Annual Report on Zoonoses in Denmark 2001

Ministry of Food, Agriculture and Fisheries

Annual Report on
Zoonoses in Denmark 2001

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The front page illustration:
Geographical distribution of cattle herds diagnosed with BSE in 2001.

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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 the Danish surveillance and control programmes as well as data from relevant research projects from the institutions which have contributed to the preparation of this report.

This report is available on the web (www.vetinst.dk or www.dzc.dk). The web edition also includes age and sex distributions of the major human zoonotic infections.

Demographic data

Total number of livestock and herds in Denmark, 2001

	Livestock	Herds
Cattle	1,924,507	35,337
Pigs	7,491,568	20,942
Laying hens excl. barn yard sale	3,570,261	378
Broilers	36,880,338	341
Sheep	200,596	8,286

Source: The Central Husbandry Register

***Area of Denmark:* 44,000 sq km**

Source: The Statistical Yearbook 2001, Danmarks Statistik

Approximate total number of animals slaughtered in 2001

Pigs:	21,7 million
Broilers:	136,7 million
Cattle:	595,000
Sheep, lambs and goats:	75,000
Horses:	2,650

Source: The Danish Veterinary and Food Administration

Human Population in 2001

Age group (years)	Male	Female	Total
<1	34,418	32,672	67,090
1-4	138,732	131,767	270,499
5-14	337,124	319,710	656,834
15-24	307,581	297,804	605,385
25-44	802,124	774,571	1,576,695
45-64	693,092	688,599	1,381,691
65+	331,158	460,670	791,828
Total	2,644,229	2,705,793	5,350,022

Profile of year 2001

Trends

Practically all of the bacterial zoonotic infections in humans increased from 2000 to 2001.

Following three years of marked decrease, the number of *Salmonella* infections increased by 25% (page 12). Except for a slight increase in the prevalence of *Salmonella* positive table-egg layers, there were no increases in the animal reservoirs that were able to explain this. Human salmonellosis shows a distinct seasonal variation, where the characteristic summer peak appears to be more pronounced in years with warm and sunny summers, as was the case in 2001. In contrast, the summer of 2000 was cold and rainy. This presumably contributed to the particular low number of *Salmonella* infections observed during this period. Even though the level of 2001 exceeded 2000, it was still below the level of the previous 8 years, so the results and effects of the *Salmonella* control programmes in 2001 are considered to be satisfactory.

The number of human *Campylobacter* infections has increased four-fold since 1992 and is now the most prevalent zoonotic infection in Denmark with almost 5,000 reported cases in 2001 (p. 21). *Campylobacter* is monitored in the broiler production (p. 20), but no control programmes have been established to reduce the number of infected flocks and the human incidence.

Events

A national outbreak involving 174 cases of *Salmonella* Bovismorbificans was not resolved despite three case-control studies and extended sampling and examination of suspected foods (p. 14). The outbreak began in the autumn of 2000 and cases were still observed in the beginning of 2002.

The source of an unexplained outbreak of *Salmonella* Enteritidis phage type (PT)34 observed in 2000 and 2001 was most likely identified in September 2001, when a table-egg farm was found infected with this particular type (p. 19). The eggs from the farm were diverted to pasteurisation and since October 2001 no further human cases have been reported. In total, approximately 260 cases of PT34 were reported in 2000 and 2001.

Surveillance

Adjustments have been made in the serological surveillance of slaughter-pig herds (p. 8). Also, the monitoring of *Salmonella* in pork and beef at the slaughterhouses is changed and now based on swabs of carcasses (p. 8).

Throughout 2001, a computerised outbreak detection system has been used routinely on reported numbers of human bacterial zoonotic infections. The system has on several occasions given early warnings of outbreaks (p. 28).

1. Salmonella

Feeding stuffs

The Danish Plant Directorate monitors all Danish feed compounders for presence of *Salmonella*. This monitoring includes sampling of compound feeds and feed materials, as well as raw materials of animal origin, and sampling during feed processing. Further details are described in Annual Report 2000.

In general, the *Salmonella* prevalence in feeds decreased in 2001, and the overall level is low (Table 1).

In contrast to previous years, a distinction between the results from ordinary and additional inspections during feed processing has been made. Additional inspections are carried out when *Salmonella* is detected during an ordinary inspection. The prevalence in samples collected as a part of an additional inspection is consequently much higher than in samples collected during an ordinary inspection.

For several years in the mandatory monitoring, *Salmonella* has not been detected in compound feed for poultry (Tabel 1). Danish feed compounders producing poultry feed have implemented a code of practice for poultry feed processing based on HACCP principles. The compounders must work out a Biosecurity Monitoring Programme, which among other things includes sampling plans, cleaning procedures and corrective actions in case of high counts of coliforme bacteria.

Table 1. Control of Salmonella *in compound feeds, feed processing and feed materials in 2001.*

	Number of samples/ % *Salmonella* positive			Serotypes, 2001 (Number of isolates)
	1999	2000	2001	
Compound feeds in total	2416/0.4	2516/0.3	2616/0.2	
Feed for pigs	1385/0.2	1436/0.3	1552/0.1	*S.* Agona (1)
Feed for cattle, horses, sheep and rabbits	648/1.1	721/0.6	741/0.4	*S.* Derby (1), *S.* Havana (1), *S.* Infantis (1)
Feed for poultry	269/0	249/0	262/0	-
Pet food	114/0	110/0	61/0	-
Feed materials in total[a)]	385/14.0	382/3.9	332/1.8	
Farm animals	301/5.6	293/2.4	244/0.8	*S.* Agona (1) *S.* Altona (1)
Pets	84/44.0	89/9.0	88/4.5	*S.* Derby (2) *S.* Muenster (1) *S.* Typhimurium DT12 (1)
Feed processing plants (process control)	*3306/6.3*	*2994/2.9*	2946/2.7	
Ordinary inspections [b)]	-	2006/1.4	2679/1.0	*S.* Cubana (2) *S.* Derby (2) *S.* Havana (3) *S.* Infantis (5) *S.* Lexington (1) *S.* Livingstone (1) *S.* Mbandaka (2) *S.* Putten (2) *S.* Senftenberg (5) *S.* Tennessee (6) S. 4.12:b:- (2)
Additional inspections [b)]	-	110/20.9	267/20.5	(not relevant)

Data: Danish Plant Directorate

a) The high level in 1999 was due to a screening for presence of *Salmonella* in dog treats e.g. dried pig ears.

b) Distinction between results from ordinary and additional inspections at feed processing plants began April 2000.

Rendering plants

Control of hygiene at rendering plants was carried out by the animal health section at the Danish Veterinary and Food Administration. In 2001, 269 samples were examined and none found positive.

Salmonella was found in 7 of 403 examined fishmeal samples. Of these, 5 isolates were sent for serotyping. The following serotypes were found: *S.* Cerro (3), *S.* Anatum (1) and one not typable strain.

Poultry and poultry products

In 2001, the *Salmonella* surveillance and control programme was continued as described in the Annual Report 2000. The overall sampling plan is presented in Table 2. In October 2001, the programme for barnyard flocks was changed. Hereafter only serological testing of egg-samples is required, but flocks are examined every 4 months instead of every 6 months as before. A study done by The Danish Zoonosis Centre indicated that the serological method generally is more effective in identifying infected flocks than the bacteriological method and that a positive serological result in most cirsumstances is more reliable than a negative bacteriological result. As part of the changed programme, it was also decided to financially support the sampling in

Table 2. Salmonella *surveillance of the broiler and table-egg production, 2001.*

Age or Frequency	Samples taken in 2001
	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 4 months for eggs sold at barn-yard sale	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.

barnyard and smaller table-egg flocks (<1000 hens) delivering eggs to authorised egg-packing centres. According to this, 75% of the sampling costs were paid by The Danish Veterinary and Food Administration.

The total number of establishments in the broiler and table-egg production is presented in Table 3.

Table 3. Number of establishments in the broiler production and the table-egg production in 2001.

	No. of establishments	No. of houses	No. of animals purchased per year
		Broiler production	
Central rearing	20	97	1,161,000
Broiler breeders	68	207	1.100.000
Hatcheries	10	-	-
Broilers	369	853	143,622,000
		Table-egg production	
Central rearing	6	7	70 - 80,000
Layer breeders	9	18	66,623
Hatcheries	6	-	-
Rearing	108	170	3,500,000
Layers, except barn-yard sale	322	545	3,624,787

Data: The Danish Veterinary and Food Administration and The Danish Poultry Council

Table-egg production

In 2001, no central-rearing flocks or layer breeders (hatching-egg production) were found infected with *Salmonella*. A total of 4 (1.2 %) of 339 rearing flocks examined were found infected with *Salmonella*, but no flocks were infected with *S.* Enteritidis or *S.* Typhimurium (Table 4).

In flocks producing table-eggs for authorised egg-packing centres, 35 (5.8%) of 607 flocks were infected with *Salmonella,* and all flocks were infected with *S.* Enteritidis (Table 4). In flocks producing table-eggs for barnyard sale, 5 (0.9%) of 575 flocks were confirmed infected with *Salmonella*. Of these, 4 flocks were declared infected based on the serological results only. The prevalence was higher among battery (4.5%) and deep-litter (5.3%) flocks than among the other production types (0.0-1.0%) (Figure 1).

The prevalence of *Salmonella* has not been reduced compared to last year, which is probably due to reinfection of new flocks placed in previously infected houses. By the end of 2001, 9 producers were identified as having repeated infection in their establishments, although cleaning and disinfection between flocks had been done as prescribed. The industry is currently working out a plan for each of the 9 establishments to reduce the prevalence of *Salmonella*. The plans will be approved by The Danish Veterinary and Food Administration.

Broiler production

No central-rearing flocks were infected with *Salmonella* in 2001. Among broiler breeders 325 houses were examined and 12 houses were declared infected. The 12 houses represent 6 flocks (2.2%, Table 5), because one flock was divided between 6 houses. Two flocks were infected with *S.* Typhimurium either DT12 or DT41. Three flocks were infected with *S.* Enteritidis, of which one isolate was PT1b, whereas the remaining two were not typable. The flock consisting of 6 houses was found infected with both *S.* Abony and *S.* Hull.

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 0.2% to 3.1% per month with a mean of 1.5% (Table 5, Figure 2). The most frequently occurring serotype was *S.* Typhimurium. The sero- and phage-type distributions are presented in Table 15, 16 and 17.

In 2001, the mandatory examination of broilers after slaughter

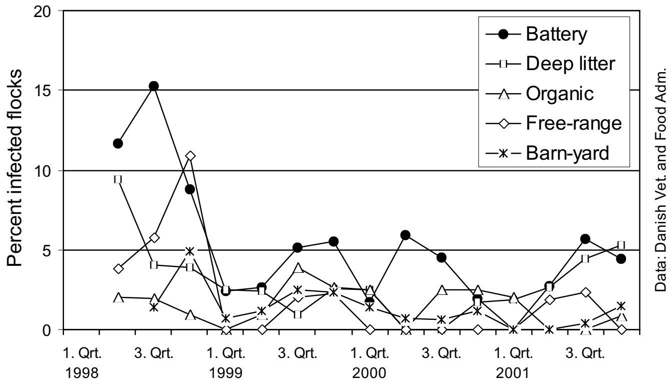

Figure 1. Number of flocks infected with Salmonella *according to type of table-egg production, 2001.*

Number of herds by type of production, 2001: Battery: 129; deep litter: 122; free-range: 46; organic: 137.
Source: Danish Veterinary and Food Administration

Table 4. Occurrence of Salmonella *in the table-egg production in 2001.*

	Central rearing[a]		Layer breeders[a]		Rearing		Table-egg production	
Zoonotic pathogen	Examined flocks	Positive flocks (%)	Examined flocks	Positive flocks (%)	Examined flocks	Positive flocks (%)	Examined flocks	Positive flocks (%)
Salmonella spp.	14	0	22	0	339	4 (1.2)	607	35 (5.8)
S. Enteritidis	-	-	-	-	-	0	-	35 (5.8)
S. Typhimurium	-	-	-	-	-	0	-	0
Other serotypes	-	-	-	-	-	4[b] (1.2)	-	0

Data: Danish Veterinary Laboratory and Danish Veterinary and Food Administration.
a) One flock may be placed into several houses.
b) Three rearing flocks were infected with two different serotypes.

