

# Annual Report on Zoonoses in Denmark 2002

Human Salmonellosis in Denmark



Ministry of Food, Agriculture and Fisheries

#### **Annual Report on** Zoonoses in Denmark 2002

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

Data in this report were provided by The Danish Plant Directorate

- (www.pdir.dk)
- The Danish Veterinary and Food Administration (www.fdir.dk)
- Danish Veterinary Institute (www.vetinst.dk)
- Statens Serum Institut (www.ssi.dk)
- The Royal Veterinary and Agricultural University (www.kvl.dk)

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Suggested citation: Anonymous, 2003. Annual Report on Zoonoses in Denmark 2002, Ministry of Food, Agriculture and Fisheries.

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

The report is also available from: www.vetinst.dk

The cover page illustration: Estimated major sources of human salmonellosis in Denmark, 1988-2002

## **Contents**

4.

1.	Salmonella	4
	- Feeding stuffs	4
	- Rendering plants	4
	- Poultry and poultry products	4
	- Pigs and pork	7
	- Cattle and beef	8
	- National surveillance of S. Dublin in cattle	9
	- Wildlife and pet animals	10
	- Products from retail outlets	10
	- Occurence of multi-drug resistant S. Typhimurium DT104	11
	- Salmonellosis in humans	11
	- Risk assessment of sources of human salmonellosis	12
	- Outbreaks of zoonotic gastrointestinal infections	13
	- Tracing sources of human salmonellosis	15
2.	Campylobacter spp.	18
	- Poultry	18
	- Pigs and cattle	18
	- Wildlife and pet animals	18
	- Products from retail outlets	18
	- Campylobacteriosis in humans	19
	- Intensive surveillance of human Campylobacter	
	infections by typing	19
	- Serotyping of Campylobacter	20
3.	Yersinia enterocolitica	21
	- Pigs	21
	- Products from retail outlets	21
	- Yersiniosis in humans	21
4.	Listeria monocytogenes	22
	- Products from retail outlets	22
	- Listeriosis in humans	22
5.	Verocytotoxigenic Escherichia coli	23
	- Cattle	23
	- Products from retail outlets	23
	- Human infections	23
6.	Transmissible Spongiform Encephalopathy	24
	- Cattle	24
	- Sheep and goats	25
	- Humans	25
7.	Cryptosporidium parvum	26
8.	Mycobacterium bovis/tuberculosis	26
9.	Brucella abortus/melitensis	26
10.	Leptospira spp.	27
11	Trichinella spiralis/nativa	27
12	Febinococcus granulosus/multilocularis	~1 97
12.	Toxonlasma gondii	~1 28
1/	Rahios	20 29
14. App	andiv	20 20
лрр		29

## 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). The web edition also includes age and gender distributions of the major human zoonotic infections.

#### **Demographic data**

Human population in 2002						
Age group (years)	Male	Female	Total			
<1	33,479	31,991	65,470			
1-4	138,343	131,694	270,037			
5-14	343,842	325,854	669,696			
15-24	303,817	294,701	598,518			
25-44	798,988	773,220	1,572,208			
45-64	701,827	696,014	1,397,841			
>65	333,850	460,734	794,584			
Total	2,654,146	2,714,208	5,368,354			
Sources The Statistical Veenhaals 2002						

Average number of livestock and herds in Denmark, 2002

	Livestock	Herds
Cattle	1,835,960	34,693
Pigs	7,646,553	20,151
Laying hens excl. barn yard sale	3,548,165	361
Broilers	36,367,339	329
Sheep	199,405	8,654

Source: The Central Husbandry Register

### Approximate total number of animals slaughtered in 2002

Pigs:	22,2 million
Broilers:	138,7 million
Cattle:	607,100
Sheep, lambs and goats:	65,700
Horses:	2,889
Source: The Danish Veterina Administration	ry and Food

Area of Denmark: 44,000 sq km

#### **Profile of the year**

Danmarks Statistik

#### Trends

Compared to 2001, a general decrease was seen in the number of human infections caused by some of the most important zoonotic pathogens, including S. Enteritidis and S. Typhimurium. This is suggestive of a continuing positive effect of the Salmonella control programmes that are currently in force in Denmark. In contrast to infections caused by most other Salmonella serotypes, the number of human S. Dublin infections increased in 2002. While the number of cattle herds clinically infected with this serotype has remained at the same level for the past decade, the number of human infections doubled from 2001 to 2002. The number of human *Campylobacter* infections was slightly lower (5%) than the previous year. A similar reduction was not observed in the primary production.

#### **Events**

Two relatively large generalised outbreaks occurred in Denmark in 2002. An outbreak of *S*. Java infections occurred during the summer of 2002, comprising 25 cases. The outbreak lasted only a couple of weeks and cases were seen throughout the country. Despite investigations, no definite source was identified. Another nation-wide outbreak involving *S*. Typhimurium DT120 with a specific antimicrobial resistance profile was observed. From mid August to mid October 25 cases were registered. Through the *Salmonella* surveillance programme for poultry in Denmark and through patient interviews, a batch of sliced smoked turkey was identified as the most likely source of the outbreak.

#### Surveillance

Minor adjustments were made to the Salmonella surveillance programme for poultry (p. 4) and to the serological surveillance of slaughter pigs (pp. 7-8). The administration of the latter programme was transferred from the Danish Veterinary and Food Administration to the Danish Bacon and Meat Council. Furthermore, a national surveillance programme for S. Dublin in cattle was implemented in October 2002 (p. 9) and the national surveillance programme for BSE in cattle was expanded to include scrapie in sheep and goats (pp. 24-25).

## 1. Salmonella

#### **Feeding stuffs**

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

In general, the Salmonella prevalence in compound feed decreased from 2001 to 2002, and the overall level is low (Table 1).

However, the incidence of Salmonella in feed materials for pets (dried pigs' ears etc.) increased from 4.5% in 2001 to 10.0% in 2002. The occurrence of Salmonella in 2002 was connected to imported dried pigs' ears produced at one specific factory.

#### **Rendering plants**

Control of the hygiene at rendering plants is carried out by

Table 1. Control of Salmonella in compound feeds, feed processing and feed materials in 2002.

	Nui % S	Jumber of samples/ Serotypes, 2002 6 Salmonella positive (Number of isolates		Serotypes, 2002 (Number of isolates)
	2000	2001	2002	
% samples where Salmonella was detected	N/%	N/%	N/%	
Compound feeds in total	2,516/0.3	2,616/0.2	2,708/0.1	
Feed for pigs	1,436/0.3	1,552/0.1	1,498/0.0	
Feed for cattle, horses, sheep and rabbits	721/0.6	741/0.4	754/0.3	S. Infantis (2)
Feed for poultry	249/0	262/0	350/0	-
Pet food	110/0	61/0	106/0	-
Feed materials in total	382/3.9	332/1.8	349/3.7	
Farm animals	293/2.4	244/0.8	269/1.9	S. Mbandaka (1), S. Senftenberg (1), S. Agona (1), S. Infantis (1), S. Montevideo (1)
Pets	89/9.0	88/4.5	80/10	S. 4.12:1- (monofatisk) (1), S. Anatum (1), S. Derby (1), S. Give (1), S. Kedougou (1), S. Livingstone (1), S. Ohio (1), S. Typhimurium DT1(14 (1)
Feed processing plants (process control)	2,994/2.9	2,946/2.7	3,002/2.7	<i>c.</i> 1 <i>jpiiiiaiaiiiiiiiiiiiii</i>
Ordinary inspections	2,006/1.4	2,679/1.0	2,740/1.2	S. 4.12:b:- (1), S. Idikan (5), S. Infantis (5), S. Poona (1), S. Putten (6), S. Tennessee (3), S. Cubana (2), S. Othmarschen (1), S. Senftenberg (3), S. Enteritidis (1), S. Godesberg (1), S. Liverpool (3), S. Meleagridis (1)
Additional inspections	110/20.9	267/20.5	262/18.3	(not relevant)

Distinction between results from ordinary and additional inspections at feed processing plants began April 2000. Source: Danish Plant Directorate

the Animal Health Section of the Danish Veterinary and Food Administration. In 2002, 269 samples of meat and bone meal were examined for Salmonella and six samples (2.2%) were found positive. Isolates from four of these were serotyped and the following serotypes were found: S. Havana (2), S. Livingstone (1) and S. Montevideo (1).

Furthermore, 406 samples of fishmeal were examined. One sample (0.25%) was found positive for S. Tennessee.

#### **Poultry and poultry** products

In 2002, the Danish Salmonella surveillance and control programme was generally continued as described in the Annual Report on zoonoses in Denmark 2000 and 2001. The sampling scheme is summarised in Table 2 and the numbers of establishments in the broiler and table-egg production are shown in Table 3.

An alteration of the programme, concerning only central rearing and breeder flocks, was implemented in September 2002. This allowed flocks, which had been found infected with exotic Salmonella serotypes (serotypes other than S. Typhimurium or S. Enteritidis) to be declared free of Salmonella following further testing and assessment. The regulations apply specifically to flocks where discrepancies occur between the results obtained from the initial samples and the follow-up samples. The change was made based on the fact that the exotic serotypes are rarely isolated from the followup samples and could not be isolated from the broiler and layer flocks.

#### **Table-egg production**

In 2002, no central rearing flocks or layer breeders (hatching-egg production) were found infected with Salmonella. Out of 330 examined rearing flocks, 9 (2.7 %) were found infected with Salmonella. Of the positive rearing flocks, 5 were infected with S. Enteritidis, 2 with S. Typhimurium and 2 with S. Lexington (Table 4).

In flocks producing table-eggs for authorised egg-packing centres, 16 (2.6%) of 619 tested flocks were infected with Salmonella and 15 of these were infected Table 3. Number of farms in the broiler production and the tableegg production in 2002.

	No. of farms	No. of houses	No. of animals
		Broiler production	
Central rearing	28	117	1,244,000
Broiler breeders	68	207	1,180,000
Hatcheries	7	-	-
Broilers	350	839	143,287,000 <sup>a)</sup>
		Table-egg production	
Central rearing	7	8	60,000
Layer breeders	7	12	60,000
Hatcheries	5	-	-
Rearing	112	177	3,050,000 <sup>a)</sup>
Layers, excl. barn-			
vard sale	305	429	3.500.000 <sup>b)</sup>

a) Hatched for use in Denmark.

b) Including hens imported as day-old chickens.

