	26 (16-36)	0.7
Table eggs	28 (18-41)	1.8	262 (245-280)	12.3	116 (91-143)	3.2
Broilers	8 (4-14)	0.5	7 (0-21)	0.3	47 (25-133)	1.3
Ducks	2 (0-7)	0.1	7 (0-19)	0.3	38 (2-99)	1.0
Imported pork	86 (59-115)	5.4	43 (22-66)	2.0	39 (12-70)	1.1
Imported beef	30 (4-51)	2.0	65 (47-86)	3.1	12 (3-25)	0.3
Imported broilers	5 (0-17)	0.2	30 (8-60)	1.4	191 (120-250)	5.2
Imported turkey	17 (2-37)	1.0	42 (11-74)	2.0	87 (8-151)	2.4
Imported duck	21 (10-37)	1.3	29 (10-50)	1.4	-	-
Travels	749 (740-758)	46.9	658 (647-669)	30.9	853 (843-864)	23.3
Unknown source	316 (275-354)	19.8	375 (322-422)	17.6	480 (413-547)	13.1
Outbreaks, unknown source	82	5.1	445	20.9	1,447	39.6
Total	1,598		2,129		3,656	

a) The model is based on a Bayesian framework which gives 95% credibility intervals.
Source: Danish Zoonosis Centre, National Food Institute

Appendix B

Human disease and outbreak data

Table A2. Zoonoses in humans, number of laboratory-confirmed cases, 2001 and 2006-2010

	Incidence per 100,000 inhabitants	Reported no. of cases					
Zoonotic pathogen	2010	2010	2009	2008	2007	2006	2001
Bacteria							
Brucella abortus/melitensis[a,c]	-	6	7	8	20	9	18
Campylobacter coli/jejuni[b]	72.6	4,035	3,352	3,454	3,868	3,242	4,620
Chlamydia psittaci[b]	0.2	9	14	6	11	7	9
Leptospira spp.[b]	0.2	10	12	13	10	15	6
Listeria monocytogenes[b]	1.1	62	97	51	58	56	38
Mycobacterium bovis[b]	0.03	2	0	1	1	3	4
Salmonella total[b]	28.7	1,598	2,129	3,656	1,647	1,658	2,918
S. Enteritidis[b]	7.0	388	600	638	566	562	1,416
S. Typhimurium[b]	9.4	521	767	2,002	343	411	589
Other serotypes[b]	12.4	689	762	1,016	740	687	913
VTEC total[b]	3.3	185	165	161	161	146	90
O157	0.4	25	24	15	25	19	24
other or non-typeable	2.6	146	141	143	136	127	66
Yersinia enterocolitica[b]	3.5	192	238	330	270	215	286
Parasites							
Cryptosporidium spp.[a,c]	-	25	35	92	49	-	-
Echinococcus multilocularis[a,d]	-	1	0	0	3	-	-
Echinococcus granulosus[a,d]	-	10	11	5	9	-	-
Toxoplasma gondii[a,e]	-	-	-	-	-	14	19
Trichinella spp.[a,c,d]	-	0	0	0	1	-	-
Viruses							
Lyssavirus[b]	-	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 Institut) only, representing a proportion of the Danish population (approximately 1/3 in 2010). 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.
d) The cases were imported.
e) The nation-wide neonatal screening for congenital toxoplasmosis stopped in 2007.
Source: Statens Serum Institut

Table A3. Foodborne disease outbreaks[a] reported in the Food- and waterborne Outbreak Database (FUD) (n=77), 2010

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
Bacillus cereus	5	.	Restaurant/catering	Beef (lasagne)	989
Bacillus cereus	112	.	Canteen	Composite meal (White peber)	1022
Campylobacter jejuni	37	6	Canteen	Chicken	1006
Campylobacter jejuni	9	4	Restaurant/catering	Chicken	1007
Campylobacter spp.	400	61	Town	Drinking water	1001
Campylobacter spp., mix of patogenes	400	8	Sea	Sea water	1015
Campylobacter spp.	2	2	Restaurant/catering	Unknown	1016
Clostridium perfringens	87	.	Restaurant/catering	Beef	1014
Clostridium perfringens	20	.	Private party	Beef	1033
VTEC O157	3	3	Private home	Unknown	997
Listeria monocytogenes	9	9	National	Fish	1035
S. Enteritidis	.	7	Tourists in Egypt	Unknown	977
S. Enteritidis	.	5	Tourists in Spain	Unknown	1038
S. Typhimurium DT104	.	8	Regional	Unknown	967
S. Typhimurium U292	.	19	National	Unknown	1010
S. Typhimurium DT10	.	7	Restaurant, Bulgaria	Unknown	1027
S. Typhimurium DT41	.	9	Tourists in Egypt	Unknown	1044
S. Typhimurium U323	.	172	National	Pork/pork products	979
S. Typhimurium DT120/DT7	.	20	National	Pork/deer product	996
S. 4,5,12:i:- U311	.	9	National	Unknown	1045
S. 4,5,12:i:- DT120	.	13	National	Unknown	995
S. Infantis	87	19	Hotel	Composite meal	1039
S. Virchow	3	3	Private party	Chicken	994
S. Umbilo	.	4	National	Unknown	1000
Staphylococcus aureus	150	.	Other	Composite meal	1011
Norovirus+ETEC	16	1	School	Lollo Bionda Lettuce	984
Norovirus+ETEC	3	1	School	Lollo Bionda Lettuce	982
Norovirus+ETEC	5	1	Shop	Lollo Bionda Lettuce	985
Norovirus+ETEC	6	1	Shop	Lollo Bionda Lettuce	981
Norovirus+ETEC	28	1	Canteen	Lollo Bionda Lettuce	986
Norovirus+ETEC	2	.	Restaurant/catering	Lollo Bionda Lettuce	970
Norovirus+ETEC	11	.	Restaurant/catering	Lollo Bionda Lettuce	959
Norovirus+ETEC	26	.	Restaurant/catering	Lollo Bionda Lettuce	963
Norovirus+ETEC	26	.	Restaurant/catering	Lollo Bionda Lettuce	956
Norovirus+ETEC	13	.	Shop	Lollo Bionda Lettuce	955
Norovirus+ETEC	62	.	Shop	Lollo Bionda Lettuce	953
Norovirus+ETEC	3	.	Restaurant/catering	Lollo Bionda Lettuce	972
Norovirus+ETEC	35	.	Restaurant/catering	Lollo Bionda Lettuce	961
Norovirus+ETEC	50	.	Canteen	Lollo Bionda Lettuce	957
Norovirus+ETEC	3	.	Restaurant/catering	Lollo Bionda Lettuce	971
Norovirus+ETEC	6	.	Restaurant/catering	Lollo Bionda Lettuce	964
Norovirus+ETEC	10	.	Shop	Lollo Bionda Lettuce	958
Norovirus+ETEC	21	.	Shop	Lollo Bionda Lettuce	960
Norovirus+ETEC	4	2	Canteen	Lollo Bionda Lettuce	952
Norovirus+ETEC	75	.	Institution	Lollo Bionda Lettuce	968

Continued on the next page

Table A3. Foodborne disease outbreaks[a] reported in the Food- and waterborne Outbreak Database (FUD), 2010 (Continued from page 30)

Pathogen	No. of patients	Patients laboratory confirmed	Setting	Source	FUD no.
Norovirus	27	2	Restaurant/catering	Molluscs, shellfish, oysters	973
Norovirus	22	1	Canteen	Buffet meals	954
Norovirus	70	1	Canteen	Buffet meals	969
Norovirus	41	16	Restaurant/catering	Buffet meals	1041
Norovirus	21	3	Canteen	Buffet meals	975
Norovirus	20	.	Restaurant/catering	Confectionary products	988
Norovirus	5	.	Private party	Raspberries	992
Norovirus	15	.	Restaurant/catering	Fresh vegetables	990
Norovirus	21	.	School	Composite meal	991
Norovirus	15	.	Restaurant/catering	Composite meal	999
Norovirus	5	.	Restaurant/catering	Fish	1023
Norovirus	24	.	Restaurant/catering	Buffet meals	1002
Norovirus	20	.	Restaurant/catering	Buffet meals	1024
Norovirus	16	.	Private home	Composite meal	998
Norovirus	116	5	Restaurant/catering	Composite meal	1003
Norovirus	14	4	Canteens	Romaine lettuce	1008
Norovirus	30	3	Canteen	Raspberries	1020
Norovirus	6	.	Private party	Fresh Fruit	1021
Norovirus	4	.	Restaurant/catering	Fish	1026
Norovirus	4	4	Restaurant/catering	Composite meal	1029
Norovirus	60	4	Restaurant/catering	Raspberries	1030
Norovirus	19	.	Private party	Buffet meals	1042
Norovirus	28	.	Restaurant/catering	Composite meal	1040
Norovirus	42	.	Shop	Composite meal	1043
Norovirus	18	.	Private party	Fresh vegetables	987
Norovirus	180	.	Canteen	Unknown	1036
Norovirus	18	2	Sport event	Unknown	978
Histamin	7	.	Restaurant/catering	Fish	976
Histamin	36	.	Institution	Fish (Macherel)	1028
Histamin	2	.	Restaurant/catering	Fish (Tuna)	1034
Lectins	105	.	Restaurant/catering	Beans	1005
Lectins	16	.	Restaurant/catering	Beans	1025
Total	2,756	440			

a) In addition, 1 confirmed household outbreak was registered (FUD 983). It was caused by Norovirus+ETEC involving 4 cases (1 case was laboratory confirmed).