Table 5. Occurrence of Salmonella *and* Campylobacter *in the broiler production in Denmark in 2001.*

	Flock level				Slaughter-house		Retail - broilers and products of broiler meat						
	Broiler breeders		Broiler flocks		Chicken cuts		Not heat treated Danish		Imported		Heat treated		
Zoonotic pathogen	No. of houses examined	% positive flocks	Flocks examined	% positive flocks	N	% positive batches	N	% positive samples	N	% positive samples	N	% positive samples	Note:
Salmonella spp.	325	2.2	4,504	1.5	1,695	4.1	40	7.5	-	-	141	0	a
S. Enteritidis	-	0.9	-	0.1	-	-	-	-	-	-	-	-	-
S. Typhimurium	-	0.9	-	0.3	-	-	-	-	-	-	-	-	-
Other serotypes	-	0.3	-	1.1	-	-	-	-	-	-	-	-	-
Campylobacter spp.	-	-	6,054	41.9	-	-	808	33.2	170	51.2	129	0	b
C. jejuni	-	-	-	37.0	-	-	-	-	-	-	-	-	-
C. coli	-	-	-	3.0	-	-	-	-	-	-	-	-	-
C. lari	-	-	-	0.05	-	-	-	-	-	-	-	-	-
Other species	-	-	-	1.8	-	-	-	-	-	-	-	-	-

Data: Danish Veterinary Institute and Danish Veterinary and Food Administration
a) At the broiler breeder level, one flock can be placed in several houses. Parent flocks were examined according to Table 2. Broiler flocks were monitored by sock-samples 2-3 weeks prior to slaughter.
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.

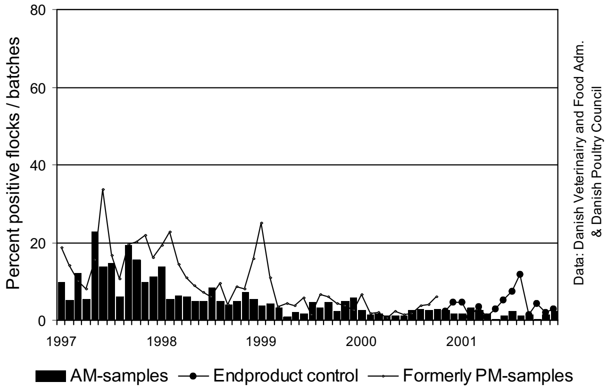

Figure 2. Percent Salmonell*a positive broiler flocks detected at the mandatory ante-mortem and end-product examination, 1997-2001. Post-mortem examination stopped in November, 2000.*

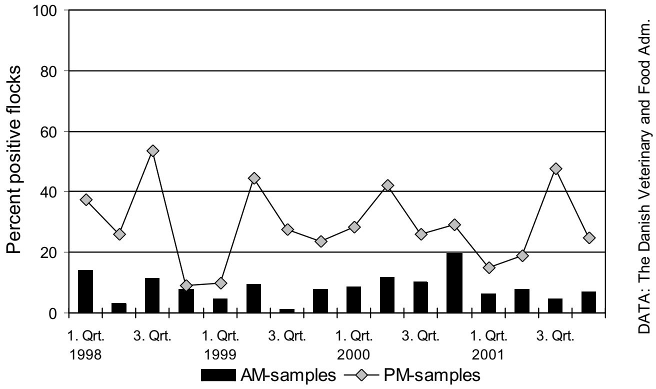

Figure 3. Percent Salmonella *positive turkey flocks detected at the ante-mortem and post-mortem examination, 1998-2001.*

was done by sampling batches of chicken cuts close to packaging. The definition of batches is described in the Annual Report 2000.

Salmonella was detected in 69 (4.1%) of 1,695 batches of chicken meat. Approximately one third of the positive batches originated from the same slaughterhouse and was due to cross-contamination with *S*. Infantis residing in the scalder.

Turkey production

All turkey flocks were monitored for *Salmonella* by mandatory ante-mortem examination in 2001. Three weeks prior to slaughter, five pairs of sock samples were collected from each flock. *Salmonella* was detected in 22 (6.6%) of 335 flocks investigated. (Table 6, Figure 3). Of these, 7 flocks were infected with *S*. Agona, 4 flocks with *S*. Hadar and 11 flocks with other serotypes. *S*. Enteritidis and *S*. Typhimurium were not isolated from turkey flocks in 2001. But S. Typhimurium was found at slaughter a few times at the end of the year.

The serotype distribution is shown in Table 15.

Salmonella was detected in a total of 94 (26.0%) out of 362 flocks/ batches examined after slaughter by five pools of 10 neck-skin samples (Table 6, Figure 3).

Duck production

Duck flocks were monitored by ante-mortem examination 3 weeks prior to slaughter in 2001. *Salmonella* was isolated from 142 (73.6%) of the 193 flocks examined. In 28 flocks more than one serotype was isolated. *S*. Anatum, the most frequently isolated serotype was found in approximately 32.9% of the infected flocks.

Table 6. Occurrence of Salmonella *and* Campylobacter *in the turkey production in Denmark in 2001.*

	Flock level		Slaughterhouse		Retail - cuts and products of turkey meat						
	Turkey flocks		Neck skin		Not heat treated				Heat treated		
					Danish		Imported				
	Flocks examined	% positive flocks	N	% positive flocks	N	% positive samples	N	% positive samples	N	% positive samples	Note:
Salmonella spp.	335	6.6	362	26.0	49	10.2	-	-	48	0	a
S. Enteritidis	-	0	-	0	-	-	-	-	-	-	-
S. Typhimurium	-	0	-	1.4	-	-	-	-	-	-	-
Other serotypes	-	6.6	-	24.6	-	-	-	-	-	-	-
Campylobacter spp.	238	37.8	-	-	502	17.7	203	33.5	71	0	b
C. jejuni	-	29.4	-	-	-	-	-	-	-	-	-
C. coli	-	6.3	-	-	-	-	-	-	-	-	-
C. lari	-	0.4	-	-	-	-	-	-	-	-	-

Data: Danish Veterinary Institute 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. One flock may be slaughtered in several batches.
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.

Pigs and pork

A serological surveillance programme for detection of *Salmonella* infection in slaughter-pig herds was implemented at the beginning of 1995. Until August 2001, the programme carried out was as described in the Annual Report 2000. The new programme differs in the following ways. First, herds producing less than 200 pigs for slaughter per year are no longer included in the surveillance. This leaves 1.6% of the slaughter pigs outside the surveillance scheme. Second, the level at which a serological test is considered positive was reduced resulting in twice as many seropositive test results as before. Third, the categorisation of herds into three levels is still based on the proportion of seropositive meat-juice samples during the last 3 months, but now the samples are weighted (0.2:0.2:0.6), so that the results of the most recent month are given more weight. The weighted average is called *the serological* Salmonella *index for slaughter-pig herds*. Finally, owners of herds placed in level 3 are no longer required to seek advice on how to reduce the prevalence of *Salmonella*. Instead, with the objective of improving the *Salmonella* level in positive herds, the Danish Bacon and Meat Council has introduced a financial penalty system with levels corresponding to the *Salmonella* level of the herd.

By the end of 2001, 97.3% of the herds fell within Level 1; 2.3% within Level 2 and 0.9% within Level 3 (Table 7). The sero- and phage type distributions are presented in Table 15 and 17.

Breeding and multiplying herds are monitored monthly by serological testing of blood samples. If a specific cut-off level 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 lifestock 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.

Clinical salmonellosis was recorded in 39 herds (Table 8). This figure represents the number of herds submitting material from clinically affected animals to the laboratory. Of these, 10 herds were placed under official veterinary supervision by the district veterinary officer.

The method for monitoring *Salmonella* in pork was changed by January 1st 2001. Previously, monitoring was based on meat samples from different cuts. Now swab samples are taken from three designated areas of chilled half-carcasses. The samples are pooled, each pool consistsing of samples from 5 carcasses, except in the smaller slaughterhouses where the samples are analysed

Table 7. Occurrence of zoonotic pathogens in pigs and pork in Denmark in 2001.

	Herd level			Slaughterhouse		Retail				
	Examined			Carcass samples		Not heat treated		Heat treated		
Zoonotic pathogen	Herds	Animals	% positive herds	N	% positive samples	N	% positive samples	N	% positive samples	Note:
Mycobacterium bovis	20,942	21.7 mill	0	-	-	-	-	-	-	a
Brucella abortus	-	-	0	-	-	-	-	-	-	b
Trichinella spp.	20,942	21.5 mill	0	-	-	-	-	-	-	c
Salmonella spp.	14,694	692,378	3.2	36,559	1.3	715	1.7	976	0.1	d e
S. Enteritidis	-	-	-	-	0	-	-	-	-	-
S. Typhimurium	-	-	-	-	0.7	-	-	-	-	-
Other serotypes	-	-	-	-	0.6	-	-	-	-	-
Campylobacter spp.	238	238	76.9	-	-	-	-	-	-	f
C. jejuni	-	-	2.9	-	-	-	-	-	-	-
C. coli	-	-	68.5	-	-	-	-	-	-	-
C. lari	-	-	0.1	-	-	-	-	-	-	-
Y. enterocolitica	411	411	14.6	-	-	14	0	5	0	f

Data: Danish Veterinary Institute and Danish Veterinary and Food Administration
a) All slaughter pigs were examined in connection with meat inspection.
b) Serological examination of boars 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) At the slaughterhouse swabs are taken from three areas of the half-carcass. Five samples are pooled except at slaughterhouses where less than five pigs are slaughtered per day, then samples are analysed individually.
f) Herds examined by caecal samples from one animal per herd collected at slaughter (from the DANMAP-programme)

individually. Slaughterhouses approved for export collect one pooled sample per day whereas minor slaughterhouses collect one pooled sample per 200 animals slaughtered, but at least one sample per month.

In 2001, 36,460 samples were pooled into 7,328 pools. *Salmonella* was found in 285 of these. Furthermore, 99 samples were collected and analysed individually, and *Salmonella* was found in one of these samples.

When determining the prevalence on the basis of pooled samples, the loss of sensitivity and the probability of more than one sample being positive in each pool have to be taken into consideration. During 2000, comparative examinations were carried out, where a number of slaughterhouses collected 10 samples per day. Five of these were analysed individually and the other five were analysed as a pool. These examinations showed that in order to estimate the prevalence at a single carcass level, the prevalence of *Salmonella* in pooled samples should be divided by a factor of 3.

On this basis, the overall prevalence for 2001 was 1.3%. The prevalence of *Salmonella* positive carcasses per month ranged from 0.9% to 1.7% (Table 7, Figure 4). The sero- and phage type distributions are presented in Table 15 and 17.

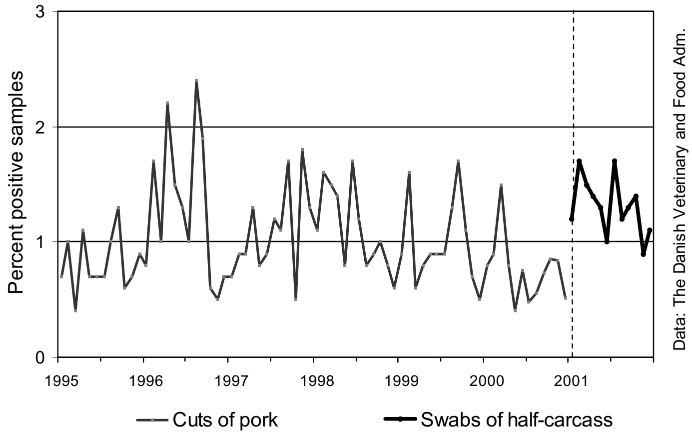

Figure 4. Pork meat monitored at slaughterhouses, 1995-2001. Since January 2001 monitored by swab samples of half-carcasses. A parallel study showed the swab method to be 1.9 times as sensitive as the previously used examination of meat cuts.

Cattle and beef

In 2001, a national programme monitoring the occurrence of *S.* Dublin and *S.* Typhimurium antibodies in dairy herds was initiated. In contrast to the serological programme running in the pig production, this programme aims at identifying herds that are free of *S.* Dublin infection. This approach was chosen, because an epidemiological study indicated that the predictive value of a negative serological test is high, whereas the positive predictive value is low. In other words, the serological method is much better at identifying truly non-infected herds than truly infected herds. The overall objective is therefore to keep non-infected herds free from infection by avoiding purchase of animals from herds that are either infected or where the *Salmonella* status is unknown.

All dairy herds are tested by bulk-milk samples collected every 3 months. Based on the serological results, the herds are divided into three levels: Level 1: Most likely free of *S.* Dublin; Level 2: *S.* Dublin is most likely present; Level 3: *S.* Dublin is isolated from the herd and clinical symptoms (salmonellosis) are present. For a dairy farmer to be placed in Level 1, the following criteria should be met: 1) The results of the latest four bulk-milk test may not exceed an average antibody level of 25 OD% (adjusted Optical Density), 2) the latest bulk-milk sample may not exceed the average of the 3 previous samples with more than 20 OD%, 3) salmonellosis caused by *S.* Dublin has not been diagnosed for at least half a year, and 4) *S.* Dublin has not been isolated from any samples collected from the farm for at least 3 months. The ELISA-results are presented in OD%.