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

Table 2. Salmonella	a surveillance o	of the broiler	and table-egg	production,	2002.
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	CENT Bro	RAL - REARING STATIONS iler and table-egg sector
Age	Samples taken	Material
Day-old chickens	Per delivery	10 samples of crate material and 20 dead chickens $^{\rm a)}$
1 week	Per unit	40 chickens
2 weeks	Per unit	2 pairs of sock samples
4 weeks	Per unit	60 faecal samples <sup>a)</sup>
8 weeks	Per unit	2 pairs of sock samples
2 weeks prior to moving	Per unit	60 faecal samples and 60 blood samples $^{a)\astb)}$
	BREEDERS Bro	(HATCHING-EGG PRODUCTION) iler and table-egg sector
Every 2 <sup>nd</sup> week	Per flock	50 dead chickens or meconium from 250 chickenstaken from the hatchery $^{\rm a)+c)}$
Every week	Per unit	2 pairs of sock samples <sup>d)</sup>
		HATCHERY
After each hatching	1-4 hatchers may be pooled	Wet dust
	REARINO	G - TABLE-EGG PRODUCTION
Age	Samples taken	Material
Day-old chickens	Per delivery	10 samples of crate material and 20 dead chickens
3 weeks	Per flock	5 pairs of sock samples or 300 faecal samples <200 animals/flock: 2 pairs of sock samples or 60 faecal samples
12 weeks - (2 weeks prior to moving)	Per flock	>500 animals/flock: 5 pairs of sock samples or 300 faecal samples, and 60 blood samples 200-499 animals/flock: 5 pairs of sock samples or 300 faecal samples, and 55 blood samples <200 animals/flock: 2 pairs of sock samples or 60 faecal samples, and blood samples <sup>b) + e)</sup>
	TABLE-EGG PRODUCTION	I for authorised egg packing/product business
Every 9 <sup>th</sup> week	Per flock	2 pairs of sock samples or faecal samples and eggs
	Barn-yard	sale or hobby poultry keeping
Every 18 <sup>th</sup> week	Per flock	Egg samples <sup>e)</sup>
a) Requirements of the Zoonos	sis 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.

e) According to Table 1 in Order no. 863, Oct  $1^{st}$ , 2001.

Source: The Danish Veterinary and Food Administration

with S. Enteritidis (Table 4).

In flocks producing table-eggs for barnyard sale, 10 (2.1%) of 479 examined flocks were confirmed infected with Salmonella. All flocks were declared infected based on the serological results only.

The overall prevalence of Salmonella was higher among battery flocks (5.5%) than among flocks from other production types (2.0-3.1%) in 2002. The number of infected flocks within the different types of production is shown in Figure 1.

In 2002, the overall prevalence of Salmonella in the table-egg production was reduced, compared to the previous year. This may be explained by the fact that a number of farms, which previously had problems with Salmonella infections in consecutive flocks, delivered eggs for heat treatment only. Therefore, they contributed with no newly infected flocks in 2002.

#### **Broiler production**

One central rearing flock was found infected with Salmonella in 2002 and the serotype and phagetype was determined to be *S*. Typhimurium DT12. Among broiler breeders, 330 houses were examined and 4 houses were found infected. The 4 houses represented 2 flocks and both flocks were infected with *S*. Typhimurium DT41 (Appendix, Table A1).



Figure 1. Number of layer flocks infected with Salmonella shown according to type of production, 2002. Number of flocks by type of production, 2002: Battery: 127; deep litter: 123; organic: 130; free-range: 49; barnyard: 479. Source: The Danish Veterinary and Food Administration

All production broiler flocks were monitored for Salmonella by the mandatory ante-mortem examination. Two-three weeks prior to slaughter, five pairs of sock samples were collected from each flock. The percentage of positive flocks ranged from 0.5% to 2.7% per month with a mean of 1.5% (Table A1, Figure 2). The most frequently occuring serotype was S. Indiana, which was found in 22.4% of the infected flocks. The serotype distribution is presented in Table 11.

In 2002, the mandatory examination of broilers post slaughter was done by sampling batches of chicken cuts close to packaging. The definition of batches has been described in Annual Report on zoonoses in Denmark 2000. Salmonella was detected in 91 (5.5%) of 1,667 investigated batches of chicken meat. Approximately one half of the positive batches originated from one small slaughterhouse and was mainly the result of cross-contamination from slaughtered hens infected with S. Enteritidis. This particular slaughterhouse is now closed.

#### **Turkey production**

All turkey flocks were monitored for Salmonella by the mandatory ante-mortem examination. Salmonella was detected in 27 (8.4%) of 323 investigated flocks (Table A2). Of these, 13 flocks were infected with S. 4.12.d, 6 flocks with S. Saintpaul, 5 flocks with S. Typhimurium and 3 flocks with other serotypes. S. Enteritidis was not isolated from any turkey

Table 4. Occurrence of Salmonella in the table-egg production in 2002.

	Central re	earing <sup>a)</sup>	Layer br	reeders <sup>a)</sup>	Rear	ring	Table-egg p	production
Zoonotic pathogen	Examined houses	Positive houses	Examined houses	Positive houses	Examined flocks	Positive flocks (%)	Examined flocks	Positive flocks (%)
Salmonella spp.	15	0	22	0	330	9 (2.7%) <sup>b)</sup>	619	16 (2.6%)
S. Enteritidis	-	-	-	-	-	5 (1.5%) <sup>c)</sup>	-	15 <sup>c)</sup> (2.4%)
S. Typhimurium	-	-	-	-	-	2 (0.6%)	-	1 (0.3%)
Other serotypes	-	-	-	-	-	$2 (0.6\%) d^{-1}$	-	0

a) One flock may be placed into several houses.

b) Three rearing flocks were infected with two different serotypes.

c) For one flock, the serotype was diagnosed as S. Typ./S. Ent based on serology alone.

d) One farm had 2 houses infected with *S*. Lexington at the same time.

Source: The Danish Veterinary and Food Administration



Figure 2. Percent Salmonella positive broiler flocks detected at the mandatory ante-mortem and endproduct examination, 1998-2002. Post-mortem examinations was replaced by end-product examinations in November, 2000. Source: The Danish Veterinary and Food Administration and the Danish Poultry Council



Figure 3. Percent Salmonella positive turkey flocks detected at the mandatory ante-mortem and post-mortem examinations, 1998-2002. Source: The Danish Veterinary and Food Administration

### flocks in 2002. The serotype distribution is shown in Table 11.

Salmonella was detected in 128 (39.2%) of 323 flocks examined after slaughter by testing five pools of 10 neck-skin samples from each flock (Table A2, Figure 3). In May 2002, the percentage of Salmonella positive neck skin samples exceeded 70%, due to a temporary problem with S. Typhimurium DT 120 contamination of a newly installed steamscalder at a major turkey slaughterhouse in Denmark. A similar increase was seen again in September, but no specific cause for this increase was identified. In November and December all antemortem Salmonella positive flocks were exported prior to slaughter, and Salmonella was not found in the neck skin samples during these two months.

#### **Duck production**

Duck flocks were monitored by the mandatory ante-mortem examination three weeks prior to slaughter. In 2002, 190 flocks were examined. Salmonella was isolated from 104 (54.7%) of the flocks. In several cases, more than one serotype was isolated from each flock (Table 11). S. Anatum was the most frequently isolated serotype found in the infected flocks.

#### **Pigs and pork**

A serological surveillance programme for detection of Salmonella infection in slaughter pig herds was implemented at the beginning of 1995. The programme was changed in 2001, and has previously been described in Annual Report on zoonoses in Denmark 2000 and 2001. Originally, the Danish Veterinary and Food Administration handled the administration of the programme. However, since May 2002, this task has been taken over by the Danish Bacon and Meat Council.

Herds producing more than 200 slaughter pigs per year are monitored by serological testing of meat juice. Herds are assigned to one of three levels based on the proportion of seropositive meat juice samples over the last three months. The samples are weighted (0.2:0.2:0.6), so that results from the most recent month are given more weight. The weighted average is referred to as the serological Salmonella index of the slaughter pig herd. Level 1: herds with no or few reactors. Level 2: herds with a higher proportion of reactors. Level 3: herds with an unacceptably high proportion of reactors from which



Figure 4. Serological surveillance of Salmonella in slaughter pig herds, percent herds within level 2 and 3, 1996-2002. The criteria for assignment to levels 2 and 3 was changed in August 2001. Source: The Danish Veterinary and Food Administration

pigs must be slaughtered under special hygienic precautions. Herds in Level 2 and 3 are obligated to collect pen-faecal samples in order to clarify the distribution and type of the Salmonella infection in the herd. Furthermore, the Danish Bacon and Meat Council employs a financial penalty system, in order to encourage implementation of control measures in the infected herds.

By the end of 2002, 96.8% of the herds fell within level 1: 2.3% within level 2 and 0.9% within level 3 (Figure 4).

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 obligated to collect pen-faecal samples. Formerly, movement of livestock from herds with serological levels exceeding a specific high level was restricted. However, these restrictions were discontinued in May 2002.

Sow herds supplying piglets to slaughter pig herds in level 2 or 3 are also obligated to collect penfaecal 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.

Since April 2002, sow herds have been exempt from collecting the above mentioned pen faecal samples in cases where only negative results have been obtained from five or more meat juice samples from slaughter pigs originating from the sow herd from the previous three months of surveillance.

Clinical salmonellosis was recorded in 54 herds (Table 5). This figure represents the number of herds submitting material from clinically affected animals to the laboratory. Of these, 11 herds were placed under official veterinary supervision including two herds that were placed under Zoonosis Supervision, due to isolation of multi-drug resistant Salmonella Typhimurium DT104.

All data concerning the surveillance of Salmonella in pigs are registered in a database, the Zoonosis Register. This register is a part of the Central Husbandry Register and is administered by The Danish Veterinary and Food Administration.

Monitoring of Salmonella in pork is based on swab samples taken from three designated areas of chilled half-carcasses.



Cuts of pork -Swabs of half-carcass

Figure 5. Salmonella in pork monitored at slaughterhouses, 1995-2002. From January 2001 monitored by swab samples of halfcarcasses

Source: The Danish Veterinary and Food Administration

The samples are pooled, each pool consisting of samples from 5 carcasses, except in the smallest slaughterhouses, where the samples are analysed individually.

In 2002, 36,690 samples were pooled into 7,338 pools, which were then analysed. Salmonella was found in 302 of these. Furthermore, 97 samples were collected and analysed individually, and Salmonella was found in two of these samples (Table A3).

When determining the prevalence of pooled samples, the loss of sensitivity and the probability of more than one sample being positive in each pool has to be taken into consideration. A conversion factor, 3, has been determined on the basis of comparative examinations, as described in Annual Report, 2001.

On this basis, the overall prevalence for 2002 was 1.4%. The prevalence of Salmonella positive carcasses per month ranged from 1.0% to 1.8% (Figure 5).