Table A4. VTEC *O-group distribution in humans[a], 2010*

O-group	Number of episodes	O-group	Number of episodes
O157	25	O128ab	9
O103	24	O145	6
O117	16	O156	5
O26	14	O91	5
O146	9	Notification[b]	18
O-rough	10	Other O-groups or not-typed	44
Continued in the next column		Total	185

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

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

Source: Statens Serum Institut

Appendix C

Monitoring and surveillance data

Table A5. Top 10 (humans) serotype distribution (%) of Salmonella *from humans, animals, carcasses at slaughterhouse and imported meat, 2010*

	Human	Pig[a]	Pork[b]	Beef[b]	Layer[c]	Broiler[c]	Duck[c]	Imported meat (batch)[d]				CKL[e]
		herds	batch	batch	flocks	flocks	flocks	Pork	Beef	Broiler	Turkey	Duck
Serotype	N=1,598	N=604	N=154	N=13	N=8	N=45	N=58	N=50	N=6	N=65	N=70	N=124
Typhimurium	32.6	57.6	31.2	15.4	12.5	22.2	17.2	40.0	0	15.4	15.7	46.8
Enteritidis	24.3	0	0	0	50.0	2.2	0	2.0	0	6.2	1.4	2.4
O:4,5,12; H:i:-	6.0	7.5	0	0	0	2.2	0	10.0	0	1.5	5.7	0.8
Dublin	3.1	0	0	38.5	0	0	0	0	50.0	0	0	0
Infantis	2.4	2.0	6.5	0	37.5	13.3	0	2.0	0	30.8	0	0
Newport	2.1	0	0	0	0	0	0	0	0	1.5	5.7	0
Virchow	2.0	0	0	0	0	0	0	0	0	0	0	0
Stanley	1.9	0	0	0	0	0	0	0	0	0	0	0
O:4,12:H:i:-	1.6	2.0	0	0	0	2.2	0	4.0	0	0	0	0
Java	1.4	0	0	0	0	0	0	0	0	0	0	0
Others	22.2	30.5	40.9	7.7	0	57.8	72.4	40.0	50.0	44.6	70.0	40.3
Unknown	0.4	0.5	21.4	38.5	0	0	10.3	2.0	0	0	1.4	9.7
Total	100	100	100	100	100	100	100	100	100	100	100	100

a) Isolates obtained from sampling of slaughter pig herds placed in level 2 and 3 (Table A34 describes the surveillance programme). The isolates are biased towards herds positive with *S.* Typhimurium as the ELISA method used to analyse the meat juice samples forming the bases for assignment of herds into level 1-3 primarily focus on detection of *S.* Typhimurium antibodies.
b) Sampling of beef and pork carcasses at slaughterhouses according to surveillance programmes (Tables A33 and A34).
c) Sampling in prodution flocks prior to slaughter according to surveillance programmes (Tables A30-A32).
d) Case-by-case monitoring of imported meat and meat products. For further information regarding case-by-case monitoring, see Annual Report on Zoonoses in Denmark 2007.
e) Imported duck meat sampled at retail (centrally coordinated studies, Table A25).
Source: Danish Veterinary and Food Administration, Statens Serum Institut and National Food Institute

Table A6. Top 10 (humans) phagetype distribution (%) of S. *Typhimurium[f] from humans, animals and imported meat, 2010*

	Human	Pig[a]	Pork[b]	Beef[b]	Layer[c]	Broiler[c]	Duck[c]	Imported meat (batch)[d]			CKL[e]
		herds	batch	batch	flocks	flocks	flocks	Pork	Broiler	Turkey	Duck
Phagetype	n=521	n=348	n=48	n=2	n=1	n=10	n=10	n=20	n=10	n=11	n=58
U323	33.8	1.1	0	0	0	0	0	5.0	0	0	0
RDNC	9.0	9.5	4.2	0	0	0	0	0	10.0	27.3	1.7
DT 120	8.8	23.6	14.6	0	0	50.0	0	35.0	10.0	9.1	0
U292	8.6	1.4	0	0	0	0	0	0	0	0	0
DT 104	6.7	7.5	2.1	0	0	10.0	0	10.0	0	18.2	1.7
DT 193	5.2	10.3	2.1	50.0	0	10.0	0	25.0	0	18.2	0
DT 7	2.9	1.1	2.1	0	0	0	0	0	0	0	0
DT 12	2.5	11.2	10.4	0	0	0	0	0	0	0	0
DT 135	2.5	0.9	0	0	0	0	0	0	0	0	0
DT 8	2.3	0	0	0	0	0	0	0	0	0	82.8
Others	12.5	31.9	8.3	0	100	30.0	80.0	10.0	80.0	18.2	12.1
Unknown	5.2	1.4	56.3	50.0	0	0	20.0	15.0	0	9.1	1.7
Total	100	100	100	100	100	100	100	100	100	100	100

a-e) See Table A5.
f) Total number of samples may differ between Tables A5-A7, since isolates of one serotype may contain more than one phage type.
Source: Danish Veterinary and Food Administration, Statens Serum Institut and National Food Institute

Table A7. Top 10 (humans) phage type distribution (%) of S. Enteritidis[a] *from humans, animals and imported meat, 2010*

	Human	Layer[c] flocks	Broiler[c] flocks	Imported meat (batch)[d]			CKL[e]
				Pork	Broiler	Turkey	Duck
Phagetype	n=388	n=4	n=1	n=1	n=4	n=1	n=3
PT 8	8.8	100	0	0	0	0	0
PT RDNC	4.9	0	0	0	0	0	0
PT 9C	3.4	0	0	0	0	0	0
PT 21	2.8	0	0	0	25.0	0	66.7
PT 4	2.8	0	0	0	50.0	100	0
PT 15A	2.3	0	0	0	0	0	0
PT 14B	2.1	0	0	0	0	0	0
PT 1	1.8	0	0	0	0	0	0
PT 11	1.3	0	0	0	0	0	0
PT 6	1.3	0	0	0	0	0	0
Others	6.7	0	100	100	25.0	0	33.3
Unknown	61.9	0	0	0	0	0	0
Total	100	100	100	100	100	100	100

a) Total number of samples may differ between Tables A5-A7, since isolates of one serotype may contain more than one phage type.
c-e): See Table A5.
Source: Danish Veterinary and Food Administration, Statens Serum Institut and National Food Institute

Table A8. Occurrence of Salmonella *in the table egg production[a], 2001-2010*

	Rearing period (parent flocks)		Adult period (parent flocks)		Pullet-rearing flocks		Table egg layer flocks	
	N	Positive	N	Positive	N	Positive	N	Positive
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	9	0	368	1	641	5
2005	16	0	9	0	355	6	655	7
2006	17	0	11	0	289	2	565	2
2007	11	0	12	0	326	0	510	5
2008	10	0	6	0	258	1	508	4
2009	13	0	6	0	253	0	454	8
2010	15	0	9	0	225	0	455	8[b]

a) See Tables A29 and A31 for description of the surveillance programmes.
b) Four flocks positive with *S.* Enteritidis PT 8, one with *S.* Typhimurium DT 41and three with *S.* Infantis.
Source: Danish Agriculture and Food Council and Danish Veterinary and Food Administration

