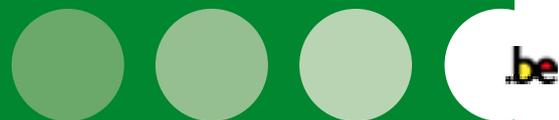




# report on zoonotic agents in belgium

working group on foodborne infections and intoxications

- Federal Agency for the Safety of the Food Chain (FASFC)
- Scientific Institute of Public Health (WIV-ISP)
- Veterinary and Agrochemical Research Centre (CODA-CERVA)



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# Preface

In February 2003, the Veterinary and Agrochemical Research centre and the Scientific Institute of Public Health published for the first time the report on zoonotic agents in Belgium, with data available from 2002. Since professionals involved in feed production and control, animal health, food safety and human infections, both from public and private organisations from Belgium and from abroad responded encouragingly to this first publication, a new edition was prepared. As was the case for the first edition, the basis for this booklet was the official Belgian “Trends and Sources” document that was transmitted to the European Commission in June 2004.

Almost all data, mainly from official monitoring programmes or laboratory findings, from primary production, from food and human (reference) laboratories available today in Belgium can be found in this report. Therefore, we are convinced that the figures presented here are useful for the professional reader. In addition, some general information on the selected zoonotic infections, on the monitoring programmes themselves and on the relevant laboratory methodology are given, which makes the document even more useful, also for those with a more general interest in animal and human infections.

As can be concluded from the number of collaborators, this compilation is the combined effort of many people, laboratories and institutions. We therefore explicitly express our gratitude to those who made this publication possible, not in the least the Federal Agency for the Safety of the Food Chain and the different National Reference Laboratories.

We wish the reader a pleasant time reading this second edition of the report on zoonotic agents.

The working group on zoonosis, foodborne infections and intoxications

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# Introduction

This publication is based on the official “Trends and Sources” document that was transmitted to the European Commission in June 2004 and contains data from the year 2003. The submission of the official report is according to European Directive 92/117/EEC concerning measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications.

The report on zoonotic agents in Belgium lists the reference laboratories active in the fields of feedstuffs, primary production and foods and some human reference laboratories. In addition, some plain figures on the human population in Belgium and on the animal populations are provided, as well as the number of animals slaughtered during 2003.

In addition to the bare listing of the available data, some general information on the clinical aspects of the zoonotic infection, the route of infection and some feasible recommendations are clarified. For each pathogenic agent the same information, if relevant, is presented, e.g. if vaccination is allowed, whether a monitoring is conducted, or what laboratory methodology is used. Finally, the brochure summarises the evolution of the main zoonotic agents among animals and in foodstuffs.

Most of the data in this report are from the following sources:

- Federal Agency for the Safety of the Food Chain (FASFC);
- National Reference Laboratory for Food Microbiology (NRLFM);
- Scientific Institute of Public Health (WIV - ISP);
- Veterinary and Agrochemical Research Centre (CODA - CERVA).

This report was co-ordinated by K. Dierick (WIV - ISP), Y. Ghafir (NRLFM), H. Imberechts (CODA - CERVA) and M. Jouret (FASFC), and, with the collaborative help of (alphabetical order):

- J. - M. Collard, National Reference Laboratory for Salmonella and Shigella, Scientific Institute of Public Health, Bacteriology Section;

- F. Costy, National Reference Laboratory for Rabies, Scientific Institute of Public Health, Pasteur Institute Department;
- P. Butaye, National Reference Laboratory for Salmonella, Antibiotic Resistance, CODA-CERVA
- G. Daube, National Reference Laboratory for Food Microbiology for the FASFC, Faculty of Veterinary Medicine, Université de Liège;
- J. De Borghrave and P. Dorny, National Reference Laboratory for Trichinella and Cysticercus, ITG-Diergeneeskunde Antwerpen;
- P. Dechamps and Ph. Dodion, Federal Agency for the Safety of the Food Chain, Control Division;
- K. De Schrijver, Ministry of the Flemish Community, Dept Hygiene and Health Inspection;
- L. De Zutter, Laboratory of Food Microbiology, Faculty of Veterinary Medicine, Universiteit Gent;
- G. Ducoffre and S. Quoilin, Epidemiology Section, Scientific Institute of Public Health;
- J. Godfroid, K. Walravens and M. Govaerts, Veterinary and Agrochemical Research Centre, National Reference Laboratory for Brucellosis, Laboratory of Bacterial Diseases and Immunology;
- M. Dauville-Dufaux, National Reference Laboratory for tuberculosis and mycobacterium, Scientific Institute of Public Health, Pasteur Institute Department;
- D. Pierard, National Reference Laboratory for Enterohemorrhagic Escherichia coli, AZ-VUB, Microbiology Section;
- C. Saegerman, Federal Agency for the Safety of the Food Chain, Control Policy Division, Scientific Secretariat;
- L. Vanholme and J-P. Maudoux, Federal Agency for the Safety of the Food Chain, Control Policy Division;
- M. Yde, National Reference Laboratory for Listeria, Scientific Institute of Public Health, Bacteriology Section.