#### **Cattle and beef**

In July 2002 new regulations regarding handling of cattle and carcasses from herds infected

Table 5. Isolation of Salmonella from outbreaks of clinical disease in pig and cattle herds in 2002.

Serotype	Pig herds	Cattle herds
Bovismorbificans		1
Brandenburg	1	
Derby	10	
Dublin		72
Infantis	2	
Stanley		1
Wien		1
Virchow	1	
Typhimurium DT104	6	4
Other Typhimurium	33	25
Worthington	1	
Rough, not typable		3
Total	54	107

Source: The Danish Veterinary and Food Administration

### National surveillance of Salmonella Dublin in cattle

In October 2002, the Danish Veterinary and Food Administration implemented a national surveillance programme for S. Dublin. The programme is based on Order no. 974 and was developed in cooperation with the Danish Veterinary Institute and the cattle industry.

The programme is based on serological testing of blood and milk samples collected for the BVD and IBR surveillance programmes. The samples are tested by a S. Dublin ELISA-test. In contrast to the serological programme in the pig production, this programme aims at identifying herds free of S. Dublin infection. This approach was chosen, because epidemiological analyses indicated that the serological test is better at identifying truly non-infected herds than truly infected herds. The overall objective is, therefore, to keep noninfected herds free of infection by avoiding purchase of animals from infected or possibly infected herds.

All dairy herds are tested by bulkmilk samples collected every three months. Based on the serological results, 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 of salmonellosis are present. For dairy herds 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% (calibrated Optical Density %), 2) the latest bulk-milk sample may not exceed the average of the three previous samples with more than 20 OD%, 3) S. Dublin has not been isolated from any samples collected from the farm within the previous three months. The S. Dublin status of a cattle farm is revised at least once every three months. Furthermore, all animals from farms diagnosed with salmonellosis caused by S. Dublin are slaughtered under special hygienic precautions and each carcass must be swabbed and tested bacteriologically. Meat from positive carcasses must be heat-treated.

## Occurrence in the primary production

In 2002, 30,868 bulk-milk samples and 54,458 blood samples were analysed. The percentage of herds in level 1-3 are shown in Table 6. Salmonellosis was diagnosed in 107 cattle herds in 2002. Of these, 72 herds were found infected with S. Dublin. The predominant serotypes isolated from clinical cases in cattle in 2002 were S. Dublin (67%) and S. Typhimurium (27%) (Table 5).

#### **Occurrence** in beef

The occurrence of Salmonella in beef is monitored at the slaugherhouse by collecting swab samples from chilled half-carcasses. The overall prevalence of Salmonella in beef increased from 0.1% in 2001 to 0.2% in 2002. In 2002, 13 out of 21 isolates were identified as S. Dublin, whereas 7 out of 12 isolates were S. Dublin in 2001.

#### **Human infections**

In contrast to infections caused by other Salmonella serotypes such as S. Enteritidis and S. Typhimurium, the number of infections caused by S. Dublin increased in 2002. In 2001, 26 cases were registered, whereas 43 cases were registered in 2002 (Figure 7). The number of human S. Dublin cases is monitored closely because of the high invasiveness of this serotype.



Figure 7: Number of human cases and cattle herds with clinical salmonellosis caused by S. Dublin, 1979-2002 Source: The Danish Veterinary and Food Administration and Statens serum Institut

Table 6. Percentage of cattle farms in each level at the start of the S. Dublin surveillance programme, October 2002

S. Dublin Level	Milk producing herds (Approx. %)	Non-milk producing herds (Approx. %)
Level 1	76	52
Level 2	23	47 <sup>a)</sup>
Level 3	0,4	0,05

a) Not all non-milk producing herds had to be tested from the starting point of the programme. Source: The Danish Veterinary and Food Administration with *S.* Dublin were implemented. The *S.* Dublin programme has previously been described in Annual Report, 2001 and further details are given on page 9.

Monitoring of Salmonella in beef and veal at slaughterhouses is based on swab samples taken from three designated areas of chilled half-carcasses. The samples are pooled, each pool consisting of samples from five carcasses, except in the smallest slaughterhouses, where the samples are analysed individually.

In 2002, 12,700 samples were pooled into 2,540 pools, which were then analysed. *Salmonella* was found in 18 of these. Furthermore, 813 samples were collected and analysed individually, and *Salmonella* was found in three of these samples.

Using the 3 as correction factor in the samme manner as reported for pork, the overall sample prevalence for 2002 was estimated to be 0.2% (Table A4). The prevalence of Salmonella positive carcasses per month ranged from 0% to 0.7%.

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 nine (3.6%) of 251 caecal samples collected from one animal per herd at slaughter (Table A4).

## Wildlife and pet animals

The Danish Veterinary Institute 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 investi-



Figure 6. Registered cases of human S. Typhimurium DT104 (including DT104b) in Denmark, 1995-2002. Source: Statens Serum Institut

gated on clinical indication only. The prevalence of *Salmonella* in pets, wildlife and zoo animals in 2002 is shown in Table A5.

## **Products from retail** outlets

At the retail level, the Danish Veterinary and Food Authorities collect samples for routine surveillance of meat and products hereof. As part of a new strategy, the number of samples collected for control purposes at this level was reduced in 2002 compared to previous years. The Danish Food Authorities have decided that 20 % of the resources, previously used for microbiological examinations of foods at retail, should be allocated to so-called "centrally coordinated projects". These projects focus on collecting data on prevalence and concentration of specific pathogens in foods during

processing and at retail, and the purpose of the new strategy is to provide the necessary data for ongoing and future risk assessment analyses.

A total of 153 broiler and broiler products, 48 samples of turkey cuts and turkey products, 8,120 samples of pork and pork products, and 1,635 samples of beef and beef products were examined in 2002. In samples from non heat-treated meat and meat products the prevalence was 0%, 0%, 1.3%, and 1.0 % respectively. Salmonella was not isolated from any heat-treated products (Tables A1, A2, A3, A4).

In 2002, Salmonella screening of Danish and imported shell eggs was carried out by the Danish Veterinary and Food Administration. Out of 10,300 sampled Danish eggs, 6 (0.06%) were infected on the shell, and 2 (0.02%) were

Table 7.	Number	of Salmo	onella po	sitive	samples
obtained	from im	ported poi	ıltry, pork	and	beef, 2002

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Imported product	Number of samples	Positive (%)	Positive for DT104 (%)
Poultry	2,215	322 (14.5)	3 (0.1)
Pork	710	22 (3.1)	5 (0.7)
Beef	643	1 (0.2)	1 (0.2)
Others	80	2 (2,5)	0
Total	3,648	347 (9.5)	9 (0.3)

Source: Danish Veterinary and Food Administration

infected in the yolk/white. All Danish eggs were grade A, and therefore qualified for retail sale. Of 4,660 imported eggs, 27 (0.58%) were found infected on the shell, and 12 (0.26%) were infected in the yolk/white. The imported eggs were both grade A and B. It should be noted that the imported eggs originated from very few packing companies and may, therefore, not be representative of the Salmonella status of the country of origin.

#### Occurrence of multidrug resistant *S.* Typhimurium DT104

In 2002, the occurrence of multidrug resistant *S*. Typhimurium DT104 (MRDT104) decreased in the primary production. A total of 22 herds were found infected with MRDT104 and this was approximately half as many as the previous year.

Monitoring of MRDT104 in fresh meat imported from the EU and third countries continued in 2002. The overall prevalence of MRDT104 in imported meat was 0.25% (9 of 3,648 samples), which is similar to what was seen in 2001. In contrast, the prevalence of S. enterica decreased slightly from 13.3% to 9.51% (Table 7).

## Salmonellosis in humans

The number of human Salmonella infections reached an all time high in 1997, but has since then decreased every year, except for the year 2001. In 2002, a total of 2,071 laboratory confirmed episodes of salmonellosis were reported (38.6 cases per 100,000 inhabitants, Table A6). The incidence thus reached the lowest level since 1986 (Figure 8) representing a decrease of 29% compared to 2001 and of 10% compared to 2000. The decline was observed in of almost all Salmonella serotypes compared to the two previous years, including S. Enteritidis and S. Typhimurium. Based on data from eight out of 15 counties, 74% of the Salmonella infections were estimated to be domestically acquired.

In 2002, the number of *S*. Enteritidis episodes was 1,104 (20.6 cases per 100,000, Table A6), which was 22% lower than in 2001 and 9% lower than in 2000. The phage type (PT) distribution among 633 arbitrarily selected *S*. Enteritidis isolates from human infections is presented in Table 12. The most common phage types were PT8 (37%), PT4 (24%), PT6 (13%), PT1 (5%) and PT21 (5%). The proportion of the two dominating types, PT8 and PT4, has increased since 1999. The proportion of PT6, which was markedly reduced in 2000 and 2001, increased in 2002. Figure 10 shows the geographical distribution of the *S*. Enteritidis cases.

The number of S. Typhimurium episodes was 378 (7.0 per 100,000) in 2002, 36% lower than in 2001 and 13% lower than in year 2000. The phage type (DT) distribution of 370 cases is presented in Table 13. The most common phage types were DT120 (15%), DT104 (12%), DT12 (10%) and DTU302 (5%). This distribution resembled that of previous years. A total of 51 DT104 cases were registered in 2002 and 43 (84%) of these were caused by multi-drug resistant DT104. Thirteen DT104 cases (25%) were acquired abroad (Figure 6) and 12 (92%) of these were multi-drug resistant. Figure 11 shows the geographical distribution of S. Typhimurium cases.

The remaining 589 (11.0 cases per 100,000) zoonotic Salmonella cases were distributed among 96 different serotypes. Overall, cases caused by serotypes other than S. Typhimurium and S. Enteritidis decreased by 38% compared to 2001 and by 15% compared to 2000. The most commonly encountered serotypes among these cases were S. Java (48 cases), S. Dublin (43 cases), S. Agona (42



Figure 8. Registered cases of human salmonellosis in Denmark 1980-2002. Source: Statens Serum Institut



Figure 9. Seasonal variation in registered cases of human salmonellosis, 1997-2002. Source: Statens Serum Institut

cases), *S.* Bovismorbificans (41 cases), *S.* Stanley (33 cases) *S.* Virchow (31 cases), and *S.* Hadar, *S.* Infantis and *S.* Newport with 27 cases each (Table 11).

#### Risk assessment of sources of human salmonellosis

To obtain a better understanding of the dynamics of the occurrence of human Salmonella infections, the Danish Zoonosis Centre has applied a risk assessment model to estimate the contribution of the major animal and food sources to human cases of salmonellosis. The model is based on 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. From 2000 and onwards, the method has evolved from being purely deterministic to becoming stochastic. This new method of estimation allows for

the inclusion of uncertainty of the estimated parameters and takes differences in the ability to cause infection between types of Salmonella, as well as types of animal food sources into account.