In 2001, 35,157 bulk-milk samples from 8,844 herds were analysed both by a *S.* Dublin and a *S.* Typhimurium ELISA-test. Based on the results, the herds were divided into the following categories: 71.6% were in level 1, 20.0% were in either level 2 or 3, 3.2% went from either level 2 or 3 to level 1, 4.0% went from level 1 to level 2 or 3, and finally 1.1% of the herds changed level twice during 2001.

Table 8. Isolation of Salmonella *from outbreaks of clinical disease in pig and cattle herds in 2001.*

Serotype	Pig herds	Cattle herds
Agona	1	
Bovismorbificans	1	
Derby	3	
Dublin		32
S. 3.10:-:-	1	
Enteritidis	1	1
Infantis	2	
Livingstone	1	
Stanley	1	
Typhimurium DT104	7	3
Other Typhimurium	21	31
Rough, non typable		4
Total	39	71

Data: Danish Veterinary and Food Administration.

Multi-drug resistant *Salmonella* Typhimurium DT104

Control in the primary production

An order issued by the Danish Veterinary and Food Administration in 1997 and revised in 1999 and 2001 made the detection of multi-drug resistant *Salmonella* Typhimurium DT104 in pig and cattle herds notifiable. Animals from infected herds are slaughtered under special hygienic precautions, and there is an epidemiological investigation of the herd and its trade contacts.

In the latest revision of the order, a new kind of official veterinary supervision was introduced – The Zoonosis Supervision. Owners of herds placed under Zoonosis Supervision are obliged to prepare a plan for, how they will reduce the level of *Salmonella* in the herd, and the plan must be sanctioned by the district veterinary officer and run for a minimum of 12 months. To lift the sanctions resulting from the Zoonosis Supervision, either two negative herd examinations at 30 days interval or a serological *Salmonella* index for slaughter pig herds below 20 for a 4 month period, is required. Sanctions may also be lifted, if the herd is destroyed.

Detection of DT104 in slaughter-poultry flocks will according to an order from 1996 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 layers and eggs will be destroyed. Breeding or rearing flocks found infected with *S. enterica* are destroyed.

Occurrence in the primary production

In 2001, 43 herds (33 pig herds and 10 combined cattle and pig herds) were found infected with DT104. This was 25% less than the previous year (Figure 5). Still, the number of infected herds with only pig production increased by more than 50%.

Monitoring of imported products

Since July 1998, the occurrence of DT104 in fresh meat imported from the EU and third countries has been monitored. In 2001, the overall prevalence of DT104 in imported meat was 0.15% (5 of 3,247 samples), which is a decrease from 2000 when the prevalence was 0.4%. Meanwhile the *S. enterica* prevalence increased from 10.0% to 13.3%.

Imported product	Number of samples	Positives (%)	Positive for DT104 (%)
Poultry	2,070	404 (19.5)	3 (0.1)
Pork	451	25 (5.5)	2 (0.4)
Beef	726	4 (0.6)	0 (0)

Human infections

The number of sporadic cases of human *S.* Typhimurium DT104 increased in 2001 (Figure 6). There were no increases in the occurrence of DT104 in domestic or imported meat that could explain this. However, the increased number of infected herds with only pig production may suggest that domestically produced pork may be a source, even though the prevalence of DT104 on pork carcassess does not reflect this.

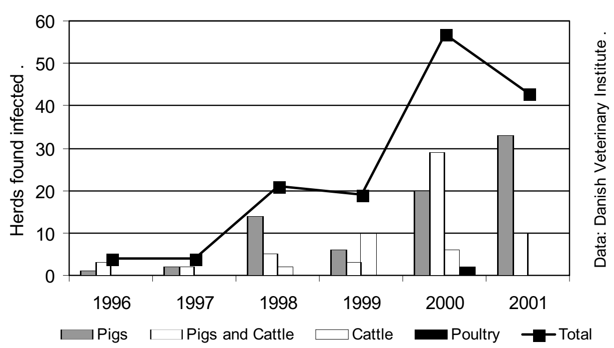

Figure 5. Herds found infected with multi-drug resistant S. *Typhimurium DT104 in Denmark 1996-2001.*

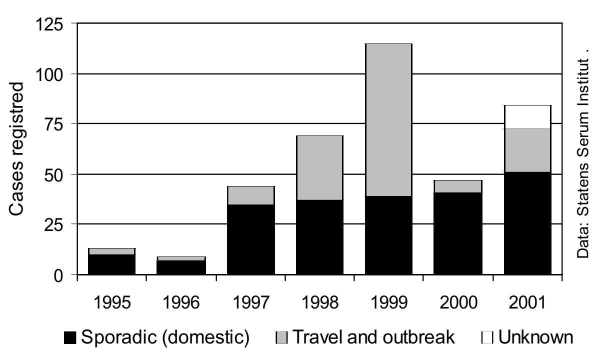

Figure 6. Registered cases of human S. *Typhimurium DT104 (including DT104b) in Denmark, 1995-2001.*

At present only dairy herds are included in the programme. However, the intention is to include beef cattle as well by using the results of blood samples collected at slaughter. The outline of such a programme is currently being discussed.

Salmonellosis was diagnosed in 71 cattle herds in 2001. Of these, 23 herds were placed under official veterinary supervision by the district veterinary officer. The predominant serotypes isolated from clinical cases in cattle in 2001 were *S.* Typhimurium (48%) and *S.* Dublin (45%) (Table 8).

Small-scale bacteriological monitoring of *Salmonella* in cattle herds is also done as part of a monitoring programme for the occurrence of antimicrobial resistance in zoonotic bacteria (DANMAP). According to this, *Salmonella* was isolated from 5 (2.2%) of 231 caecal samples collected from one animal per herd at slaughter (Table 9).

The method for monitoring *Salmonella* in beef and veal at slaughterhouses was changed on the 1st of January 2001. As reported for pork, swab samples are now taken from three designated areas of the chilled half-carcass. In 2001, 10,455 samples were collected and pooled into 2,091 pools, which were then analysed. *Salmonella* was found in 8 of these. Furthermore, 435 samples were collected and analysed individually, and *Salmonella* was not found in any of these samples.

Using the same estimated factor of 3 as reported for pork, the overall prevalence for 2001 was 0.1%. The prevalence of *Salmonella* positive carcasses per month ranged from 0% to 0.4% (Table 9).

Wildlife and pet animals

The Danish Veterinary Laboratory monitors the occurrence of *Salmonella* in pet animals and wild mammals and birds. The group of wild mammals and birds consists mainly of dead animals submitted by hunters, veterinarians and others. Pet animals were investigated on clinical indication only. The prevalence of *Salmonella* in pets and wildlife in 2001 was below 5% (Table 10).

Products from retail outlets

The Regional Veterinary and Food Authorities collect samples for routine surveillance of meat and meat products at the retail level. A total of 182 broiler and broiler products, 100 samples of turkey cuts and turkey products, 1,691 samples of pork and pork products, and 848 samples of beef and beef products were examined in 2001. In non-heat treated samples the prevalences were 7.5%, 10.2%, 1.7%, and 2.0%, respectively. The prevalence in heat-treated products ranged between 0% and 0.5% (Tables 5, 6, 7, and 9). The total number of *Salmonella* isolates is relatively small, however, serotypes repre-

Table 9. Occurrence of zoonotic pathogens in cattle and beef in Denmark in 2001.

	Herd level			Slaughterhouse		Retail				
	Examined			Carcass samples		Not heat treated		Heat treated		
Zoonotic pathogen	Herds	Animals	% positive herds	N	% positive samples	N	% positive samples	N	% positive samples	Note:
Mycobacterium bovis	-	595,500	0	-	-	-	-	-	-	a
Brucella abortus	-	-	0	-	-	-	-	-	-	b
Salmonella spp.	231	231	2.2	10,890	0.1	642	2.0	206	0.5	c d
S. Enteritidis	-	-	0	-	0.01	-	-	-	-	-
S. Typhimurium	-	-	0.4	-	0.03	-	-	-	-	-
S. Dublin	-	-	1.7	-	0.04	-	-	-	-	-
Other serotypes	-	-	0	-	0.02	-	-	-	-	-
Campylobacter spp.	76	76	72.4	-	-	-	-	-	-	c
C. jejuni	-	-	56.6	-	-	-	-	-	-	-
C. coli	-	-	6.6	-	-	-	-	-	-	-
C. lari	-	-	0	-	-	-	-	-	-	-
E. coli O157 (VT+)	186	186	3.2	-	-	-	-	-	-	c

Data: Danish Veterinary Institute 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 (from the DANMAP-programme).
d) At the slaughterhouse swabs are taken from three areas of the half-carcass. Five samples are pooled except at slaughterhouses where less than five pigs are slaughtered per day, then samples are analysed individually.

sented in chickens were *S.* Enteritidis, and *S.* Heidelberg; in turkeys: *S.* Hadar, *S.* Newport, *S.* Agona, and *S.* Saigon; in pork: *S.* Typhimurium and *S.* Dublin and in cattle: *S.* Dublin, *S.* Heidelberg and *S.* Derby.

In a survey of 712 samples of imported and 437 samples of Danish vegetables and fruit, *S.* Saintpaul was isolated from one sample of imported lemongrass and *S.* Augustenborg was isolated from one sample of imported green asparagus. Both products were imported from Thailand.

In 2001 and 2002 a *Salmonella* screening of 10,000 Danish and 10,000 imported shell eggs is being carried out by the Danish Veterinary and Food Administration. Preliminary results show that 6 (0.06%) of 9,820 Danish eggs tested were positive. Of these, there were 4 shell infections and 2 yolk/white infections. All Danish eggs were Grade A (retail sale). Of 1,480 imported eggs 10 (0.7%) were positive. Of these, there were 7 shell infections, 2 yolk/white infections and 1 both. Imported eggs were both Grade A and B, where the latter are used for industrial production. All findings were *S.* Enteritidis.

Salmonellosis in humans

In 2001, the registered number of human infections with zoonotic *Salmonella* serotypes was 2,918 (54.5 cases per 100,000 inhabitants, Table 11). Compared with 2000, this represents an increase of 24.7% (Figure 7). However, the level of 2001 is still lower than in the previous nine years, and the increase may in part be explained by climate conditions. Human salmonellosis shows a distinct seasonal variation, whith a characteristic summer peak. This appears to be more pronounced in warm and sunny summers, as in 2001 (Figure 8). In contrast, the summer of 2000 was cold and rainy, which presumably contributed to the particular low number of *Salmonella* infections observed that year. In addition, there were more reported outbreaks of *Salmonella* in 2001 than in 2000.

A travel association was reported for 263 *Salmonella* cases. For 7 of 15 counties no information on travel status was available. Based on information from the remaining 8 counties, it is estimated that 84% of the *Salmonella* infections were domestically acquired.

The number of *S.* Enteritidis cases increased from 1,212 in 2000 to 1,416 (26.5 cases per 100,000, Table 11) in 2001. This increase of 16.8% is less than the increase of *Salmonella* in total, and apart from year 2000, the level is the lowest in seven years.

The phage type (PT) distribution of 796 *S.* Enteritidis isolates from human infections is presented in Table 16. The major types were PT8 (29%), PT4 (25%), PT34 (11%), PT6 (5%) and PT1 (5%). The proportion of the two dominant types, PT8 and PT4, has increased since 1999, whereas PT6, which accounted for 29% of the infections in 1999, has been markedly reduced.

It is estimated that 15% of the *S.* Enteritidis cases are associated with travelling abroad. There are, however, some differences between phage types. Among the frequent phage types, PT34 cases appear to be mainly domestically acquired (94%), whereas PT4 infections includes a larger proportion of travellers (24%). Also PT14b and PT21 infections seem to be highly related to travel (47% and 58%, respectively).

Figure 9 shows the geographical distribution of the *S.* Enteritidis cases.

Table 10. Occurrence of Salmonella *and* Campylobacter *in pet animals, wild mammals and birds in Denmark in 2001.*

	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	220	0.5	100	0	24	4.2	17	0	35	2.9	22	0	349	0.9	52	0	272	1.5
S. Enteritidis		0		-		0		-		0		-		0.3		-		0
S. Typhimurium		0.5		-		0		-		0		-		0		-		1.5
Others/not typeable/not typed		0		-		4.2		-		2.9		-		0.6		-		0
Campylobacter spp.	109	14.7	52	23.1	21	4.8	7	0	18	5.6	15	0	33	9.1	33	27.3	35	11.4
C. *jejuni*		1.8		3.8		0		-		0		-		6.1		6.1		5.7
C. coli		0		0		0		-		0		-		3.0		0		2.9
C. upsaliensis		10.1		15.4		0		-		0		-		0		0		0
Others/not speciated		2.8		3.8		4.8		-		5.6		-		0		21.2		2.9

Data: Danish Veterinary Institute

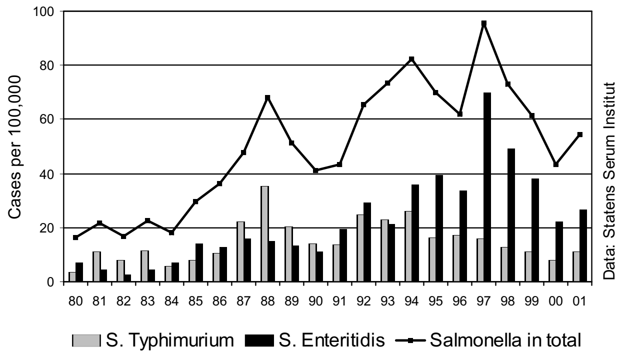

Figure 7. Registered cases of human salmonellosis in Denmark 1980-2001.