## Belgian reference laboratories for zoonotic agents

Zoonotic agent	Contact	Address	E-mail
Brucella sp	J. Godfroid <i>As from September 2004:</i> K. Walravens	CODA - CERVA Groeselenberg 99, 1180 Brussels	Karl.Walravens@var.fgov.be
Escherichia coli VTEC and EHEC, animal health	H. Imberechts	CODA - CERVA Groeselenberg 99, 1180 Brussels	Hein.Imberechts@var.fgov.be
Escherichia coli VTEC and EHEC, public health	D. Pierard	AZ-VUB, Microbiologie Laarbeeklaan 101, 1090 Brussels	labomicro@az.vub.ac.be
Food Microbiology	G. Daube	Université de Liège Fac. de méd. vétérinaire, Bat. B43bis, Sart Tilman, 4000 Liège	Georges.Daube@ulg.ac.be
Listeria monocytogenes	M. Yde	WIV, Bacteriologie, J. Wijtmanstraat 14, 1050 Brussel	Marc.Yde@iph.fgov.be
Mycobacterium sp.	M. Fauville-Dufaux F. Portael  <i>As from September 2004:</i> K. Walravens	ISP-Dept Institut Pasteur, Rue Engeland 642, 1180 Brussels  ITG-Mycobacteriologie Nationalestraat 155, 2000 Antwerpen CODA - CERVA, Groeselenberg 99, 1180 Brussels	Mfauville@pasteur.be Fportael@itg.be  Karl.Walravens@var.fgov.be
Phage typing centre	C. Godard	ISP-Dept Institut Pasteur, Rue Engeland 642, 1180 Brussels	Cgodard@pasteur.be
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Salmonella, public health	J.M. Collard	ISP-Bacteriologie, Rue J. Wijtsman 14, 1050 Brussels	Jean-Marc.Collard@iph.fgov.be
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Clostridium botulinum	M. Turneer	ISP-Dept Institut Pasteur rue Engeland, 642, 1180 Bruxelles	mturneer@pasteur.be
Trichinella and other zoonotic parasites	J. de Borchgrave P. Dorny	ITG-Diergeneeskunde Nationalestraat, 155, 2000 Antwerpen	Jdeborchgrave@itg.be Pdorny@itg.be



## general information

# Susceptible human population

The total human population in Belgium: 10.355.844 on 1 January 2003 is shown in Table A.

	Flanders	Brussels	Wallonia	Belgium
<b>Female</b>	3.038.995	515.349	1.734.615	5.288.959
0-19	658.029	114.608	404.349	1.176.986
20-64	1.783.085	302.732	989.490	3.075.307
65+	597.881	98.009	340.776	1.036.666
<b>Male</b>	2.956.558	476.692	1.633.635	5.066.885
0-19	687.656	119.392	423.334	1.230.382
20-64	1.826.229	298.043	986.507	3.110.779
65+	442.673	59.257	223.794	725.724
<b>Total</b>				10.355.844
0-19	1.345.685	234.000	827.683	2.407.368
20-64	3.609.314	600.775	1.975.997	6.186.086
65+	1.040.554	157.266	564.570	1.762.390

**Table A:** Total human population in Belgium: 10 355 844 on 1 January 2003. Source: National Institute for Statistics

- Susceptible human population
- Susceptible animal populations
- Animals slaughtered in 2003

# Susceptible animal populations

## Ruminants and pigs

The origin of the following figures is Sanitel, the computerised registration and identification system for farm animals (dd 17 May 2004).