Table A9. Occurrence of Salmonella *in the table egg layer flocks sorted by type of production, 2001-2010*

	Deep litter		Free range		Organic		Battery	
	N	Positive	N	Positive	N	Positive	N	Positive
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	145	1	135	1
2009	133	1	78	0	130	4	110	3
2010	117	0	45	2[a]	136	1[b]	157	5[c]

a) One flock positive with *S.* Enteritidis PT 8 and one flock positive with *S.* Infantis.
b) One flock positive with *S.* Typhimurium DT 41.
c) Three flocks positive with S. Enteritidis PT 8 and two positive with *S.* Infantis.
Source: Danish Agriculture and Food Council and Danish Veterinary and Food Administration

Table A10. Occurrence of Salmonella *in the broiler production[a], 2001-2010*

	Rearing period (parent flocks)		Adult period (parent flocks)		Broiler flocks		Slaughterhouse (flocks/batches)	
	N	Positive	N	Positive	N	Positive	N	Positive
2001	243	0	325	7	4,571	76	1,695[b]	69
2002	241	2	330	2	4,443	68	1,667	92
2003	265	2	182[c]	4	4,414	77	1,552	77
2004	275	1	155[c]	6	4,246	64	1,472	24
2005	214	0	185[c]	0	4,034	87	1,174	27
2006	190	0	282	5	3,621	71	875[d]	17
2007	152	0	258	3	3,703	60	884	10
2008	146	0	293	2	3,845	43	518[e]	3
2009	140	0	225	4	3,767	35	375	3
2010	126	0	200	5[f]	3,773[g]	43	346	1[h]

a) See Tables A29 and A30 for description of the surveillance programmes.
b) PM sampling at the slaughterhouse were changed from pooled neck skin samples of flocks to chicken cuts sampling of batches.
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) From 2006, data cover only samples taken following the *Salmonella* programme. Verification samples taken once a week by producers of poultry meat approved to market *Salmonella*-free poultry meat are not included, this sampling started in middle of 2005.
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.
f) 3 flocks positive with *S.* Typhimurium and 2 flocks positive with *S.* Infantis.
g) In total, 8 flocks were positive with *S.* Typhimurium and 1 flock was positive with S. Enteritidis. Data includes 57 organic flocks.
h) One flock positive with S. Enteritidis PT 1.
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, 2004-2010*

Year	Broiler flocks		Chilled broiler meat[a]	
	N	% pos	N	% pos
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	4,950	26.3	484[b]	14.7[b]
2009	4,591	29.4	1,179[c]	15.4[c]
2010	3,132[d]	16.5[d]	1,177[c]	10.4[c]

a) Centrally coordinated studies (see section 7.4 for describtion). Detection limit <10 cfu/g.
b) Data are not compareable with other years as they represent the last two quarters of the year, which is the high prevalent period.
c) Data are not directly comparable to previous years, as additional small slaughterhouses has been included in the monitoring. The prevalence has been weighted according to the Danish market share.
d) Data are not comparable to previous years, as the sampling has been changed from cloacal swabs at slaughter to boot swabs in the stable 7-10 days before slaughter. Changes came into action from January 2010, according to Regulation No. 1469 of 15/12/2010 as ammened.
Source: Danish Agriculture and Food Council, Danish Veterinary and Food Administration, and National Veterinary Institute

Table A12. Occurrence of Campylobacter *in non-heat treated broiler meat at retail[a], 2003-2010*

	Chilled broiler meat (samples)				Frozen broiler meat (samples)			
Year	Denmark		Import		Denmark		Import	
	N	% pos[b]	N	% pos[b]	N	% pos[b]	N	% pos[b]
2003-2004	334	27.2	170	65.7	566	10.9	272	19.6
2004-2005	517	31.1	299	73.2	937	12.2	391	25.9
2005-2006	401	29.8	854	56.3	1,087	13.5	698	31.3
2006-2007	363	31.0	1,128	51.1	897	19.0	812	33.9
2007-2008	1,058	32.8	1,067	53.9	655	29.6	577	44.4
2008-2009	1,459	33.8	1,316	46.7	847	26.1	773	27.7
2009-2010	1,469	35.6	1,292	46.9	1,026	32.4	676	23.6

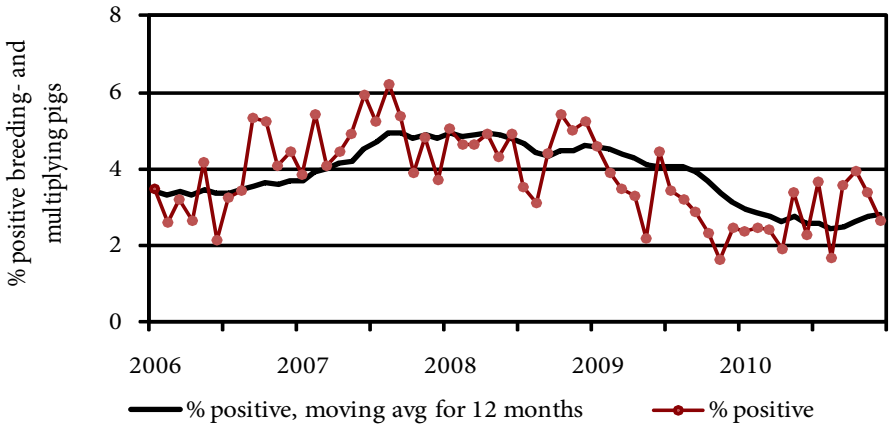
a) Centrally coordinated studies, retail samples (see section 7.4 for describtion). Detection limit <0.1 cfu/g.
b) The prevalence is calculated as a mean of quarterly prevalences based on the sum of data from the two years specified.
Source: National Food Institute

Table A13. Occurrence of Salmonella *in turkey and duck flocks[a], 2006-2010*

	Duck flocks		Turkey flocks	
Year	N	% pos	N	% pos
2006	266	80.5	11	0
2007	-	-	13	0
2008	68	64.7	10	10.0
2009	85	63.5	15	0
2010	108	56.5	24	4.2[b]

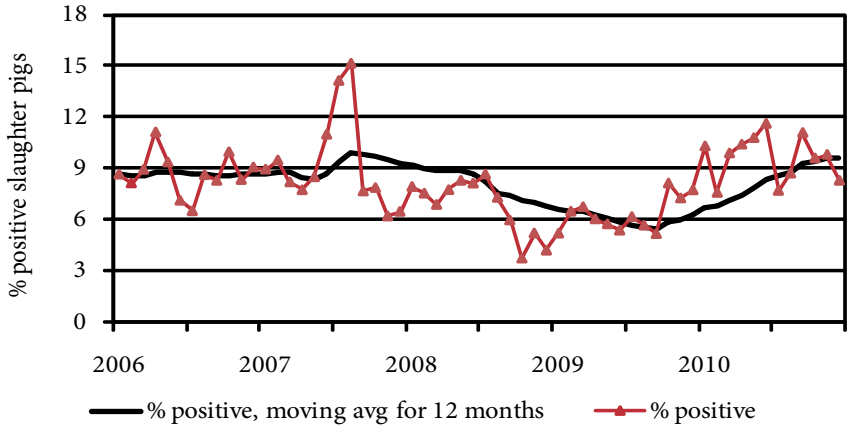
a) See Table A32 for description of the surveillance programmes. The two major turkey and duck slaughterhouses in Denmark closed down in 2004 and 2007, respectively. Therefore, most commercially reared duck and turkey flocks are transported abroad for slaughter.
b) One flock positive with *S.* Saintpaul.
Source: Danish Agriculture and Food Council

Figure A1. Serological surveillance of Salmonella *in breeding and multiplying pigs[a] based on monthly testing of blood samples, 2006-2010*



a) For more information about the surveillance programme, see Table A34
Source: Danish Agriculture and Food Council

Figure A2. Serological surveillance of Salmonella *in slaughter pigs[a], 2006-2010. Percentage of seropositive meat juice samples (first sample per herd per month)[b]*



