Annual Report on Zoonoses in Denmark 2000

CAMPYLOBACTER

Ministry of Food, Agriculture and Fisheries

Contents

Annual Report on Zoonoses in Denmark 2000

Edited by:
Tine Hald and Therese Brøndsted,
Danish Zoonosis Centre, Danish Veterinary Laboratory

Birgitte Beck Jørgensen,
Danish Veterinary and Food Administration

Kåre Mølbak,
Statens Serum Institut

This is an official publication of the Danish Zoonosis Centre, the Danish Veterinary and Food Administration and the Statens Serum Institut.

Data in this report were provided by
- The Danish Plant Directorate (www.plantedir.dk)
- The Danish Veterinary and Food Administration (www.fdir.dk)
- The Danish Veterinary Laboratory (www.svs.dk)
- Statens Serum Institut (www.ssi.dk)
- Danish Veterinary Institute for Virus Research (www.vetvirus.dk)
- The Royal Veterinary and Agricultural University (www.kvl.dk)

Suggested citation:
Anonymous, 2001. Annual Report on Zoonoses in Denmark 2000, Ministry of Food, Agriculture and Fisheries.

Reprints can be ordered from:
Danish Zoonosis Centre
Danish Veterinary Laboratory
Bülowsvej 27
DK - 1790 Copenhagen V, Denmark
Phone: +45 35 30 01 48
Fax: +45 35 30 03 77
E-mail: dzc@dzc.dk
Printing: Datagraf Auning
ISSN 0909-4172

The report is also available from:
http://www.svs.dk, and
the Danish website on zoonoses:
http://www.dzc.dk

The front page illustration:
Resemblance in seasonal variation of *Campylobacter* in broilers and turkeys, 1998 (Sept. 1999)-2000.

Introduction

This report on zoonoses presents a summary of the occurrence of zoonotic agents in feeding stuffs, animals, food stuffs and humans in Denmark. The report is based on data compiled according to the zoonoses directive 92/117/EEC, supplemented by data obtained from Danish surveillance and control programmes as well as data from relevant research projects from the different institutions that have contributed to the preparation of this report.

Demographic data

Total number of livestock and herds in Denmark, 2000

	Livestock	Herds
Cattle	1,944,197	35,498
Pigs	7,322,026	21,406
Laying hens excl. barn yard sale	3,532,130	392
Broilers	37,575,735	344
Sheep	189,826	7652

Source: The Central Husbandry Register

Approximate total number of animals slaughtered in 2000

Pigs:	21 million
Broilers:	135 million
Cattle:	604,000
Sheep, lambs and goats:	72,000
Horses:	2,700

Source: The Danish Veterinary and Food Administration

Human population in 2000

Age group (years)	Male	Female	Total
<1	33,906	32,396	66,302
1-4	140,923	133,368	274,291
5-14	328,536	312,019	640,555
15-24	315,611	305,509	621,120
25-44	802,212	772,225	1,574,437
45-64	683,729	679,184	1,362,913
65+	329,205	461,197	790,402
Total	2,634,122	2,695,898	5,330,020

Area of Denmark:
44,000 sq km

Source: The Statistical Yearbook 2000, Danmarks Statistik

1. Salmonella

Feeding stuffs

All Danish feed compounders are monitored for *Salmonella* by the Danish Plant Directorate. Monitoring includes sampling of compound feeds and feed materials, including raw materials of animal origin, as well as sampling during feed processing. Table 1 shows the overall results of the monitoring in 2000.

Samples of feeding stuffs

The Danish Plant Directorate collects samples of feeding stuffs from the production plants and retailers. The number of samples depends on the size of the production, but is increased if *Salmonella* is detected during the feed processing control or in the feeding stuffs.

The number of *Salmonella* samples in compound feeding stuffs in 2000 is listed in Table 1. Compared to the previous years, the good hygienic quality of compound feeding stuffs has stabilised at a very low level (Figure 1).

Samples from feed processing

Inspectors carry out hygiene control at feed processing plants at least four times a year. Samples are collected for microbiological examination at the critical control points of the production process.

From compounders producing heat treated feeding stuff (>81°C), the samples are collected at critical control

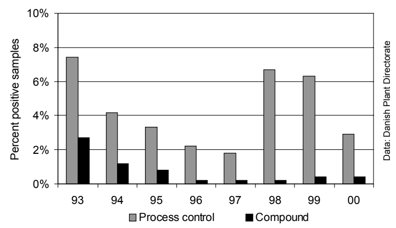

Figure 1. Percent Salmonella *positive samples of process control and compound feed, 2000.*

points after the heat treatment. When there is no heat treatment, the samples are collected during the entire process as well as from the feed materials.

The plants are inspected in order to check their compliance with a national set of rules for good production hygiene. This includes control of the heat treatment, which is monitored every second hour during the process.

Additional inspections of the plants are implemented when *Salmonella* is detected in the samples or critical hygiene conditions are reported.

The prevalence of *Salmonella* in samples from the process control at the plants has decreased from 6.3% in 1999 to 2.9% in 2000 (Figure 1).

Serotypes

The serotypes found in feeding stuffs and samples collected during feed processing appear from Table 1. The *Salmonella* serotypes found consisted mainly of the exotic serotypes usually associated with feeding stuffs. For the first time *Salmonella* Typhimurium DT104 has been found in a sample of feeding stuffs for farm animals (pigs). A rodent or a wild bird most likely caused the contamination.

Summary

The results of the *Salmonella* control by the Danish Plant Directorate indicate that the good hygienic quality of compound feeding stuffs has stabilised. *Salmonella* was found in only a small percentage of the compound feeding stuffs samples.

However, the results of the inspection of the feed processing and the incidence of *Salmonella* positive samples in feed materials have shown that *Salmonella* infected feed materials constitute a risk for contamination of feeding stuffs plants. The results also make it clear that the hygienic standard of the plants can be further improved.

Rendering Plants

Control of hygiene at rendering plants is carried out by the animal health section of the Danish Veterinary and Food Administration. In 2000, 516 samples of the final products from rendering plants were examined for *Salmonella*. One sample (0.2%) was positive for S. Livingstone.

Table 1. Control of Salmonella *in compound feeds, feed processing and feed materials in 2000.*

	Control of compound feeds				Process control	Feed materials
	Pig feed	Cattle feed 1)	Poultry feed	Others		
Total number of samples	1,436	721	249	180	2,994	382
Salmonella detected	4	4	0	2	88	15
Percent positive	0.3	0.6	0	1.1	2.9	3.9
Serotypes	S. Tennesee 2	S. Derby 2	-	S. Infantis 1	S. Tennessee 29	S. Tennessee 3
	S. Derby 1	S. Agona 1		S. Liverpool 1	S. Liverpool 11	S. Agona 1
	S. Typhimurium DT 104 1	S. Senftenberg 1			S. 4.12:b:- 9	S. Goldcoast 1
					S. Infantis 7	S. Havana 1
					S. Havana 4	S. Infantis 1
					S. Muenster 4	S. Kapemba 1
					S. Muenster var. 15+ 4	S. Mbandaka 1
					S. Senftenberg 4	S. Ohio 1
					S. Agona 2	S. Senftenberg 1
					S. Alachua 2	S. Typhimurium 1
					S. Meleagridis 2	S. Weltevreden 1
					S. Anatum 1	S. 4.23:b:- 1
					S. Hadar 1	S. 9.12:lv:- (monophasic) 1
					S. Kentucky 1	
					S. Livingstone 1	
					S. London 1	
					S. Mbandaka 1	
					S. Putten 1	
					S. Rissen 1	
					S. Stanley 1	
					S 9.46:-:- 1	

Data: Danish Plant Directorate.
1) Includes feed for cattle, horses, sheep and rabbits.

In 2000, 234 samples of fishmeal were examined and 2 (0.9%) were found to be contaminated with *Salmonella*. The serotypes found were S. Cerro and one was untypable.

Poultry and poultry products

In December 1996, the Danish Ministry of Agriculture and Fisheries implemented an extended surveillance and control programme for the prevention of *Salmonella* in the broiler- and table-egg production. Initially, the programme was financed for a three-year period, but by the end of 1999, it was decided to continue the programme for another period. The basic strategy of the programme was maintained, but specific initiatives for the prevention and early recognition of *Salmonella* in poultry flocks were adopted. These included changes in methods of sampling and intervals between testing, and constituted an improvement in the protection of the consumers. In June 2000, the original method of collection, 60 faecal samples in parent flocks, changed to 2 pairs of "sock-samples“, where practically feasible. A "sock-sample“ consists of elastic cotton tubes pulled over the collector’s boots. While walking through the poultry house, the cotton tubes absorb faecal droppings. Two pairs of „sock-samples“ analysed as one pool has shown to be just as effective in detecting *Salmonella* as the 60 faecal samples. In addition, the sampling method is easier to perform. In accordance with the zoonosis directive, 60 faecal samples were taken from pullets at four weeks of age and two weeks prior to entering the laying phase. In breeders, the serological samples collected every 4th week were replaced with weekly "sock-samples“. In central-rearing flocks, serological testing for *Salmonella* Infantis was abandoned. At the hatcheries, hatchers were tested after each hatching instead of once weekly as was the case earlier. The most important problem today is considered to be reinfection in previously infected houses as a result of persisting infection in the environment. Consequently, research into new methods of cleaning and disinfection of infected houses, as well as determination of criteria for cleaning and disinfection, were initiated.

Parent flocks, rearing and table-egg layer flocks were tested for all *Salmonella* serotypes. The number of establishments in the broiler and table-egg production and the frequency of testing are listed in Table 2 and 3. Flocks testing positive for *Salmonella* or *Salmonella* antibodies in the routinely collected samples (Table 3) were put under suspicion of infection. As a consequence, the district veterinary officer (DVO) collected additional samples. If the second set of samples was positive, the flock was declared infected with *Salmonella*. Breeder and rearing flocks infected with *Salmonella* were slaughtered. Eggs from layer flocks under suspicion and eggs from infected flocks were directed to heat treatment (pasteurisation). On farms with infected layer flocks, the examination of non-infected flocks was intensified. Eggs and faeces from such flocks were tested

Table 2. Number of establishments in the broiler production and the table-egg production in 2000.

	No. of establishments	No. of houses	No. of animals
	Broiler production		
Central rearing	20	96	1,223,000 purchased per year
Broiler breeders	69	210	
Hatcheries	4	149 a)	
Broilers	361	825	140,410,000
	Table-egg production		
Central rearing	6	7	70 - 80,000 purchased per year
Layer breeders	9	18	
Hatcheries	3	65 a)	-
Rearing	106	164	3,899,000
Layers, except barn-yard sale	308	465	4,090,000

Data: Danish Veterinary and Food Administration and Danish Poultry Council
a) Number of hatchers.

Table 3. Salmonella *surveillance of the broiler and table-egg production, 2000.*

Age or Frequency	Samples taken in 2000
	CENTRAL - REARING STATIONS Broiler and table-egg sector
Day-old chickens	10 samples of cratematerial, · 20 dead/destroyed chickens [a]
1 week	40 dead chickens
2 weeks	2 pairs of sock samples
4 weeks	60 faecal samples [a]
8 weeks	2 pairs of sock samples
2 weeks prior to moving	60 faecal samples and 60 blood samples [a] [b]
	BREEDERS (HATCHING-EGG PRODUCTION) Broiler and table-egg sector
Every 2 weeks	50 dead chickens or meconium from 250 chickens taken from the hatchery [a] [c]
Every week	2 pairs of sock samples [d]
	HATCHERY
After each hatching	Wet dust
	REARING - TABLE-EGG PRODUCTION
Day-old chickens	10 samples of cratematerial and 20 dead chickens
3 weeks	5x2 sock samples in floor production units or 300 faecal samples
12 weeks	5x2 sock samples in floor production units or 300 faecal samples, and 60 blood samples [b]
	TABLE-EGG PRODUCTION
Every 9th week for eggs sold to authorized egg - packing centres	2 pairs of sock samples in floor production units or faecal samples, and egg samples
Every 6 months for eggs sold at barn-yard sale	2 pairs of sock samples or faecal samples and egg samples

Data: Danish Veterinary and Food Administration.
a) Requirements of the EU Zoonosis Directive (92/117/EEC).
b) Samples taken by the district veterinary officer.
c) Samples taken by the district veterinary officer every 8 weeks.
d) Samples taken by the district veterinary officer every 3 months.

every 4th week instead of every 9th week. No new flocks were permitted in the houses where infected flocks had been kept, before the cleaning and disinfection had been approved by the DVO.

Table-egg production

In 2000, no central rearing flocks or layer breeders (hatching-egg production) were found infected with *Salmonella*. A total of 8 (2.1%) of 374 rearing flocks examined were found infected with *Salmonella*. Of these, 6 flocks were infected with S. Enteritidis, 1 flock with S. Enteritidis and S. Senftenberg and 1 flock with S. Typhimurium (Table 4).

In flocks producing table-eggs for authorised egg-packing centres, 31 (4.6%) of 668 flocks tested were confirmed as being infected with *Salmonella*. Of these, 26 flocks were infected with S. Enteritidis, 1 flock was infected with S. Typhimurium, 1 flock was infected with S. Infantis and 3 flocks were declared infected based on the serological results only (Table 4).

In flocks producing table-eggs for barnyard sale, 13 (1.8%) of 740 flocks were confirmed as being infected with *Salmonella*. Of these, 12 flocks were declared infected based on the serological

Table 4. Occurrence of Salmonella *in the table-egg production in 2000.*

	Central rearing		Layer breeders		Rearing		Table-egg production	
	Examined flocks	Positive flocks	Examined flocks	Positive flocks	Examined flocks	Positive flocks (%)	Examined flocks	Positive flocks (%)
Salmonella spp.	15	0	29	0	374	8 (2.1)	668	31 [1] (4.6)
S. Enteritidis	-	-	-	0	-	7 [2] (1.9)	-	26 (3.9)
S. Typhimurium	-	-	-	0	-	1 (0.2)	-	1 (0.1)
Other serotypes	-	-	-		-	1[2] (0.2)	-	1 (0.1)

Data: Danish Veterinary Laboratory and Danish Veterinary and Food Administration.
1) Three flocks found infected based on serological confirmation only.
2) One flock infected with both S. Senftenberg and S. Enteritidis

results only.

The percentage of infected layer flocks by type of table-egg production is shown in Figure 2. In the beginning of 1998, a considerable difference between types of production was noticed: battery: 12%, deep litter: 9%, free-range: 4%, organic: 2%. However, this difference was not considered to be due to the production type, but rather was dependent on the hatchery, since many battery and deep litter producers were supplied by the same hatchery which had *Salmonella* problems. As a consequence of the control programme, the prevalence in all production types was reduced to less than 3% by the end of 2000.