		Herds	Animals
Pigs	Number of herds	10.986	
	sows		688.908
	finishing pigs		5.115.683
Cattle	Herds	44.595	
	total cattle		2.752.974
Sheep	Number of herds	31.762	
	total sheep		221.434
Goats	Number of herds	13.522	
	total goats		43.130
Deer	Number of herds	2.907	
	total deer		16.588

*Table B: Number of animals is estimated as the number of available places in a herd*

## Poultry

Poultry	Herds	Animals
Layers	516	12.952.585
Broilers	1145	28.441.044
Other	337	5.242.193
<b>Total</b>	1998	46.635.822

*Table C: Number of animals is estimated as the number of available places in a herd*

### Animals slaughtered in 2003

	Number
Cattle	570.099
Calves	317.000
Pigs	11.609.933
Solipeds	12.304
Sheep	8.311
Goats	2.514
Deer	230
Broiler	222.327.256
Layer	19.711.279

*Table D: Number of animals slaughtered in Belgium in 2003  
(Source: Data are from the Federal Agency for the Safety of the Food Chain)*





## zoonotic tuberculosis

## Zoonotic tuberculosis (*Mycobacterium bovis*)

Tuberculosis in humans caused by *M. bovis* is rare. In regions where *M. bovis* infections in cattle are largely eliminated, only few residual cases occur among elderly persons as a result of the reactivation of dormant *M. bovis* within old lesions, and among migrants from high-prevalence countries. Agricultural workers may acquire infection by *M. bovis* by inhaling cough aerosols from infected cattle and may subsequently develop typical pulmonary or genito-urinary tuberculosis. Such patients may infect cattle through cough or urine, but evidence for human-to-human transmission is only limited.

In developing countries where *M. bovis* is largely prevalent among cattle, some studies reported that 3-6% of all diagnosed tuberculosis cases are due to *M. bovis* and that mostly young persons get infected by contaminated raw milk. In human, the disease caused by *M. bovis* is clinically indistinguishable from that caused by *M. tuberculosis*. Pulmonary tuberculosis is frequently observed but cervical lymphadenopathy, intestinal lesions, chronic skin tuberculosis and other nonpulmonary forms are particularly common. In Belgium, 5 human cases of bovine tuberculosis were identified in 2003. Molecular typing of strains isolated from cattle and human cases is on going in order to evaluate the presence of similar strains in both species.

Belgium is officially free from bovine tuberculosis since 25 June 2003 (Decision 2003/467/EC).

- *Mycobacterium bovis* in cattle
- *Mycobacterium* in other animals
- *Mycobacterium* in humans

# Mycobacterium bovis in cattle

## Surveillance system

The control of tuberculosis is based on European Directive 64/432/EEC, which is implemented and adapted in the national legislation since 1963 and last adapted by Royal Decree of October 2002. The control implies skin testing of animals at the occasion of trade and in the context of tracing contact animals. The Federal Agency for the Safety of the Food Chain is informed about any doubtful or positive result of the skin test and may decide to re-examine (additional tests) the animals or to kill them (test slaughter). Systematic post mortem examinations at the slaughterhouse are performed as well. In case a suspected lesion is identified, a sample is sent to the reference laboratory for analysis. Consequently, if *Mycobacterium bovis* is isolated, all animals in the herd of origin are skin tested, and a complete epidemiological investigation is made.

Isolation of *M. bovis* and biochemical testing is exclusively performed in the National Reference Laboratory where also IFN-gamma and molecular typing by means of RFLP are done.

No vaccination is allowed in Belgium.

## Epidemiological history and results of 2003 surveillance

Belgium is officially free from bovine tuberculosis since 25 June 2003 (Decision 2003/467/EC). A total of 7 infected herds were recognised in Belgium in 2003 (cumulative incidence over the year), which is comparable to the 10 herds notified in 2002. Stamping out was done in 5 herds. A total of 409 animals reacted after tuberculation, which is less than the 799 reactors in 2002. This number corresponds to the intensive testing of infected and contact farms. In total 3 799 herds and 337 260 animals were included in epidemiological investigations. The Federal Agency for the Safety of the Food Chain instructed the slaughter of 1014 animals. During 2003, more than 950 000 € was spent on compensation.

At the end of December 2003, 44 588 herds (99.98%) with 2 752 327 animals (99.97%) were officially free of tuberculosis.

Figures from the Federal Agency for the Safety of the Food Chain show that in abattoirs in 2003, 6 whole carcasses and 49 parts were rejected on a total of 570 099. Not a single calf carcass was rejected (317 000 inspections).