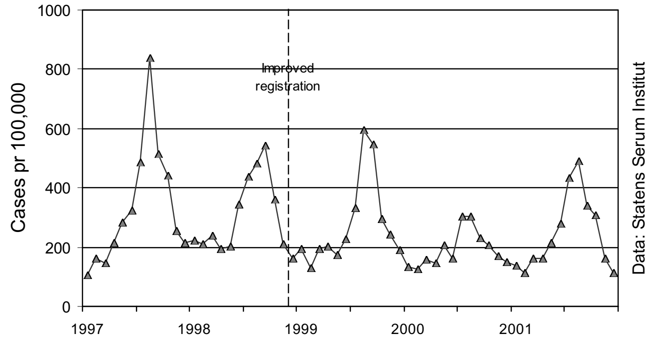

Figure 8. Seasonal variation in registered cases of human salmonellosis, 1997-2001.

The number of *S.* Typhimurium cases increased with 34.8% from 436 in 2000 to 589 (11 cases per 100,000) in 2001, which is the same level as in 1999.

The phage type (DT) distribution of 567 cases is presented in Table 17. The most common phage types were DT12 (20%), DT104 (14%), DT120 (7%) and DT193 (5%). This distribution is fairly similar to previous years. The number of multiple drug resistant DT104 cases was 84 in 2001. The number of domestic sporadic cases was at least 51. This is an increase compared to the period from 1997 to 2000 where a more or less constant level around 40 cases per year was seen (Figure 6).

Approximately 90% of *S.* Typhimurium infections are assumed to be domestically acquired. Infections with DT104 and DT3 appear to be more frequently associated with travel (28%) than other *S.* Typhimurium phage types.

Figure 10 shows the geographical distribution of *S.* Typhimurium cases.

The remaining 913 (17.1 cases per 100,000 inh.) zoonotic *Salmo-*

Table 11. Zoonoses in humans 2001 - incidence and trends of 5 and 10 years.

	2001		Five years trend					10 years	
Agent	Cases per 100,000 inh.	Registered cases	2000	1999	1998	1997	1996	1991	Note:
Mycobacterium bovis	0.1	4	12	2	8	11	11	3	a
Brucella abortus/melitensis	0.3	18	-	-	-	-	-	-	b
Trichinella spiralis/nativa	-	-	-	-	-	-	-	-	c
Salmonella spp.	54.5	2,918	2,308	3,268	3,880	5,015	3,259	2,238	d
S. Enteritidis	26.5	1,416	1,182	2,025	2,607	3,674	1,771	1,013	
S. Typhimurium	11.0	589	436	584	678	841	907	700	
Other zoonotic serotypes	17.1	913	690	659	595	500	581	520	
Campylobacter coli/jejuni	86.4	4,620	4,386	4,164	3,372	2,666	2,973	1,261	e
E. multilocularis/granulosus	-	-	-	-	-	-	-	-	c
Leptospira spp.	0.2	9	18	30	11	9	23	4	
Listeria monocytogenes	0.7	38	39	44	41	33	39	32	f
Rabies	0	0	0	0	0	0	0	0	g
Toxoplasma gondii	-	-	-	-	-	-	-	-	b
Cryptosporidium parvum	1.6	84	-	-	-	-	-	-	h
Yersinia enterocolitica	5.3	286	265	339	464	430	532	929	
Escherichia coli (VTEC)	1.7	92	60	51	34	33	5	49	
O157 (VTEC)	0.4	24	18	10	6	12	3	6	

Data: Statens Serum Institut.
a) Notification mandatory. Cases of tuberculosis are due to reactivation of latent infections in elderly or imported disease.
b) Notification not mandatory.
c) Notification not mandatory. A few imported cases occur.
d) Only first isolations registered.
e) A sample (n=802) of the isolates were identified to the species level: 94% *C. jejuni* and 6% *C. coli*. In the years 1996-2000 the percentage of *C. coli* were 5%, 6%, 4%, 4% and 11%, respectively.
f) Notification mandatory (since 1986).
g) Notification mandatory. No domestical or imported cases.
h) Notification not mandatory. Cases diagnosed at SSI, but it is estimated that approximately 200 cases are diagnosed annually.

nella cases were distributed over almost 100 different serotypes. The most common among these were *S.* Bovismorbificans (174 cases), *S.* Agona (130 cases), *S.* Hadar (62 cases), *S.* Thompson (41 cases), *S.* Virchow (39 cases), and *S.* Derby (38 cases) (Table 15). The number of 'exotic' serotypes rose 32% from 690 in 2000. Overall, travel association among cases with exotic serotypes is estimated at 20%, but varies greatly between serotypes. Among the dominant serotypes, travel association was most frequently reported for *S.* Virchow (74%) and *S.* Hadar (29%).

Outbreaks of zoonotic gastrointestinal infections

In Denmark, outbreaks of food- and water-borne infections caused by zoonotic agents are registered in three different systems. First, GP's and hospitals are obliged to notify all infections suspected to be food-borne, without awaiting microbiological analysis. These early notifications of suspected outbreaks are submitted to the Regional Medical Officer of Health with a copy to the Department of Epidemiology at Statens Serum Institut (Table 12).

Second, 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 13).

Third, individuals who experience food poisoning often report these incidents to the Regional Veterinary and Food Authorities. Such reports and results of the outbreak investigations are collated at the Danish Veterinary and Food Administration (Table 14).

There is at present no systematic evaluation of the overlap between the three parallel systems, nor has the completeness of these systems been formally evaluated. However, a new unified outbreak reporting system is scheduled to be put into place during 2002.

Outbreaks reported by physicians increased from 92 in 2000 to 116 in 2001. The increase is mainly due to household outbreaks, in particular among the groups of 'Others' and 'Unknown' (Table 12).

The number of laboratory detected outbreaks was about the same as in 2000, but included more laboratory-confirmed cases (230) than in 2000 (30 cases) (Tabel 13).

In 2001, 30 outbreaks reported by the Regional Veterinary and Food Authorities were investigated. Of these, 13 were caused by zoonotic bacteria, 17 outbreaks were of unknown origin and in 2 outbreaks other causes were found (Table 14). In total 349 persons became ill. For 27 of the outbreaks, the suspected foodstuff or meal was produced in approved food establishments (general outbreaks) and for 5 outbreaks produced in private homes (family outbreaks). The number of reported outbreaks in

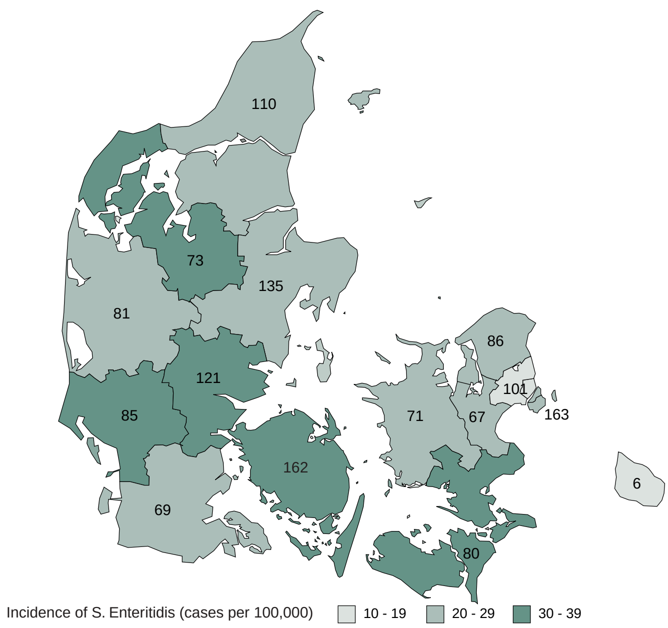

Figure 9. Geographical distribution of the number of cases per county and incidence of human infections with S. Enteritidis in 2001.
Data: Statens Serum Institut.

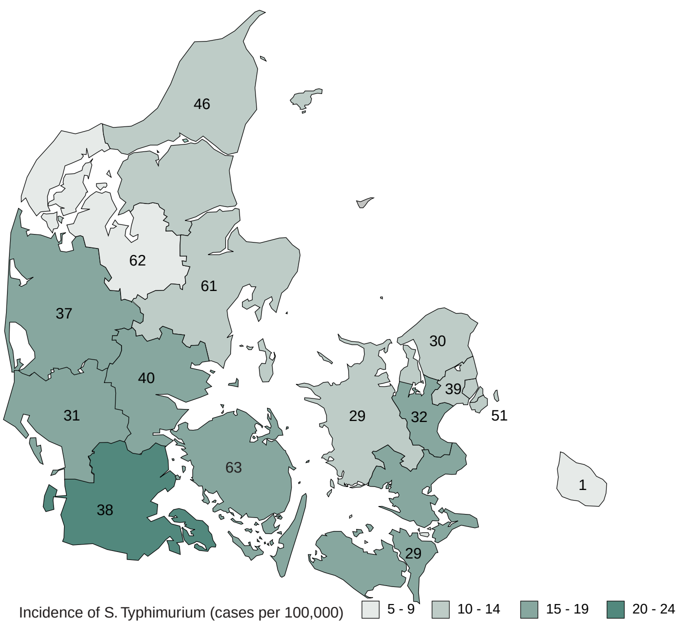

Figure 10. Geographical distribution of the number of cases per county and incidence of human infections with S. Typhimurium in 2001.
Data: Statens Serum Institut.

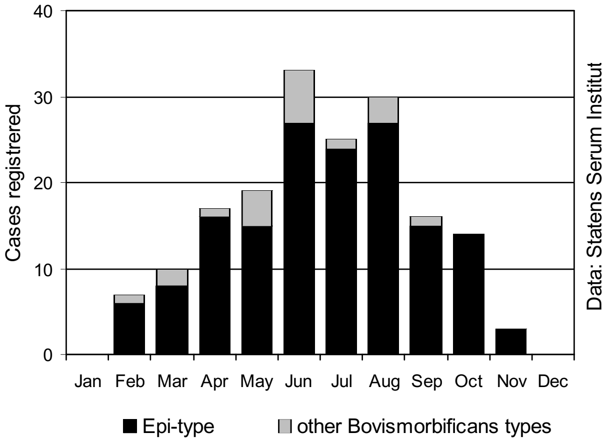

Figure 11. National outbreak with Salmonella *Bovismorbificans, 2001. Human isolates were charaterised by pulsed-field gel electrophoresis which made distinction between the epi-type and other types possible.*

2001 was at the same level as in 2000.

In 2001, Denmark witnessed a large general outbreak with the otherwise rare *Salmonella* serotype Bovismorbificans. There were 174 laboratory-confirmed cases in 2001, of which 154 belonged to the same pulsed-field gel-electrophoresis subtype. Of these 25% were children less than five years of age and 59% were female. There were relatively few patients who lived in the area in or near Copenhagen and relatively many who lived in North Jutland. The number of patients peaked during summer (Figure 11). A large number of investigatory patient interviews were done, as well as three independent case-control studies, testing hypotheses regarding different types of chicken and pork products as the source. Also, cohort studies were performed on two occasions when several people became infected after attending confined social gatherings. In spite of these efforts, the source remains undetected. Subtyping of *S.* Bovismorbificans isolates from neighbouring countries indicates that this outbreak is of domestic origin.

A cluster of 17 cases of *S.* Oranienburg in October to December was part of an international outbreak caused by German chocolate.

Among local outbreaks, the largest took place at an agricultural school in August (Table 13). Approximately 50 persons had clinical symptoms, and 18 of these were diagnosed with salmonellosis caused by *S.* Enteritidis PT34. The source was not identified, but a cohort study pointed at a meal which included eggs.

Tracing sources of human salmonellosis

As described above, the number of human *Salmonella* infections caused by exotic serotypes increased by 32% in 2001. A major part of this increase (28%) is explained by the outbreaks of *S.* Bovismorbificans and *S.* Oranienburg. However, sporadic infections with *S.* Agona and *S.* Derby also increased in 2001. In order to improve the knowledge as to sources of some of the more frequently occurring infections,

Table 12. Clinical based surveillance of suspected outbreaks of food-borne zoonotic diseases notified to Statens Serum Institut, 2001.