In 2002, the estimated number of human cases (per 100,000 inhabitants) that could be attributed to various sources, was as follows: table eggs: 11.9; broilers: 0.8; pork: 1.4; turkeys: 0.5; ducks: 0.1; beef: 0.7; imported poultry products: 4.9; imported beef: 0.2; imported pork: 0.4; travel: 10.3; cases related to an outbreak of unknown origin: 0.5 (Figure 12). Approximately 359 cases (6.7 per 100,000) could not be associated with any specific source. Figure 13 shows the estimated number of cases caused by three major sources of infection (broilers, eggs and pork) from 1988 to 2002. Compared to 2001, the number of cases caused by eggs and pork decreased, while a slight increase was observed in cases caused by broilers.

Furthermore, 271 (25%) of the S. Enteritidis cases were estimated to be associated with travelling abroad. As reported in previous years, differences are observed between phage types acquired abroad and those that are acquired domestically. Thus, among the frequently encountered phage types, PT8 cases appear to be mainly domestically acquired (83%), while PT1 infections were mainly isolated from travellers (95%). Also, 53% of human PT4 infections and 48% of the PT6 infections were found to be travel associated.

Approximately 59% of *S*. Typhimurium infections were estimated to be domestically acquired, and infections caused by DT104 appeared to be more frequently associated with travel (64%) than other *S*. Typhimurium phage types. In contrast, infections caused by DT135 were almost exclusively acquired domestically (97%).

Overall, 22% of cases caused by serotypes other than *S*.



Figure 10. Geographical distribution of the number of cases per county and incidence of human infections with S. Enteritidis in 2002. Source: Statens Serum Institut



Figure 11. Geographical distribution of the number of cases per county and incidence of human infections with S. Typhimurium in 2002. Source: Statens Serum Institut

Enteritidis and *S*. Typhimurium were estimated to be travel associated, but estimates varied greatly between serotypes. Among the dominant serotypes, travel association was most frequently reported for *S*. Virchow (40%), *S*. Newport (30%) and *S*. Hadar (28%).

#### Outbreaks of zoonotic gastrointestinal infections

In Denmark, outbreaks of foodand water-borne infections caused by zoonotic agents are registered in three different systems. First, general practioners and hospitals are obligated 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 8).

Secondly, gastrointestinal pathogens identified at clinical microbiology laboratories are



Figure 13. Estimated major sources of human salmonellosis in Denmark, 1988-2002. Source: Danish Zoonosis Centre

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 9).

Thirdly, 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 10).

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.

In general, there were fewer outbreaks reported in 2002 than in the preceding years. This is in accordance with the general decrease in the number of zoonotic infections and Salmonella infections in particular. Outbreaks reported by



Figure 12. Estimated sources of 2071 human salmonellosis in Denmark in 2002. The estimated mean number of cases per source: 636 from table eggs, 550 travel associated, 146 from imported chicken, 100 from imported turkey, 77 from pork, 22 from imported pork, 29 from turkey, 41 from broilers, 37 from beef, 18 from imported duck, 11 from imported beef, 8 from ducks, 28 from S. Java outbreak and 359 of unknown source. Source: Danish Zoonosis Centre

physicians decreased from 116 in 2001 to 81 in 2002 (Table 8). Three nationwide outbreaks were found through the laboratory based surveillance system (see below) including 61 laboratory confirmed cases (Table 9). This compares to 230 cases in 2001 where there was a large general outbreak with *S*. Bovismorbificans.

In 2002, 30 outbreaks reported by the Regional Veterinary and Food Authorities were investigated. Of these, three were caused by zoonotic bacteria, 17 outbreaks were of unknown origin and in 10 outbreaks other causes were identified (Table 10). In total, 465 persons became ill. For 27 of the outbreaks, the suspected food was produced in approved food establishments (general outbreaks) and for three outbreaks produced in private homes (family outbreaks).

Table 8. Clinical based surveillance of suspected outbreaks of food-borne zoonotic diseases notified to Statens Serum Institut, 2002.

		General outbreaks		Outbreaks within household
Zoonotic pathogen	No. of outbreaks	Suspected source	No. of outbreaks	Suspected source
S. Enteritidis	10	Pancakes, omelet with chicken, eggs, sushi, turkey	11	Chicken, eggs, pizza, sausages
S. Typhimurium	4	Chicken salad	2	
Other zoonotic Salmonella spp.	4	Pork	4	
Campylobacter	10	Pizza, chicken	12	Meat, chicken, minced beef, eggs, pizza
Yersinia	0		1	
Virus or food toxin	2		1	
Unknown	13	Buffet, potato salad with beef, sausages, ice cream, pizza, soup, pork, chicken	7	Chicken, eggs, cake
Total	43		38	

Source: Statens Serum Institut

### Table 9. Outbreaks identified in the laboratory-based surveillance of zoonotic diseases, Statens Serum Institut, 2002.

Occasion and pathogen	No. of cases confirmed	Suspected source
General outbreak, S. Java	28	Unknown
General outbreak, S. Infantis	11	Pork
General outbreak, S. Typhimurium DT120, R-type ASSuT	22	Smoked turkey
Total	61	

Source: Statens Serum Institut

Table 10. Outbreaks of food-borne zoonotic diseases causes by zoonotic bacteria registered by the Regional Veterinary and Food Authorities in 2002.

Zoonotic pathogen	No. of out- breaks	Total number of sick persons	Suspected source (No. of outbreaks)	Confirmed by culture in foodstuffs/patients
Salmonella Enteritidis	1	14	Japanese omelette	-/+
Campylobacter	2	25	Fried chicken with potatoes and salad (1) Steaks of beef, turkey and pork and pasta salad (1)	-/+ -/+
Calicivirus	5	110	Various foodstuffs	
Other microorganisms	5	40	Various foodstuffs	
Unknown	17	276	Various foodstuffs	
Total	30	465		

Source: Danish Veterinary and Food Administration

In the summer 2002 there was an outbreak with S. Java encompassing 28 cases with an identical PFGE profile. The outbreak affected the entire country though half of the cases lived in one county. The outbreak came and went quickly and appeared to be a point-source outbreak. A case-control investigation performed after the outbreak had passed failed to reveal the source, but berries or vegetables produced in the county that was primarily affected may have been the source. The PFGE profile was different from the S. Java profile found in imported poultry (Table 11).

Another nation-wide outbreak was caused by S. Typhimurium DT120 with the resistance profile Amp, Strep, Sulfa and Tet. From mid August to mid October there were 25 cases with this otherwise unusual combination of phage type/resistance pattern of which 22 had identical PFGE profiles. Through the Salmonella surveillance program for poultry in Denmark and through patient interviews, a batch of sliced smoked turkey was identified as the likely source of infection.

#### Tracing sources of human salmonellosis by use of pulsed-field gel electrophoresis (PFGE)

The number of human Salmonella infections caused by serotypes other than S. Enteritidis and S. Typhimurium, decreased by 36% in 2002 compared to 2001 and 15% compared to 2000 (Table A6). However, the proportion of human Salmonella infections caused by these serotypes remained comparable to previous years; approximately 30%. In order to determine the source of human Salmonella serotypes that frequently occur in various animal sources, additional typing methods need to be applied. For this purpose, selected isolates, representing patients and each potential source, were genotyped by PFGE. The serotypes selected for PFGE typing were: S. Dublin, S. Agona, S. Hadar, S. Infantis, S. Saintpaul and S. Heidelberg. A maximum of 20 human isolates as well as non-human isolates from each source within the official Salmonella surveillance programmes were selected proportionally to their frequency in occurrence (i.e. representing a possible seasonal variation) and clustering of samples within herds/flocks were taken into account. Where no isolates were available from fresh products, isolates from the animal reservoir were used. The restriction enzyme XbaI was used for all analyses.

#### S. Dublin

Following a steady decrease in the registered number of human infections caused by S. Dublin between 1988 and 1995, an increase has been observed the last few years (69% from 2001 to 2002) (Figure 7). In 2002 it was ranked as the fourth most commonly isolated serotype among humans (third if an outbreak with S. Java involving 28 registered cases is omitted). S. Dublin was isolated from cattle herds, beef, finishing pig herds and pork (Table 11). Among the S. Dublin isolates. 33 were selected for PFGE (20 human isolates, 11 isolates from beef and 2 from pork). The results of the investigation showed five unique PFGE patterns. In general, the differences between the unique patterns were small, making the distinction between patterns difficult. In the Danish Salmonella surveillance programme, the majority of S. Dublin isolates have been isolated

from beef samples. In combination with PFGE typing results, this indicated that beef was the major source of human salmonellosis caused by *S*. Dublin, but pork could not be ruled out as a source.

#### S. Agona

Between 1998 and 2001, the human incidence of S. Agona more than quadrupled, reaching a total of 130 cases in 2001. In 2002 S. Agona was the fifth most prevalent serotype with an annual incidence of 42 cases (Table 11). S. Agona was isolated from nonhuman sources including pig herds, pork, broiler flocks, imported chicken and turkey meat (post-mortem samples). A total of 20 human and 24 non-human isolates were compared by PFGE. The results showed 27 unique PFGE patterns. The results suggested that Danish pork and turkey meat were important sources of human infections caused by S. Agona.

#### S. Hadar

Another frequently occurring serotype in humans was S. Hadar. During the previous five years, the annual incidence ranged from 58 to 74 cases, but with 27 registered cases in 2002, S. Hadar ranks 9th, along with the serotypes S. Infantis and S. Newport. S. Hadar was isolated from ducks and imported poultry (chickens, turkey and duck meat). A sample of 31 strains was selected for PFGE typing (20 human isolates and 11 isolates from various animal sources). The PFGE results indicated that imported poultry was an important source of human infections with S. Hadar in 2002.

#### S. Infantis

With 27 registered cases, *S*. Infantis was ranked among the 9<sup>th</sup> most registered serotypes in 2002. However, subtraction of 19 cases linked to a pork-related outbreak reduced the number of cases caused by S. Infantis to 8 cases in 2002 which was similar to the level in 2001 (10 cases). S. Infantis was further isolated from pig herds, pork, cattle herds, beef, broiler flocks and imported pork and poultry (turkey, chicken and duck meat). A sample of 48 strains was selected for PFGE typing (20 human isolates and 28 isolates from various animal sources). A total of 19 unique PFGE patterns were observed. Twelve non-human isolates, representing 8 unique PFGE patterns could not be matched with any of the selected human isolates. The typing identified S. Infantis isolates with a unique or closely related PFGE

pattern in a variety of sources. However, in conjunction with the observed prevalence of *S*. Infantis in the sources, these findings confirm that pork and domestic as well as imported poultry were the most common sources.