## **Mycobacterium in other animals**

During slaughter, the Federal Agency for the Safety of the Food Chain rejected 1 complete carcass and 87 partial carcasses of pigs due to tuberculosis (more than 11 million inspections). No tuberculosis was registered in solipeds (mainly horses), sheep or goats.

## **Mycobacterium bovis in humans**

In 2003, 5 cases of *M. bovis* infection were detected in humans.



## brucellosis

# Zoonotic brucellosis

**(Brucella melitensis, Brucella abortus, Brucella suis)**

Bacteria of the genus *Brucella* may infect sheep, goats, cattle, deer, elk, pigs, dogs, and several other animals, where they cause disease. Humans become infected by coming in contact with infected animals or with contaminated animal products. *Brucella* infections in humans may cause a range of symptoms that are similar to that of flu and may include fever, sweats, headaches, back pains, and physical weakness. Several infections of the central nervous systems or lining of the heart may occur.

In the non-“Officially Brucellosis Free” Mediterranean countries, the consumption of raw milk or raw cheese from sheep and goats is thought to be the major source of contamination. In Northern European countries, besides some occupational human cases of *B. abortus* infections, the majority of brucellosis cases are imported and are caused by *B. melitensis*. In Belgium, less than 10 cases/year of imported *B. melitensis* infections have been reported over the past few years. Last year no single case was reported. Belgian pigs and small ruminants are free of brucellosis and since the publication of Decision 2003/467/EC in June 2003, Belgium is also officially free from bovine brucellosis. *B. suis* biovar 2 is responsible for an enzootic brucellosis in wild boars (*Sus scrofa*) throughout continental EU, but is not considered to be an important source of human brucellosis.

- Brucellosis in cattle
- Brucellosis in sheep
- Brucellosis in pigs
- Brucellosis in humans

# Brucellosis in cattle

## Surveillance system and methods used

Belgium is officially free from bovine brucellosis since the 25 June 2003 (Decision 2003/467/EC). For this reason, the eradication programme has been changed in a surveillance programme. Beef cattle older than 2 years are monitored once every three years by means of serological tests. The herds for serological examination are selected by geographical localisation. Dairy cattle are checked at least 4 times a year via tank milk. Furthermore, all animals are serologically tested at trade. Each abortion or premature birth in animals at risk is subject to compulsory notification to the Federal Agency for the Safety of the Food Chain, and testing for brucellosis is obligatory. Aborting females should be kept in isolation until the results of the investigation exclude *Brucella* infections. Pooled tank milk is examined by means of the milk ring test. For animals older than 2 years, serology (i.e.–micro-agglutination as screening test; in case of a positive result, an indirect ELISA test is performed in parallel) is used if no sufficient milk ring tests are done (at least 4 ring tests a year). Bacteriological examination is done when serological and/or epidemiological suspicion is present. Allergic (brucellin) and IFN-gamma test may be carried out if serological cross-reactions are suspected. These tests are performed by the Federal Agency for the Safety of the Food Chain in collaboration with the National Reference Laboratory.

An animal is legally suspected of brucellosis in case of a positive ELISA. If, according to the epidemiology and the results of the skin test, an animal or herd is found to be at risk, a bacteriological investigation always takes place. Hence, a brucellosis animal is defined as an animal in which *Brucella* has been isolated, and cattle herd is legally positive if one of its animals is bacteriologically positive for brucellosis.

Vaccination has been prohibited in Belgium since 1992.

## Epidemiological history

The intensified bovine brucellosis control programme started in Belgium in 1988. In case of active brucellosis, i.e. excretion of *Brucella*, the plan consisted in the culling of all animals of the infected herd (total depopulation), and was compensated for based on the replacement value of the animals. The annual herd prevalence notified at the year end was 1,13% in 1988 and has fallen below 0,01% since 1998. In March 2000, the last case of bovine brucellosis was identified. No infected herd was recognised in Belgium in 2003. A total of 44 586 herds (99,98%) covering 2 820 258 animals (99,95%) were officially free of brucellosis. The Federal Agency for the Safety of the Food Chain instructed the slaughter of 58 animals during 2003, which is less than the 85 animals in 2002, 239 animals in 2001 and the 436 animals in 2000.