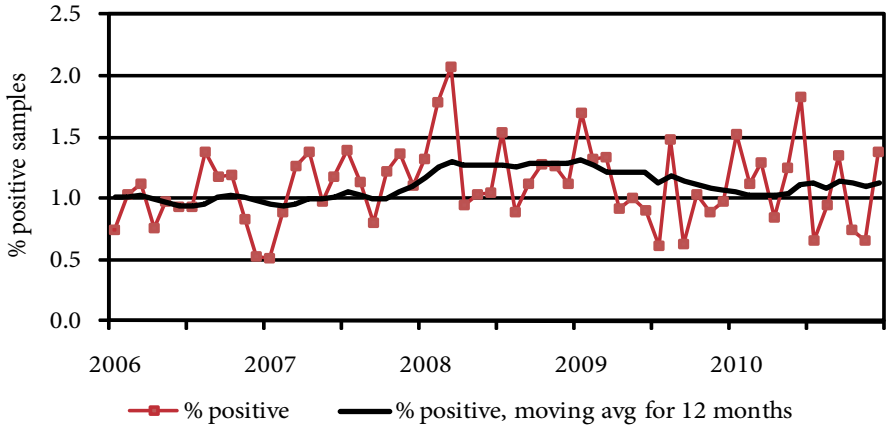
a) For more information about the surveillance programme, see Table A34
b) The peak in late summer 2007 and the very low level during 2008 were due to technical problems in the laboratory.
Source: Danish Agriculture and Food Council

Table A14. Occurrence of zoonotic pathogens in pigs and pork in Denmark, 2010

	Herds		Animals/Samples		
Zoonotic pathogen	N	Pos	N	Pos	% pos
At farm					
Brucella abortus[a]	-	-	25,150	0	-
Leptospira[b]	38	5	111	15	-
At slaughterhouse					
Salmonella spp.[c,d]	7,823	330	-	-	-
Salmonella spp.[c,e] (slaughtering >50 pigs/month)	-	-	22,485	-	1.2[f]
Salmonella spp.[c,e] (slaughtering 50 or less pigs/month)	-	-	223	-	1.8[f]
Trichinella spp.[g]	-	-	22,878,200	0	-
Mycobacterium bovis[h]	-	-	19,793,743	0	-
Echinococcus granulosis/multilocularis[h]	-	-	19,793,743	0	-

a) Including samples from boars (examined at pre-entry, every 18 month, and prior to release from semen collection centres) (14,743 samples), samples collected in connection with export (10,213 samples), import (11 samples) or fertility problems (105 samples). 5-8 ml blood samples were analysed using either the SAT, RBT, CFT or ELISA methods.
b) Sampling is based on suspicion of leptosporosis due to increased abortions or other reproductive problems in a herd. Samples are investigated using immunoflourescence techniques.
c) See Table A34 for describtion of the surveillance programme.
d) Data are from December 2010. Slaughter pig herds monitored using serological testing of meatjuice samples collected at slaughter. Herds belonging to level 2 and 3 were defined as *Salmonella* positive.
e) Swab samples from three designated areas of the half-carcass were collected at the slaughterhouse after min. 12 h chilling. Sample size is 3x100 cm^2. Samples from five animals were pooled, except at slaughterhouses where 50 pigs or less were slaughtered per month, in which case samples were analysed individually.
f) 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.
g) 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.
h) Slaughtered pigs were examined by slaughterhouse meat inspectors.
Source: Danish Veterinary and Food Administration, National Veterinary Institute and National Food Institute

Figure A3. Salmonella *in pork, monitored at slaughterhouses, 2006-2010*



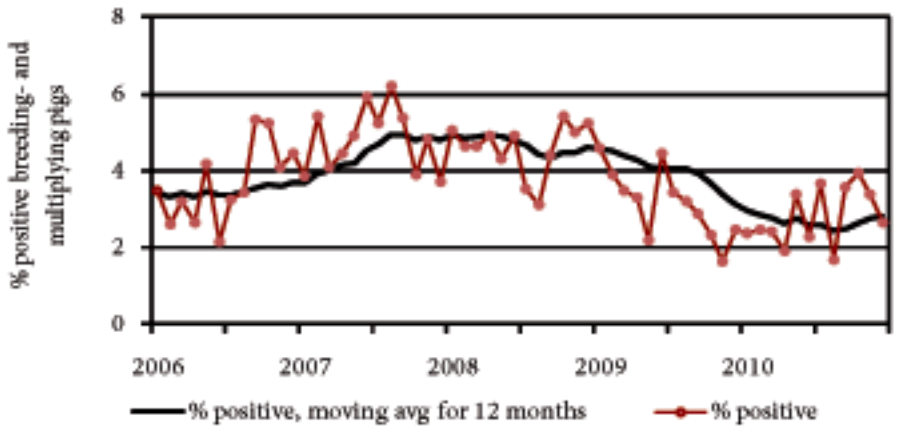
Source: Danish Veterinary and Food Administration

Table A15. Occurrence of zoonotic pathogens in cattle and beef in Denmark, 2010

	Herds		Animals/Samples		
Zoonotic pathogen	N	Pos	N	Pos	% pos
At farm					
Brucella abortus[a]	-	-	2,197	0	-
Coxiella brunetii	88[b]	66	62[c]	18	-
At slaughterhouse					
Salmonella spp.[d] (slaughtering >50 pigs/month)	-	-	7,660	-	0.3[e]
Salmonella spp.[d] (slaughtering 50 or less pigs/month)	-	-	162	-	0[e]
Mycobacterium bovis[f,g]	-	-	496,494	0	-
VTEC O157[h]	260	5	-	-	-
Echinococcusus granulosis/multilocularis[g]	-	-	496,494	0	-

a) Denmark has been declared officially brucelosis free since 1979. The last outbreak was recorded in 1962. Including samples from boars (examined at pre-entry, every year, and prior to release from semen collection centres) (1,667 samples), samples collected in connection with export (478 samples), import (1 sample) or fertility problems (36 samples). 5-8 ml blood samples were analysed using either the SAT, RBT, CFT or ELISA methods.
b) Bulk tank milk samples taken for diagnostic testing and analysed using an ELISA method.
c) Serum samples taken for diagnostic testing and analysed using an ELISA method. An additional 11 samples from placenta was analysed using the FISH method, one sample was positive.
d) See Table A33 for describtion of the surveillance programme. Swab samples from three designated areas of the half-carcass were collected at the slaughterhouse after min. 12 h chilling. Sample size is 3x100 cm^2. Samples from five animals were pooled, except at slaughterhouses where 50 cattle or less were slaughtered per month, in which case samples were analysed individually.
e) 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.
f) Denmark has been declared officially tuberculosis free since 1980. The last case of TB in cattle was diagnosed in 1988.
g) Slaughtered cattle were examined by the slaughterhouse meat inspectors.
h) Caecal content are tested from one animal per herd, collected at slaughter (DANMAP programme). A 25 g faecal sample from one slaughter calf per herd is examined using overnight enrichment, immunomagnetic separation method and plating on CT-SMAC plates for O157.
Source: Danish Veterinary and Food Administration, National Veterinary Institute and National Food Institute

Figure A4. Salmonella *in beef, monitored at slaughterhouses[a,b], 2006-2010*



Source: Danish Veterinary and Food Administration

Table A16. Cattle herds in the S. Dublin *surveillance programme[a], January 2011*

Salmonella Dublin level			Non-milk producing herds N	Non-milk producing herds %	Milk producing herds N	Milk producing herds %
Level 1	1a	On the basis of milk samples	861	6.2	3,622	89.7
	1b	On the basis of blood samples	11,732	84.8	10	0.2
	Total	Probably *Salmonella* Dublin free	12,593	91.0	3,632	89.9
Level 2	2	Titer high in blood- or milk samples	115	0.8	183	4.5
	2R	Titer high, official restrictions	271	2.0	215	5.3
	2	Contact with herds in level 2 or 3	449	3.2	6	0.1
	Total	Non *Salmonella* Dublin free	835	6.0	404	10.0
Level 3	Total	Salmonellosis, official supervision	3	0.02	2	0.05
Unknown		Too few blood samples	405	2.9	0	0
Total number of herds sampled			13,836	100	4,038	100

a) See Table A33 for describtion of the surveillance programme.
Source: Danish Agriculture and Food Council and Danish Veterinary and Food Administration

Table A17. Results from the intensified control of Salmonella *and* Campylobacter *in fresh meat based on a case-by-case risk assessment, 2010*