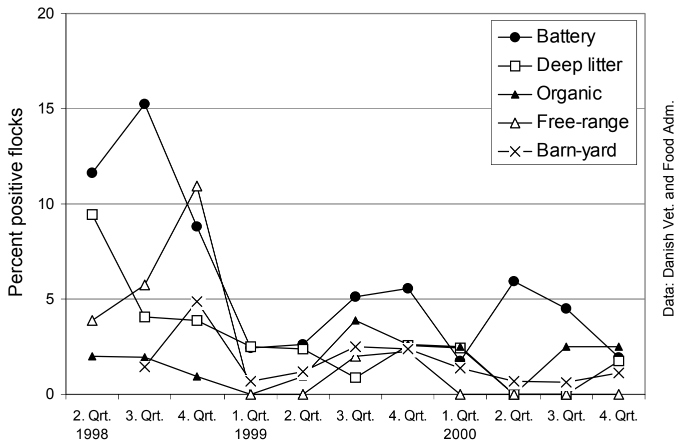

Figure 2. Number of flocks infected with Salmonella *according to type of table-egg production, 2000.*

Number of herds by type of production, 2000: Battery: 79; Deep litter: 86; Free range: 48; Organic: 111.
Source: Danish Veterinary and Food Administration

Broiler production

For the first time during the programme, *Salmonella* was found in a grand-parent flock. The flock was found infected with S. Enteritidis phage type (PT) 6. Also for the first time during the programme, *Salmonella* was found in one of the 246 examined central-rearing flocks. This flock was found to be infected with S. Tennessee. Among the broiler breeders (hatching-egg production), 3 (0.9%) of 345 flocks were found infected with *Salmonella* (1 S. Enteritidis PT 1b, 1 S. Typhimurium PT 41 and 1 S. Typhimurium PT 12) (Table 5).

All production flocks were monitored for *Salmonella* by mandatory ante-mortem examination. Three weeks prior to slaughter, five pairs of sock-samples were collected from each flock. The percentage of positive flocks ranged from 1.1% to 2.8% per month with a mean of 2.1% (Table 5, Figure 3). The most frequently encountered serotype was S. Typhimurium, which was found in 18.1% of the infected flocks. S. Enteritidis was isolated from 11.7% of the infected flocks. The sero- and phage-type distributions are shown in Table 15, 16 and 17.

The monitoring of broiler flocks after slaughter (post

Table 5. Occurrence of Salmonella *and* Campylobacter *in the broiler production in Denmark in 2000.*

	Flock level				Slaughterhouse		Retail - broilers and products of broiler meat				
	Broiler breeders		Broiler flocks		Neck skin		Not heat treated		Heat treated		
Zoonotic pathogen	Flocks examined	% positive flocks	Flocks examined	% positive flocks	N	% positive flocks	N	% positive samples	N	% positive samples	Note:
Salmonella spp.	345	0.9	4,567	2.1	4,543	2.9	94	4.3	216	0.5	a
S. Enteritidis	-	0.3	-	0.2	-	-	-	-	-	-	-
S. Typhimurium	-	0.6	-	0.4	-	-	-	-	-	-	-
Other serotypes	-	0	-	1.5	-	-	-	-	-	-	-
Campylobacter spp.	-	-	6,160	37.7	-	-	708	41.1	82	1.2	b
C. jejuni	-	-	-	34.3	-	-	-	-	-	-	-
C. coli	-	-	-	2.7	-	-	-	-	-	-	-
C. lari	-	-	-	0.1	-	-	-	-	-	-	-
Other species	-	-	-	0.6	-	-	-	-	-	-	-

Data: Danish Veterinary Laboratory and Danish Veterinary and Food Administration
a) Parent flocks were examined according to Table 3. Broiler flocks were monitored by sock-samples 2-3 weeks prior to slaughter and by 50 neck-skin samples at slaughter. This post-mortem monitoring was terminated by November 2000.
b) Flocks investigated by cloacal swabs collected at slaughter, ten birds per flock were examined. Summed up in batches, where one flock is slaughtered in up to 6 batches.

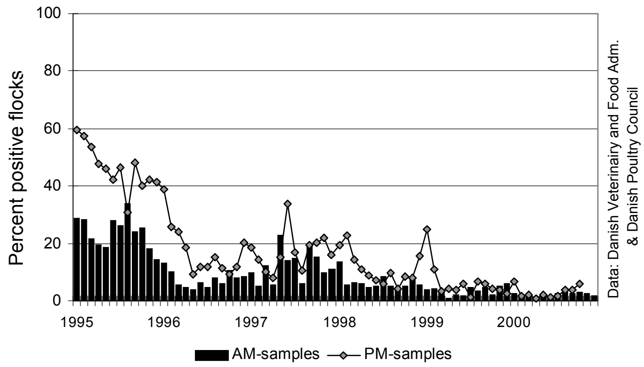

Figure 3. Percent Salmonella *positive broiler flocks detected at the mandatory ante-mortem and post-mortem examination, 1995-2000. Post-mortem examination stopped in November, 2000.*

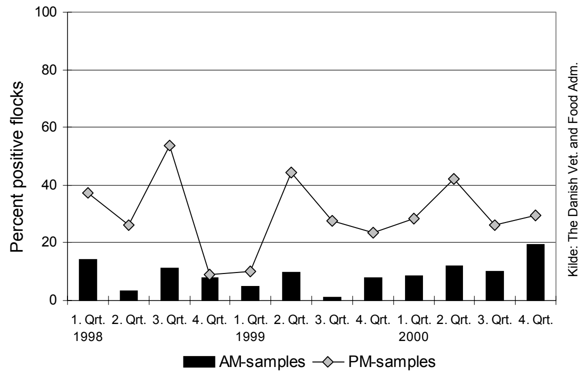

Figure 4. Percent Salmonella *positive turkey flocks detected at the ante-mortem and post-mortem examination, 1998-2000.*

mortem) detected *Salmonella* in 2.9% of the flocks by examination of 5 pooled samples each consisting of 10 neck-skin samples from each slaughter flock (Table 5, Figure 3). This is a reduction compared to 6.6% in 1999 and 11.1% in 1998.

From the middle of November 2000, the mandatory examination of broilers after slaughter was changed from swab samples of neck skin to samples of batches of poultry parts close to the packaging stage. A batch is defined as the amount of meat from animals slaughtered between two cleanings and disinfections. The new monitoring programme aims to give an indication of the level of cross-contamination in the slaughterhouse and a better picture of the contamination level of broiler meat as close to the consumption as possible. The programme is temporary and runs until January 1st 2002. The results will be used in the planning of the future post-mortem control for *Salmonella* in broilers.

Turkey production

All turkey flocks were monitored for *Salmonella* by mandatory ante-mortem examination in 2000. Three weeks prior to slaughter, five pairs of sock samples were collected from each flock. *Salmonella* was detected in 44 (12.8%) of 345 flocks investigated (Table 6, Figure 4). Of these, 11 flocks were infected with S. Agona, 10 flocks with S. Heidelberg and 23 flocks with other serotypes. One flock was found infected with S. Typhimurium DT104 on the

Table 6. Occurrence of Salmonella *and* Campylobacter *in the turkey production in Denmark in 2000.*

	Flock level		Slaughterhouse		Retail - cuts and products of turkey meat				
	Turkey flocks		Neck skin		Not heat treated		Heat treated		
	Flocks examined	% positive flocks	N	% positive flocks	N	% positive samples	N	% positive samples	Note:
Salmonella spp.	345	12.8	363	31.4	69	8.7	50	0	a
S. Enteritidis	-	0	-	-	-	-	-	-	-
S. Typhimurium	-	0	-	-	-	-	-	-	-
Other serotypes	-	12.8	-	-	-	-	-	-	-
Campylobacter spp.	367	47.4	-	-	303	30.4	34	0	b
C. jejuni	-	39.8	-	-	-	-	-	-	-
C. coli	-	4.4	-	-	-	-	-	-	-
C. lari	-	0	-	-	-	-	-	-	-

Data: Danish Veterinary Laboratory and Danish Veterinary and Food Administration
a) Flocks monitored by sock samples 2-3 weeks prior to slaughter and by 50 neck-skin samples at slaughter.
b) Flocks monitored by cloacal swabs at slaughter, ten birds per flock were examined. Summed up in batches, where one flock is slaughtered in 2-4 batches. Because one flock may be tested for *Campylobacter* more than once; more than one species of *Campylobacter* may be detected in the same flock.

farm, but not at slaugther. S. Enteritidis was not isolated from any turkey flocks in 2000. The serotype distribution is shown in Table 15.

Salmonella was detected in a total of 114 (31.4%) out of 363 flocks examined after slaughter (Table 6, Figure 4).

Duck and goose production

In 1999, *Salmonella* monitoring was started in duck and goose flocks by ante-mortem examination. Three weeks prior to slaughter, five pairs of sock samples were collected from each flock.

In 2000, 171 duck flocks were examined. *Salmonella* was isolated from 166 (97.1%) of the flocks. For 103 flocks (62%) more than one serotype was isolated. S. Hadar was the most frequently isolated serotype found in approximately 28% of the infected flocks (Table 15). No results from goose flocks were reported in 2000.

Pigs and pork

A serological surveillance programme for detection of *Salmonella* infection in slaughter-pig herds was implemented at the beginning of 1995. All herds producing more than 100 pigs for slaughter per year are monitored by serological testing of meat juice. The herds are assigned to one of three levels based on the proportion of samples with a positive serological reaction. Level 1: a herd with no or few reactors where intervention is not required; Level 2: a herd with a higher proportion of reactors and where the owner is required to seek advice on how to reduce the prevalence of *Salmonella*; Level 3: the proportion of reactors in the herd is unacceptably high and the owner is required to seek advice and slaughter of pigs from the herd has to be carried out under special hygienic precautions. From August 1996, it became mandatory for herds in Level 2 and 3 to collect a sufficient number of pen-faecal samples in order to clarify the distribution of *Salmonella* in the herd. Based on the results, an intervention plan must be prepared by the owner and farm advisors.

The proportion of *Salmonella* positive herds declined during the first six months of 2000, but in July the proportion began to increase and this increase continued throughout the autumn of 2000 with a peak in October. The increase in the proportion of *Salmonella* positive herds reflects a similar increase in the proportion of individual meat-juice samples with a serological reaction during the same period (Figure 5). No cause of the increase was found. At the end of 2000, the proportion of *Salmonella* positive herds again started to decline. In December 2000, 95.9%

Table 7. Occurrence of zoonotic pathogens in pigs and pork in Denmark in 2000.

	Herd level			Slaughterhouse				Retail				
	Examined			Cuts of pork		Plucks		Not heat treated		Heat treated		
Zoonotic pathogen	Herds	Animals	% positive herds	N	% positive samples	N	% positive samples	N	% positive samples	N	% positive samples	Note:
Mycobacterium bovis	21,406	21 mill	0	-	-	-	-	-	-	-	-	a
Brucella abortus	-	-	0	-	-	-	-	-	-	-	-	b
Trichinella spp.	21,406	20.5 mill	0	-	-	-	-	-	-	-	-	c
Salmonella spp.	15,494	766,892	4.1	17,954	0.8	4,139	3.3	1,782	1.1	1,228	0.08	d
S. Enteritidis	-	-	-	-	0.01	-	0	-	-	-	-	-
S. Typhimurium	-	-	-	-	0.4	-	2.0	-	-	-	-	-
Other serotypes	-	-	-	-	0.4	-	1.3	-	-	-	-	-
Campylobacter spp.	310	310	64.2	-	-	-	-	-	-	-	-	e
C. jejuni	-	-	4.2	-	-	-	-	-	-	-	-	-
C. coli	-	-	59.4	-	-	-	-	-	-	-	-	-
C. lari	-	-	0.6	-	-	-	-	-	-	-	-	-
Y. enterocolitica O:3	316	316	13.0	-	-	-	-	96	6.3	-	-	e f

Data: Danish Veterinary Laboratory and Danish Veterinary and Food Administration
a) All slaughter pigs were examined in connection with meat inspection.
b) Boars examined on admission to semen collection centres and before leaving the station.
c) All pigs slaughtered at export slaughterhouses were examined in connection with meat inspection
d) Herds were monitored by serological testing. Herds belonging to Level 2 and 3 were defined as *Salmonella* positive.
e) Herds examined by caecal samples from one animal per herd collected at slaughter.
f) All isolates from food belonged to apathogenic types.

Multi-drug resistant *Salmonella* Typhimurium DT104

Control in the primary production

In October 1997, the Danish Veterinary and Food Administration (DVFA) issued an order making the detection of multi-drug resistant *Salmonella* Typhimurium DT104 notifiable in pig and cattle herds. Infected herds are put under official veterinary supervision including special hygiene at slaughter, and there is epidemiological investigation of the herd and its trade contacts. Two negative herd examinations at 45 day intervals are required to lift the sanctions. Sanctions can also be lifted if the herd is destroyed. The order was issued to prevent spread of DT104 between herds as well as from animals to humans. In August 1999, the order was replaced with a new order extending the authorities powers to investigate the spread of DT104. According to this, all animal species on an infected farm and herds associated with the infected herd by e.g. trade of live animals or geographical location, can be ordered examined.

The pig industry's strategy of stamping out infected pig herds as described in the Annual Report 1999 was terminated in June 2000 as a rapidly increasing number of infected pig herds made this approach economically unfeasible. In December 2000, the DVFA released a report describing a new strategy for the control of DT104 in animal and food production. A working group representing relevant trade organisations, research institutions and authorities, prepared the report. The report points out a number of initiatives to be taken in the primary production and at slaughterhouses. Basically the surveillance of pig and cattle herds must be improved, while the aim at the slaughterhouses is to improve the hygiene and reduce cross contamination.

In poultry production, the surveillance for DT104 is a part of the extended *Salmonella* surveillance and control programme implemented in December 1996. Detection of DT104 in slaughter-poultry flocks will lead to slaughtering and heat treatment or destruction of the flock. DT104 has never been found in the Danish table-egg production. But in the case of a positive finding, the flock and eggs will be destroyed. Breeding or rearing flocks found infected with S. *enterica* are destroyed.

Occurrence in the primary production

The number of herds infected with DT104 increased markedly in 2000 (Figure 6). Over the past four years a total of 48 herds were identified. In 2000, 57 herds (20 pig herds, 6 cattle herds, 29 combined cattle and pig herds, 1 turkey and 1 chicken flock) were identified. The first poultry flocks infected with DT104 in Denmark were found by the end of 2000. First in a turkey flock originating from Germany, and later in a broiler flock raised near two DT104 infected pig herds.