#### S. Saintpaul

S. Saintpaul was the 12<sup>th</sup> most commonly isolated serotype among humans in 2002, together with S. Oranienburg (13 registered cases). Both in 2000 and 2001, the number of registered human cases with S. Saintpaul was 18. In non-human reservoirs, it was most frequently isolated from poultry, domestic as well as imported. In an effort to distinguish the human cases attributed to domestic poultry from those attributed to imported poultry products, 24 isolates were analysed by PFGE (13 human isolates, 11 from non-human sources). Two human isolates could not be typed, but the remaining isolates resulted in 18 unique PFGE patterns. These PFGE results indicated that imported poultry and domestic turkeys were important sources. However, the source of a large proportion of human cases remains unexplained.

#### S. Heidelberg

The final serotype subjected to PFGE typing was *S*. Heidelberg. With 10 registered cases it ranked as the 16<sup>th</sup> most commonly isolated serotype in 2002, along with *S*. Braenderup and *S*. Derby. As in previous years, *S*. Heidelberg was most frequently isolated from

Table 11. Serotype distribution (%) of Salmonella from animals, carcasses at slaughterhouses, imported meat and humans in Denmark, 2002. 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 <sup>f)</sup>	
Serotype		Pig		Cattle		Layer	Broiler	Turkey	Duck				
	Human	herds <sup>a)</sup>	Pork <sup>b)</sup>	herds <sup>c)</sup>	Beef <sup>b)</sup>	flocks <sup>d)</sup>	flocks <sup>e)</sup>	flocks <sup>e)</sup>	flocks <sup>e)</sup>	Pork	Chicken	Turkey	Duck
	n=2072	n=713	n=306	n=117	n=21	n=16	n=67	n=29	n=127	n=39	n=152	n=95	n=56
S. Enteritidis	53.3	0.3	0.0	1.7	0.0	93.8	1.5	0.0	2.4	0.0	21.1	1.1	0.0
S. Typhimurium	18.5	72.7	42.8	26.5	14.3	6.3	14.9	6.9	0.0	46.2	1.3	3.2	57.1
S. Java	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
S. Dublin	2.1	0.1	0.7	64.1	61.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. Agona	2.0	0.8	1.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0	1.3	5.3	0.0
S. Bovismorbificans	1.9	0.0	0.0	0.9	0.0	0.0	1.5	0.0	0.0	0.0	0.0	5.3	1.8
S. Virchow	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.9	0.0	0.0
S. Newport	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	1.8
S. Hadar	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	2.6	21.1	12.5
S. Infantis	1.3	4.6	14.7	0.9	4.8	0.0	20.9	0.0	0.0	5.1	3.9	1.1	7.1
S. Saintpaul	0.6	0.0	0.0	0.0	0.0	0.0	0.0	24.1	1.6	0.0	3.9	3.2	8.9
S. Blockley	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0
S. Thompson	0.5	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
S. Derby	0.5	12.3	15.7	0.0	0.0	0.0	4.5	0.0	0.0	2.6	0.7	10.5	0.0
S. Heidelberg	0.5	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.9	17.9	0.0
S. Kentucky	0.4	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
S. Kottbus	0.4	0.0	0.0	0.0	0.0	0.0	3.0	0.0	25.2	0.0	0.7	4.2	5.4
S. Indiana	0.3	0.0	0.0	0.0	0.0	0.0	22.4	0.0	7.9	0.0	2.6	2.1	1.8
S. Panama	0.3	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0
S. Rissen	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.9	0.0	0.0	0.0
Others	12.1	5.3	10.5	2.6	4.8	0.0	17.9	62.1	59.1	20.5	17.1	21.1	1.8
Not typable	0.0	3.5	14.1	3.4	14.3	0.0	4.5	6.9	3.1	5.1	2.0	1.1	1.8
Total	100	100	100	100	100	100	100	100	100	100	100	100	100

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. Only one S. Typhimurium isolate was obtained from imported beef in 2002.

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

imported poultry (chicken and turkey meat) in 2000 and 2001. Furthermore, S. Heidelberg was isolated from domestic turkey flocks and imported duck. A total of 28 isolates were analysed by PFGE (10 human and 18 nonhuman isolates). The results showed 7 unique PFGE patterns. In combination with the observed prevalence in the various sources these results identified imported poultry meat as the most important source for human salmonellosis caused by S. Heidelberg.

Table 13. Phage-type distribution (%) of S. Typhimurium from humans, animals, carcasses at slaughterhouses and imported meat, 2002.

						Imported	meat <sup>f)</sup>
		Pig		Cattle			
Phage type	Human	herds <sup>a)</sup>	Pork <sup>b)</sup>	herds <sup>c)</sup>	Broilers <sup>e)</sup>	Pork	Duck
	n=370	n=535	n=131	n=31	n=10	n=18	n=32
DT120	15.4	9.2	8.4	3.2	0.0	11.1	0.0
DT104	11.6	3.7	2.3	6.5	10.0	33.3	3.1
DT12	10.3	37.9	28.2	61.3	30.0	5.6	0.0
DTU302	4.9	0.9	1.5	0.0	0.0	5.6	9.4
DT193	4.6	4.9	1.5	3.2	0.0	5.6	0.0
DT170	4.3	8.8	6.9	0.0	20.0	0.0	0.0
DT3	4.3	0.6	0.8	0.0	0.0	0.0	0.0
DT135	2.4	1.5	0.0	0.0	0.0	0.0	0.0
DT104b	2.2	0.7	0.0	0.0	0.0	11.1	0.0
DT66	1.9	4.1	12.2	0.0	20.0	0.0	0.0
NT	21.1	7.7	16.8	9.7	0.0	16.7	3.1
Others	17.0	20.0	21.4	16.1	20.0	11.1	84.4
Total	100	100	100	100	100	100	100
NI-to- Cool	T-1-1- 10						

Notes: See Table 15.

Other phage typed S. Typhimurium isolates: 3 beef isolates (DT120), 2 turkey flock isolates (DT120, NT), 2 imported chicken isolates (DT104, DTU302), 3 imported turkey isolates (DT104, 2 DT135).

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

Table 12. Phage-type distribution (%) of S. Enteritidis from humans, animals, carcasses and slaughterhouses and imported meat, 2002

			Imported meat <sup>f]</sup>
Phage type	Human	Layer	Chicken
	n=633	n=15	n=30
PT8	37.4	53.3	0.0
PT4	23.9	13.3	30.0
PT6	13.0	13.3	6.7
PT1	5.2	0.0	33.3
PT21	5.1	6.7	13.3
PT14B	4.3	0.0	0.0
PT11	1.1	0.0	0.0
PT6A	0.9	0.0	16.7
NT	3.5	6.7	0.0
Others	5.7	6.7	0.0
Total	100	100	100

Notes: See table 11.

Other phage typed S. Enteritidis isolates: 1 broiler flock (PT6), 1 cattle herd (PT8), 2 duck flock isolates (PT4, PT9B), 2 pig herd isolates (PT2).

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

## 2. Campylobacter spp.

#### Poultry

In 2002, the national surveillance of Campylobacter spp. in broilers was continued. Ten cloacal swabs were collected from each broiler flock/batch at the time of slaughter (a flock may be slaughtered in several batches) and cloacal swabs from individual flocks/batches were pooled to one sample. A total of 6,255 pooled samples were analysed by use of a polymerase chain reaction (PCR) detection method. Overall, the prevalence was 42.6% (Table A1) with percentages of positive batches per month ranging from 20.7% to 74.9% (Figure 14). In 2002, the prevalence of Campylobacter in hens was 40% (12 of 30 examined batches) and in ducks 100% (24 of 24 examined batches). It should be noted that hens and ducks were not examined for Campylobacter after 1<sup>st</sup> of April 2002.

As part of the monitoring programme for the occurrence of antimicrobial resistance in zoonotic bacteria from broilers (DANMAP), one flock from each broiler house in Denmark was examined for Campylobacter. Each sample, consisting of a pool of ten cloacal swabs, was analysed by conventional microbiological methods. Out of 489 investigated samples, 178 (36.8%) were found positive for Campylobacter. 93.1% of the obtained isolates were identified as C. jejuni and 6.9% were C. coli.

No turkey flocks were tested for Campylobacter in 2002.

#### **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 80,4% (Table A3) and in cattle 65,5% (Table A4).

### Wildlife and pet animals

The Danish Veterinary Institute monitoring of *Campylobacter* in wild mammals and birds, previously carried out by the



Figure 14. Percent Campylobacter positive poultry flocks, 1998-2002 Source: The Danish Veterinary Institute

Danish Veterinary Institute, was discontinued in 2002.

In 2002, pet samples were not routinely monitored for *Campylo*bacter. Only samples submitted specifically for *Campylobacter* analysis (i.e. clinical cases) were examined. *Campylobacter* were found in four of 10 samples from dogs and in four of 13 examined cats (Table A5). The predominant species in dogs and cats was *C*. upsaliensis.

## Products from retail outlets

The national surveillance programme for thermophilic *Campylobacter* spp. in foods from retail outlets was established in 1996 by the Danish Veterinary and Food Administration, and was continued in 2002. As in 2001, the samples were analysed by a semiquantitative method based on pre-enrichment in Mueller-Hinton broth supplemented with trimethoprim and cefaperazone followed by plating on mCCDA.

The food represented in the survey in 2002 included Danish and imported poultry products, mainly raw chicken and turkey products. In total, 816 samples of raw poultry were analysed. The prevalence of thermophilic Campylobacter spp. are shown in Table A1 and A2. In 2002, the prevalence in raw chicken and turkey products was 42% (N=712) and 21% (N=104), respectively. For chicken products, this represents a slight increase compared to 2001 where the prevalence was 35% (N=1,096). For turkey products the level of contaminated samples remained almost unchanged compared to 2001. The number of thermophilic Campylobacter spp.



Figure 15. The number of thermophilic Campylobacter in Danish produced and imported chicken products from retail outlets, 2002. Source: The Danish Veterinary and Food Administration



Figure 16. The number of thermophilic Campylobacter in Danish produced and imported turkey products from retail outlets, 2002. Source: The Danish Veterinary and Food Administration

per gram sample (CFU/g) is shown in Figures 15 and 16.

In 2001-2002, minced meat of pork and beef was included in the surveillance. In total, thermophilic Campylobacter spp. were found in 0.2% of 2413 samples of minced pork (at levels of 0.4-40 cfu/g) and in 0.1% of 3046 samples of minced beef (at levels of 0.4-4 cfu/g) (Table A3 and A4).