		No. of batches tested	No. of batches positive	No. of batches sanctioned	Mean prevalence in positive batches[a]	Mean relative human risk in positive batches
Campylobacter						
Danish	Broiler	301	22	3	34.9%	3.4
Imported	Broiler	490	86	1	28.5%	2.0
	Turkey	592	59	0	17.5%	1.1
Salmonella						
Danish	Beef	125	3	1	13.9%	103.7
	Pork	300	37	10	19.3%	9.6
	Broiler	97	0	-	-	-
Imported	Beef	127	4	4	24.5%	485.7
	Pork	296	40	11	6.5%	7.2
	Broiler	490	58	15	8.6%	0.7
	Turkey	592	56	8	10.3%	0.7

a) 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.
Source: Danish Veterinary and Food Administration and National Food Institute

Table A18. Feed business operators own sampling of Salmonella *in compound feeds, feed processing and feed material (batch-based data), 2007 and 2009-2010*

	2010		2009		2007	
	N	Positive	N	Positive	N	Positive
Feed processing plants (process control)[a]:						
Ordinary inspections - clean zone	7,963	12[d]	7,781	3	6,865	9
Ordinary inspections - dirty zone	548	58[e]	340	28	-	-
Compound feed, farm animals	390	0	384	0	424	6
Feed materials, farm animals[b]	1,285	49[f]	1,051	85	1,408	35
Transport vehicles, clean zone/hygiene samples[c]	963	0	1,176	1	949	2
Transport vehicles, dirty zone/hygiene samples[c]	224	1[g]	29	0	-	-

a) Presence of *Salmonella* in compound feed is indirectly monitored by environmental samples collected during feed processing.
b) Sampling of feed materials (predominantly soy bean meal and rapeseed cake).
c) Samples from transport vehicles (hygiene samples) prior to loading of feed compounds.
d) *S.* 4.12:b:-, *S.* Liverpool, *S.* Minnesota, *S.* Schleissheim.
e) *S.* 4.12:b:-, *S.* Derby, *S.* Falkensee, *S.* Havana, *S.* Mbandaka, *S.* Ohio, *S.* Putten, *S.* Senftenberg.
f) *S.* 1.3.19;L, *S.* 4.12:d:-, *S.* Agona, *S.* Banana, *S.* Cerro, *S.* Falkensee, *S.* Kentucky, *S.* Lexington, *S.* Livingstone, *S.* Mbandaka, *S.* Minnesota, *S.* Orion var 15, *S.* Putten, *S.* Rissen, *S.* Senftenberg, *S.* Tennessee.
g) *S.* Havana.
Source: Danish Plant Directorate / the feed business operators

Table A19. Control of Salmonella *in compound feeds, feed processing and feed material (batch-based data), 2007-2010*

	2010		2009		2008		2007	
	N	Positive	N	Positive	N	Positive	N	Positive
Feed processing plants (process control)[a]:								
Ordinary inspections	558	5[d]	907	18	1,085	18	976	17
Additional inspections	-	-	-	-	-	-	-	-
Feed materials, farm animals[b]	379	24[e]	186	4	174	12	71	3
Transport vehicles, hygiene samples[c]	-	-	-	-	3	0	95	0

a) See footnote to Table A18. Companies are sampled one to four times per year.
b-c) See footnotes to Table A18.
d) *S.* Liverpool, *S.* London, *S.* Putten.
e) *S.* Agona, *S.* California, *S.* Havana, *S.* Kentucky, *S.* Livingstone, *S.* London, *S.* Mbandaka, *S.* Minnesota, *S.* Montevideo, *S.* Putten, *S.* Rissen, *S.* Senftenberg, one non-typeable.
Source: Danish Plant Directorate

Table A20. Salmonella *in three categories of meat and bone meal by-products not intended for human consumption[a], 2010*

Category of processing plant		Own-check samples		Product samples	
		N	Positive	N	Positive
1	By-products of this material cannot be used for feeding purposes	-	-	140	0
2	By-product of this material may be used for feed for fur animals	-	-	197	0
3	By-products from healthy animals slaughtered in a slaughterhouse. Products of these may be used for petfood[b] and for feed for fur animals	1,426	33	1,812	28
	Total	1,426	33	2,149	28

a) Regulation No. 1774 of 03/10/2002.
b) For cats and dogs. Only by-products from pigs are used in this petfood.
Source: Danish Veterinary and Food Administration

Table A21. Occurrence of zoonotic pathogens in pets, zoo animals and wild life in Denmark[a], 2010

	Pet animals						Zoo animals				Wildlife			
	Dogs		Cats		Others		Mammals & reptiles		Birds		Mammals		Birds	
Zoonotic pathogen	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos	N	Pos
Salmonella spp.	9	1	1	0	0	-	18	5[b]	4	1	95	31[c]	37	4[d]
Campylobacter spp.	1	0	0	-	0	-	0	-	0	-	0	-	0	-
Brucella canis/abortus	0	-	0	-	0	-	0	-	0	-	1	0	0	-
Chlamydia psittaci	0	-	4	2	15	0	7	0	59	16[e]	0	-	8	0
Cryptosporidium spp.	15	2	12	3	0	-	41	2[f]	2	0	215	30[g]	0	-
Trichinella spp.[h]	0	-	0	-	0	-	0	-	0	-	317	0	30	0
Lyssavirus (classical)	0	-	1	0	2	0	0	-	0	-	1	0	0	-
European Bat *Lyssavirus*	0	-	0	-	0	-	1	0	0	-	10	0	0	-

a) All samples are analysed based on suspision of disease and does not reflect the country prevalence.
b) 1 sloth of 1 tested, 4 unspec. reptiles of 12 tested.
c) 3 raccoon dogs of 24 tested, 1 mink of 7 tested, 26 hedgehogs of 34 tested, 1 fox of 16 tested.
d) 2 herring gulls of 4 tested, 1 brambling of 1 tested, 1 pigeon of 6 tested.
e) 6 parakits of 10 tested, 10 unspec. zoo birds of 39 tested.
f) 2 unspec. zoo mammals of 34 tested.
g) 5 squirrels of 5 tested, 3 hedgehogs of 3 tested, 22 roe deer of 203 tested.
h) 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 and National Veterinary Institute

Table A22. The Bovine Spongiform Encephalopathy (BSE) surveillance programme[a] for cattle, 2010

Type of surveillance	N[b]	Positive
Active surveillance		
Healthy slaughtered animals (>48 months)	144,391	0
Risk categories:		
Emergency slaugthers (>48 months)	606	0
Slaughterhouse antemortem inspection revealed suspicion or signs of disease (>48 months)	0	0
Fallen stock (>48 months)	24,766	0
Animals from herds under restriction	0	0
Passive surveillance		
Animals suspected of having clinical BSE	1	0
Total	169,764	0

a) According to the EU Regulation (EC) 999/2001 as amended, Commission Decision 2009/719/EC as amended and Danish Order no. 1361 of 19/12/2008.
b) Samples (brain stem material) are tested using a IDEXX technique or Prionics-Check PrioStrip. Confirmatory testing is carried out using Western blot (definitive diagnosis if positive case), else with histopathology or immunohistochemistry. Further confirmation on autolysed material is performed at the Community TSE reference laboratory.
Source: Danish Veterinary and Food Administration

Table A23. The Transmissible Spongiform Encephalopathy (TSE) surveillance programme[a] for sheep and goats, 2010

Type of Surveillance	N[b]	Positive
Active surveillance		
Fallen stock (>18 months)	7,882	0
Animals from herds under restriction	4	0
Passive surveillance		
Animals suspected of having clinical TSE	3	0
Total	7,889	0

a) According to the EU Regulation (EC) 999/2001 as amended and Danish Order no. 930 of 07/08/2006.
b) Samples (brain stem material) are tested using a IDEXX technique or Prionics-Check PrioStrip. Confirmatory testing is carried out using Western blot (definitive diagnosis if positive case), else with histopathology or immunohistochemistry. Further confirmation on autolysed material is performed at the Community TSE reference laboratory.
Source: Danish Veterinary and Food Administration

Table A24. Distribution[a] (%) of prion protein genotype of sheep randomly selected, 2010