Monitoring of imported products

Since July 1998, the occurrence of DT104 in fresh meat imported from the EU and third countries has been monitored. The overall prevalence of DT104 in imported meat is the same in 2000 by 0.4% (14 of 3,219 samples) as in 1999 by 0.4% (21 of 5,217 samples).

Imported product	Number of samples	Positives (%)	Positive for DT104 (%)
Poultry	1,776	291 (16.4)	5 (0.3)
Pork	439	37 (8.4)	4 (0.4)
Beef	1,004	8 (0.8)	5 (0.5)

Human infections

Despite the rise in the domestic primary production, the number of registered sporadic cases of human S. Typhimurium DT104 has remained constant since 1997 (Figure 7). In contrast to the past two years there were no outbreaks of DT104 in 2000.

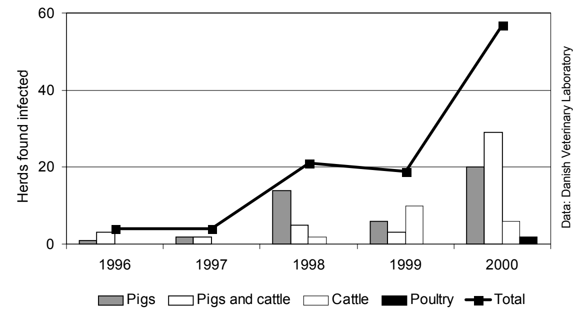

Figure 6. Herds found infected with multi-drug resistant S. Typhimurium DT104 in Denmark 1996-2000.

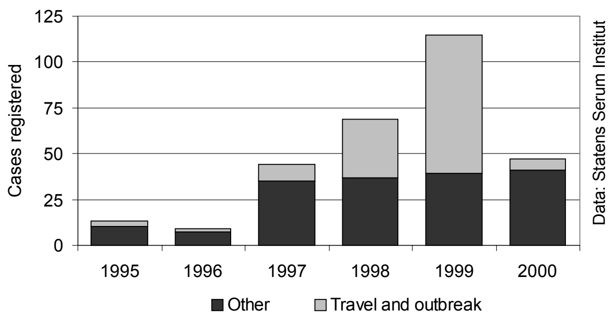

Figure 7. Registered cases of human S. Typhimurium DT104 (including DT104b) in Denmark, 1995-2000

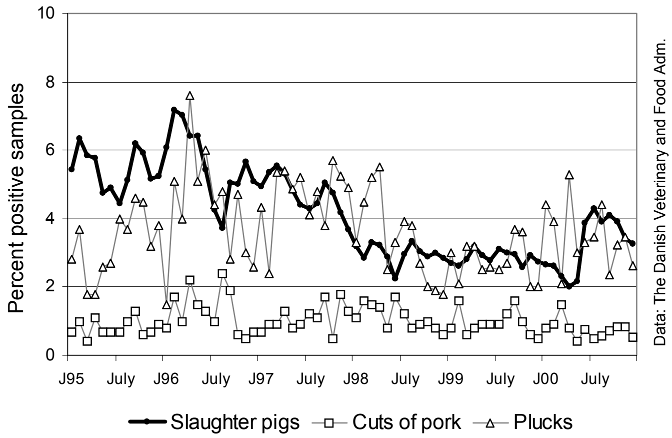

Figure 5. Percent seropositive meat-juice samples and percent Salmonella *positive samples of cuts of pork and pluck, 1995-2000.*

of the herds fell within Level 1, 3.1% within Level 2 and 1.0% within Level 3.

Breeding and multiplying herds are monitored monthly by serological testing of blood samples. If a specific cut-off level in these samples is reached, the herd owner is obliged to collect pen-faecal samples. Further, if the serological reactions exceed a specific high level, all movement of livestock from the herd is restricted.

Sow herds producing piglets for slaughter-pig herds placed in Level 2 or 3 are also obliged to collect pen-faecal samples in order to determine the distribution of *Salmonella* within the herd, and to clarify possible transmission of *Salmonella* from the sow herd to the slaughter-pig herd.

A programme for monitoring *Salmonella* in pork at the slaughterhouses was initiated in July 1993. Approximately 2,250 samples were analysed every month. For each slaughterhouse the number of samples collected is determined by the actual number of animals slaughtered. In 2000, the proportion of *Salmonella* positive cuts of pork varied between 0.4% and 1.5% with a mean of 0.8%, which is slightly lower than in 1999 (1.0%). For samples of the plucks (incl. lungs, liver and heart), the proportion of positive samples ranged from 2.1% to 5.3% with a mean of 3.3% (Table 7, Figure 5).

Clinical salmonellosis was recorded in 76 herds (Table 8). This figure represents the number of herds submitting material from clinically affected animals to the laboratory. Of these, 67 herds were placed under official veterinary supervision by the district veterinary officer. In the past 50 years, S. Choleraesuis has rarely been found in Danish pig herds. Until 1999, the last finding was in a pig herd in Zealand in 1972. However, in 1999, S. Choleraesuis var kunzendorf was isolated from a pig herd in Northern Jutland and furthermore in two pig herds supplied by the infected herd. In October 2000, S. Choleraesuis var kunzendorf was detected in another pig herd. The herd, which was situated in Central Jutland, was like the herd infected the year before, detected through the serological surveillance programme, and S. Choleraesuis was subsequently isolated from the obligatory pen-faecal samples. A pig herd supplied by the infected herd was also found positive for S. Choleraesuis. Both herds infected in 2000 were stamped out and the farmers were partly compensated for their losses by the Danish Bacon and Meat Council. The source of the infection was not discovered, but DNA-analyses of the isolates from 1999 and 2000 showed, that an epidemiological connection between the outbreaks could not be excluded.

Cattle and beef

Herds of cattle are investigated for *Salmonella* on clinical indications and as part of a

Table 8. Isolation of Salmonella *from outbreaks of clinical disease in pig and cattle herds in 2000*

Serotype	Pig herds	Cattle herds
Brandenburg	1	
Choleraesuis	2	
Derby	3	
Dublin	1	38
Duesseldorf		1
Enteritidis		1
Heidelberg	2	
Infantis	3	1
Livingstone	1	
Livingstone/Stanley	1	
Mbandaka		1
Orion var. 34+	1	
Tennessee		1
Typhimurium DT104	7	3
Typhimurium/4.5.12:i:-	1	
Other Typhimurium	50	33
Worthington	1	
9.12:lv	1	
9.12:-:-		1
Rough, non typable	1	2
Total	76	82

Data: Danish Veterinary and Food Administration.

monitoring programme for the occurrence of antimicrobial resistance in zoonotic bacteria. Salmonellosis was diagnosed in 82 cattle herds in 2000 (Table 8). Of these, 37 herds were placed under official veterinary supervision by the district veterinary officer. The predominant serotypes isolated from clinical cases in cattle in 2000 were S. Dublin (46%) and S. Typhimurium (40%) (Tables 8). As part of the monitoring at the slaughterhouses, *Salmonella* was isolated from 7 (2.7%) of 262 caecal samples collected from one animal per herd (Table 9).

The Danish Ministry of Food, Agriculture and Fisheries has initiated the work of developing a national surveillance and control programme for S. Dublin and S. Typhimurium in Danish cattle herds. The programme will be based on continuous serological testing of milk samples and blood samples of slaughter animals. In 2000, 51,226 milk samples and 54,854 blood samples were analysed by a S. Dublin ELISA-test and 28,223 milk samples were analysed by a S. Typhimurium-ELISA. The interpretation of the results was at the time of finishing this report not completed.

A programme for monitoring the occurrence of *Salmonella* in beef at the slaughterhouses was initiated in July 1993. For each slaughterhouse the number of samples collected is determined by the actual number of animals slaughtered. Approximately 250 samples were collected each month from a representative sample of beef cuts and plucks. The proportion of positive samples of beef cuts per month ranged from 0.0% to 1.2% during 2000 with a mean of 0.5% (Table 9), which is comparable with 0.4% in 1999. The predominant serotype was S. Dublin (56%) (Table 15).

Pet animals, wild mammals and birds

The Danish Veterinary Laboratory monitors the occurrence of *Salmonella* in pet animals and wildlife. The group of wild mammals and birds consists mainly of road kills and animals otherwise dead submitted by hunters, veterinarians and others. Pet animals were investigated on clinical indication only. The prevalence of *Salmonella* in pets and wild life in 2000 was low (<2%). An exception was in the group of other pets, where 2 (9.5%) of 21 examined pets were positive (Table 10). The two *Salmonella* positive pets were both reptiles; a king grass snake and a monitor lizard (*Varanous*).

Products from retail outlets

The Regional Veterinary and Food Authorities collect samples for monitoring of meat and meat products at the retail level. A total of 310 broiler and broiler products, 119 samples of turkey cuts and turkey products, 3,010 samples of pork and pork products, and 1,971 samples

Table 9. Occurrence of zoonotic pathogens in cattle and beef in Denmark in 2000.

	Herd level			Slaughterhouse				Retail				
	Examined			Cuts of beef		Plucks		Not heat treated		Heat treated		
Zoonotic pathogen	Herds	Animals	% positive herds	N	% positive samples	N	% positive samples	N	% positive samples	N	% positive samples	Note:
Mycobacterium bovis	-	604,000	0	-	-	-	-	-	-	-	-	a
Brucella abortus	-	-	0	-	-	-	-	-	-	-	-	b
Salmonella spp.	262	262	2.7	2,079	0.5	588	1.7	1,599	1.2	372	0.3	c
S. Enteritidis	-	-	0	-	0.1	-	0	-	-	-	-	-
S. Typhimurium	-	-	0.4	-	0.05	-	0	-	-	-	-	-
S. Dublin	-	-	1.5	-	0.1	-	1.4	-	-	-	-	-
Other serotypes	-	-	0.8	-	0.2	-	0.3	-	-	-	-	-
Campylobacter spp.	90	90	61.0	-	-	-	-	-	-	-	-	c
C. jejuni	-	-	56.7	-	-	-	-	-	-	-	-	-
C. coli	-	-	1.1	-	-	-	-	-	-	-	-	-
C. lari	-	-	3.3	-	-	-	-	-	-	-	-	-
E. coli O157 (VT+)	214	214	2.8	-	-	-	-	-	-	-	-	c

Data: Danish Veterinary Laboratory and Danish Veterinary and Food Administration.
a) Bulls at semen collection centres were examined by TB test. Slaughter animals examined in connection with meat inspection. Notifiable disease.
b) Bulls examined on admission to semen collection centres and annually after entry. Clusters of abortions are notifiable. Notifiable disease in cattle.
c) Herds were investigated by caecal samples from one animal per herd collected at slaughter.

of beef and beef products were examined in 2000. In non heat-treated samples the prevalences were 4.3% (Table 5), 8.7% (Table 6), 1.1% (Table 7), and 1.2% (Table 9) respectively. The prevalence in heat-treated products ranged between 0% and 0.5% (Tables 5, 6, 7 and 9).

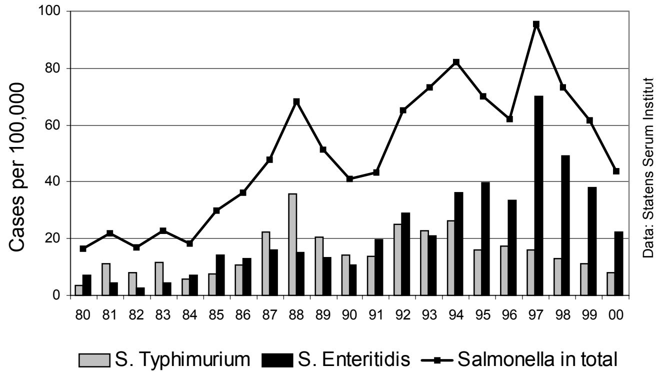

Figure 8. Registered cases of human salmonellosis in Denmark 1980-2000.

Table 10. Occurrence of Salmonella *and* Campylobacter *in pet animals, wild mammals and birds in Denmark in 2000*

	Pet animals						Wild mammals								Wild birds			
	Dog		Cat		Others		Hare		Ruminants		Fox		Others		Water fowl		Others	
Zoonotic patogen	Animals	% positive	Animals	% positive	Animals	% positive	Animals	% positive	Animals	% positive	Animals	% positive	Animals	% positive	Birds	% positive	Birds	% positive
Salmonella	318	1.3	105	1.0	21	9.5	32	0	21	0	55	1.8	113	1.8	17	0	259	1.2
S. Enteritidis		-		-		0		-		-		0		0.9		0		-
S. Typhimurium		-		-		0		-		-		0		0		0		0.8
Others/not typeable/not typed		1.3		-		9.5		-		-		1.8		0.9		0		0.4
Campylobacter spp.	286	16.1	102	10.8	50	2.0	25	0	25	20.0	54	1.9	107	4.7	12	16.7	185	12.4
C. jejuni		4.2		1.0		2.0		-		12.0		0		3.7		16.7		8.6
C. coli		0		0		-		-		8.0		0		0		0		3.2
C. upsaliensis		6.3		6.9		-		-		0		0		0		0		0
Others/not speciated		5.6		2.9		-		-		0		1.9		0.9		0		0.5

Table 11. Zoonoses in humans 2000 - incidence and trends of 5 and 10 years

	2000		Five years trend					10 years	
Agent	Cases per 100,000 inh.	Registered cases	1999	1998	1997	1996	1995	1990	Note:
Mycobacterium bovis	0.2	12	2	8	11	11	9	5	a
Brucella abortus/melitensis	0	0	0	0	0	0	0	0	a
Trichinella spiralis/nativa	0	0	0	0	0	0	0	0	a
Salmonella spp.	43.3	2,308	3,268	3,880	5,015	3,259	3,654	2,112	b
S. Enteritidis	22.2	1,182	2,025	2,607	3,674	1,771	2,070	562	
S. Typhimurium	8.1	436	584	678	841	907	848	728	
Other zoonotic serotypes	13.0	690	659	595	500	581	736	822	
C. coli/jejuni	82.3	4,386	4,164	3,372	2,666	2,973	2,601	1,367	c
E. multilocularis/granulosus	0	0	0	0	0	0	0	0	d
Listeria monocytogenes	0.7	39	44	41	33	39	29	37	e
Rabies	0	0	0	0	0	0	0	0	f
Toxoplasma gondii	-	-	-	-	-	-	-	-	g
Cryptosporidium parvum	-	-	-	-	-	-	-	-	h
Yersinia enterocolitica	5.0	265	339	464	430	532	779	967	
Escherichia coli (VTEC)	1.1	60	51	34	33	5	2	32	
O157 (VTEC)	0.3	18	10	6	12	3	2	2	

Data: Statens Serum Institut.
a) Notification not mandatory. Cases of tuberculosis are due to reactivation of latent infections in elderly or imported disease.
b) Only first isolations registered.
c) A sample(n=157) of the isolates were identified to the species level: 89% *C. jejuni* and 11% *C. coli*. In the years 1996-1999 the percentage of *C. coli* in similar samples were 5%, 6%, 4% and 4%, respectively.
d) Notification not mandatory. A few imported cases occur.
e) Notification mandatory (since 1986).
f) Notification mandatory. No domestical or imported cases.
g) Notification not mandatory
h) Notification not mandatory. Approximately 200 cases are diagnosed annually, most are imported cases.