	General outbreaks		Outbreaks within household	
Zoonotic pathogen	No. of outbreaks	Suspected source	No. of outbreaks	Suspected source
S. Enteritidis	13	Eggs, chicken, shrimps, grilled food, buffet, shellfish	18	Turkey, eggs, minced meat, grilled food, shrimps, slice of meat, chicken
S. Typhimurium	7	Chicken, pork, beef	5	Eggs, chicken, pizza
Campylobacter	9	Pizza, chicken, ice cream, turkey, fish, handling of poultry	12	Eggs, chicken
Others [a)]	11	Fish, poultry, chicken, chocolate	10	Sausages, turkey, eggs, chicken, shellfish
Unknown	16	Oysters, fish, potato salad, burger, chicken, buffet, pancakes with stuffing, pizza	15	Eggs, chicken, sausages, shellfish, pizza, fish, turkey, marinated lamb, poultry

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

molecular typing of selected isolates representing patients and each potential source was performed.

During the last four years, the human incidence of *S.* Agona has more than quadrupled, reaching a total of 130 cases in 2001. From 2000 to 2001 the number of cases increased by 78%, making *S.* Agona the fourth most prevalent serotype in 2001 (Table 15). A large proportion (24%) of the patients were children less than two years of age.

S. Agona was also isolated a substantial number of times from non-human sources including pig herds, pork, turkey flocks and Danish and imported turkey meat (Table 15). A total of 24 human and 26 non-human isolates were compared by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *BlnI*. The results showed that half of the isolates could be divided into 3 different groups each representing an unique PFGE pattern. The first group included 5 human isolates as well as isolates from pig herds and pork. The second group was the largest and included 8 human isolates and 5 isolates from turkey meat imported from France. The final group did not include any human isolates, but only isolates from the Danish turkey production. A total of 11 human isolates was shown to have PFGE patterns not found among the non-human isolates, whereas 18 isolates originating from pig herds, pork and Danish turkeys 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 imported turkey meat are important sources of human infections caused by *S.* Agona. However, Danish produced turkey meat can by no means be ruled out as an important source, since only 24

Table 13. Outbreaks identified in the laboratory-based surveillance of zoonotic diseases, Statens Serum Institut, 2001.

Occasion and pathogen	No. of cases confirmed (suspected)	Suspected source
Farming school, *S.* Enteritidis PT34	18 (50)	Eggs
Private party, *S.* Thompson	5 (9)	Fruit gel
Secondary school camp, *S.* Bovismorbificans	3 (14)	Pig or chicken product
Family party, *S.* Bovismorbificans	5 (8)	Unknown
General outbreak, *S.* Bovismorbificans	154	Unknown
Community outbreak, *S.* Typhimurium DT12	29	Unknown
General outbreak (part of international outbreak), *S.* Oranienburg	17	Two specific brands of German chocolate

Data: Statens Serum Institut

Table 14. Outbreaks of food-borne zoonotic diseases registered by the Regional Veterinary and Food Authorities in 2001.

Zoonotic pathogen	No. of outbreaks	Total number of sick persons	Suspected source (No. of outbreaks)	Confirmed by culture in foodstuffs/patients
S. Enteritidis	6	105	Pancakes filled with salmon, minced meat, cheese and shrimps (1)	-/+
			grilled whole pork, potato salad, green salad and ice cream (1)	-/+
			Chicken sandwich (1)	-/+
			Marinated salmon, beefsteak and potatoes (1)	-/+
			Eggs and shrimps (1)	-/+
			Sweet made of raw eggs (1)	-/+
Salmonella Bovismorbificans [a)]	2	22	Coctail with shrimps, veal steak and fresh fruit salad (1)	-/+
			Vegetable pie, moussaka Eggs-, and tuna salad	-/+
S. Agona and *S.* Uganda	1	2	Egg sandwich with bacon (1)	+/-
Salmonella [b)]	1	8	Fried eel, potato stew and sauce with chopped parsley (1)	-/+
Campylobacter	3	13	Sandwich (1)	-/+
			Pasta salad with turkey (1)	-/+
			Chicken burger (1)	-/+
Unknown	17	199	Many different foodstuffs involved	

Data: Danish Veterinary and Food Administration

a) These 2 outbreaks are part of a countrywide outbreak.
b) Unknown type.

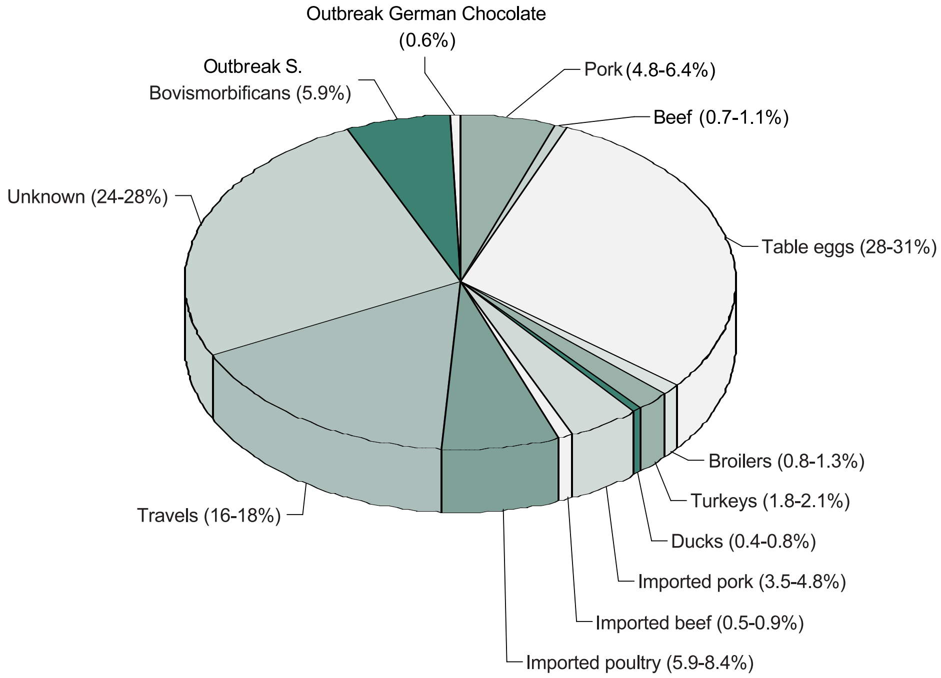

Figure 12. Estimated sources of human salmonellosis in Denmark in 2001. Estimated mean number of cases per source: 852 cases from table eggs, 482 travel associated, 208 from imported poultry, 163 from pork, 122 from imported pork, 57 from turkey, 29 from broilers, 25 from beef, 20 from imported beef, 17 from ducks, 174 from S. *Bovismorbificans outbreak, 18 from* the *outbreak with German chocolate and 750 of unknown sources. Data: Danish Zoonosis Centre.*

(18%) of the 130 *S.* Agona cases were typed by PFGE. Finally, the findings of unique PFGE patterns among the human isolates indicate that some sources still need to be identified.

Another frequently occurring serotype in humans is *S.* Hadar. During the last five years, the annual incidence has ranged from 58 to 74 cases, making *S.* Hadar a permanent member of the human top five. In 2001, 62 cases were reported, and *S.* Hadar was also found in pig herds, pork, beef, broilers and chicken meat, turkeys and turkey meat, ducks and imported poultry products. A sample of 50 strains was selected for PFGE using the restriction enzymes *Xba*I and *Bln*I. The sample included 22 human isolates plus 28 isolates from various animal sources. The largest group of isolates with indistinguishable PFGE patterns included 10 human isolates, 8 isolates from poultry imported from France and 1 isolate from duck meat imported from Germany. Two other groups, each including a single human isolate and 4 isolates from Danish poultry and pigs were also identified. The remaining 10 human isolates were distributed on 8 different PFGE types, of which all were human isolates. Likewise, 11 non-human isolates were divided into 7 different PFGE patterns, which were not shared with any of the human isolates examined. The results indicate that imported poultry in particular, but also pork and poultry products of Danish origin are important sources of human infections with *S.* Hadar. In addition, the findings of unique PFGE patterns among the human strains indicate that not all sources have been identified.

S. Derby is a serotype normally occurring at a low frequency in humans in Denmark. Except for 1998, where 23 cases were reported, the incidence has been around 10 cases per year. In 2001, however, this figure increased to 38 cases. Among the animal reservoirs, *S.* Derby was found in slaughter-pig herds, pork, beef and imported poultry products (Table 15).

A number of these isolates were selected for PFGE using *Xba*I as the restriction enzyme. The selected isolates included 13 human isolates plus 17 non-human isolates (12 from pig carcasses, 2 from cattle herds and 3 from imported poultry). The results of the investigation showed a large strain diversity with only a few isolates having similar PFGE patterns and no human strains were found to be identical to non-human strains. Consequently, it was not possible to link any of the examined food products as sources of human infection based on the typing results, and further research into the stability of the genome of *S.* Derby seems to be needed. However, other epidemiological evidence suggests that at least a part of the increase in humans may be explained by Danish produced pork, as the *S.* Derby prevalence in pork also increased significantly in 2001.

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, a risk assessment model quantifying the contribution of the major animal-food sources to human salmonellosis is used. The principle behind the model is a comparison of the number of human cases caused by different *Salmonella* sero- and phage types with the prevalence of the *Salmonella* types isolated from the various animal-food sources, weighted by the amount of food source consumed.

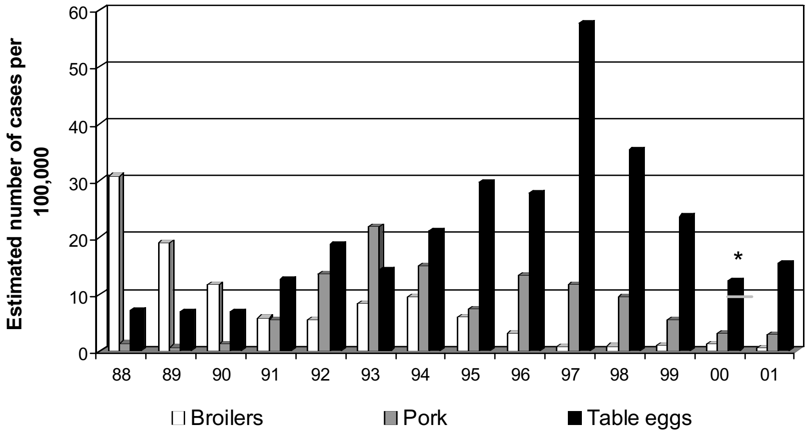

*Figure 13. Estimated major sources of human salmonellosis in Denmark, 1988-2001. *)In 2000, table eggs were estimated to account for 10.1 cases pr 100,000, during 2001 the source of 124 S. Enteritidis PT34 cases was most likely found to be eggs as well. Data: Danish Zoonosis Centre.*

In 2001, the estimated number of human cases per 100,000 inhabitants that could be attributed to various sources, was as follows: table eggs: 15.9; broilers: 0.5; pork: 3.0; turkeys: 1.1; ducks: 0.3; beef: 0.5; imported poultry products: 3.9; imported beef: 0.4; imported pork: 2.3; travel: 9.0; outbreak related cases: 3.6 (Figure 12). Approximately 750 cases (14.0 per 100,000) could not be associated with any specific source, but a part of these infections are probably related to pet animals or foodstuffs that are not presently monitored, e.g. imported table eggs and vegetables.