## Campylobacteriosis in humans

Campylobacteriosis remains the most common cause of bacterial gastrointestinal disease. There were 4,378 laboratory confirmed episodes of campylobacteriosis in 2002 (82 cases per 100,000 inhabitants), which is 5% less than the year before. The number of human Campylobacter infections has risen continuously from 1992 to 2001 (Figure 17), but the 2002 figure suggests that this increase may now have stopped. Consumption of poultry and poultry products is believed to be the primary source of human campylobacteriosis in Denmark, though other sources also exist. It is assumed that approximately 80% of the infections are domestically acquired. The geographical distribution of human infections caused by Campylobacter spp. is shown in Figure 18.

Outbreaks of human campylobacteriosis are relatively rare. They are identified and recorded in the same manner as Salmonella outbreaks and summarised in Tables 9, 10 and 11.

#### Intensive surveillance of human Campylobacter infections by typing

From May 2001 to June 2002, a research project based on intensive surveillance of *Campylobacter* in two regions of Denmark was performed in order to reveal the occurrence of small unnoticed outbreaks (e.g. family outbreaks). The surveillance was based on typing using two definitive methods: Penner serotyping and RiboPrinting (automated ribotyping). All *Campylobacter* isolates from two counties (Funen and Copenhagen) were typed. Further-



Figure 17. Incidence per 100,000 of human campylobacteriosis in Denmark, 1980-2002. Source: Statens Serum Institut



Figure 18. Geographical distribution of the number of cases per county and incidence of human campylobacteriosis in 2002. Source: Statens Serum Institut

Serotype	Human n=113	Cattle n=55	Broilers n=114	Food n=41
2	24.8	23.6	27.2	24.4
4-complex <sup>a)</sup>	24.8	12.7	13.2	4.9
1,44	11.5	3.6	9.7	9.8
19	9.7	9.1	2.6	7.3
11	3.5	9.1	5.3	12.2
3	3.5	3.5	0.9	0
42	2.7	3.6	0.9	2,4
6.7	1.8	1.8	13.2	9.8
23,36	0.9	18.2	0.9	0
12	0.9	0	2.6	4.9
Others	15.0	11.2	21.7	14.5
Not typable	0.9	3.6	1.8	9.8
Total	100	100	100	100

Table 14. Serotype distribution (%) of Campylobacter jejuni from humans, food and animals in 2002.

a) 4-complex: Reaction with one or more of the following antisera: 4, 13, 16, 43, 50, 64, 65.

Source: The Danish Veterinary Institute

Table 15. Serotype distribution (%) of Campylobacter coli from humans, food and animals in 2002.

C	Human	Pigs	Broilers	Food
Serotype	n=12	n=109	C1=11	n=7
46	16.7	17.4	13.3	0
48	16.7	0.9	0	0
24	8.3	15.6	0	0
14	8.3	0	0	14.3
59	8.3	7.3	20.0	14.3
49	8.3	0	13.3	0
5	0	13.8	6.7	0
30	0	8.3	6.7	14.3
49	0	0	13.3	0
26	0	9.2	6.7	0
Others	33.4	22.9	20.0	57.1
Not typable	0	4.6	0	0
Total	100	100	100	100

Source: The Danish Veterinary Institute

more, the patients were asked to fill in a short questionnaire concerning symptoms, travel, restaurant visits, contacts to other infected persons, drinking water, and general description of food consumption in the week before onset of symptoms. *Campylobacter* isolated from food at the retail level (mainly poultry) in these regions were included in the study. In total 1,204 isolates were included in the study, and 975 of these were clinical isolates obtained from humans. The project was performed in collaboration between Statens Serum Institut, the Danish Veterinary Institute, and the Department of Clinical Microbiology at Herlev Hospital.

The results showed that clustering of cases were quite common: typically 5-15 persons shed the same sero-/ribotype in a time frame of a few weeks. More than a quarter of all clinical isolates could be assigned to such clusters of cases. In addition, one or more poultry food isolates with a matching sero-/ ribotype were identified for many of these clusters. This clustering of cases is hardly coincidental. A common origin of the isolates and thereby a common source of infection is likely. However, on the basis of the questionnaires, it was not possible to identify a common source or a link between patients in any cluster.

#### Serotyping of Campylobacter

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 C. jejuni serotypes among human isolates (Table 14). These serotypes are also common in most other sources of human infections. 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 2002, 3% 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 15). Very few C. coli isolates were obtained from other sources.

## 3. Yersinia enterocolitica

#### Pigs

As part of the monitoring programme for the occurrence of antimicrobial resistance in zoonotic bacteria from pigs (DANMAP), caecal contents were sampled at slaughterhouses and examined for Yersinia enterocolitica. One animal per herd was sampled. In 2002, 65 (17%) of 383 examined animals were found positive for Y. enterocolitica. All of the obtained isolates were serotype O:3 (Table A3).

## 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 Administration. Therefore, information on the prevalence of this organism in various types of food is scarce. Only 20 samples of not heattreated pork were analysed in 2002. One sample was found positive, but the bio- and serotype was not determined (Table A3).



Figure 20. Geographical distribution of the number of cases per county and incidence of human yersinosis 2002. Source: Statens Serum Institut.

#### Yersinosis in humans

A total of 240 cases of infection with Y. enterocolitica were registered in year 2002 (4.5 cases per 100,000 inhabitants), 16% fewer than in 2001. Overall, the number of infections with Y. enterocolitica has decreased steadily since 1985 where more than 1,500 cases were reported (Figure 19, and Table A6). As in previous years, the majority of isolates (92%) were serotype O:3. The vast majority of the infections were domestically acquired. The primary source of yersiniosis in Denmark is thought to be pork. The geographical distribution of the human Y. enterocolitica cases in 2002 is presented in Figure 20.



Figure 19. Incidence per 100,000 of human yersiniosis in Denmark, 1980-2002. Source: Statens Serum Institut

## 4. Listeria monocytogenes

## Products from retail outlets

Data describing the occurrence of Listeria monocytogenes in food at the retail level in Denmark in 2002 were reported by the regional Veterinary and Food Authorities to the Danish Veterinary and Food Administration (Table 16). From 2001 to 2002, the number of samples containing more than 100 L. monocytogenes per gram decreased from 2% to 0% in gravad, smoked, salted, not heattreated or slightly heat-treated fish products. Otherwise no major changes were observed.

It should be noted that the number of samples tested for L. monocytogenes has declined, especially in the group of heattreated products and products containing mayonnaise (Table 16).



Figure 21. Incidence per 100,000 of human listeriosis in Denmark, 1981-2002. Source: Statens Serum Institut

This is a reflection of the previously described change in strategy in the use of microbiological examinations at the retail level (p. 10).

#### Listeriosis in humans

In 2002, 28 cases of listeriosis were registered (Table A6, Figure 21). Nineteen cases presented with septicaemia, five with meningitis, and two were classical maternofoetal cases. One case presented with peritonitis and one with cystitis. Geographically, the patients were spread all over the country and no outbreaks were detected. Sixteen cases were caused by strains of serogroup 1 and twelve by serogroup 4.

Table 16. Percenta	ge distribution	of the numb	er of Listeria	monocytoge	enes in selecte	ed foods, sa	mpled at retail
level in Denmark	by the Regional	Veterinary a	and Food Auth	norities in 199	9-2002.		1

	2002		2001			2000	1999	
	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 of samples with cfu>100 per g	Number of samples	Percent samples with cfu>100 per g
Heat-treated products of pork, beef, chicken and turkey handled after heat treatment	1,331	0.2	2,952	0.2	3,861	0.4	5,534	0.5
Preserved, not heat - treated or slightly heat- treated products of pork, beef, chicken and turkey	244	0.8	115	0.9	162	2.5	212	0.9
Gravad, smoked, salted, not heat-treated or slightly heat -treated fish products	157	0	152	2.0	120	0.8	178	0.6
Sprouts or sliced vegetables	71	0	87	0	160	0	398	0.3
Products containing mayonnaise	573	0.3	1,664	0.1	2,163	0.2	2,393	0.2
Cheese and cheese products	34	0	31	0	44	0	53	0.0
Ready to-eat dishes	482	0	1,239	0.2	1,410	0.2	1,816	0.0

Source: The Danish Veterinary and Food Administration.

## 5. Verocytotoxigenic Escherichia coli

#### Cattle

As part of the DANMAPprogramme the occurrence of verocytotoxigenic Escherichia coli O157 (VTEC O157) has been monitored since 1995 on faecal samples from cattle collected at the slaughterhouse (one sample per herd, monthly sampling). In 2002, VTEC O157 was detected in 5.5% (13/237) faecal samples from cattle (Table A4).

## Products from retail outlets

In 2002 the Danish Veterinary and Food Administration investigated 444 samples of fresh beef (cuts of beef or minced beef) for the presence of VTEC 0157. Most of the samples were taken at cutting plants and a few samples originated from retail outlets. All samples were negative for VTEC 0157.

In another study, faecal samples from 125 cattle at slaughter and 50 samples of fresh meat cuts were examined for the presence of the *E. coli* serotypes O26, O103, O111, and O145. The samples were analysed by a method, which included an



23. Geographical distribution of the number of cases per county a

Figure 23. Geographical distribution of the number of cases per county and incidence of human infections with verocytotoxigenic E. coli (VTEC), 2002. Source: Statens Serum Institut.

immunomagnetic separation step. One VT negative strain of *E. coli* O26 was isolated from a faecal sample.

#### **Human infections**

In 2002, 141 episodes of VTEC infections from 137 individuals were identified (incidence 2.6 per 100,000). Of these, 16% were O157 (Figure 22, Table 17) and 40% of



Figure 22. Incidence of human infections with verocytotoxigenic E. coli, 1992-2002. Source: Statens Serum Institut

cases were five years old or less. No large outbreaks were identified, but in eight instances two members of the same family were infected with the same serotype and in one instance two children from the same day-care facility were infected with the same serotype. The steep increase in VTEC infections observed over the past few years (Figure 22) to a large degree reflect an increase in awareness and in diagnostic effort.

Haemolytic uraemic syndrome (HUS) has been notifiable in Denmark since spring 2000. In 2002 four cases were reported, none of them fatal. Only two of the cases tested positive by microbiological examination. From these cases VTEC strains of serotype O26:H- and O149:H1 were isolated.

The method used for microbiological diagnosis was slide agglutination of suspect colonies with OK-antisera against the Table 17. Human VTEC serotypes resulting in more then 5 episodes in 2002. A total of 26 different serotypes were registered

Serotype	Number of episodes
O157	23
O26	22
O103	20
Rough	14
O146	13
O145	8
Other	41
Total	141

Source: Statens Serum Institut

most common VTEC and EPEC serotypes. In counties where diagnostics are performed by Statens Serum Institut (covering about half of the Danish population) colony hybridisation using probes for verocytotoxin- and eae-genes was used for screening of all diarrhoeagenic *E. coli* prior to slide agglutination. In these counties, examination for VTEC was performed on stool samples from all patients less than seven years old or presenting with bloody diarrhoea, traveller's or persistent diarrhoea.