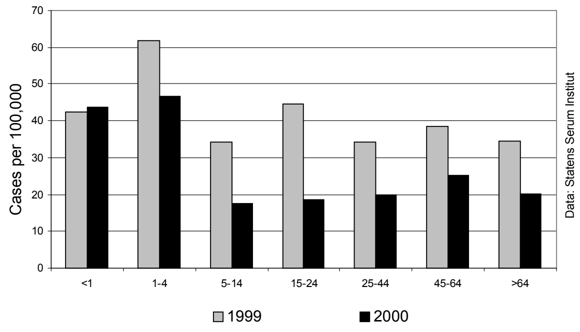

Figure 9. Incidence of infections with S. Enteritidis by age. 1999-2000.

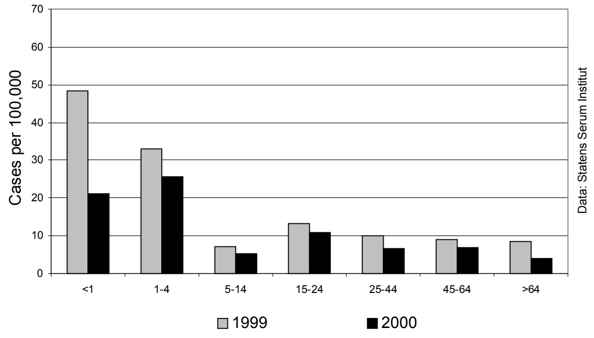

Figure 11. Incidence of infections with S. Typhimurium by age, 1999-2000.

Salmonellosis in humans

In 2000, the registered number of human infections with zoonotic *Salmonella* serotypes was 2,308 (43.3 cases per 100,000 inhabitants, Table 11). This number represents a continued decline from 1997 when 5,015 cases were registered. The numbers of *Salmonella* cases in 2000 fell by 29% compared with 1999 (3,268 cases) and has reached almost the same level as seen in 1991 when 2,203 cases were registered (Figure 8).

The decrease was mainly due to a lower number of S. Enteritidis cases in 2000 (1,182 compared with 2,025 in 1999 and as many as 3,674 in 1997). Thus, the number of S. Enteritids infections has been reduced by 67% over three years. The number of S. Enteritidis infections is now at same level as in 1993 (1,186 cases) but still higher than the 1980s when a maximum of 825 cases were registered in 1987. Table 16 shows the phage type (PT) distribution of 786 S. Enteritidis strains from human infections. The major types were PT4 (24%), PT8 (22%), PT34 (11%), PT6 (9%) and PT1 (8%). In 1997, PT6 and PT8 accounted for 72% of the S. Enteritidis cases. According to the laboratory reports, more than 90% of PT6, PT8 and PT34 cases were domestically acquired, whereas around 20% of both the cases of PT1 and PT4 had a history of foreign travel. It is estimated that approximately 80% of all S. Enteritidis cases are domestically acquired.

Figure 9 shows the age-specific distribution of S. Enteritidis cases in 1999 and 2000. Figure 10 shows the geographical distribution of the S. Enteritidis cases.

The number of S. Typhimurium cases decreased from 584 in 1999 to 436 in

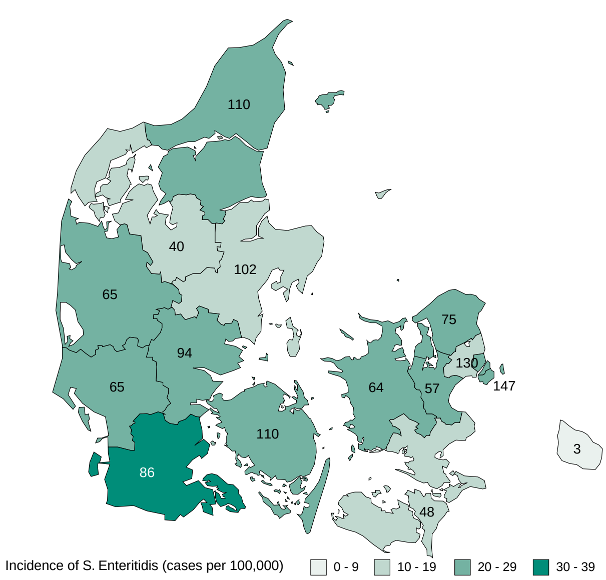

Figure 10. Geographical distribution of the number of cases per county and incidence of human infections with S. Enteritidis in 2000. Data: Statens Serum Institut.

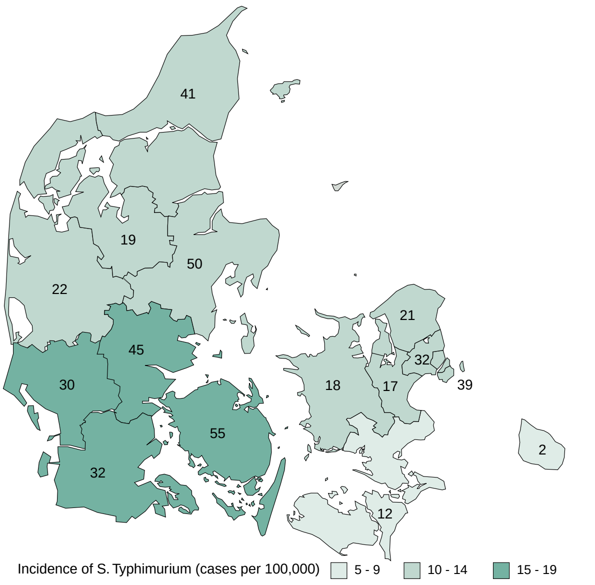

Figure 12. Geographical distribution of the number of cases per county and incidence of human infections with S. Typhimurium in 2000. Data: Statens Serum Institut.

2000, a 26% reduction. The incidence of sporadic cases of S. Typhimurium in Denmark has gradually declined since 1994. Table 17 shows the phage type (DT) distribution of 419 cases. The most common phage types were DT12 (19%), DT104 (11%), DT120 (8%) and DT1 (7%). In 1999, DT104 was the most frequent phage type, accounting for 25% of the infections. The high incidence of DT104 in 1999 was mainly due to two outbreaks comprising 58 of the registered cases (see the Annual Report 1999). The number of sporadic cases of multiple drug resistant DT104 infections has been more or less constant and at a low level between 1997 and 2000. There was an increase in multi-drug resistant S. Typhimurium U302 in 2000, primarily due to an outbreak with 9 cases. In total 16 cases of U302 infections were registered in 2000.

The distribution of phage types in 2000 represents a gradual change in the predominating types since 1996 where DT12 accounted for around 60% of the S. Typhimurium from humans.

Approximately 90% of S. Typhimurium infections are domestically acquired.

Figure 11 shows the age-specific incidence of S. Typhimurium in 1999 and 2000 and Figure 12 shows the geographical distribution of this serotype.

The remaining 690 zoonotic *Salmonella* cases were distributed over more than 100 different serotypes. Among these were S. Agona (73 cases), S. Hadar (62 cases), S. Virchow (41 cases), S. Thompson (37 cases), and S. Senftenberg (34 cases) the most common. The numbers of the 'exotic' serotypes rose from 659 in 1999, i.e. a 5% increase. Table 15 shows the distribution of the major serotypes.

Outbreaks of zoonotic gastrointestinal infections

In Denmark, outbreaks of food- and waterborne infections caused by zoonotic agents are detected in three different systems. *First*, physicians in general practice and hospitals are obliged to report all infections which are suspected to be foodborne. In principle, this should be carried out without awaiting a microbiological diagnosis. These early notifications of suspected outbreaks are submitted to the Medical Officer of Health in the county with a copy to the department of epidemiology at Statens Serum Institut (Ministry of Health) (Table 12).

Second, individuals who experience food poisoning often report these incidents to the Regional Veterinary and Food Authorities. These reports as well as the result of the outbreak investigations

Table 12. Clinical based surveillance of suspected outbreaks of food-borne zoonotic diseases reported to Statens Serum Institut, 2000.

	General outbreaks		Outbreaks within household	
Zoonotic pathogen	No. of outbreaks	Suspected source	No. of outbreaks	Suspected source
S. Enteritidis	14	Homemade ice cream, soufflé, wild boar, vegetable pie, meat balls, shrimps, open sandwiches, oysters	14	Eggs, home made ice cream and custard with non-pasteurized eggs, fried eggs, chicken, lamb
S. Typhimurium	3	Unknown	1	Unknown
S. Typhimurium DT 104	-		2	Pizza
Campylobacter	12	Chicken, minced beef, pizza, turkey, pork, open sandwiches, fish	10	Eggs, meat balls (pork), omelette, turkey, shellfish
Others [a)]	6	Pizza, smoked fillet, ice cream, raw eggs	2	Tainted meat
Unknown	20	Chicken, shrimps, pizza, oysters, beef, duck, sausages, soufflé, home made ice cream, turkey, fish, salmon	8	Shrimps, chicken, lasagna, turkey, gyros

Data: Dept. of Epidemiology, Statens Serum Institut
a) Other zoonotic *Salmonella* spp. and food toxins

are collated at the Danish Veterinary and Food Administration (Table 13).

Third, gastrointestinal pathogens identified at clinical microbiology laboratories are reported to the department of gastrointestinal infections at Statens Serum Institut, which is the reference laboratory for enteric pathogens and in charge of the laboratory surveillance system (Table 14).

There is at the present time no systematic evaluation of the overlap between the three parallel systems, nor has the completeness of these systems been formally evaluated.

Table 14. Outbreaks identified in the laboratory based surveillance of zoonotic diseases, Statens Serum Instiut, 2000.

Zoonotic pathogen	No. of cases confirmed (suspected)	Suspected source
Family outbreak S. Enteritidis PT6	3,2 fatal	Cake, made from raw eggs
Boarding school in Haslev S. Enteritidis PT4	8(12)	Mayonnaise, beef, sausages, tomatoes, mixed salad
Restaurant in Sønderjylland S. Enteritidis PT1	4	Tiramisu, made from raw eggs
Mixed general/nosocomial S. Typhimurium U302 MR	9,1 fatal	Imported vegetables
Family outbreak VTEC O26:H11	2	Raw milk, one secondary case
Private day care VTEC O26:H11	4	Person-to-person spread

Data: Statens Serum Institut.

No large general outbreaks were registrered in 2000. Outbreaks reported by physicians (Table 12) declined from 164 in 1999 to 92 in 2000. The largest reduction was accounted for by the S. Enteritidis outbreaks decreasing from 69 to 28. Laboratory detected outbreaks, showed in Table 14, declined from 15 in 1999 to 6 in 2000. Again, the largest decline was in S. Enteritidis outbreaks, decreasing from 9 in 1999 to 3 in 2000.

In 2000, 27 outbreaks involving 335 reported illnesses were investigated by the Regional Veterinary and Food Authorities (Table 13). The suspected food or meal in 19 outbreaks was produced in approved food establishments (general outbreaks). In 8 outbreaks food was prepared in private homes (family outbreaks). The number of reported outbreaks has declined in 2000 compared to previous years, in particular the number of outbreaks caused by S. Enteritidis.

Table 13. Outbreaks of food-borne zoonotic diseases registered by the Regional Veterinary and Food Authorities in 2000.

Zoonotic agent	No. of outbreaks	Total number of sick persons	Suspected source (No. of outbreaks)	Confirmed by culture in foodstuffs/patients
S. Enteritidis	4	42	Grilled beef, sausages, potato salad and green salad (1)	-/+
			Cake with raw eggs (1)	+/+
			Ice-cream with raw eggs (1)	-/+
			Dish with buttermilk (1)	+/+
S. Typhimurium U 302, multiresistent	1	9	Raw vegetables	-/+
Campylobacter	2	11	Beef, ham, potato salad and green salad (1)	-/+
			Shellfish cocktail and chicken (1)	-/+
L. monocytogenes	1	2	Hamburger steak (1)	+/+
Norwalk-like Virus	4	45	Live oysters (1)	+/-
			Live oysters (1)	-/+
			Buffet supper (2)	-/+
Unknown	15	226	Many different foodstuffs involved	

Data: Danish Veterinary and Food Administration.

Tracing sources of human salmonellosis

As described above, the number of human *Salmonella* infections caused by exotic serotypes increased by 5% in 2000. In order to improve the knowledge of the sources of some of the more frequently occurring infections, different epidemiological investigations including molecular typing were performed.

In the last few years the frequency of human cases of S. Agona has increased significantly in Denmark. There were 30 cases of S. Agona in 1998, 58 in 1999 and 73 in 2000, making S. Agona the third most prevalent serotype (Table 15). A large proportion of the patients was children less than two years of age. A case-control study was carried out, where the parents were questioned about their children's behaviour, incl. their food intake, contact with animals, etc. Unfortunately, it was not possible to identify any source of infection by this approach.

S. Agona was also isolated a substantial number of times from non-human sources including animal feed, pig herds, pork, turkeys and imported poultry in 2000 (Table 1 and 15). The majority of the S. Agona isolates was compared by pulsed-field gel electrophoresis (PFGE). All 73 human strains and 55 strains isolated from non-human sources were included. Two restriction enzymes *Xba*I and *Bln*I were used. The results of the investigation showed that the majority of the strains (63) were grouped in 3 different groups each represented by a unique PFGE pattern. In each of these groups strains originating from humans as well as from pigs and poultry were represented. A total of 23 human isolates was shown to have PFGE patterns that were not found among the non-human isolates. Finally, 18 isolates originating from animal feed, pork and poultry were shown to have PFGE patterns that were not reflected in the patterns found among the human isolates. The results suggest that Danish pork and turkey meat, and imported poultry products are potential sources of human infections caused by S. Agona.

The number of human cases caused by S. Thompson has also increased over the last years. There were 6 laboratory verified cases in 1998, 15 in 1999 and 37 in 2000. During the same period S. Thompson was also found in 8 Danish broiler flocks (5 in 1998, 1 in 1999, 2 in 2000), one batch of imported beef (2000) and 4 batches of imported poultry (2 in 1998, 1 in 1999, 1 in 2000).