Figure 13 shows that Denmark has experienced three waves of human salmonellosis, where the majority of cases have been caused by three

Table 15. Serotype distribution (%) of Salmonella *from animals, carcasses at slaughterhouses, imported meat and humans in Denmark, 2001. In some cases more than one serotype was found per positive herd/batch and therefore the number of typed units may be greater than the number of positive herds/batches.*

										Imported meat f)				
Serotype	Humans n=2918	Pig herds a) n=845	Pork b) n=300	Cattle herds c) n=71	Beef b) n=12	Layer flocks d) n=35	Broilers e) n=71	Turkey flocks e) n=25	Duck flocks e) n=170	Pork n=21	Beef n=3	Chicken n=193	Turkey n=139	Duck n=52
S. Enteritidis	48.5	0.2	0.0	1.4	0.0	100	7.0	0.0	4.1	0.0	0.0	0.5	0.0	0.0
S. Typhimurium	20.2	74.7	54.0	47.9	8.3	0.0	19.7	0.0	0.0	28.6	0.0	2.6	1.4	46.2
S. Bovismorbificans	5.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
S. Agona	4.5	0.9	0.3	0.0	0.0	0.0	0.0	28.0	0.0	0.0	0.0	1.0	8.6	0.0
S. Hadar	2.1	0.1	0.0	0.0	0.0	0.0	0.0	16.0	12.4	0.0	0.0	11.4	13.7	3.8
S. Thompson	1.4	0.1	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. Virchow	1.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	0.0	0.0
S. Derby	1.3	0.0	17.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.5	4.3	0.0
S. Newport	1.2	8.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.3	10.8	0.0
S. Dublin	0.9	0.0	1.0	45.1	58.3	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0
S. Stanley	0.9	0.1	0.3	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. Senftenberg	0.8	0.2	0.0	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
S. Braenderup	0.7	0.2	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	0.6	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. Oranienburg	0.6	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. Infantis	0.6	5.0	7.7	0.0	0.0	0.0	11.3	12.0	0.0	9.5	0.0	4.1	0.7	3.8
S. Saintpaul	0.6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	15.8	23.1
S. Heidelberg	0.5	0.4	0.3	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	44.0	10.8	1.9
Others	5.7	5.9	6.0	0.0	8.3	0.0	45.1	24.0	60.0	28.6	66.7	17.6	31.7	15.4
Not typable	1.0	3.5	13.4	5.6	16.8	0.0	15.5	16.0	23.5	0.0	0.0	4.8	1.5	5.8
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Data: Danish Veterinary Institute, 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 swab samples of pork and beef carcass from the surveillance programme at slaughterhouses.
c) Cattle herds examined on clinical indications. The data is 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.

distinct sources: broilers in the late 80's, pork in the mid 90's and eggs in the mid/late 90's. At each peak, a new control programme was implemented resulting in a decline of human cases attributable to that particular source. Compared to 2000, there has been an increase in the number of egg-borne infections. This may at least to some extent be explained by a simultaneous increase in the number of *Salmonella* infected table-egg flocks (Figure 1). Furthermore, in 2000, a substantial part of the unaccounted cases was *S.* Enteritidis PT34, and this particular type continued to be a dominant cause of human infections in the first 9 months of 2001. However, in September 2001 the primary source was most likely identified, when a table-egg-producing farm was found infected with this phage type. The eggs from the farm were diverted to pasteurisation and since October 2001 no further human cases have been reported. As illustrated in Figure 14, this finding means that the number of egg-borne infections in 2000 was higher than reported last year. A total of 260 cases of PT34 reported in 2000 and 2001 are estimated to be associated with eggs from the producer in question.

Table 16. Phage-type distribution (%) of S. Enteritidis from humans, animals, carcasses at slaughterhouses and imported meat, 2001.

					Imported meat [f]
Phage type	Humans	Layer flocks [d]	Broiler flocks [e]	Duck flocks [e]	Chicken
	n=796	n=35	n=5	n=6	n=100
PT8	29.3	51.4	0.0	0.0	0.0
PT4	25.3	11.4	0.0	0.0	0.0
PT34	11.2	8.6	0.0	0.0	0.0
PT6	4.8	14.3	0.0	0.0	0.0
PT1	4.5	0.0	0.0	0.0	0.0
PT14b	4.1	0.0	0.0	0.0	0.0
PT21	2.9	11.4	0.0	0.0	0.0
PT6a	2.1	0.0	0.0	0.0	0.0
PT13a	1.5	0.0	0.0	0.0	0.0
Not typable	8.3	2.9	20.0	33.3	0.0
Others	6.0	0.0	80.0	66.7	0.0
Total	100	100	100	100	100

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

Table 17. Phage-type distribution (%) of S. *Typhimurium from humans, animals, carcasses at slaughterhouses and imported meat, 2001.*

							Imported meat [f]			
Phage type	Humans	Pig herds [a]	Pork [b]	Cattle herds [c]	Beef [b]	Broiler flocks [e]	Pork	Chicken	Turkey	Duck
	n=567	n=649	n=162	n=27	n=1	n=14	n=6	n=5	n=2	n=24
DT12	20.3	35.6	37.7	25.9	0.0	21.4	0.0	20.0	0.0	0.0
DT104	13.8	5.5	1.2	11.1	100	0.0	33.3	0.0	100.0	16.7
DT120	6.7	2.5	2.5	14.8	0.0	0.0	16.7	0.0	0.0	0.0
DT193	4.8	4.9	3.7	0.0	0.0	0.0	16.7	0.0	0.0	0.0
DT17	3.5	8.0	10.5	7.4	0.0	7.1	16.7	0.0	0.0	0.0
DT3	3.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DT170	3.2	9.4	10.5	25.9	0.0	14.3	0.0	0.0	0.0	0.0
DT1	3.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DTU292	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DT89	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DT135	2.1	1.5	0.0	3.7	0.0	0.0	0.0	0.0	0.0	16.7
DT110	1.2	1.4	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0
DT15A	1.2	1.8	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DTU302	1.2	0.6	0.0	0.0	0.0	0.0	0.0	80.0	0.0	20.8
DT66	1.1	7.2	10.5	3.7	0.0	0.0	0.0	0.0	0.0	0.0
DT104b	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Not typable	17.6	2.9	10.5	7.4	0.0	14.3	0.0	0.0	0.0	4.2
Others	9.7	18.2	11.7	0.0	0.0	35.8	16.6	0.0	0.0	41.6
Total	100	100	100	100	100	100	100	100	100	100

Data: Danish Veterinary Institute, Danish Veterinary and Food Administration and Statens Serum Institut.
Notes: See Table 15.

2. Campylobacter jejuni/coli

Poultry

National monitoring of thermophilic *Campylobacter* in broilers, hens and ducks was initiated in 1998. 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 41.9% in 2001 (Table 5). The percentage of positive batches ranged from 18.8% to 82.8% per month, and a distinct seasonal variation was observed (Figure 14). In 2001, the prevalence in hens was 56.6% (47 of 83 batches examined) and in ducks 95.9% (117 of 122 batches examined).

A project on national monitoring of thermophilic *Campylobacter* in Danish turkey flocks was initiated in September 1999 and was continued until the beginning of September 2001. All turkey flocks were tested for *Campylobacter* at the time of slaughter. As for the monitoring in broilers, cloacal swab samples were collected at the slaughterhouse. However, unlike broilers, most turkey flocks are 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. The prevalence ranged from 3.6% in April to 87.1% in August with a mean of 37.8% (Table 6, Figure 14).

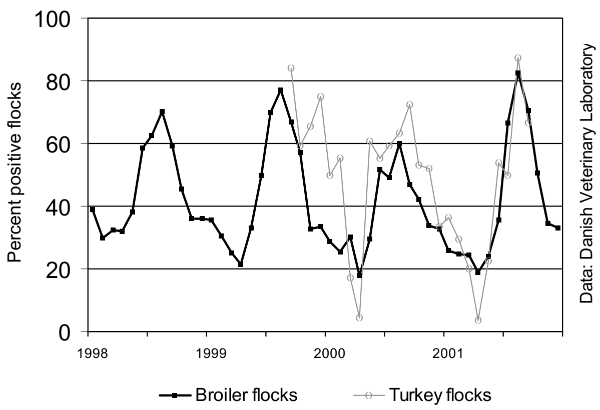

Figure 14. Percent Campylobacter *positive poultry flocks, 1998-2001*

Pigs and cattle

As part of a monitoring programme (DANMAP) for 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 76.9% (Table 7) and in cattle 72.4% (Table 9).

Wildlife and pets animals

The Danish Veterinary Institute monitors the occurrence of *Campylobacter* in pet animals, wild mammals and birds. The group of wild mammals and birds consists mainly of dead animals submitted by hunters, veterinarians and others. Until June 15th, all feacal samples from pets were analysed, including samples submitted for parasitic analysis and samples from dead pets. Hereafter only samples submitted specifically for *Campylobacter* analysis were examined. In 2001, *Campylobacter* was found in 16 (14.7%) of 109 dogs examined and in 12 (23.1%) of 52 cats (Table 10). The predominant species in dogs and cats was *C. upsaliensis*.

Products from retail outlets

In 1996, the Danish Veterinary and Food Administration established a nation-wide surveillance program for thermophilic *Campylobacter* spp. in foods from retail outlets. This program was continued in 2001. As in 2000, the samples have been analysed by a semi-quantitative method based on pre-enrichment in Mueller-Hinton broth supplemented with trimethoprim and cefaperazone followed by plating on mCCDA.

The food included in the survey in 2001 includes imported and Danish poultry products, mainly raw chicken and turkey products. In total, 1,896 samples of raw poultry have been analysed. The prevalences of thermophilic *Campylobacter* spp. in Danish and imported products are shown in Table 5 and Table 6. The origin is known for 89% and 88%, respectively. In 2001, the total prevalences in raw chicken and turkey products were 35% (N=1,096) and 22% (N=800), respectively. This is a decrease compared to 2000 where the prevalences were 41.1% (N=708) and 30.4% (N=303), respectively. The numbers of thermophilic *Campylobacter* spp. per gram sample (CFU/g) are shown in Figure 15 and 16. In 2001, the samples investigated had lower counts of thermophilic *Campylobacter* and more samples were negative (<0.4 CFU/g) as compared to the findings in 2000.

In 2001, fruit and vegetables were included in the surveillance. Thermophilic *Campylobacter* spp. were found in 0.21% of 954 samples.

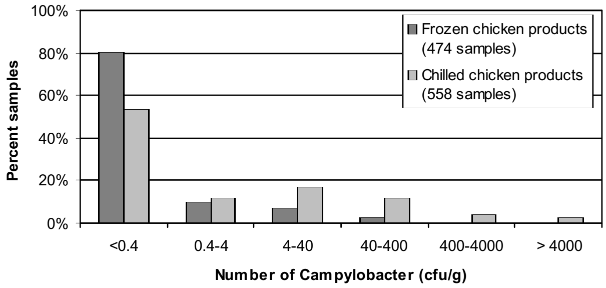

Figure 15. The number of thermophilic Campylobacter *in Danish produced and imported chicken products from retail outlets, 2001. Data: Danish Veterinary and Food Administration.*

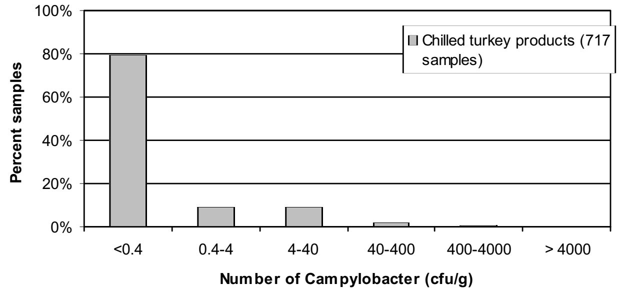

Figure 16. The number of thermophilic Campylobacter *in Danish produced and imported turkey products from retail outlets, 2001. Data: Danish Veterinary and Food Administration.*

Campylobacteriosis in humans

The number of human *Campylobacter* infections continued the steady increase observed since 1992 (Figure 17). There were 4,620 laboratory confirmed episodes of campylobacteriosis in 2001 (86 cases per 100,000 inhabitants), which is 5% more than the year before. The same trend is seen in other industrialised countries and poses a serious public health problem. There are many ill-defined sources of human campylobacteriosis, but consumption of poultry and poultry products is believed to be a major risk factor in Denmark. Approximately 80% of the *Campylobacter* infections are assumed to be domestically acquired and the rise in infections cannot be explained by an increase in the number of travel-related infections.

Outbreaks of human campylobacteriosis are relatively rare. They are identified and recorded in the same manner as *Salmonella* outbreaks and summarised in Table 12, 13 and 14.

Figure 18 shows the geographical distribution of infections with *Campylobacter* spp.

Intensive surveillance of human Campylobacter infections by typing

Systematic typing of clinical *Campylobacter* isolates has not previously been performed in Denmark. A one-year research project was initiated in May 2001 with the purpose of intensive surveillance of *Campylobacter* in two regions of Denmark. The surveillance is based on using two definitive typing methods: Penner serotyping and RiboPrinting (automated ribotyping). Two counties, Funen and Copenhagen, were chosen, and all *Campylobacter* isolates from these counties are typed in real-time. The patients are asked to fill out a short questionnaire concerning symptoms, travel, restaurant visits, contacts to other infected persons, drinking water, and general description of food consumption prior to onset of disease. *Campylobacter* isolated from food (mainly poultry) in these regions are included in the study. The expectation was that the project would make it possible to identify small outbreaks through typing and in some cases to identify a common source by analysing the responses in the questionnaires. The preliminary results show that

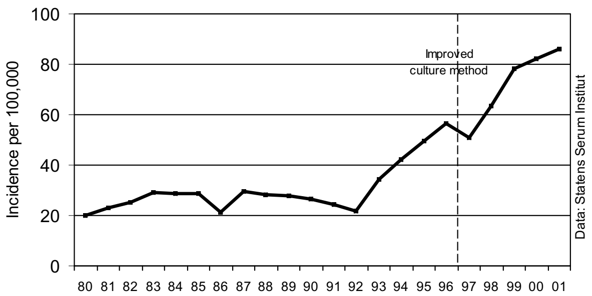

Figure 17. Incidence per 100,000 of human campylobacteriosis in Denmark, 1980-2001. Data: Statens Serum Institute.