	Genotype	Sheep n=102
NSP 1	ARR/ARR	26.0
NSP 2	ARR/AHQ	1.0
	ARR/ARQ	19.0
	ARR/ARH/Q	0
NSP 3 (ARQ/ARQ)	ARQ/ARQ	34.0
NSP 3 (Other)	AHQ/AHQ	1.0
	AHQ/ARQ	8.0
	ARH/ARH	1.0
	ARH/ARQ	1.0
	ARQ/ARH	0
	ARQ/AHQ	0
NSP4	ARR/VRQ	2.0
NSP5	ARQ/VRQ	6.0
	AHQ/VRQ	1.0
Total		100

a) The genotypes were grouped in the NSP classification system according to their different susceptibility: NSP 1: Genetically most resistant, NSP 2: Genetically resistant, NSP 3: Genetically little resistance, NSP 4: Genetically susceptible, and NSP 5: Genetically highly susceptible.
Source: National Veterinary Institute

Table A25. Centrally coordinated studies conducted in 2010

Title of project	No. of samples	Pathogen surveyed	Futher information
DANMAP, antimicrobial resistance in Danish and imported broiler, beef and pork	1,000	*Salmonella* spp., *Campylobacter, E. coli, Enterococcus faesium, Enterococcus faecalis*	Results are presented in the DANMAP Report 2010
Listeria monocytogenes, *Salmonella* spp., *E. coli* and *Staphylococci* in fish goods from Greenland	50	*Salmonella* spp., *E. coli, Staphylococci, L. monocytogenes*	Results are being processed
Microbiological classification of mussel production areas in Denmark 2010	20	*Salmonella* spp., *E. coli*	Results are being processed
Virus in mussel production areas in Denmark 2010	100	Virus	Results are being processed
MRSA, ESC and *Clostridium difficile* in pigs, broilers and cattle	1,600	*E. coli, Staphylococcus aureus, Clostridium difficile*	Continues in 2011
Campylobacter spp. in fresh, chilled Danish broiler meat	1,200	*Campylobacter* spp.	Appendix C, Table A11
Campylobacter spp. in fresh, chilled and frozen Danish and imported broiler meat	2,800	*Campylobacter* spp.	Appendix C, Table A12
Salmonella spp. and *Campylobacter* spp. in fresh imported duck and turkey meat	800	*Campylobacter* spp., *Salmonella* spp.	Results are being processed
Intensified control for *Salmonella* spp. and *Campylobacter* spp. in fresh Danish and imported meat	2225[a]	*Salmonella* spp., *Campylobacter* spp.	Appendix C, Table A17
Salmonella spp. in pork, during cutting/ retail	2,725	*Salmonella* spp.	Continues in 2011
Salmonella spp. in ready-to-eat meat products (not fermented sausages)	1,300	*Salmonella* spp.	Results are being processed
Salmonella spp. in table eggs - trade	Not defined	*Salmonella* spp.	Results are being processed
Pathogens in slightly preserved fermented Danish and imported sausages	500	*Salmonella* spp., VTEC[b], *L. monocytogenes, enterobactericeae, enterococcus*	Results are being processed
Salmonella spp. in dry snack nuts and fruits		*Salmonella* spp.	Results are being processed
Microbiological quality of meat products with risk of recontamination	1,000	Total viable counts, coliforms, *E. coli, S. aureus*	Results are being processed
Microbiological quality of minced meat - wholesale	275	Total viable count, *Salmonella* spp., *E. coli*	Results are being processed
Microbiological quality in minced meat - retail	1,000	Total viable count, *Salmonella* spp., *E.coli*	Continues in 2011
Listeria monocytogenes in smoked and gravad fish	150	*L. monocytogenes*	Results are being processed
EU-baseline, *Listeria monocytogenes*	80	*L. monocytogenes*	Continues in 2011
Salmonella spp. and *E. coli* in raw, frozen scallop from Greenland	50	*Salmonella* spp., *E. coli*	Results are being processed

Continued on the next page

Table A25. Centrally coordinated studies conducted in 2010 (Continued from page 44)

Title of project	No. of samples	Pathogen surveyed	Futher information
Pathogens in Danish and imported ready-to-eat vegetables	500	*Salmonella* spp., *E. coli, Campylobacter* spp.	Results are being processed
Microbiological quality of meals ready to eat	1,300	Total viable counts, coliforms, *B. cereus, Staphylococcus aureus, C. perfringens, L. monocytogenes*	Results are being processed
Microbiological quality of brawn production at butchers	200	Total viable counts, lactic acid-producing bacteria, coliforms, sulphite-reducing bacteria	Continues in 2011
Hygiene in small slaughterhouses	500		Continues in 2011
Milk and dairy, pathogens and hygiene	800	*Salmonella* spp., *L. monocytogenes, S. aureus, Enterobacteriaceae/E. coli*	Continues in 2011
Pathogens in imported cheese	250	*Salmonella* spp., *L. monocytogenes, S. aureus*	Continues in 2011
Pathogens in salatbars	750	*Salmonella* spp., *L. monocytogenes, E. coli*	Continues in 2011
Pathogens in cut salat	100		Continues in 2011

a) Batches.
b) Verotoxin-producing *Escherichia coli*.
Source: Danish Veterinary and Food Administartion and National Food Institute

Table A26. Listeria monocytogenes *in Danish produced ready-to-eat foods[a], 2010*

		Samples analysed by a qualitative method[c]				Samples analysed by a quantitative method			
		Batches[b]		Single samples		Batches[b]		Single samples	
Food category	Sampling place	N	Pos	N	Pos	N	Pos	N	Pos
Products of meat origin,RTE	At processing	70	3	15	0	167	0	29	0
	At retail	-	-	65	1	16	1[d]	86	0
Cheese, RTE	At processing	71	0	12	0	64	0	2	0
	At retail	-	-	7	0	-	-	-	-
Milk and dairy products, RTE									
	At processing	72	0	14	0	49	0	-	-
	At retail	-	-	7	0	-	-	7	0
Fishery products, RTE	At processing	80	10	-	-	97	4[e]	-	-
	At retail	4	2	19	1	-	-	43	6[e]
Fruit and vegetables, RTE	At processing	12	0	-	-	22	0	-	-
	At retail	-	-	4	0	-	-	2	0
Other RTE products	At processing	20	0	-	-	33	0	-	-
	At retail	-	-	114	4	-	-	241	0

a) Samples are collected by the Regional Veterinary and Food Control Authorities according to European Regulation (EC) No 2073/2005.
Source: Danish Veterinary and Food Administration
b) 5 samples from each batch, analysed individually.
c) *Listeria monocytogenes* present in a 25 g sample of the product.
d) Detected 10-100 cfu/g cfu: Coloni forming units.
e) Detected >100 cfu/g.

Appendix D

Monitoring and surveillance programmes

Table A27. Overview of notifiable and non-notifiable human diseases presented in this report, 2010

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

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

Table A28. Overview of notifiable and non-notifiable animal diseases presented in this report, 2010

Patogen	Notifiable	EU legislation	Danish legislation
Bacteria			
Brucella spp.	1920[a]		
Cattle	OBF in 1979[b]	Decision 2004/320/EC	Order no 305 of 3/5 2000
Sheep and goats	ObmF in 1995[c]	Decision 2004/320/EC	Order no. 739 of 21/8 2001
Pigs	No cases since 1999	Directive 2003/99/EC	Order no. 205 of 28/3 2009
Campylobacter spp.	no	-	-
Chlamydophila psittaci	1920	-	
Birds and poultry			Order no. 78 of 30/1 1997
Listeria monocytogenes	no	-	-
Leptospira spp. (only in production animals)	2003	-	Act no. 432 of 09/06/2004
Mycobacterium bovis/tuberculosis	1920[a]		
Cattle	OTF since 1980[d]	Decision 2004/320/EC	Order no. 1417 of 11/12 2007
Coxiella burnetii	2005	-	Act no. 432 of 09/06/2004
Salmonella spp.	1993[e]	-	
Cattle			Order no. 1723 of 22/12/2010
Swine			Order no. 1722 of 22/12/2010
Poultry			Order no. 1462 of 16/10/2009
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[a]	Regulation 2075/2005/EC	Order no. 412 of 28/05/2008
Viruses			
Lyssavirus	1920	-	Order no. 14 of 11/01/1999 and Order no. 914 of 15/12/1987
Prions			
TSE			
Sheep and goats	yes	Regulation 999/2001/EC (as amended)	Order no. 930 of 07/09/2006
BSE			
Cattle	yes	Regulation 999/2001/EC (as amended)	Order no. 1361 of 19/12/2008

a) Clinical cases, observations during the meat inspection at the slaughterhouse, positive blood samples or finding of agens are notifiable.
b) Officially Brucellosis Free (OBF) according to Council Directive 64/432/EC as amended and Commision Decision 2004/320/EC. No cases in cattle since 1962.
c) Officially *B. melitensis* Free (ObmF) according to Council Directive 91/68/EC and Commision Decision 2004/320/EC. Never detected in sheep or goat.
d) Officially Tuberculosis Free (OTF) according to Council Directive 64/432/EC as amended and Regulation (EC) 1226/2002, and Commission Decision 2003/467/EC. No cases in cattle since 1988 or in deer since 1994.
e) Only clinical cases notifiable.
Source: Danish Veterinary and Food Administration