A number of these isolates was selected for PFGE using *Xba*I and *Bln*I as restriction enzymes. The selected isolates included 36 human strains plus 27 non-human strains (26 strains from Danish and imported poultry and one strain from imported beef). The results of the investigation showed that a majority of the human strains belonged to a single PFGE pattern, using either enzyme. The PFGE pattern of this group (24 strains) was indistinguishable from the PFGE pattern that was found among 25 of the 27 non-human strains (from Danish poultry as well as from imported poultry and beef). The 7 different PFGE patterns identified among the remaining 12 human strains included in the investigation, were not found among any of the non-human isolates. Based on the lack of discrimination among the majority of the included strains, it was not possible to exclude any of the examined food products as sources of human infection. However, the results also indicate that not all sources of S. Thompson have been identified as there has been no observable increase in the prevalence of S. Thompson among non-human isolates that can explain the increase seen in humans. This may be supported by the fact that several of the PFGE patterns identified among the human isolates were not found among the non-human isolates.

Over the last years the human incidence of S. Senftenberg has approximately doubled each year reaching a total of 34 human cases in 2000. S. Senftenberg has also been found in animal feed, pig herds, broilers and imported poultry during the same period. A sample of 50 strains was selected for PFGE using the restriction enzymes, *Xba*I and *Bln*I. The sample included 33 human strains from 2000 plus 17 strains isolated from a variety of sources during the years 1998, 1999 and 2000. The majority of the human strains (20 strains) had identical PFGE patterns. This

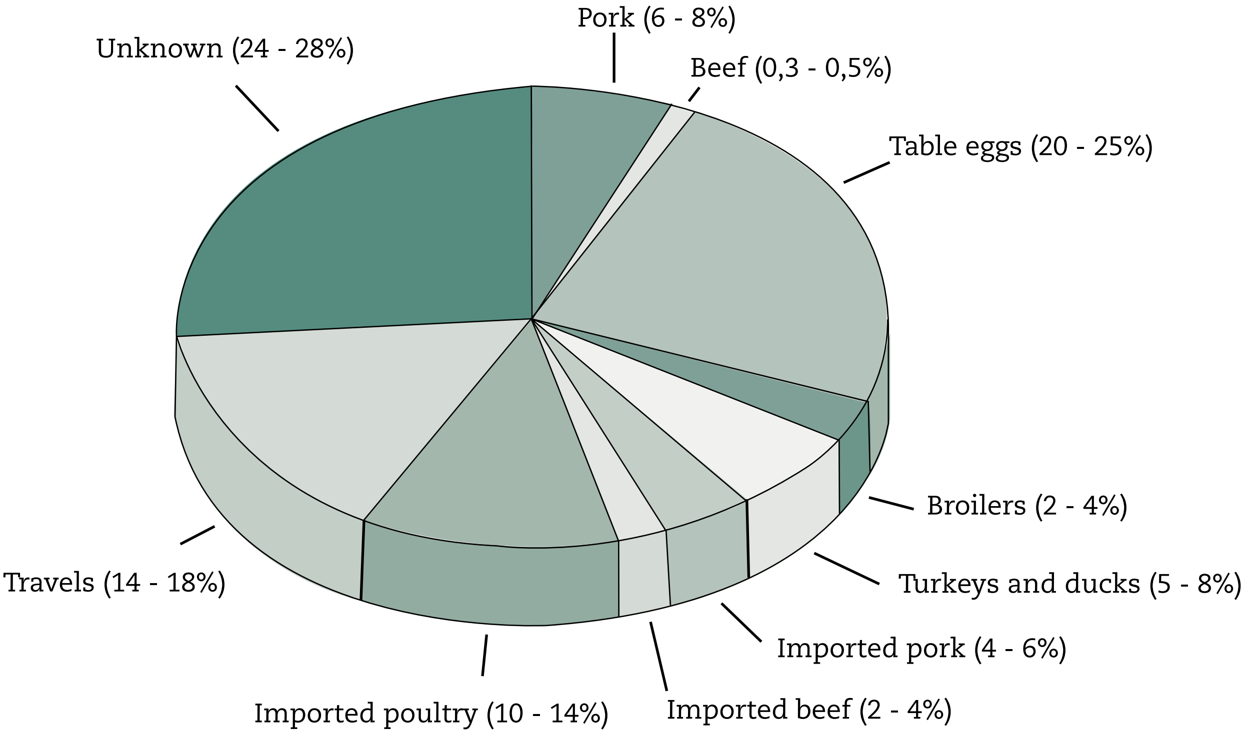

Figure 13. Estimated sources for human salmonellosis in Denmark in 2000. Estimated number of cases per source: 536 cases from table eggs, 370 travel associated, 276 from imported poultry, 166 from pork, 135 from turkey and duck, 99 from imported pork, 71 from broilers, 49 from imported beef, 8 from beef and 597 of unknown sources. Data: Danish Zoonosis Centre.

Table 15. Serotype distribution (%) of Salmonella *from animals, meat at slaughterhouses, imported meat and humans in Denmark, 2000.*

										Imported meat f)				
Serotype	Humans	Pig herds a)	Pork b)	Cattle herds c)	Beef b)	Layer flocks d)	Broilers e)	Turkey flocks e)	Duck flocks e)	Pork	Beef	Chicken	Turkey	Duck
S. Enteritidis	51.6	0.4	0.5	0.7	8.0	83.9	11.7	0.0	5.4	0.0	0.0	9.9	1.5	7.0
S. Typhimurium	18.7	62.7	58.2	37.2	4.0	3.2	18.1	0.0	0.6	47.3	12.5	2.6	3.1	4.7
S. Agona	3.1	0.8	0.5	0.0	0.0	0.0	1.1	25.0	0.6	0.0	0.0	1.5	0.5	0.0
S. Hadar	2.7	0.1	0.5	0.68	0.0	0.0	1.1	0.0	27.7	0.0	0.0	2.0	14.3	20.9
S. Virchow	1.8	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1	3.1	0.0
S. Thompson	1.6	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	6.3	0.5	0.0	0.0
S. Senftenberg	1.5	0.2	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.8	1.0	0.0
S. Newport	1.1	0.1	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	17.1	17.3	4.7
S. Infantis	1.0	5.2	5.1	0.0	0.0	3.2	17.0	9.1	0.0	0.0	6.3	6.9	1.0	4.7
S. Stanley	0.9	0.4	0.0	0.7	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0
S. Bovismorbifica	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
S. Saintpaul	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.5	10.2	9.3
S. Braenderup	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. Java	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. Blockley	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.4	1.0	0.0
S. Heidelberg	0.6	0.6	0.3	0.0	0.0	0.0	0.0	25.0	0.0	1.8	0.0	29.8	14.3	4.7
S. Dublin	0.6	0.2	0.0	46.0	56.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
S. Panama	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0
Not typable	1.1	5.5	20.2	5.4	8.0	9.7	2.1	0.0	0.0	1.8	6.3	3.1	3.1	0.0
Others	9.4	23.1	14.4	9.3	24.0	0.0	45.7	38.6	63.3	45.5	43.6	14.8	29.1	44.0
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Number typed	2324	1557	392	148	25	31	94	44	166	55	16	392	196	443

Data: Danish Veterinary Laboratory, Danish Veterinary and Food Administration and Statens Serum Institut.
a) Isolates obtained from sampling in slaughter-pig herds placed in Level 2 or 3.
b) Representative meat samples from the surveillance programme at slaughterhouses.
c) Cattle herds examined on clinical indications, data not representative for the Danish cattle population.
d) Representative samples from the surveillance programme in production flocks.
e) Representative faecal or sock samples from the mandatory ante-mortem inspection.
f) Monitoring of imported meat and meat products.

Table 16. Phage-type distribution (%) of S. Enteritidis from humans, animals, meat at slaughterhouses and imported meat, 2000.

Phage type	Humans	Layer flocks d)	Broiler flocks e)	Duck flocks e)	Beef b)	Pig herds a)	Cattle herds c)	Imported meat f) Chicken	Turkey	Duck
4	24.3	11.5	0.0	0.0	0.0	0.0	0.0	71.8	0.0	33.3
8	22.5	42.3	18.2	11.1	33.3	20.0	0.0	0.0	100	0.0
34	10.7	0.0	0.0	0.0	66.7	0.0	0.0	0.0	0.0	0.0
6	9.3	11.5	18.2	0.0	0.0	0.0	0.0	5.1	0.0	0.0
1	8.1	7.7	63.6	0.0	0.0	0.0	0.0	5.1	0.0	0.0
21	3.7	11.5	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0
6a	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13a	1.5	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
44	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14B	1.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7	0.0	0.0
1b	0.9	0.0	0.0	0.0	0.0	80.0	0.0	0.0	0.0	0.0
25	0.9	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9A	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Not typable	5.7	0.0	0.0	0.0	0.0	0.0	100	7.7	0.0	66.7
Others	5.3	4.0	0.0	88.9	0.0	0.0	0.0	0.0	0.0	0.0
Total	100	100	100	100	100	100	100	100	100	100
Number typed	786	26	11	9	3	5	1	39	3	3

Data: Danish Veterinary Laboratory, Danish Veterinary and Food Administration and Statens Serum Institut.
Notes: see Table 15.

Table 17. Phage-type distribution (%) of S. Typhimurium from humans, animals, meat at slaughterhouses and imported meat, 2000.

Phage type	Humans	Pig herds a)	Pork b)	Cattle herds c)	Beef b)	Broiler flocks e)	Layer flocks d)	Ducks e)	Imported meat f) Pork	Beef	Chicken	Turkey	Duck
12	19.3	39.7	41.6	27.0	0.0	23.5	0.0	100	3.7	0.0	0.0	0.0	0.0
104	10.5	3.4	1.4	30.2	0.0	0.0	0.0	0.0	55.6	0.0	50.0	33.3	0.0
120	7.6	1.9	0.7	3.2	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.0
1	7.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
66	5.3	8.9	18.9	1.6	0.0	17.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
U302	3.8	1.0	1.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0
170	3.6	6.4	4.1	7.9	0.0	17.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
193	3.6	5.0	3.8	3.2	0.0	0.0	0.0	0.0	7.4	0.0	10.0	16.7	0.0
U292	3.6	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
135	3.3	1.9	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
U312	1.7	1.7	0.7	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	1.4	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	1.2	7.0	6.2	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
104B	1.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15A	0.7	2.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
141	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
U288	0.7	1.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Not Typable	17.2	11.1	12.0	11.1	100	17.6	0.0	0.0	7.4	100	10.0	50.0	0.0
Others	6.7	8.8	6.9	6.2	0.0	23.7	100	0.0	22.2	0.0	20.0	0.0	100
Total	100	100	100	100	100	100	100	100	100	100	100	100.0	100
Number typed	419	931	291	63	1	17	1	1	27	1	10	6	2

Data: Danish Veterinary Laboratory, Danish Veterinary and Food Administration and Statens Serum Institut.
Notes: see Table 15.

pattern was shared by the majority (13) of the strains isolated from food products, including Danish broilers, imported poultry and spices. A few of the non-human isolates (e.g. from animal feed) had a different PFGE pattern. The results indicate that Danish and imported poultry and spices (of unknown origin) are potential sources of human cases of S. Senftenberg.

Risk assessment of sources of human salmonellosis

In order to get a better understanding of the mechanisms behind the dynamics in the occurrence of *Salmonella* infections in humans, the previously described risk assessment model (see Annual Report 1998) quantifying the importance of the major foodborne sources of domestic and sporadic cases of human salmonellosis, was further developed in 2000. As before, the principle behind the model is to compare the number of human cases caused by different *Salmonella* sero- and phage types with the *Salmonella* types isolated from the various food sources. However, as something new, the model now also takes into account the prevalence of the different *Salmonella* types in the various sources as well as the amount of food source consumed per year.

In 2000 in Denmark, the estimated number of human cases per 100,000 inhabitants that could be attributed to various sources, was as follows: table eggs: 10.1; broilers: 1.3; pork: 3.1; turkeys and ducks: 2.5; beef: 0.2; imported poultry products: 5.2; imported beef: 0.9; imported pork: 1.9; travel: 6.9 (Figure 13). Approximately 597 cases (11.2 per 100,000) could not be associated with any specific source. Some of these infections may be related to pet animals or food stuffs, which are not presently monitored.

A substantial part of the unaccounted cases was S. Enteritidis PT34. This phage type increased from an insignificant level (apart from an outbreak in 1999) to the third most frequent S. Enteritidis phage type in 2000 by 10.7%, i.e. estimated 126 cases. The phage type was not detected in the animal production except for two isolates from beef samples collected at the slaughterhouse in the fall of 2000. These findings, however, can

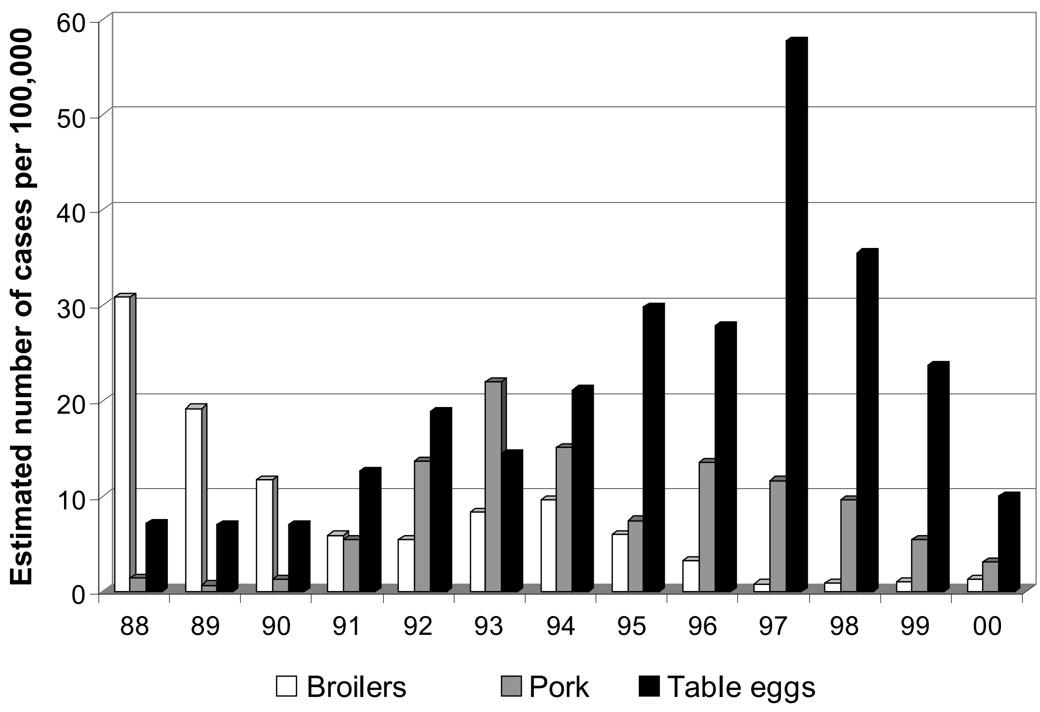

Figure 14. Estimated major sources for human salmonellosis in Denmark, 1988-2000. Data: Danish Zoonosis Centre.

not explain the majority of the cases.