# Brucellosis in sheep

## Surveillance system

Serum samples taken in the framework of national monitoring for Visna-Maedi and at export were examined for *Brucella melitensis* specific antibodies by means of ELISA. Positive samples were subsequently tested in Rose Bengal and in complement fixation test. A sample is classified as positive for brucellosis only if positive in all three tests. If this is the case, a skin test should be performed on the seropositive animals and the congeners. A positive skin test leads to the bacteriological investigation of the animal. In 2001, 2002 and 2003 about 7 000 serum samples were tested at the National Reference Laboratory. In addition, 388 serum samples from sheep for export were analysed. The results confirmed those of previous years, i.e. the absence of any epidemiological or bacteriological evidence of ovine brucellosis in Belgium.

# Brucellosis in pigs

## Surveillance system and epidemiological history

Serological screening for *Brucella* is done for breeding pigs that are gathered (at a fair, for example), at artificial insemination centres and in animals intended for trade. The methods used are Rose Bengal, Slow Agglutination test according to Wright, complement fixation test and ELISA. Bacteriological examination for *Brucella* and *Yersinia* is done in case of positive serology. Regularly, false positive serological reactions are reported. These are due to a *Yersinia enterocolitica* O9 infection and are confirmed by *Yersinia* spp. isolation in the absence of *Brucella* spp. isolation. *B. suis* biovar 2 may be isolated from wild boars (*Sus scrofa*). The infection seems to be enzootic in wild boar in Europe.

The domestic pig population is free of brucellosis (last *Brucella* isolation in pigs in Belgium was in 1969). It is interesting to note that the Office International des Epizooties (<http://www.oie.int>) considers that the value of any brucellosis serological test in pigs is questionable.

# Brucellosis in humans

Probably due to the absence of a domestic animal reservoir of *Brucella* in Belgium, no single autochthon human brucellosis case was reported in 2003. In addition, no case of imported *B. melitensis* infection was registered at the National Reference Centre. It is helpful to note that *B. suis* biovar 2, circulating among wild boars, shows only limited pathogenicity for human, if pathogenic at all.



## salmonellosis

# Salmonellosis

Salmonella is the major cause of bacterial food-borne infections, both in individuals and in communities. It may cause gastro-intestinal infection with nausea, vomiting, abdominal cramps, diarrhoea and fever. In susceptible persons bacteraemia and septicaemia may occur. Often, food prepared with contaminated raw eggs, egg products or insufficiently heated poultry meat or pork are the source of the human Salmonella infection. Therefore, surveillance programmes that timely detect Salmonella contaminations in the whole food chain (feed, living animals, slaughterhouses, cutting plants, retail sector, restaurants) together with sanitary measures to reduce contamination are essential. Good hygienic practices during food preparation in the kitchen and adequate refrigeration and heating also help to prevent Salmonella infections.

In 2003, a total number of 12 894 Salmonella cases were recorded in humans, which is 28% more than in 2002. This rise was due to the significant increase of serotype Enteritidis isolates that thus surpassed for the first time 70% of the total number of serotyped strains. The source of human *S. Enteritidis* infection was not clarified: serotype Enteritidis is mainly associated with poultry (laying hens) and poultry products (especially eggs), but available figures did not indicate a significant rise at these parts of the food chain. On the contrary, the increase in the serotype Virchow in poultry from 20 to about 30% was striking.

As for Salmonella contamination of meat, contamination of pork in retail cuts and of minced pork decreased, whereas contamination of pig carcasses, broilers and spent hens remained at the same level of 2002. In pork as well as in pigs serotypes Typhimurium and Derby were predominant, whereas in poultry meat and in broilers, serotypes Virchow, Hadar and Paratyphi B were mainly found. Minced beef was rarely contaminated; serotypes Dublin and Typhimurium were the most prevalent in this meat.

In animal, food and human Salmonella isolates, antibiotic resistance was most apparent against tetracycline (25 to 31%), ampicillin (29%), sulfonamides (17 to 23%), nalidixic acid (24 to 29%) and the combination trimethoprim-sulfonamides (11 to 17%). Resistance to cefotaxime was found in 14% of the human Virchow isolates. The majority of *S. Enteritidis* isolates remained susceptible to all antimicrobials tested. On the contrary, multi-resistance was commonly found in *S. Typhimurium*. These isolates, from animals, food and humans, showed in 21 to 51% of cases resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline, a resistance profile frequently associated with phage type DT 104. Indeed, 73% of human *S. Typhimurium* isolates with this pentaresistance profile belong to phage type DT 104. Multi-resistance was remarkable among isolates belonging to serotypes Hadar and Virchow.