Table A29. Salmonella *surveillance programme for the rearing flocks and adult flocks of the grandparent and parent generation of the broiler and table egg production, 2010*

Time	Samples taken	Material	Material
Rearing flocks		*Grandparent generation*	*Parent generation*
Day-old[a,b]	Per delivery	5 transport crates from one delivery: crate liners (>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.
1st & 2nd week[b, c]	Per unit	-	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g.
4th week[b,c]	Per unit	5 pairs of boot swaps (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g.	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g.
8th week[b,c]	Per unit	2 pairs of boot swabs (analysed as one pooled sample). Cage birds: 60 samples of fresh droppings (1g). Analysed as one pool.	2 pairs of boot swabs (analysed as one pooled sample). Cage birds: 60 samples of fresh droppings (1g). Analysed as one pool.
2 weeks prior to moving[a,d]	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g.	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g.
Adult flocks		*Grandparent generation*	*Parent generation*
Every two weeks[b] (Every 16th week)[e]	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[b]	Per hatch	Wet dust samples. Up to four hatchers of the same flock can be pooled.	Wet dust samples. Up to four hatchers of the same flock can be pooled.
Every week[b]	Per unit	-	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample of 60g.
0-4 weeks after moving, 8-0 weeks before slaughter[d]	Per unit	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g.	5 pairs of boot swabs (analysed as two pooled samples), or 1 faeces sample consisting of 2x150g.
After positive findings[d]	Per unit	5 pairs of boot swabs (analysed as two pooled samples), 2 dust samples (250 ml) and 5 birds (analysed for antimicrobial substances.	5 pairs of boot swabs (analysed as two pooled samples), 2 dust samples (250 ml) and 5 birds (analysed for antimicrobial substances).

a) Sampling requirements set out by Regulation (EC) 2160/2003.
b) Samples collected by the food business operator.
c) Order no 1259 of 15/12/2008.
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.
Source: Danish Veterinary and Food Administration

Table A30. Salmonella *and* Campylobacter *surveillance programme for the broiler flocks, 2010*

Time	Samples taken	Material
Broiler production - *Salmonella*		
15 - 21 days before slaughter[a,c,d]	Per flock	5 pairs of boot swabs. Analysed individually.
7 - 10 days before slaughter[b,e]	Per flock	5 pairs of boot swabs. Analysed individually.
After slaughter[b,c]	Per batch	300 neck skin samples of 1 gram, analysed in pools of max. 60 grams. Sampling is depending on whether the slaughterhouse slaughters only AM-negative flocks or AM-negative as well as AM-positive flocks.
Broiler production - *Campylobacter*		
7 - 10 days before slaughter[b,f]	Per flock	1 pair of boot swabs.

a) Regulation (EC) 2160/2003.
b) Order no 1462 of 16/12/2009.
c) Samples collected by the food business operator.
d) Once a year, the samples are collected by the Regional Veterinary and Food Control Administration.
e) Samples are collected by a representative of the slaughterhouse, laboratorium or the Regional Veterinary and Food Control Administration.
Source: Danish Veterinary and Food Administration

Table A31. Salmonella *surveillance programme for the pullet-rearing, table egg layer and barnyard/hobby flocks in the table egg production, 2010*

Time	Samples taken	Material
Pullet- rearing		
Day-old[a,d]	Per delivery	5 transport crates from one delivery: Crate liner (> 1 m^2 in total) or swab samples (> 1 m^2 in total) (Analysed as one pooled sample).
4 weeks old[b,d]	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples of 60 gram.
2 weeks before moving[a,c]	Per flock	5 pairs of boot swabs (analysed as two pooled samples) or 5 faeces samples of 60 gram. 60 blood samples (serology).
Table egg layers (Production for certified packing stations)		
24 weeks old[a,c]	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 gram. 250 ml (100 g) dust or 1 pair of boot swabs. 60 eggs[b] (serology).
Every 9 weeks[a,d,e]	Per flock	2 pairs of boot swabs (analysed as one pooled sample) or 1 faeces sample consisting of 2x150 gram. 60 eggs[b] (serology).
Barnyard and hobby flocks		
Every 18 weeks[d]	Per folck	Egg samples.

a) Sampling requirements set out by Regulation (EC) 2160/2003.
b) 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 A32. Salmonella *surveillance programmes[a] for the duck and turkey flocks, 2010*

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

a) Order no 1261 of 15/12/2008.
b) Samples collected by the food business operator.
Source: Danish Veterinary and Food Administration

Table A33. Salmonella *Dublin surveillance programme[a] for the cattle herds and* Salmonella *surveillance programme at slaughter, 2010*

No. of samples	Samples taken	Comment
Milk producing herds		
4 samples distributed over 13 months	Bulk tank samples	Calculation of herd level[b]
10 samples	Blood samples	If the owner wants a herd moved from level 2 to 1b
Non-milk producing herds		
1 sample[c]	Blood samples	Calculation of herd level[b]
4-8 samples	Blood samples	Consecutive negative samples required for level 1b[d]
Beef carcasses at the slaughterhouse		
5 samples daily, pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering more than 200 cattle per day
5 samples per 200 slaughtered cattle, pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering more than 200 cattle per month but 200 or less cattle per day
5 samples every 3rd month, pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering 50-200 cattle per month
1 sample every 3rd month	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering less than 50 cattle per month

a) Order no. 1723 of 22/12/2010 as ammended. In 2010, the programme for eradication of *Salmonella* Dublin from the Danish cattle production was intensified. This implies a new category of level 2 (level 2R) where the most contagious herds in this level are placed under official restrictions by the veterinary authorities.
b) Herd levels based on serological testing (blood and milk).
Level 1a: Milk producing-herd assumed free of infection (based on bulk tank samples),
Level 1b: Non-milk producing-herd or milk producing-herd assumed free of infection (based on blood samples),
Level 2: Herd not assumed free of infection,
Level 3: Herd infected, and
Unknown level: insufficient number of blood samples have been taken from herd and no samples had antibody levels above the limit value.
c) No samples are taken, 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.
d) Number of samples equals total number of animals in the herd minus 2 (max. 8 animals, min. 4 animals).
Source: Danish Veterinary and Food Administration