The geographical distribution of human infections with VTEC is presented in Figure 23. The apparent differences in incidences between the various counties may, in part, be due to the differences in diagnostic practices mentioned above.

## 6. Transmissible Spongiform Encephalopathy

The Danish Surveillance programme continued throughout 2002. The major changes included the TSE Regulations/Decisions (nr. 270/2002, 1494/2002, 1003/2002) being put into force. One of the consequences of this was that sheep and goats have to be tested, beginning with testing of fallen stock from 1<sup>st</sup> of January 2002 and testing of slaughter animals from 1<sup>st</sup> of April 2002.

#### Cattle

BSE testing of samples from normal slaughter animals is performed at three approved private laboratories in Denmark. Two of these laboratories use the Enfer Test (ELISA) with spinal cord as test material, while the third uses the Prionics Check Test (western blotting) with brain stem as test material. All risk animals in Denmark are tested with western blotting (bovine categories presented in Table 18). Fallen stock is generally tested at the approved



Figure 24. BSE farms in Denmark, 2000-2002 Source: The Danish Veterinary and Food Administration.

private laboratory using western blotting. The remaining samples from the Danish cattle population at risk are examined at the Danish Veterinary Institute (DVI). Furthermore, a small part of the fallen stock tests are examined at DVI to maintain a testing routine, since it is the national reference laboratory.

When rapid tests show

Table 18. The BSE surveillance p	programme in Denmark,	2002.
----------------------------------	-----------------------	-------

Active surveillance	No. of tests	No. of positive animals
Healthy slaughtered animals (>30 mo.)	249,250	1
Risk categories		
Emergency slaughters (>24 mo.)	1,680	0
Animals >24 mo., where ante-mortem inspection at the slaughterhouse reveals signs of disease or zoonotic infection	24	0
Fallen stock (>24 mo.)	34,291	2
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)	2,630	0
Imported UK animals	10	0
Animals from herds under restriction	4	0
Passive surveillance	No. of tests	No. of positive animals
Animal clinically suspected of having BSE	38	0
Total	287,927	3

Source: The Danish Veterinary and Food Administration

inconclusive or positive results, material is subjected to confirmatory testing at DVI by histopathology and immunohistochemistry (IHC). In case of severely autolysed material, or an inconclusive IHC, DVI forwards test material to the Veterinary Laboratories Agency, Weybridge, UK for SAF (scrapie associated fibrils) testing.

In 2002, Denmark tested a total of 287,927 bovine animals. Among these, three were positive for BSE (Table 18). The geographical distribution of BSE positive herds in Denmark, 2000-2002 is shown in Figure 24. In addition, a Danish born animal exported to Italy and a Danish born animal exported to Portugal was tested positive in the importing country (these are not included in Table 18 or Figure 24, because cases of BSE are reported to EU from the country of diagnosis).

Of the three cases identified in Denmark in 2002, one was born in December 1998, after implementation of the real feed ban in Denmark in January 1997. Such animals are referred to as BARB animals (born after real ban: BARB). Investigations concerning this case had not been concluded by the deadline of this report.

Of the exported animals, the one exported to Portugal was also a BARB animal (born in March 1999). Investigations regarding this case indicated that contamination with meat-and-bone meal (MBM) could not be ruled out as a possible source of infection.

#### Sheep and goats

It has been demonstrated that sheep can contract BSE under experimental conditions and there is, therefore, concern that this may also occur under field conditions. Some genotypes of sheep are resistant to scrapie and - although based on less evidence - also to BSE. In order to limit the spread of scrapie and to be prepared for potential occurrence of BSE in sheep, the European Commission is planning a breeding programme for resistance against scrapie. Denmark is required to test 100 animals per year for their prionproteingenotype.

The general procedures for BSE/ scrapie testing of slaughter animals are carried out as described for cattle. Brain stem is used as material in all cases and all fallen stock is tested at DVI.

In 2002, a total of 1,179 sheep and goats were tested for scrapie (Table 19). To date scrapie has never been detected in sheep and goats in Denmark.

#### Humans

Since 1997, the human form of the disease, variant Creutzfeldt-Jakob Disease (vCJD) has been notifiable in Denmark. No cases of vCJD have been reported to date.

Table	19.	The	TSE su	irveil	lance	
progra Denm	amr Iark	ne oi 200	f sheep 2	and	goats	in

	No. of tests sheep and goats	No. of positive tests
Active surveillance	492	0
Fallen stock (>18 mo.) Slaughter animals (>18 mo.)	675	0
Passive surveillance Animals clinically suspected of having TSE	12	0
Total	1,179	0

Source: The Danish Veterinary and Food Administration

#### 7. Cryptosporidium Mammals

At present, 13 species of Cryptosporidium are regarded as valid. Of these, the most extensively studied species, *C.* parvum, has a very wide host spectrum, whereas some of the other species appear to be more host-specific. Currently, genotyping of Cryptosporidium species is not offered as a routine diagnostic tool in Denmark, but has been completed as a part of research projects funded by the EU and the Danish Directorate for Development.

In 2002, 5,708 faecal samples from mammals were screened for Cryptosporidium. Cryptosporidium was detected in 10.9% of the bovine samples. Among other animal species the occurrence of Cryptosporidium did not exceed 2%. Results are presented in Table 20.

All mammalian samples (from all age groups), submitted to DVI for routine parasitological analysis, were screened for Cryptosporidium. Thus many samples from animals outside the group of 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, 29.2 % of the analysed samples were positive for Cryptosporidium.

So far all isolates from Danish cattle have been characterized as the 'bovine' genotype of *C. par*vum. Although this genotype is known to infect a wide range of animals including pigs as well as man, the 'bovine' genotype has not so far been identified in Danish pigs. However, studies have identified two distinct and apparently host-adapted genotypes of *Cryptosporidium* in pigs. One of these (*C. parvum* pig genotype I) has also been reported in humans, whereas the other (*C. parvum* pig genotype II) has been detected in human sewage. Thus, these potentially new species appear to have zoonotic potential.

#### Humans

In humans, cryptosporidiosis is mainly caused by the zoonotic species *C.* parvum and the anthroponotic species *C.* hominis, but other species have also been shown to infect immunocompetent as well as immunocompromised hosts. In Denmark, *C.* parvum and *C.* hominis have been detected in humans in addition to a few cases caused by *C.* meleagridis.

Cryptosporidiosis is not a notifiable disease in Denmark. The incidence in humans is therefore unknown. At most diagnostic laboratories in Denmark only patients with persistent diarrhoea or a history of recent travel are routinely examined for cryptosporidiosis. At Statens Serum Institut 38 cases of cryptosporidiosis were diagnosed in 2002 (Table A6). Previous surveys have shown that approx. 80% of the cases are acquired abroad.

### 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.

#### Humans

Bovine tuberculosis in humans has been a notifiable disease since May 1st 2000. In year 2002, two cases (<0.1 case per 100,000 inhabitants) of human tuberculosis caused by M. bovis were registered (Table A6). One patient was an elderly Dane and the disease was considered a reactivation of a latent infection acquired prior to the eradication of bovine tuberculosis. The second patient was of foreign origin (M. bovis isolated from the intestine) and in this case, the disease was believed to have been acquired outside of Denmark.

#### 9. Brucella abortus/ melitensis Cattle

In accordance with Commission Decision 99/466/EEC as amended by Decision 2000/69/EEC

Table 20. Occurrence of Cryptosporidium in faecal samples, 2002.

	No. examined	No. positive	% positive
Cattle	2,825	307	10.9
Sheep/goat	625	1	0.2
Pig	933	8	0.9
Horse	397	0	0
Dog	199	2	1.0
Cat	107	2	1.9
Others	622	3	5.7
Total	5.708	323	5.7

Source: The Danish Veterinary Institute

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 preentry testing, followed by testing at least every 18 months and before they leave the centre. Due to serological cross-reactions with Yersinia enterocolitica serotype O:9 a surveillance programme for this infection has been implemented in breeding herds with monthly serologic analyses. With this programme potential false positive Brucella reactive herds are identified prior to testing of pigs in quarantine for export or entry into semen collection centres. Brucellosis in pigs was not recorded in 2002.

#### Sheep and goats

In accordance with Commission Decision 94/877/EEC Denmark has been declared officially free from brucellosis. 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 2002, 5,862 blood samples from 833 herds were examined.

#### Humans

Infection in humans is not notifiable in Denmark. At Statens Serum Institut 16 persons were found positive by serology in 2002 (9 positive for B. abortus, 3 for B. melitensis and 4 for both). No information on travel association was avaiable.

#### **10.** Leptospira spp.

Leptospirosis in animals is not a notifiable disease in Denmark.

#### Pigs

Examination for leptospires in pigs is performed by antigen detection, culture and serology. Suspicion of leptospirosis is often based on increased incidence of abortions or other reproductive problems in a herd. In 2002, a total of 300 samples were investigated by immunoflourescens testing and leptospires were detected in two herds. One herd was infected with L. pomona 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.

#### Humans

Leptospirosis in humans is notifiable in Denmark. In year 2002, 13 patients were diagnosed by serology; all recovered. Infections caused by L. interrogans serovar ichterohaemorrhagiae are most common (four cases in year 2002), but infections caused by serovar bratislava, ballum, hardjo, poi and hurstbridge are also detected. The number of cases of leptospirosis in earlier years has been revised. Cases of L. biflexa serovar patoc have now been included, since it has been shown that strain patoc I is agglutinated by sera from patients infected with many other L. interrogans serovars. Thus, the average number of annual cases has risen from 13 to 18 (413 cases over the past 23 years).

#### 11. Trichinella spiralis/ nativa

Infection has not been recorded in domestic animals since 1930.

#### **Pigs and wild boars**

All pigs slaughtered at Danish export approved slaughterhouses are examined for *Trichinella* spp. in accordance with Council Directive 64/433/EEC. During 2002 samples from 22,044,781 pigs were examined, and none of the samples were found to contain *Trichinella* spp.

It is also compulsory to examine slaughtered wild boars. The Danish Veterinary and Food Administration was informed of 1,354 examinations, all of which were negative.

#### Horses

All horses which are slaughtered at Danish export slaughterhouses are examined for Trichinella spp. During 2002, samples from 1,441 horses were examined, and none of the samples were found to contain Trichinella spp.

#### Humans

Human trichinosis is not a notifiable disease. No domestically acquired cases of human trichinosis were recorded in year 2002. Generally, a few cases acquired abroad occur annually.