Figure 14 shows that Denmark has experienced three waves of human salmonellosis, where the majority of cases has been caused by three distinct sources: broilers in the late 80s, pork in the mid 90s and eggs in the mid/late 90s. At each peak, a new control programme targeted at the specific source was implemented resulting in a decline of human cases attributable to that particular source. Especially, the steep decline in the number of cases associated with Danish produced table eggs is remarkable.

2. Campylobacter jejuni/coli

Poultry

In 1998, national monitoring of thermophilic *Campylobacter* in broilers, hens and ducks was initiated. At slaughter, ten birds per flock/batch were examined by cloacal swabs (a flock might be slaughtered in several batches). The prevalence in broiler flocks was 37.7% in 2000 (Table 5). The percentage of positive batches ranged from 18.0% to 60.1% per month, and a distinct seasonal variation was observed (Figure 15). This seasonal variation coincided with human campylobacteriosis in Denmark. In 2000, the prevalence in hens was 64.1% (50 of 78 examined batches) and in ducks 88.4% (84 of 94 examined batches).

Apart from the ongoing national surveillance of *Campylobacter* in broilers, a study of *Campylobacter* prevalence in broiler flocks of three different production categories was carried out during 1998-1999. One hundred and sixty broiler flocks originating from organic, conventional and extensive indoor production farms in Denmark were investigated for the presence of *Campylobacter* at the time of slaughter. *Campylobacter* spp. were isolated from all of 22 (100%) organic broiler flocks, from 29 of 79 (36.7%) conventional broiler flocks and from 29 of 59 (49.2%) extensive indoor broiler flocks. The results suggest that free-range production systems, like for instance the organic production, provide a higher prevalence of *Campylobacter* positive flocks.

The national monitoring of thermophilic *Campylobacter* in turkey flocks was initiated in September 1999 and continued throughout 2000. All turkey flocks were tested at the time of slaughter. Similar to the monitoring in broilers, cloacal swab samples were collected at the slaughterhouse and examined for thermophilic *Campylobacter*. However, unlike broilers, most turkey flocks were slaughtered over a period of 2 to 4 days. Since the cloacal swab samples were collected from 10 birds from each batch at slaughter, the same flock could be tested for *Campylobacter* 2 to 4 times.

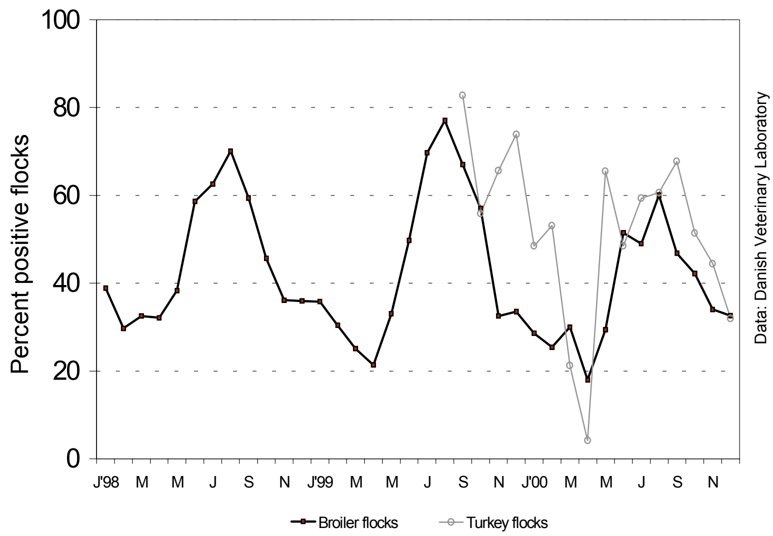

Figure 15. Percent Campylobacter *positive poultry flocks, 1998-2000.*

The prevalence in 2000 ranged from 4% to 66% per month with a mean of 47.4% (Table 6). As in broilers, there seemed to be a distinct seasonal variation (Figure 15).

Pigs and cattle

As part of a monitoring programme of the occurrence of antimicrobial resistance in zoonotic bacteria from pigs and cattle, caecal contents were sampled at slaughterhouses and examined for thermophilic *Campylobacter*. One animal per herd was sampled. In pigs the prevalence was 64.2% (Table 7) and in cattle 61.0% (Table 9).

Pet animals, wild mammals and birds

The Danish Veterinary Laboratory monitors the occurrence of *Campylobacter* in pet animals and wildlife. The group of wild mammals and birds consists mainly of road kills and animals otherwise dead submitted by hunters, veterinarians and others. Pet animals were investigated on clinical indication only. In 2000, *Campylobacter* was found in 46 (16.1%) of 286 examined dogs and in 11 (10.8%) of 102 examined cats (Table 10). The dominating species in dogs and cats was *C. upsaliensis*.

The occurrence of *Campylobacter* in pet animals and wildlife is summarised in Table 10.

Products from retail outlets

In 1996 the Danish Veterinary and Food Administration established a nationwide surveillance program for thermophilic *Campylobacter* spp. in foods from retail outlet. This program has been continued in 2000 with some modifications due to the fact that the Danish authorities has decided that management of biological hazards in foods should be based on the Food Safety Risk Analysis concept as described by WHO/FAO. The implementation of this concept has stressed the need for quantitative data describing the level of contamination by biological hazards in foods, as management options should be based mainly on quantitative risk assessment.

Therefore in 2000, the Danish Veterinary and Food Administration and the 11 regional laboratories introduced a semi-quantitative method for analysis of thermophilic *Campylobacter* spp. in foods. This method is based on pre-enrichment in Mueller-Hinton broth supplemented with trimethoprim and cefaperazone and following plating on mCCDA.

The food represented in the survey in 2000 includes raw poultry products – mainly chicken and turkey - and both imported products and products of Danish origin. In total 1,011 samples of raw poultry were analysed. The results showing the prevalence of thermophilic *Campylobacter* spp. in chicken and turkey products are shown in Table 5 and Table 6. Beef, pork, shellfish, fruits and vegetables were not included in the survey in 2000.

In raw chicken and turkey products from retail outlets the prevalences in 2000 were 41.1% (N=708) and 30.4% (N=303), respectively. This is an increase compared to 1999 when the prevalences were 34% (N=994) and 11% (N=351), respectively. The increase in the percentage of positive samples may partly be explained by the change in method of analysis, as the semi-quantitative method seems to be more sensitive than the formerly used qualitative method defined by the Nordic Committee on Food Analysis.

The number of thermophilic *Campylobacter* spp. per gram sample (CFU/g) in chicken and turkey products

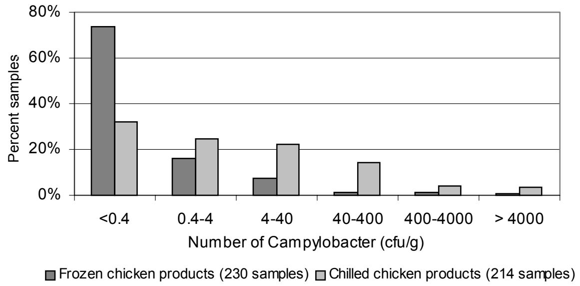

Figure 16. The number of thermophilic Campylobacter *in Danish produced and imported chicken products from retail outlets, 2000.*

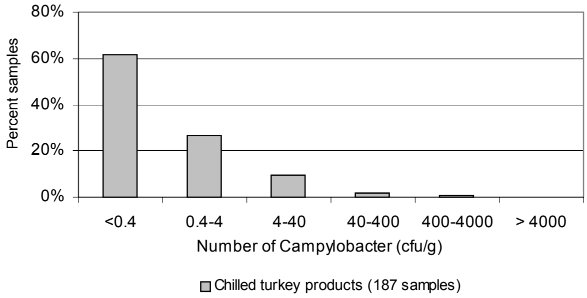

Figure 17. The number of thermophilic Campylobacter *in Danish produced and imported turkey products from retail outlets, 2000.*

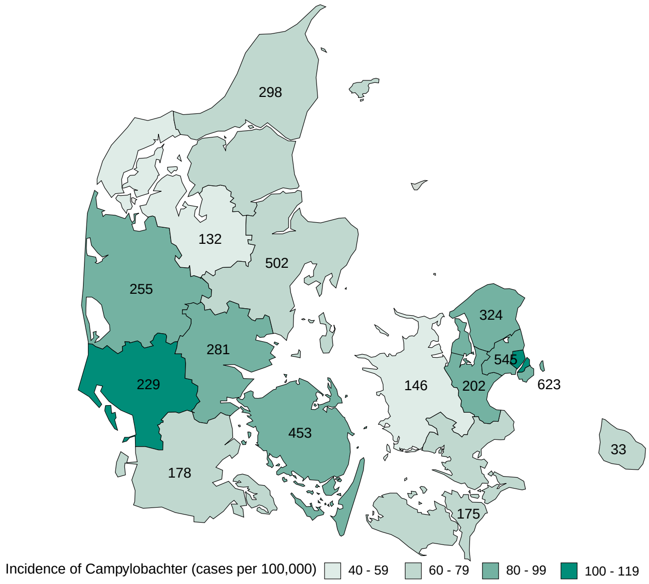

Figure 20. Geographical distribution of the number of cases per county and incidence of human campylobacteriosis in 2000. Data: Statens Serum Institut.

is shown in Figure 16. and Figure 17, respectively. Figure 16 shows how chilled chicken products more frequently and in higher concentrations are contaminated with *Campylobacters* than frozen chicken products.

Campylobacteriosis in humans

The number of human *Campylobacter* infections increased 5% from 4,164 cases in 1999 to 4,386 cases in 2000 (82 cases per 100,000 inhabitants). This trend represents a continuation of the increasing incidence of campylobacteriosis in Denmark (Table 11, Figure 18). The number of *Campylobacter* infections started to increase in 1992 (1,129 registered cases), and has now increased almost with a factor of four. Figure 19 shows the age-specific incidence of *Campylobacter* spp. in 1999-2000. The incidence was highest in children 1 to 4 years of age (182 cases per 100,000) but also high in the age group 15 to 24 years (143 per 100,000) and infants (121 per 100,000). Similar trends have been observed in other industrialised countries, and this increase is a concern for public health. The consumption of poultry and poultry products in Denmark started to increase in 1992. In addition, there has been a increasing consumer preference for fresh poultry as opposed to frozen products. This may be a principal factor behind the increasing incidence of *Campylobacter* infections. However, the possible sources of *Campylobacter* infections are multiple, and other factors may play an important role as well.

Figure 20 shows the geographical distribution of infections with *Campylobacter* spp.

Approximately 80% of the *Campylobacter*-infections are domestically acquired. The increased number of *Campylobacter* infections does not seem to be related to an increased incidence of travel related infections.

Outbreaks of human campylobacteriosis are identified in the same systems as outbreaks of salmonellosis. Outbreaks reported in 2000 are summarised in Table 12 and 13.

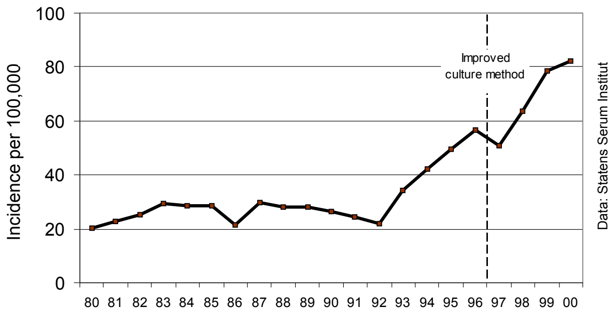

Figure 18. Incidence per 100,000 of human campylobacteriosis in Denmark, 1980-2000.

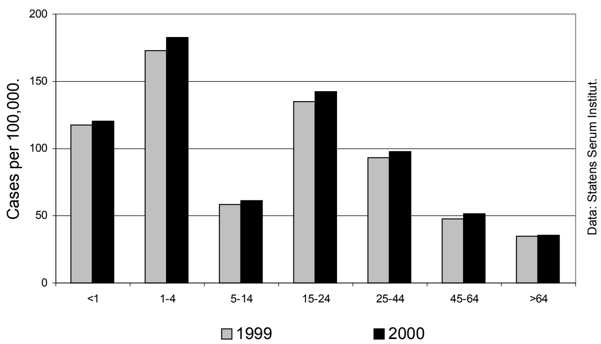

Figure 19. Incidence of infections with Campylobacter by age, 1999-2000.

Serotyping of *Campylobacter*

Isolates of *C. jejuni* and *C. coli* were serotyped using the "Penner serotyping scheme" (heat-stable antigens). *C. jejuni* is the dominant *Campylobacter* species in human patients, poultry and cattle. Among human *C. jejuni* isolates, O:2 was the most common serotype accounting for 30% of the serotyped *C. jejuni* isolates in 2000. Serotype O:4-complex and O:1,44 were also common human serotypes accounting for 19% and 12%, respectively. Since serotyping of Danish *Campylobacter* isolates was initiated in 1995, these three major serotypes have represented about 60% of all human *C. jejuni* isolates each year. In 2000, other important serotypes included O:3 (6%), O:5 (4%), and O:6,7 (4%). Other serotypes accounted for less than 3% each (Table 18).

The three most common human serotypes were also common among cattle, broiler and turkey isolates. Serotype O:2 was prevalent among isolates from cattle (40%) and turkey (33%). In addition, serotype O:6,7 (19%) and O:12 (15%) were commonly found in broilers. Among the few *C. jejuni* isolates from pigs, the most important serotypes were O:23,36 (38%) and O:35 (38%). These are rather unusual serotypes in other sources, but have consistently been seen as the dominating *C. jejuni* serotypes in pigs.

C. coli is the prevailing *Campylobacter* species in pigs, whereas it accounts for less than 10% of *Campylobacters* in most other sources. The most common *C. coli* serotypes in pigs were O:46, O:59, O:5, and O.30 (Table 19). Among *Campylobacter* isolates from human patients, an unusually high percentage, 11%, were *C. coli* in 2000 (only a small sample of 157 isolates were selected for species identification and serotyping). In earlier years, 4-7% *C. coli* were found with the remainder being *C. jejuni*. Among human *C. coli* isolates, serotype O:59 and O:56 were the most common (Table 19).