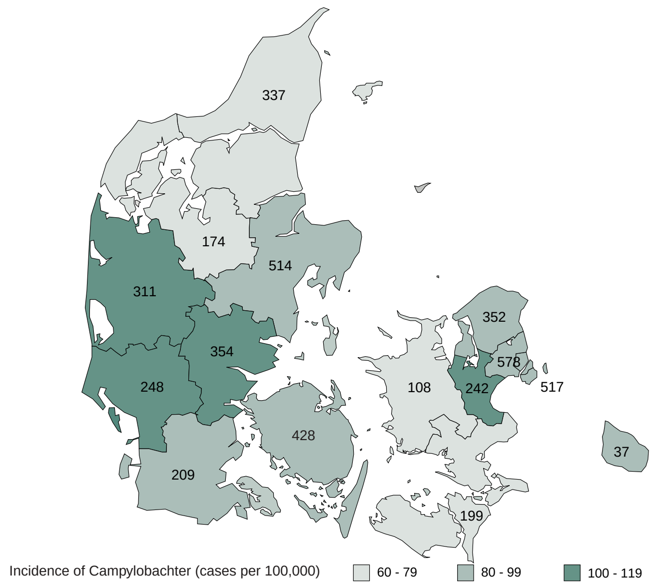

Figure 18. Geographical distribution of the number of cases per county and incidence of human campylobacteriosis in 2001.Data: Statens Serum Institut.

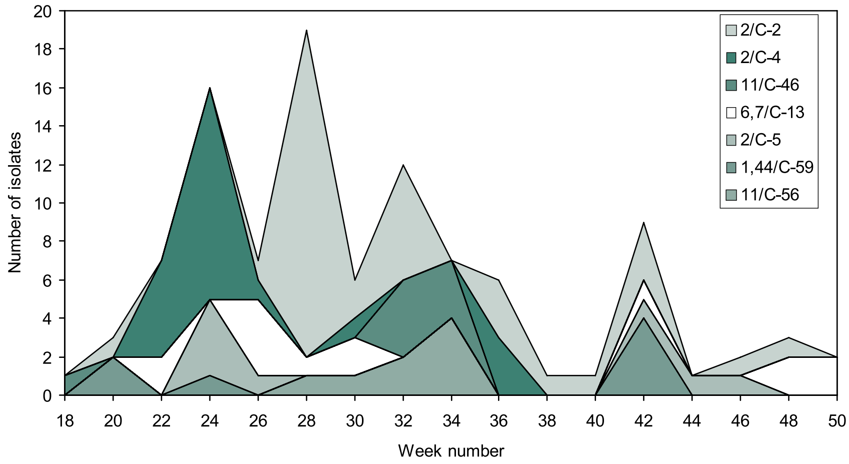

Figure 19. Human Campylobacter *jejuni cases from two counties, 2001: Occurrence over time of seven selected sero-/ribotypes that tend to be accumulated in one or two relatively short time periods. Data: Danish Veterinary Institute.*

Table 18. Serotype distribution (%) of Campylobacter jejuni *from human patients and animals in 2001.*

Serotype	Human n=232	Cattle n=32	Broilers n=133	Food n=105	Turkey n=50
2	29.3	37.5	12.0	18.1	26.0
4-complex [1)]	15.1	21.9	13.5	9.5	12.0
1,44	10.8	0.0	5.3	13.3	14.0
6,7	5.6	0.0	10.5	7.6	10.0
11	4.7	0.0	3.8	9.5	0.0
19	3.4	3.1	4.5	1.0	0.0
3	3.0	0.0	1.5	1.9	2.0
12	2.2	0.0	7.5	8.6	6.0
5	1.7	3.1	1.5	4.8	4.0
23,36	1.7	12.5	3.0	0.0	0.0
29	0.0	6.3	0.0	1.0	0.0
35	0.0	3.1	0.8	0.0	0.0
Not typable	3.9	0.0	10.5	1.9	4.0
Others	18.5	12.5	25.6	22.9	22.0

Data: Danish Veterinary Institute
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 2001.*

Serotype	Human n=24	Broilers n=21	Food n=52	Pigs n=70
24	16.7	4.8	9.6	18.6
30	16.7	9.5	5.8	7.1
34	12.5	0.0	3.8	5.7
5	4.2	9.5	9.6	7.1
46	0.0	14.3	5.8	12.9
48	0.0	14.3	1.9	7.1
49	0.0	19.0	1.9	0.0
54	0.0	0.0	13.5	11.4
59	0.0	4.8	7.7	11.4
Not typable	20.8	9.5	19.2	1.4
Others	29.2	14.3	21.2	17.1

Data: Danish Veterinary Institute

clusters of cases are quite common: typically 8-25 persons shed the same sero-/ribotype in the time frame of a few weeks (Figure 19). Such clustering of cases is hardly coincidental. A common origin of the isolates and thereby a common source of infection is likely. Based on the questionnaires, it has not yet been possible to identify a common source or a link between patients in any cluster. However, a poultry food isolate with a matching sero-/ribotype was identified for many of these clusters, thereby indicating a link. During the eight month period of the project in 2001, 781 human isolates and 134 poultry food isolates were typed.

The project is performed in collaboration between the Statens Serum Institut, the Danish Veterinary Institute, and the Department of Clinical Microbiology at Herlev Hospital.

Serotyping of Campylobacter

A sample of isolates of *C. jejuni* and *C. coli* were serotyped using the 'Penner serotyping scheme' (heat-stable antigens). *C. jejuni* was the predominant species among humans, poultry and cattle. Serotype 2, serotype 1,44 and the 4-complex were the most common serotypes among human isolates (Table 18). These serotypes are also common in most other sources. *C. coli* is the prevailing species in pigs, whereas *C. coli* accounts for less than 10% of the thermophilic *Campylobacter* species in humans, broilers, turkeys and cattle. In 2001, 6% of the human isolates that were speciated were *C. coli* and the remaining *C. jejuni*. The most common *C. coli* serotypes in pigs were serotype 24 and 46 (Table 19). In humans the most common *C. coli* serotypes were 24 and 30.

3. Yersinia enterocolitica

Pigs and cattle

As part of a monitoring programme (DANMAP) for 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 2001, 60 (14.6%) of 411 examined animals were found positive for *Y. enterocolitica*. Of these, 59 were serotype O:3 and one was serotype O:9 (Table 7).

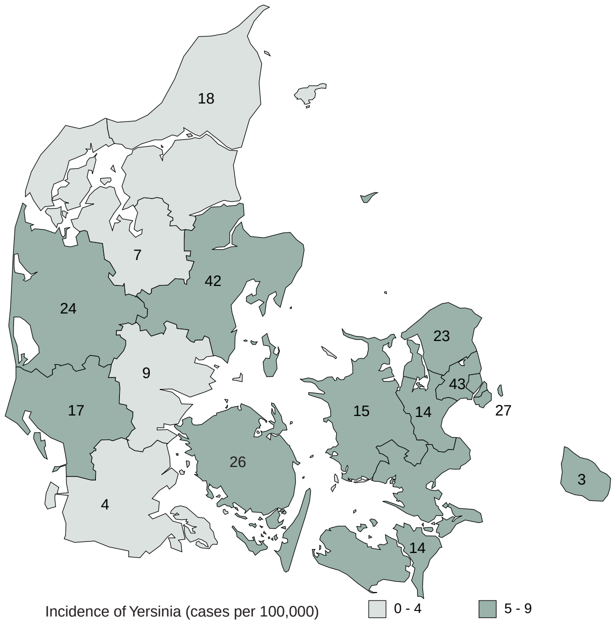

Figure 21. Geographical distribution of the number of cases per county and incidence of human yersinosis 2001. Data: Statens Serum Institut.

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 routine surveillance carried out by the Danish Veterinary and Food Authorities. Therefore the information on prevalence of this organism in various types of foods is scarce. Only 14 samples of non-heat treated pork and 5 samples of heat treated pork meat were analysed in 2001. None were positive (Table 7).

Figure 20. Incidence per 100,000 of human yersiniosis in Denmark, 1980-2001.

Yersiniosis in humans

A total of 286 cases of infection with *Y. enterocolitica* were registered in year 2001 (5.3 cases per 100,000 inhabitants, Table 11, Figure 20). Almost all isolates (277) were serotype O:3. Among the cases, 45% were children less than five years of age, and the vast majority of the infections were domestically acquired.

There were 7% more cases in 2001 compared to 2000, but 19% fewer compared to 1999 (Figure 20). Overall, the number of infections with *Y. enterocolitica* has decreased steadily since 1985 where more than 1,500 cases were reported. Because most Danish slaughter pig herds are assumed to harbour *Y. enterocolitica* serotype O:3 biotype 4, the primary source of yersiniosis is thought to be porcine.

The geographical distribution of *Y. enterocolitita* cases in 2001 is presented in Figure 21.

4. Listeria monocytogenes

Products from retail outlets

Data describing the presence of *Listeria monocytogenes* in food at retail level in Denmark in 2001 were reported by the Regional Veterinary and Food Authorities (Table 20). According to the Danish regulations, investigations of the level of *L. monocytogenes* in foods are to be performed on certain ready-to-eat foods. No significant changes in the number of foods with *L. monocytogenes* exceeding 100 cfu per gram have been observed in the period 1998-2001.

Listeriosis in humans

In 2001, 38 cases of listeriosis were registered (Table 11, Figure 22). Twenty-five cases presented with septicaemia, ten with meningitis, and three were classic materno-foetal cases. Geographically, the patients were spread all over the country. Based on serogrouping, riboprinting and pulsed field gel electrophoresis no clustering of cases could be identified. Twenty cases were caused by strains of serogroup 1 and eight-teen by serogroup 4.

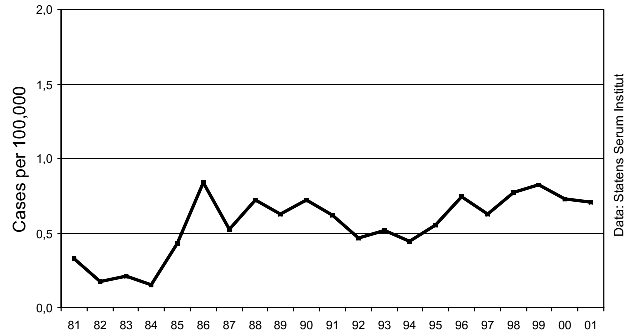

Table 20. Percentage distribution of the number of Listeria monocytogenes *in selected foods, sampled at retail level in Denmark by the Regional Veterinary and Food Authorities in 1998-2001.*

	2001		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	Number of samples	Percent of samples with cfu>100 per g
Heat-treated products of pork, beef, chicken and turkey handled after heat treatment	2,952	0.2	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	115	0.9	162	2.5	212	0.9	512	1.4
Gravad, smoked, salted, not heat-treated or slightly heat -treated fish products	152	2.0	120	0.8	178	0.6	193	0.0
Sprouts or sliced vegetables	87	0	160	0	398	0.3	505	0.2
Vegetable mayonnaise	1,664	0.1	2,163	0.2	2,393	0.2	2,283	0.5
Cheese and cheese products	31	0	44	0	53	0.0	50	0.0
Ready-prepared dishes	1,239	0.2	1,410	0.2	1,816	0.0	1,531	0.2

Data: Danish Veterinary and Food Administration.

5. Verocytotoxigenic *Escherichia coli*

Cattle

As part of the DANMAP-progamme the occurrence of verocytotoxigenic *Escherichia coli* O157 (VTEC O157) has been surveyed since 1995 on faecal samples from cattle collected at the slaughterhouse (one sample per herd). In 2001, VTEC O157 was detected in 3.2% (6 of 186) faecal samples from cattle (Table 9).

In 2001 the Danish Veterinary and Food Administration investigated 580 cattle at slaughter for the presence of VTEC O157. The analyses were performed on faecal samples taken per rectum and swab samples from carcasses. VTEC O157 was isolated from the faecal content of 25 animals (4.3%) and from 4 (0.7%) of the carcasses.

In another study, samples of faecal content from 50 cattle at slaughter and 100 samples of fresh beef were examined for the presence of the *E. coli* serotypes O26, O103, O111, and O145. The samples were analysed by a method, which included immuno-

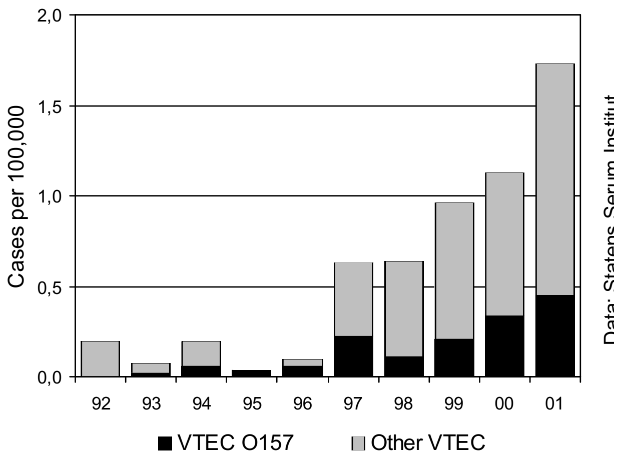

Figure 23. Incidence of human infections with verocytotoxigenic E. coli, *1992-2001.*

magnetic separation. None of the four *E. coli* serotypes were detected in this study.