Table A34. Salmonella *surveillance programme[a] for the pig production, 2010*

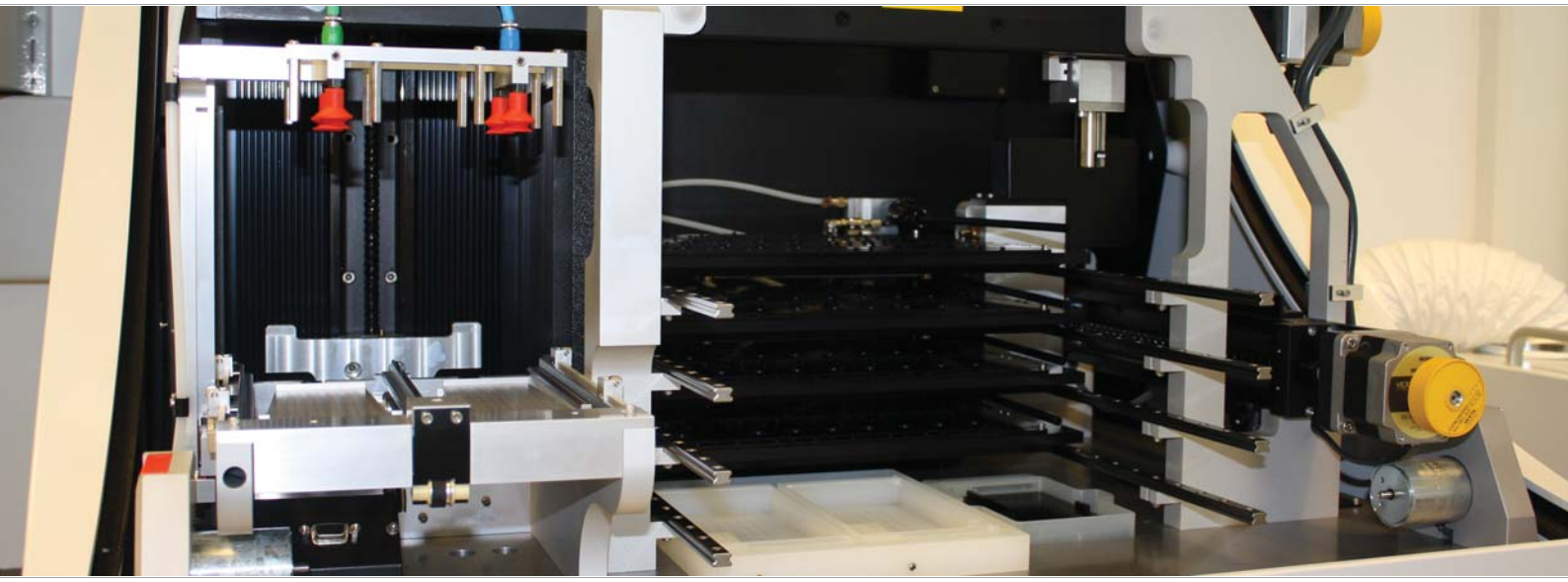
Time	Samples taken	Purpose
Breeding and multiplier herds		
Every month	10 blood samples per epidemiological unit	Calculation of *Salmonella*-index based on the mean from the last three months with most weight to the result from the more recent months (1:3:6)
Max. twice per year	Herds with *Salmonella*-index 5 or above: Pen-faecal samples[b, d]	Clarify distribution[c] and type of infection in the herd
Sow herds		
When purchaser of piglets is assigned to level 2 or 3, max. twice per year	Pen-faecal samples	Clarify distribution[c] and type of infection in the herd, and clarify possible transmission from sow herds to slaughter pig herds
Herds positive with *S.* Typhimurium, *S.* Infantis and *S.* Derby are considered positive for the following 5 years	No samples are taken for 5 years, unless herds are proven negative	Reduce pen samples in sow herds with high serology
Slaughter pig herds		
At slaughter	Meat juice, 60-100 samples per herd per year. Herds in RBOV[d, e]: one meat juice sample per month	Calculation of slaughter pig index based on the mean from the last three months with most weight to the result from the most recent month (1:1:3). Assigning herds to level 1-3 and assigning herds to risk-based surveillance (RBOV)[e]
Herds assigned to level 2 or 3, max. twice per year	Pen-faecal samples	Clarify distribution and type of infection in the herd
Pork carcasses at the slaughterhouse		
5 samples daily, pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering more than 200 pigs per day
5 samples per 200 slaughtered pig, pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering more than 200 pigs per month or 200 or less pigs per day
5 samples every 3rd month, pooled into one analysis	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering more than 50 pigs per month or less than 200 pigs per month
1 sample every 3rd month	Swab samples from 3 designated areas after 12 hours chilling (3x100m^2)	Slaughterhouses slaughtering less than 50 pigs per month

a) Order no. 1722 of 22/12/2010.
b) Herds with index above 10 have to pay a penalty for each pig sold.
c) Pigs from herds in Level 3 must be slaughtered under special hygienic precautions.
d) The herd owner must inform buyers of breeding animals about the infection level and type of *Salmonella*.
e) RBOV: risk-based surveillance where the sample size in herds with a SP-index of zero (no positive samples in the previous three months) are reduced to one sample per month.
Source: Danish Veterinary and Food Administration

Table A35. Typing methods used in the surveillance of foodborne pathogens in Denmark, 2010

Methods	Human	Food	Animal
Salmonella enterica			
Serotype	All	All	All
Phage type	*S.* Typhimurium and *S.* Enteritidis	*S.* Typhimurium and *S.* Enteritidis	*S.* Typhimurium and *S.* Enteritidis
Antimicrobial resistance	*S.* Typhimurium, 50% of *S.*Enteritidis, approx. 90% of other serotypes	*S.* Typhimurium and occasionally other serotypes	*S.* Typhimurium and occasionally other serotypes
MLVA	*S.* Typhimurium	*S.* Typhimurium (outbreak investigations), research	*S.* Typhimurium (outbreak investigations), research
PFGE	Outbreak investigations	Outbreak investigations	Outbreak investigations
Campylobacter coli/jejuni			
Antimicrobial resistance	Isolates from 3 districts for DANMAP surveillance	Only for DANMAP surveillance purposes	Only for DANMAP surveillance purposes
FlaA-SVR	Outbreak investigations	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
MLVA	All	All	None
PFGE	All	All	All
Yersinia enterocolitica			
O-group	Isolates from one district	None	None

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



Appendix E

Population and slaughter data

Table A36. Human population, 2010

Age groups (years)	Males	Females	Total
0-4	167,084	158,411	325,495
5-14	342,623	326,969	669,592
15-24	354,824	339,101	693,925
25-44	727,992	719,243	1,447,235
45-64	742,275	743,325	1,490,600
65+	416,784	516,997	933,781
Total	2,756,582	2,804,046	5,560,628

Source: Statistics Denmark

Table A37. Number of herds/flocks, livestock and animals slaughtered, 2010

	Herds/flocks[a]	Livestock[a] (capacity)	Number slaughtered
Slaughter pigs (>27 kg)	7,731	6,422,624	19,793,743
Cattle	20,829	1,631,863	496,494
Broilers	589	22,065,410	139,753,738
Layers (excl. barnyard)	271	3,270,000	-
Turkeys	44	483,237	5,334
Sheep & lambs	8,629	172,580	85,285
Goats	3,624	25,368	2,680
Horses	-	-	1,872

a) March 2011.

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

Table A38. Number of farms in the broiler production, 2010

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (grandparent)	4	14	90,000
Adult period (grandparent)	5	11	80,000
Rearing period (parent)	15	90	130,000
Adult period (parent)	44	145	710,000
Hatcheries	5	-	-
Broilers	242	589	-

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

Table A39. Number of farms in the table egg production, 2010

	No. of holdings	No. of houses/flocks	Livestock (capacity)
Rearing period (parent)	5	6	20,000
Adult period (parent)	8	9	30,000
Hatcheries	5	-	-
Pullet-rearing	83	140	1,300,000
Layers (excl. Barnyard)	207	271	3,270,000

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

Table A40. Distribution of import, export and production of fresh and frozen meat and the production of table eggs in Denmark, 2007-2010. Data is presented in tons

	Year	Pork	Beef	Broiler meat[a]	Turkey meat	Duck meat[b]	Table eggs[c]
Import	2007	40,201	80,287	30,390	8,423	3,845	-
	2008	83,057	81,427	32,480	8,264	4,494	-
	2009	83,265	88,818	30,321	7,000	4,251	-
	2010	87,304	102,612	42,667	8,740	4,875	-
Export	2007	1,263,169	61,374	105,741	1,692	454	-
	2008	1,386,849	66,690	109,725	2,345	772	-
	2009	1,321,820	78,572	108,377	1,564	534	-
	2010	1,400,251	90,385	118,046	2,969	807	-
Danish production	2007	1,447,894	134,374	168,354	34	2,956	66,800
	2008	1,602,648	149,744	157,543	49	37	67,900
	2009	1,508,640	163,068	159,723	93	0	60,600
	2010	1,582,107	182,584	171,208	78	0	62,200
Consumption[d]	2007	224,925	153,287	93,003	6,765	6,347	-
	2008	298,857	164,481	80,298	5,968	3,722	-
	2009	270,084	173,314	81,667	5,529	3,717	-
	2010	269,160	194,811	95,829	5,849	4,068	-

a) Natural-marinated chicken is included.

b) Mixed products of ducks, geese and guinea fowl are not included.

c) Consumption of table eggs is assumed to be roughly the same as the production, since import and export of table eggs is minimal.

d) Consumption = Production + import - export

Source: Statistics Denmark

Appendix F

List of Figures

List of Tables

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