Table 18. Serotype distribution (%) of Campylobacter jejuni *from human patients and animals in 2000.*

Serotype	Human n=139	Cattle n=52	Broilers n=80	Turkey n=99	Pigs n=8
2	30.2	40.4	23.2	33.3	0
4-complex [1)]	19.4	23.1	11.6	12.1	12.5
1,44	12.2	7.8	7.3	8.1	0
3	5.8	0	1.5	5.1	0
6,7	4.3	3.9	18.8	0	0
5	3.6	3.9	0	3.0	0
11	2.9	0	4.4	2.0	0
12	2.9	0	14.5	9.1	0
18	2.2	0	0	2.0	0
19	2.2	1.9	2.9	2.0	0
23,36	2.2	3.9	0	0	37.5
42	1.4	0	2.9	2.0	0
21	0.7	0	2.9	0	0
27	0.7	1.9	1.5	0	0
31	0.7	0	2.9	11.1	0
29	0	9.6	0	1.0	0
35	0	1.9	0	0	37.5
Others	10.3	3.7	2.7	11.0	12.5
Non typable	2.2	1.9	2.9	1.0	0

Data: Danish Veterinary Laboratory.
1) 4-complex: Reaction with one or more of the following antisera: 4, 13, 16, 43, 50, 64, 65.

Table 19. Serotype distribution (%) of Campylobacter coli *from human patients and animals in 2000.*

Serotype	Human n=18	Broilers n=26	Pigs n=114
56	27.8	0	6.1
59	22.2	3.9	14.9
34	11.1	7.7	1.8
46	11.1	23.1	17.5
54	5.6	7.7	2.6
5	0	3.9	14.0
24	0	0	10.5
30	0	23.1	12.3
48	0	15.4	1.8
Others	22.2	15.2	14.1
Non-typable	0	0	4.4

Data: Danish Veterinary Laboratory.

3. Yersinia enterocolitica

Figure 21. Incidence per 100,000 of human yersiniosis in Denmark, 1980-2000.

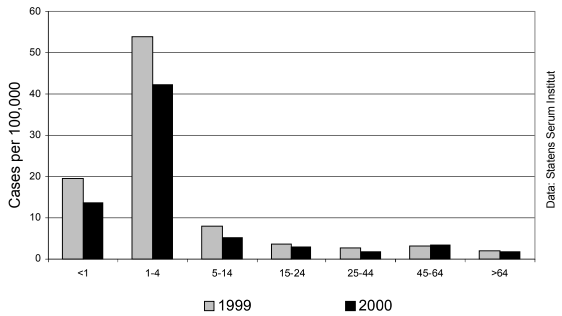

Figure 22. Incidendence of infections with Y. enterocolitica by age, 1999-2000.

Pigs and cattle

The majority of Danish slaughter-pig herds are assumed to harbour Y. *enterocolitica* O:3. A serological survey in 1993, showed that 90% of herds and 75% of slaughter-pigs were seropositive. In addition, several bacteriological investigations have shown a within-herd prevalence of approximately 80%.

As part of a monitoring programme of the occurrence of antimicrobial resistance in zoonotic bacteria from pigs, caecal contents were sampled at slaughterhouses and examined for Y. *enterocolitica*. One animal per herd was sampled. In 2000, 41 (13.0%) of 316 examined animals were found positive for Y. *enterocolitica* O:3 (Table 7).

Due to cross reaction in the serological *Brucella* test, the introduction of Y. *enterocolitica* O:9 in Danish cattle and pig herds is unwanted. The involved organisations, therefore, have decided to examine all imported animals bacteriologically. In 2000, no Y. *enterocolitica* serotype O:9 was isolated.

Products from retail outlets

Analysis of the presence of Y. *enterocolitica* in meat and meat products at the retail level is not part of the monitoring carried out by the Regional Veterinary and Food Authorities. However in 2000, one regional laboratory performed an investigation of raw pork meat. Y. *enterocolitica* was found in 6 (6.3%) of 96 samples investigated (Table 7). Further examination revealed that all isolates belonged to types without human pathogenicity.

Yersiniosis in humans

A total of 265 cases of infections with Y. *enterocolitica* were registered in 2000 (5 cases per 100,000 inhabitants (Table 11, Figure 21). Most isolates (258) were serotype O:3. In 1999, 339 cases of Y. *enterocolitica* O:3 were registered, i.e., a decrease of 22%. The number of infections with Y. *enterocolitica* has decreased sine 1985 when 1,512 cases were identified. Because most Danish slaughter pigs are assumed to harbour Y. *enterocolitica* serotype O:3 biotype 4, the primary source of yersinosis is thought to be porcine.

A total of 47% of the cases were among children less than five years of age (Figure 22), and more than 95% of the cases were domestically acquired.

Figure 23 shows the geographical distribution of Y. *enterocolitica* infections.

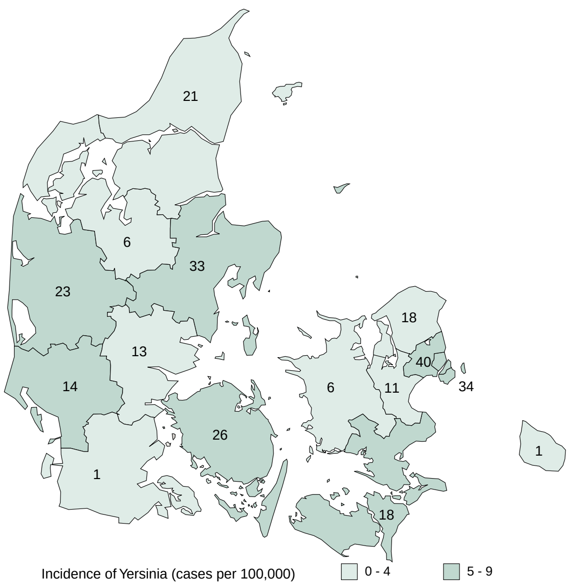

Figure 23. Geographical distribution of the number of cases per county and incidence of human yersinosis 2000.
Data: Statens Serum Institut.

4. Listeria monocytogenes

Products from retail outlets

Data describing the presence of *Listeria monocytogenes* in food at retail level in Denmark in 2000 were reported by the Regional Veterinary and Food Authorities. According to Danish regulations, investigations of the level of *L. monocytogenes* in foods are to be performed on certain ready-to-eat foods. The results of these routine examinations are reported to the Danish Veterinary and Food administration (Table 20). No significant differences were seen in the number of foods with *L. monocytogenes* exceeding 100 cfu per gram.

Listeriosis in humans

In 2000, 39 sporadic cases of listeriosis were registered. Twenty-four cases presented with septicaemia, 8 with meningitis, 6 were classical maternofoetal cases, and 1 was a septic abortion in the 18th week of gestation. The cases were spread geographical throughout the country. Based on serogrouping, ribotypings and pulsed-field gel electrophoresis no clusters of cases could be identified. Twenty-three cases were caused by strains of serogroup 1, 13 by serogroup 4, and from 3 patients, the strains were lost before further characterization could be made.

The annual incidence and the age-specific distribution of the 39 cases is given in Figure 24 and Table 21, respectively.

Table 21. Number of listeriosis cases in Denmark in 2000 by age group.

Age group (years)	Number of cases
< 1	7
1-4 years	1
5-14 years	1
15-24 years	1
25-44 years	2
45-64 years	9
> 64 years	18
Total	39

Data: Statens Serum Institut.

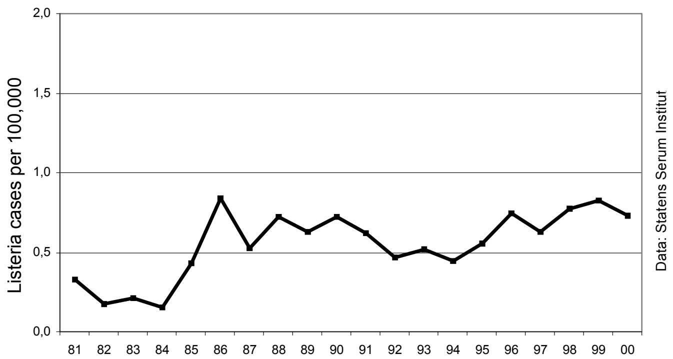

Figure 24. Incidence per 100,000 of human listeriosis in Denmark, 1981-2000.

Table 20. Percentage distribution of the number of Listeria monocytogenes *in selected foods, sampled at retail level in Denmark by the Regional Veterinary and Food Authorities in 1998-2000.*

	2000		1999		1998	
	Number of samples	Percent of samples with cfu>100 per g	Number of samples	Percent of samples with cfu>100 per g	Number of samples	Percent samples with cfu>100 per g
Heat-treated products of pork, beef, chicken and turkey handled after heat treatment	3,861	0.4	5,534	0.5	4,141	0.5
Preserved, not heat -treated or slightly heat -treated products of pork, beef, chicken and turkey	162	2.5	212	0.9	512	1.4
Gravad, smoked, salted, not heat-treated or slightly heat - treated fish products	120	0.8	178	0.6	193	0.0
Sprouts or sliced vegetables	160	0	398	0.3	505	0.2
Vegetable mayonnaise	2,163	0.2	2,393	0.2	2,283	0.5
Cheese and cheese products	44	0	53	0.0	50	0.0
Ready-prepared dishes	1,410	0.2	1,816	0.0	1,531	0.2

Data: Danish Veterinary and Food Administration.

5. Verotoxigenic Escherichia coli

Cattle

The occurrence of verotoxigenic *Escherichia coli* O157 (VTEC O157) has been surveyed since 1995 on faecal samples from cattle collected at the slaughterhouse (one sample per herd per month). In 2000, VTEC O157 was detected in 6 (2.8%) of 214 faecal samples from cattle (Table 9).

The prevalence of VTEC O157 in 60 Danish dairy cattle herds from Southern Jutland was assessed in the autumn of 1999. Ten herds with at least one excreting animal were identified (herd prevalence of 17%) and the overall animal prevalence was found to be 3.6%. It was found that cattle at the age of 2-6 months had the highest excretion rate (8.4%), whereas only 0.9% of calves less than 2 months of age excreted VTEC O157. A total of 2.4% of the cows excreted VTEC O157. The prevalence for bull calves and heifers was the same within the positive herds. By the use of register data, the influence of different herd characteristics were examined. Herd size did not influence the VTEC O157 status of the herd, but the ratio of bull calves to heifers seemed to be an important factor. Consequently all positive herds had relatively many bull calves. In 2000, a follow-up study was carried out in 8 of the 10 VTEC O157 positive herds that were identified in the prevalence study performed in 1999. The objectives of the follow-up study were to identify seasonal trends, examine persistence of strains, introduction of new strains and identify risk factors for VTEC infection. Each of the eight farms was visited four times with intervals of about three months between visits. Faecal samples from up to 60 animals were examined for VTEC O157 each time. A seasonal variation characterised by a low prevalence in winter and higher prevalence in summer and autumn was observed. Genotyping by pulsed-field gel electrophoresis (PFGE) showed that the same strain probably had persisted in several of the farms for up to one year. In some farms, new strains were introduced which replaced the original strain. Minor differences in PFGE type were found for isolates from the same herd at one sampling and over time. These could mostly be explained by one genetic event, i.e. the isolates were likely to have evolved from the same clone. At a few samplings, two clearly distinct strains were identified in different animals of the same herd. On the basis of information concerning stable conditions, change of diet, etc., attempts will be made to identify risk factors for VTEC O157 excretion. The analysis of these data is not completed.

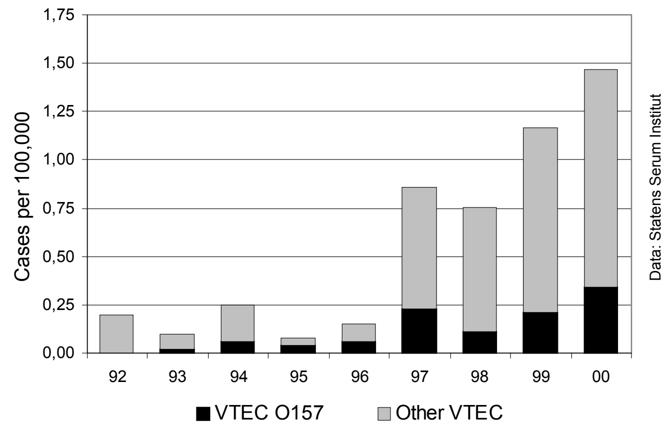

Figure 25. Incidence of human infections with verotoxigenic E. coil*, 1992-2000.*

Products from retail outlets

In 2000, no surveys on verotoxigenic *E. coli* were performed.

Human infections

The problem of zoonotic *E. coli* infections remains low in Denmark, and no general food-associated outbreaks have been identified. Before 1997, VTEC was rarely looked for in humans. In 1997, laboratories covering more than 2/3 of the stool cultures performed in Denmark changed their diagnostic practice and began to look for VTEC in all stools from patients with grossly bloody diarrhoea and in all stools from patients 4 years of age or less with a history of

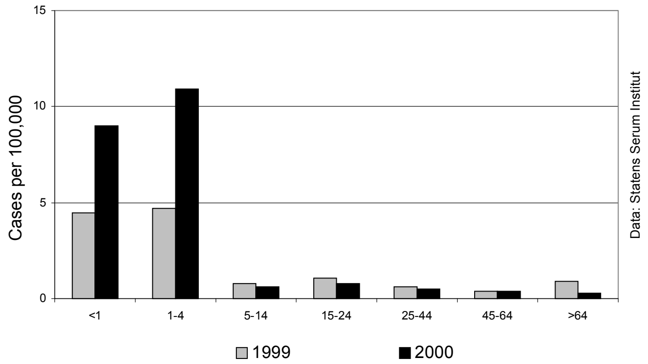

Figure 26. Incidence of infections with verotoxigenic E. coli (VTEC) by age, 1999-2000.

bloody diarrhoea. Additionally, stools from patients evaluated for traveller's or persistent diarrhoea have been cultured for VTEC. The methods used were a combination of colony hybridisation using probes for verotoxin and eae genes, and live slide agglutination of suspect colonies with O-antisera against the most common VTEC and EPEC serotypes.

In 2000, this approach resulted in the identification of 62 episodes of VTEC infections from 60 different individuals (incidence 1.1 per 100,000) (Figur 25). A total of 19 (31%) were O157:H7 (incl. 11 non-motile), 11 (18%) O26:H11 (3 non-motile), 4 (6%) O145, and 3 (5%) O103:H2. The remaining 25 strains belonged to the following O:H serotypes: O5:H-, O8:H- (two strains), O15:H8 (two strains), O28ab:H-, O55:H7, O71:H-, O91:H- (two strains), O98:H-, O101;H-, O111:H-, O117:H7 (two strains), O121:H19, O126:H20, O128ab:H-, O165:H-, and OX177H-. In addition there were three O rough strains and two non-typable strains (ONT:H25 and H41).