#### 12. Echinococcus granulosus/multilocularis

Echinococcus granulosus/multulocularis infections in all animals are notifiable. Surveillance for *E.* granulosus is performed through meat inspection. In 2002, there were no findings.

In 2002, 62 foxes were examined at the Royal Veterinary and Agricultural University. None were positive.

Infection in humans is not notifiable. No domestically acquired human cases were registered at Statens Serum Institut, but a few imported cases occurred.

#### 13. Toxoplasma gondii

Toxoplasmosis in humans is not a notifiable disease in Denmark, and the incidence of toxoplasmosis in humans is unknown. However, Denmark has a nationwide neonatal screening system for toxoplasmosis. In 2002, 12 newborns were found positive through this system.

#### 14. Rabies

Rabies is a notifiable infection in both humans and animals. In 2002, 42 wild bats were submitted for diagnosis and two were found infected with European bat lyssa virus (EBL) by the Danish Veterinary Institute. Moreover, one bat from a research facility and 17 other animals were examined. One sheep was found infected with EBL.

No human cases of rabies were reported in 2002. However, 20 people were treated by postexposure prophylaxis after suspected exposure in Denmark. Of these, 14 suffered from bat bites (none of these bats were examined) and four people were treated after exposure to a sheep that tested positive for rabies (EBL). In addition, 69 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 2002 report will be available at: www.vetinst.dk or can be ordered from the Danish Zonoosis Centre (dzc@dzc.dk) in June 2003.

		Flock	level		Slau	oducts of it					
	Broiler b	preeders	Broiler	flocks <sup>a)</sup>		Cuts	Ν	Not heat treated	He		
	Flocks examined	% positive flocks	Flocks examined	% positive flocks	Ν	% positive flocks	Ν	% positive samples	Ν	% positive samples	Note:
Salmonella spp.	-	-	-	-	-	-	-	-	-	-	а
Danish S. Enteritidis S. Typhimurium Other serotypes	330 - - -	0.6 - 0.6 -	4,378 - -	1.5 0.02 0.23 1.25	1667 - - -	5.5	14 - - -	0 - - -	75 - - -	0 - -	- - -
Imported	-	-	-	-	-	-	-	-	-	-	-
Unknown origin	-	-	-	-	-	-	12	0	52	0	-
Campylobacter spp.	-	-	-	-	-	-	712	41.7	-	-	-
Danish	489	36.8	6,255	42.6	-	-	520	38.5	24	0	b
Imported	-	-	-	-	-	-	167	52.1	1	0	-
Unknown origin	-	-	-	-	-	-	25	40.0	4	0	-
Other species	-	-	-	-	-	-	-	-	-	-	-

#### Table A1. Occurrence of Salmonella and Campylobacter in the broiler production in Denmark in 2002.

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 may be slaughtered in up to 6 batches.

Source: The Danish Poultry Council and the Danish Veterinary and Food Administration

	Flock	level	Slaught	erhouse		Retail - cuts and products og turkeymeat						
	Turkey	flocks	Neck	skins	N t	ot heat reated	Hea					
	Flocks examined	% positive flocks	Flocks examined	% positive flocks	Ν	% positive samples		% positive samples	Note:			
Salmonella spp.	-	-	-	-	8	0	40	0	а			
Danish	323	8.4	323	39.6	5	0	23	0	-			
S. Enteritidis	-	0	-	0	-	-	-	-	-			
S. Typhimurium	-	1.5	-	21.9	-	-	-	-	-			
Other serotypes	-	6.9	-	17.7	-	-	-	-	-			
Imported	-	-	-	-	-	-	2	0	-			
Unknown origin	-	-	-	-	3	0	15	0	-			
Campylobacter spp.	-	-	-	-	104	21.2	7	0	-			
Danish	-	-	-	-	77	20.8	-	-	-			
Imported	-	-	-	-	24	20.8	-	-	-			
Unknown origin	-	-	-	-	3	33.3	-	-	-			
Other species	-	-	-	-	-	-	-	-	-			

Table A2. Occurrence of Salmonella and Campylobacter in the turkey production in Denmark in 2002.

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.

Source: The Danish Poultry Council and the Danish Veterinary and Food Administration

### Appendix

		Herd level		Slaugh	terhouse	Retail					
-	Exar	nined		Carcass	samples	Not he	at treated	Heat	treated		
Zoonotic pathogen	Herds	Animals	% posi- tive herds	N	% posi- tive samples	N	% posi- tive samples	N	% posi- tive samples	Note:	
Mycobacterium bovis	20,151	22,0 mill	-	-	-	-	-	-	-	а	
Brucella abortus	-	-	-	-	-	-	-	-	-	b	
Trichinella spp.	20,151	22,0 mill	-	-	-	-	-	-	-	С	
Salmonella spp.	14,597	641,243	2.5	36,787	1.4	7,003	1.3	1,117	-	d,e	
S. Enteritidis	-	-	-	-	0.6	-	-	-	-	-	
S. Typhimurium	-	-	-	-	0.0	-	-	-	-	-	
Other serotypes	-	-	-	-	0.8	-	-	-	-	-	
Campylobacter spp.	240	240	80.4	-	-	2,413	0.2	205	-	f,g	
C. jejuni	-	-	1.6	-	-	-	-	-	-	-	
C. coli	-	-	78.8	-	-	-	-	-	-	-	
C. lari	-	-	-	-	-	-	-	-	-	-	
Y. enterocolitica	383	383	17.0	-	-	20	5.0	-	-	f,h	

Table A3. Occurrence of zoonotic pathogens in pigs and pork in Denmark in 2002.

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 slaughter

houses where less than five pigs are slaughtered per day, in which case the samples are analysed individually.

f) Herds examined by caecal samples from one animal per herd collected at slaughter (from the DANMAP-programme).

g) Retail data: surveillance data from 2001 included.

h) Isolates obtained at the retail level were not sero- or biotyped.

Source: The Danish Veterinary Institute and the Danish Veterinary and Food Administration

Tab.	le A4	. О	ccurrence of	zoonotic	patl	hogens	in	cattle	e and	be	eef	ìin	D	enmarl	< in	200	)2.

		Herd leve	el	Slaugh	nterhouse	Retail					
	Exan	nined		Carcas	s samples	Not hea	t treated	Heat	Heat treated		
Zoonotic pathogen	Herds	Animals	% posi- tive herds	N	% posi- tive samples	N	% posi- tive samples	N	% posi- tive samples	Note:	
Mycobacterium bovis	-	607,100	-	-	-	-	-	-	-	а	
Brucella abortus	-	-	-	-	-	-	-	-	-	b	
Trichinella spp.	-	-	-	-	-	-		-	-	-	
Salmonella spp.	251	251	3.6	12,700	0.2	1,400	1.0	235	-	c, d	
S. Enteritidis	-	-	-	-	0	-	-	-	-	-	
S. Typhimurium	-	-	0.8	-	0.04	-	-	-	-	-	
S. Dublin	-	-	2.8	-	0.16	-	-	-	-	-	
Campylobacter spp.	87	87	65.5	-	-	3,046	0.1	42	-	С	
C. jejuni	-	-	63.2	-	-	-	-	-	-	-	
C. coli	-	-	-	-	-	-	-	-	-	-	
Not speciated	-	-	2.3	-	-	-	-	-	-	-	
E. coli (0157)	-	237	5.5	-	-	444	0	-	-	С	

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, in which case the samples are analysed individually.

Source: The Danish Veterinary Institute and the Danish Veterinary and Food administration

			Pet an	imals				Wild mammals						Wildlife		Zoo animals		
	De	og	C	at	Oth	ners	Ha	are	Ru	mi-	Fo	ox	Oth	ners	Wate	rds r fowl		
Zoonotic patogen	Ani- mals	% posi- tive	Ani- mals	% posi- tive	Ani- mals	% posi- tive	Ani- mals	% posi- tive	Ani- mals	posi- tive	Ani- mals	% posi- tive	Ani- mals	% posi- tive	Ani- mals	% posi- tive	Animals	% positive
Salmonella spp.	189	1.1	91	1.1	17	5.9	22	0	16	0	22	0	368	2.2	127	3.2	264	5.7
S. Enteritidis	-	0	-	0	-	0	-	-	-	-	-	-	-	0.3	-	0	-	0
S. Typhimurium	-	1.1	-	1.1	-	5.9	-	-	-	-	-	-	-	1.1	-	3.2	-	1.5
Others	-	0	-	0	-	0	-	-	-	-	-	-	-	0.8	-	0	-	4.2
Campylobacter spp.	10	40.0	13	30.8	-	-	-	-	-	-	-	-	-	-	1	0	5	0
C. jejuni	-	0	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. coli	-	0	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. upsaliensis	-	40.0	-	23.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Others/not speciated	-	-	-	7.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table A5. Occurrence of Salmonella and Campylobacter in pet animals, wild mammals and birds in Denmark in 2002.

Source: The Danish Veterinary Institute

Table A6. Zoonoses in humans 2002 - incidence over a ten-year period.

	20	002							
Zoonotic Pathogen	Cases per 100,000 inh.	Registered cases	2001	2000	1999	1998	1997	1992	Note:
Mycobacterium bovis	0.03	2	4	12	2	9	11	-	а
Brucella abortus/melitensis	0.3	16	18	-	-	-	-	-	b
Trichinella spiralis/nativa	-	-	-	-	-	-	-	-	С
Salmonella spp.	38.6	2,071	2,918	2,308	3,268	3,880	5,015	3,373	-
S. Enteritidis	20.6	1,104	1,416	1,182	2,025	2,607	3,674	1,511	-
S. Typhimurium	7.0	378	589	436	584	678	841	1,289	-
Other serotypes	11.0	589	913	690	659	595	500	573	-
Campylobacter coli/jejuni	81.6	4,378	4,620	4,386	4,164	3,372	2,666	1,129	е
E. multilocularis/granulosus	-	-	-	-	-	-	-	-	С
Leptospira spp.	0.2	13	6	21	23	12	9	-	f
Listeria monocytogenes	0.5	28	38	39	44	41	33	-	f
Rabies	-	-	-	-	-	-	-	-	g
Toxoplasma gondii	-	-	-	-	-	-	-	-	b
Cryptosporidium parvum	0.7	38	84	-	-	-	-	-	b
Yersinia enterocolitica	4.5	240	286	265	339	464	430	901	-
Escherichia coli (VTEC)	2.6	141	92	60	51	34	33	-	-
O157 (VTEC)	0.4	23	24	18	10	12	12	-	-

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 travel associated cases occur.

d) Only first isolations registered.

e) A sample (n=125) of the isolates were identified to the species level: Of these 113 were C. jejuni and 12 were C. coli.

f) Notification mandatory (since 1986).

g) Notification mandatory. No domestical or imported cases.

Source: Statens Serum Institut