Products from retail outlets

In a screening of imported fresh beef cuts, 543 samples were examined for VTEC O157. The majority of meat samples were taken at cutting plants but the survey also included a few samples from retail outlets. VTEC O157 was not detected in any of the investigated samples.

VTEC O157 was not found in a survey comprising 712 samples of imported and 437 samples of Danish vegetables and fruit.

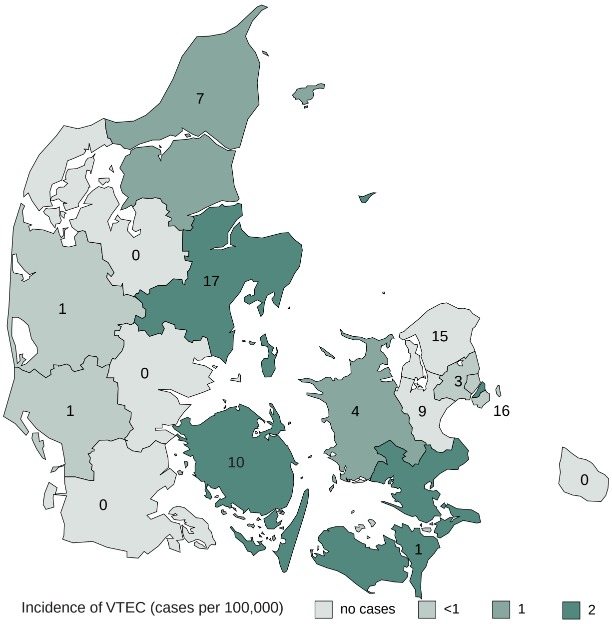

Figure 24. Geographical distribution of the number of cases per county and incidence of human infections with verocytotoxigenic E. coli *(VTEC), 2001. Data: Statens Serum Institut*

Human infections

The incidence 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 (reduced to 1/2 in the year 2001) changed their diagnostic practice and began to look for VTEC in all stools from patients with bloody diarrhoea and in all stools from patients 4 years of age or less with a history of bloody diarrhoea. In year 2001 the age limit was raised from 4 to 7 years. 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 verocytotoxigenic and eae genes, and live slide agglutination of suspect colonies with O-antisera against the most common VTEC and EPEC serotypes.

In 2001, this approach resulted in the identification of 92 episodes of VTEC infections from 90 individuals (incidence 1.7 per 100,000). Of these, 27% were O157 (Figure 23, Table 21). No outbreaks occurred, but there was one example of person-to-person transmission (father to son).

Seven cases of haemolytic uraemic syndrome (HUS) were reported. One was fatal and an *E. coli* strain of O group O157:H- was recovered during treatment. The strain did not produce verocytotoxin and did not possess the VT gene at the time of isolation. From the remaining six cases, the following serotypes were isolated: O 21:H8, O 26:H11, O111:H-, O121:H19, O157:H7 and O157:H-. All these were eae- and vtx2 positive, and the O111 strain was also positive for vtx1.

The geographical distribution of human infections with VTEC is presented in Figure 24.

Table 21. VTEC serotype distribution in 2001. All serotypes that resulted in five or more episodes are listed. A total of 22 different serotypes were registered

Serotype	Number of episodes
O157	24
O26	14
O103	8
O117	7
O145	5
Rough	5
Other	27

Data: Statens Serum Institut

6. Bovine Spongiform Encephalopathy

Cattle

The Danish BSE surveillance programme continued throughout 2001. The major changes included the TSE Regulations (No 999/2001, No 1248/2001 and No 1326/2001) being put into force and in consequence the testing of all fallen stock older than 24 months by the 1st of July 2001.

Among the animals tested, 6 were positive for BSE (Figure 25). The categorisation of the cases is presented in Table 22. In total, 276,327 animals were tested.

Table 22. The BSE surveillance programme in Denmark, 2001.

Risk category	No. of tests	No. of positive animals
Emergency slaughters (>24 mo.)	1.797	0
Animals >24 mo., where ante-mortem inspection at the slaughterhouse reveals signs of disease or zoonotic infection	99	0
Fallen stock (>24 mo.)	20.297	1
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)	3.597	0
Imported UK animals	16	0
Animals from herds under restriction	39	1
Animal clinically suspected of having BSE	71	1
Normal slaughter animals (>30 mo.)	250.411	3

Data: Danish Veterinary and Food Administration

Two of the above mentioned positive animals where BARB-animals, meaning that they were born after implementation of the real feed ban in Denmark (January 1997). This ban prohibited the use of mammalian protein as feed for ruminants. The investigation in the first case showed that contamination with meat-and-bone meal (MBM) could not be ruled out as a possible source of infection, and the investigations regarding the second case were not completed by the deadline of this report.

Samples from normal slaughter animals are tested at four approved private laboratories in Denmark. Three of these laboratories use the Enfer Test (an ELISA test) with spinal cord as test material, and the fourth uses the Prionics Check Test (Western Blotting) with brain stem as test material. When a test shows an inconclusive or a positive result, the material is sent to The Danish Veterinary Institute (DVI) for further testing. DVI also examined the remaining samples from the Danish cattle population at risk (categories presented in Table 22). DVI uses the Prionics Check Test, histopathology and immunohistochemistry (IHC) for BSE testing. In case of very damaged material or an inconclusive IHC test, material is forwarded to the Veterinary Laboratory Agency, Weybridge, UK.

Humans

The human form, variant Creutzfeldt-Jakob Disease (vCJD) is notifiable in Denmark. No cases have been reported.

Figure 25. BSE farms in Denmark, 2001

7. Cryptosporidium parvum

Mammals

As a part of an EU research project, all faecal samples from mammals (N=5,954), submitted for parasitic examination to Section for Parasitology, Danish Veterinary Institute, in 2001, were screened for *Cryptosporidium*. In cattle, *Cryptosporidium* was found in 11.4% of the samples. Among other animal species the occurrence of *Cryptosporidium* did not exceed 1.2% (Table 23).

The screening included all age groups; thus many samples from animals outside the agegroup at risk were analysed, thereby decreasing the relative prevalence of *Cryptosporidium* in the samples. In specimens from clinically affected calves in the group at risk, i.e. < 1 month of age, 21.3% of the analysed samples were positive for *Cryptosporidium*.

Table 23. Occurrence of Cryptosporidium *in faecal samples, 2001*

	No. Examined	No. positive	% positive
Cattle	2,509	286	11.4
Sheep/ goat	492	0	0
Pig	1,503	4	0.3
Horse	477	1	0.2
Dog	170	2	1.2
Cat	52	0	0
Others	751	4	0.5
Total	5,954	297	5.0

Molecular characterisation of the material is ongoing, thus it is not known whether all positive samples belong to zoonotic species. However, so far only *C. parvum*, genotype II has been detected in specimens from Danish cattle whereas the genetic variation in samples from other animal species has been larger.

Humans

Cryptosporidiosis is not a notifiable disease in Denmark and the incidence is not known. At most diagnostic laboratories in Denmark, only patients with persistent diarrhoea or a history of recent travel are routinely examined for *Cryptosporidium*. At Statens Serum Institut 84 patients were diagnosed in year 2001 (Tabel 11). It is estimated that approximately 200 patients were diagnosed in the country as a whole during 2001.

8. Mycobacterium bovis/tuberculosis

In accordance with Commission Decision 99/467/EEC as amended by Decisions 2000/69/EEC, 2000/442/EEC, 2000/694/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.

Since December 1994, bovine tuberculosis has not been diagnosed in deer in Denmark.

Bovine tuberculosis in humans has been a notifiable disease since May 1st 2000. In year 2001, four cases (<0.1 case per 100,000 inhabitants) of human tuberculosis caused by *M. bovis* were registered (Table 11). The four patients were all born before 1938, and one of them was a foreigner. Their disease is regarded as either imported or as reactivations of latent infections acquired before the eradication of bovine tuberculosis in cattle.

9. Brucella abortus/ melitensis

Cattle

In accordance with Commission Decision 99/466/EEC as amended by Decision 2000/69/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 subject to pre-entry testing, followed by testing at least every 18 months and before they leave the centre. B. abortus has not been detected in 2001.

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 is performed by testing for *Brucella* antibodies in blood samples from sheep and goats submitted as a part of a voluntary control programme for lentivirus. In 2001, 6.234 blood samples from 761 herds were examined.

Humans

Infection in humans is not notifiable in Denmark. At Statens Serum Institut 18 persons were found positive by serology in 2001 (14 positive for *B. abortus*, 2 for *B. melitensis* and 2 for both). Travel association is not known.

10. Leptospira spp.

Leptospirosis in animals is not a notifiable disease. Examination for leptospires in pigs is performed by antigen detection, culture and serology. Suspicion of leptospirosis is often based on increased incidense of abortions or other reproductive problems. In 2001, leptospires were detected by immunoflourescens test in four pig herds. Two herds were infected with *L. pomona*, one with *L. bataviae* and one possibly with *L. bratislava*. Antibodies against *L. bratislava* are frequently detected in blood samples from sows. However, *L. bratislava* has never been isolated from Danish pig herds, and the significance of this serovar is unknown.

Leptospirosis in humans is notifiable in Denmark. In year 2001, nine patients were diagnosed by serology, this number being close to the annual average of 12 patients (238 cases over the past 20 years, Table 11). All patients were hospitalised, but later recovered. Six of the nine patients were infected with the serotype *L. icterohaemorrhagiae*, indicating rats as the primary reservoir of infection. In general, *L. icterohaemorrhagiae* is responsible for about 70% of the cases. The remaining serotypes are primarily ones carried by mice, in particular *L. sejroe*.

11. Trichinella spiralis/ nativa

The disease has 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. During 2001 samples from 21,516,771 pigs were examined, and none of the samples were found to contain *Trichinella* spp. One single parasite larva resembling *Trichinella* was found. The finding could not be confirmed by further examination of the larva nor by testing of the 15 herds related to the slaughter batch.

It is also compulsory to examine slaughtered wild boars. The Danish Veterinary and Food Administration was informed of 1,678 examinations, all of which were negative.

All horses which are slaughtered at Danish slaughterhouses approved for intra-Community trade are examined for *Trichinella* spp. During 2001, samples from 1,245 horses were examined, and none of the samples were found to contain *Trichinella* spp.

No domestically acquired cases of human trichinosis were recorded in year 2001. Generally, a few imported cases occur annually. Human trichinosis is not a notifiable disease.

12. Echinococcus granulosus/multilocularis

Echinococcus granulosus/multulocularis infections in all animals are notifiable. Surveillance for *E. granulosus* is performed through meat inspection. In 2001, there were no findings.

In 2001, 145 foxe were examined at the Royal Veterinary and Agricultural University. None were positive.

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

13. Toxoplasma gondii

Toxoplasmosis in humans is not a notifiable disease in Denmark, and the incidence of toxoplasmosis in humans is unknown. A study from 1999 indicates low incidence as only 0.2% of seronegative women seroconverted during pregnancy. In 2001, 19 newborns were found to have toxoplasmosis through the national neonatal screening system.

14. Rabies

Rabies is a notifiable infection in both humans and animals. In 2001, 20 wild bats were submitted for diagnosis and 2 were found infected with European bat lyssa virus by the Danish Veterinary Institute for Virus Research. Moreover 184 bats from zoological gardens and 13 other animals were examined, but none found infected.

No human cases were reported in 2001, but 11 people were treated by prophylactic vaccination after suspected exposure in Denmark. Of these, eight suffered from bat bites (none of these bats were examined) and three from bites from other animals suspected of being infected. In addition, 56 people were treated by prophylactic vaccination after exposure abroad to bites from bats or other animals suspected of being infected.

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 2001 report will be available at:

http://www.vetinst.dk

or can be ordered from the Danish Zoonosis Centre (dzc@dzc.dk) by the end of June 2002.

Automatic early warning outbreak detection system

Throughout 2001 a computerised outbreak detection system has been used routinely. The core of the system is an outbreak algorithm, which looks for temporal and geographical clusters among humans testing positive for bacterial gastro-intestinal pathogens. This is possible because all clinical microbiological laboratories supply all data on confirmed cases in a person identifiable format to a central database at the Statens Serum Institute as soon as the data are available.

The outbreak algorithm is applied on a weekly basis and uses information about the number of patients over the past five years to calculate an outbreak threshold. If the present number of patients surpasses the threshold, the computer issues a warning and an epidemiological evaluation of the situation is then made. Edited versions of the results of the outbreak calculations are also published on the Internet each week along with the human surveillance data. The website is mainly intended for Danish food safety and public health officials, but is generally admissible and exists in an English language version at the address: http://www.germ.dk.