Three cases of haemolytic uraemic syndrome (HUS) were reported: Two one-year old children with O157:H- and O111:H- infections, respectively, and an infant infected with O165:H-.

Two small outbreaks occurred. One was caused by O26:H11, the suspected source of infection was unpasteurised milk from a cow on a family farm. O26 with a PFGE pattern similar to the outbreak strain but VT negative was recovered from the cow. In the other outbreak, the source of infection was unknown, but person-to-person spread among four children in a day-care was suspected.

Figure 26 shows the age-specific incidence and Figure 27 shows the geographical distribution. The regional differences are most likely due to different diagnostic practices.

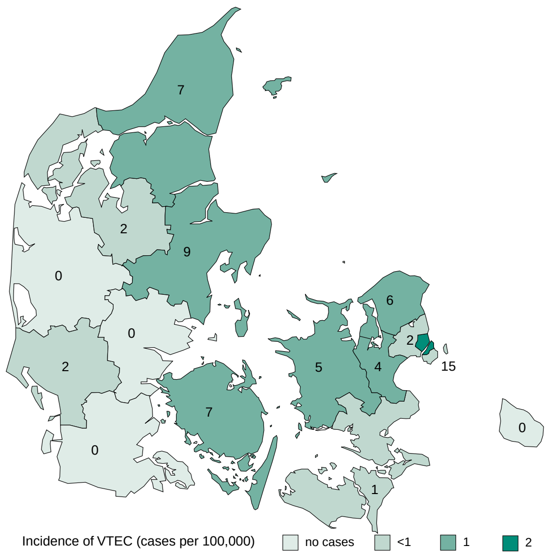

Figure 27. Geographical distribution of the number of cases per county and incidence of human infections with verotoxigenic E. coli (VTEC), 2000. Data: Statens Serum Institut

6. BSE

Surveillance of Bovine Spongiform Encephalopathy (BSE) in Denmark takes place according to the European Commissions decision on "epidemio-surveillance for transmissible spongiform encephalopathies" (98/272/EC with later amendments). A targeted surveillance programme started by October and reached full capacity by December.

Reporting of suspected cases

During the 1990's mandatory reporting of clinically suspect cases to the veterinary authority and the submission of relevant material to the Danish Veterinary Laboratory (DVL) for diagnostic examination has taken place. Because the clinical signs are relatively unspecific and also include poor general performance and decrease in production (e.g. milk yield), the farmer may be likely to send a cow to slaughter or rendering, before a concrete suspicion of BSE is formed. In low-prevalence areas, veterinarians will also tend to be less aware of the clinical signs due to a lack of experience with the manifestations of the disease. This is reflected in the low frequency of submissions to the DVL of BSE-suspected specimens during the 1990s, increasing to 96 in 2000, once the first native case was confirmed in February in a clinically suspect cow from Rebild, Jutland (Table 22).

Table 22. Examinations of cattle brains of clinical suspicion of BSE, 1997-2000.

Year	Examined	Positive	Negative
1997	6	0	6
1998	24	0	24
1999	39	0	39
2000	96	1	95

Data: Danish Veterinary Laboratory.

Targeted surveillance of cattle populations at-risk

Due to the lack of efficiency of reporting suspected cases in low-prevalence or newly affected areas, targeted programmes are gaining increasing importance in monitoring the extent of the BSE epidemic outside the U.K. The rationale behind testing cattle populations at-risk is the fact, that BSE-affected animals are more likely to be found among animals, which are emergency slaughtered animals or fallen stock. According to the experience obtained in the Swiss surveillance programme, the prevalence of BSE infection is 5-10 times higher in this population of animals than in normal slaughters. Targeted surveillance programmes require implementation of rapid monitoring tests at the national level and a confirmatory testing scheme for positive and doubtful monitoring reactions. In Denmark, the testing of at-risk cattle is performed at DVL according to the following scheme:

- The brain stem (taken by the foramen magnum technique) is received at DVL
- A sample of obex (½ gram) is tested by Western Blotting (WB). The rest of the brain stem is stored refrigerated.
- In case of a positive or inconclusive result in WB, relevant regions of the brain stem are subjected to confirmatory testing by immunohistochemistry (IHC). In case the material is severely autolysed or there is an inconclusive result of IHC, material is forwarded to the Veterinary Laboratory Agency, Weybridge, U.K.

In 2000, 930 cattle were tested in the active surveillance and none was found positive (Table 23).

The targeted surveillance programme was extended by January 1st 2001 to include all slaughter cattle over 30 months as instituted by the European Commission. The number of fallen stock animals in the test programme will be 3-4 times higher by July 1st 2001, when all such animals over 24 months of age will be included.

Table 23. The targeted BSE surveillance programme in Denmark, starting October 2000.

Risk category	Approx. annual number	No. of tests in 2000 (from Oct.)
Emergency slaughters (>24 mo.)	2,500 (all)	333
Animals >24 mo., where antemortem inspection at the slaughterhouse reveals signs of disease or zoonotic infection	1,600 (all)	-
Fallen stock (>24 mo.)	8,000 (sample) [1)]	464
Feed cohort investigation (Animals born between Aug. 1995 and July 1997 in herds receiving feed of the same origin as the first Danish BSE-case from Jan. 2000)	3,100 (all)	119
Imported UK animals	20 (all)	14
Animals from herds under restriction	variable	-

1) To be increased to all animals by July 1 2001

7. Cryptosporidium parvum

Monitoring of natural infections in mammals

The epidemiology of *Cryptosporidium* infections in humans as well as animals is still subject to much debate. A screening of faecal samples from animals was conducted at the Danish Veterinary Laboratory (DVL) to provide material for molecular characterisation studies, and to increase knowledge about the occurrence of *Cryptosporidium* in Denmark.

All clinical faecal samples from mammals (N=7,358), submitted to Section for Parasitology, DVL between January and December 2000, were screened for *Cryptosporidium*. In cattle, *Cryptosporidium* was diagnosed in 13.2% of the samples. Among other animal species the occurrence of *Cryptosporidium* did not exceed 1%. Results are presented in Table 24.

The screening included all age groups; thus many samples from animals outside the group of risk were analysed, thereby decreasing the relative prevalence of *Cryptosporidium* in the samples. Previous Danish results from routine diagnostic of *Cryptosporidium* in faecal samples revealed prevalences between 27% and 35% in calves younger than 4 weeks.

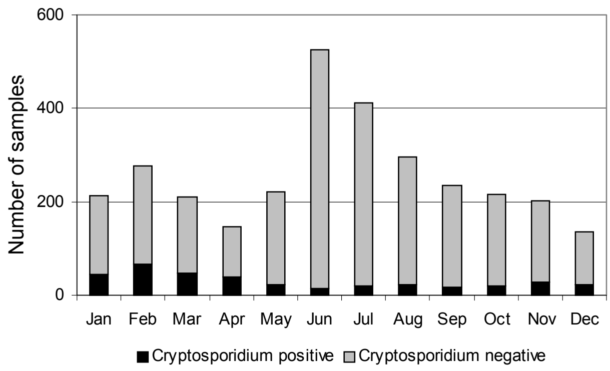

Figure 28. Faecal samples from cattle examined for Cryptosporidium parvum *at DVL from January to December 2000.*

As demonstrated earlier, shedding of oocysts in Danish calves was highest during the late winter months (Figure 28). The seosanality may be explained by certain management factors.

Cryptosporidiosis in humans

Cryptosporidiosis is not a notifiable disease in Denmark, thus the prevalence in humans is not known. At most diagnostic laboratories in Denmark only patients with persistent diarrhoea or a history of recent travel are routinely examined for cryptosporidiosis. It is estimated that approximately 200 cases were diagnosed in Denmark during 2000. Throughout this period 39 clinical cases were diagnosed at Statens Serum Institut. Of these 20 patients had a recent history of foreign travel; 11 had no history of travel and in 8 patients, the anamnesis was unknown. Seasonal variation of prevalence in humans has not been documented in Denmark.

Subgenotyping of *Cryptosporidium parvum*

Molecular methods that enable genotyping and subgenotyping of *Cryptosporidium* have only recently been developed. Until now 82 Danish isolates from humans and animals have been characterised by PCR amplifi-

Table 24. Occurrence of Cryptosporidium *in faecal samples examined at DVL during Jan. to Dec. 2000*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	No. Examined	No. positive	% positive
Cattle	45	67	46	39	22	15	20	22	16	18	28	23	2,730	361	13.2
Sheep/goat		1		4									544	5	0.9
Swine		2	2	3					1		1	2	2,390	13	0.5
Horse	2	2										1	743	5	0.7
Dog							2						197	2	1.0
Cat													38	0	0
Others										1			716	2	0.3
Total	47	73	48	46	24	15	22	22	17	19	29	26	7,358	388	5.3

cation of GAG microsatellites and sequencing. Sub-genotype H1 was found in 53.6% of the human isolates. Two isolates were identified as the "human-associated genotype" by analysis of the 18S rDNA only. Subgenotype C2 or C3 (the zoonotic genotypes) were detected in 21.4% and 14.3% of the samples, respectively, whereas *C. meleagridis* was detected in 1 (3.6%) of the samples.

In isolates from Danish livestock, sub-genotype C1 was found in 16.7%, including two mixed infections with sub-genotype C1 and C3. Sub-genotype C2 was demonstrated in 18.8% of the analysed samples, including one mixed infection with C1 and C2. Sub-genotype C3 was considerably more prevalent than the other genotypes representing 68.8% of the samples.

8. Mycobacterium bovis/tuberculosis

In accordance with Commission Decision 99/467/EEC, Danish cattle herds have been declared officially free from bovine tuberculosis (TB) since 1980. TB in cattle is a notifiable disease in Denmark. Monitoring is performed by meat inspection, which means that all slaughter animals are examined for lesions indicative of TB. Bulls at semen collection centres are subject to pre-entry and annual intradermal tuberculin testing. The last case of TB in cattle was diagnosed in 1988.

In 1988-89, 13 deer farms were found infected with bovine tuberculosis, and in 1994 another 3 farms were found affected. Since December 1994, bovine tuberculosis has not been diagnosed in deer in Denmark.

In 2000, tuberculosis caused by *Mycobacterium tuberculosis* was diagnosed in a Zoological garden in two tapirs (a grown male tapir and a nine-month old tapir). A grown female tapir as well as seven maras and nine capivars housed in the same area, were killed and examined for tuberculosis. They were all negative.

In 2000, 12 cases (0.2 cases per 100,000 inhabitants) of human tuberculosis caused by *M. bovis* were registered (Table 11). These cases are regarded as either imported or reactivation of a latent infection acquired before the eradication of bovine tuberculosis in cattle. Bovine tuberculosis in humans is no longer a notifiable disease in Denmark.

9. Brucella abortus/melitensis

Cattle

In accordance with Commission Decision 99/466/EEC Denmark has been regarded officially free from brucellosis in cattle since 1979. Brucellosis is a notifiable disease, and clusters of abortions are notifiable. Monitoring is performed by examination of abortion material. Bulls are subject to serological testing before entering bovine semen collection centres. After entry they are examined annually for brucellosis.

Pigs

Boars at porcine semen collection centres are likewise subject to pre-entry testing, followed by testing at least every 18 months and before they leave the centre. Brucella has not been detected since 1999, where one case of *B. suis* biotype 2 occurred in a free-range pig herd. This type was last seen in 1994 in another free-range pig herd in the same area, where it was also detected in wild hares.

Sheep and goats

In accordance with Commission Decision 94/877/EEC Denmark has been declared officially free from brucellose. Ovine and caprine brucellosis (*B. melitensis*) has never been recorded in Denmark. Monitoring are performed by testing for brucella antibodies in blood samples from sheep and goats send as a part of the voluntary control programme for lentivirus. In 2000, 6,540 bloodsamples from 762 herds were investigated.

Humans

No domestically acquired human cases of brucellosis, but a few imported cases occur each year. Infection in humans is not notifiable in Denmark.

10. Trichinella spiralis/nativa

Trichinella infections have not been recorded in domestic animals since 1930.

All pigs slaughtered at Danish export slaughterhouses are examined for *Trichinella spp.* in accordance with Council Directive 64/433/EEC, Annex 1, Chapter VIII. During 2000 samples from 20,501,000 pigs were examined, and none of the samples were found to contain *Trichinella* (Table 7).

It is also compulsory to examine slaughtered wild boars. The Danish Veterinary and Food Administration has been informed of 752 examinations, which of all were negative. The total number of slaugtered wild boars examined for *T. spiralis* in Denmark in 2000 is higher, as not all laboratories have submitted results.

All horses which are slaughtered at Danish export-authorised slaughterhouses and approved for intra-Community trade are examined for *T. spiralis*. During 2000, samples from 1,136 horses were examined, and none of the samples were found to contain *T. spiralis*.

No domestically acquired cases of human trichinosis were recorded in 2000. A few imported cases occur annually. Infection in humans is not notifiable.

11. Echinococcus granulosus/multilocularis

Echinococcus granulosus infections in all animals are notifiable. Surveillance for *Echinococcus* is performed through meat inspection. In 2000 there were no findings.

In 2000, 580 foxes were examined at the Royal Veterinary and Agricultural University. Three were found infected with *E. multilocularis*. Two of these were confirmed by PCR analysis, whereas the third diagnosis was based on morphological confirmation only.

No domestically acquired human cases but a few imported cases occur annually. Infection in humans is not notifiable.

12. Toxoplasma gondii

Toxoplasmosis in humans is not a notifiable disease in Denmark, and the incidence of toxoplasmosis in humans is unknown. A recent study including 65,000 seronegative women showed that the incidence is low; only 0.2% seroconverted during pregnancy (1999).

13. Rabies

Rabies is a notifiable infection in both humans and animals. In 2000, 3 bats were found infected with European bat lyssa virus by the Danish Veterinary Institute for Virus Research. One infected bat had bitten three humans. No human cases were reported in 2000, but 11 people were treated by prophylactic vaccination after exposure in Denmark to bat bites or other animals suspected of being infected. In addition 57 people were treated by prophylactic vaccination after exposure abroad to bat bites or other animals suspected of being infected. Sylvatic rabies (genotype 1 lyssa virus) was not found in domestic animals, pet animals or wildlife in 2000.

Antimicrobial resistance

For information on antimicrobial resistance in zoonotic bacteria we refer to the yearly report: "DANMAP - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark". The 2000 report will be available at:

http://www.svs.dk

or can be ordered from the Danish Zoonosis Centre (sca@svs.dk) by the end of June 2001.

Suspected case of clinical BSE. Cow's head opened with saw for removal of whole brain at the Danish Veterinary Laboratory.