- Salmonella in animal feed
- Salmonella in poultry
- Salmonella in pigeons
- Salmonella in pigs
- Salmonella in cattle
- Antibiotic resistance in strains from living animals
- Salmonella in food (meat and meat products)
- Antimicrobial resistance in strains isolated from meat
- Salmonella in humans
- Antimicrobial resistance of human isolates

## Salmonella in animal feed

An official monitoring for the detection of Salmonella in compound feedingstuffs and in raw materials was organised by the Federal Agency for the Security of the Food Chain. The microbiological testing on 25g of sample was done at the CODA - CERVA and the laboratory of FASFC Gembloux. In case of isolation of Salmonella in official samples no certification was provided by the FASFC.

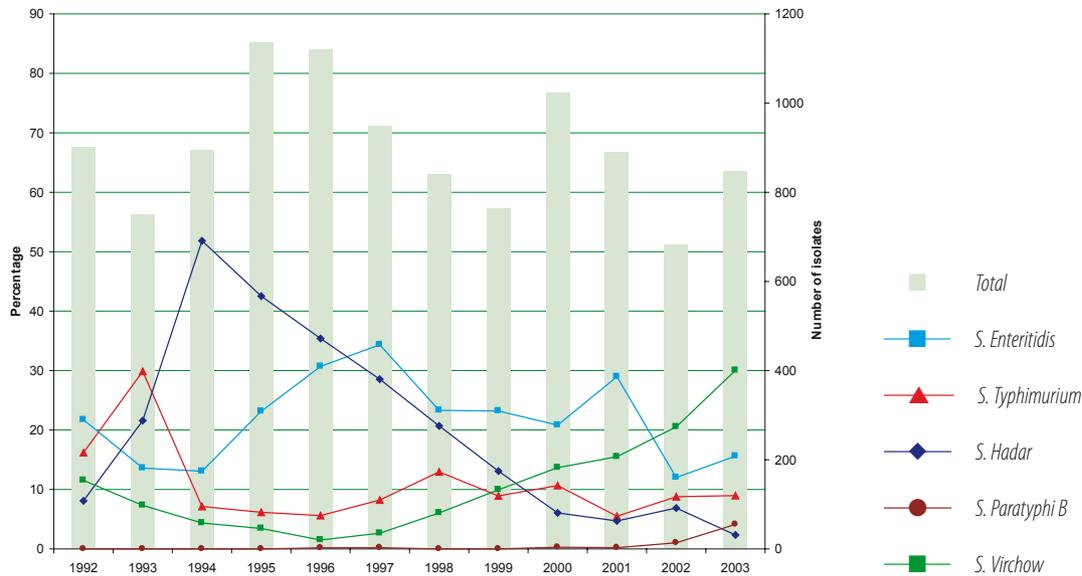
A total of 519 raw feed materials and compound feedingstuffs were analysed. Six of these samples were found positive. The serotypes were as follows: one Salmonella O<sub>3</sub>,10 in feed material of animal origin (fish meal), and 5 Salmonella in compound feedingstuffs (1 S. Mbandaka in final product for cattle, 1 S. Thompson and 1 S. Brandenburg in final product for pigs, 2 S. Enteritidis in final product for poultry layers).

## Salmonella in poultry

A total of 846 Salmonella strains from poultry origin were serotyped at the CODA - CERVA in 2003, which is 24% more than in 2002 and somewhat the same number as in 2001 (n=890). Serotypes Virchow (30,0%), Enteritidis (15,6%), Typhimurium (9,0%) and Agona (5,6%) were the most frequent.

From 403 isolates (47,6%) the production level was known. Among the breeder isolates four S. Enteritidis isolates (9,1% of breeder strains) were detected, but 22,7% and 20,5% were serotypes Typhimurium and Virchow, respectively. Eleven isolates from hatcheries were analysed, including 3 S. Typhimurium and 4 S. Virchow isolates. Almost 63% of layer isolates were serotype Enteritidis, while also Typhimurium (10,4%) and to a lesser extent Virchow and Senftenberg (both 6,0%) were found. Two S. Gallinarum strains were identified on one of the herds that were found infected in 2002. As for broilers, especially Virchow (25,3%) but also Typhimurium (9,6%), Agona and Paratyphi B (both 8,9%) and Enteritidis (6,8%) represented a large number of isolates.

Figure A shows the evolution of the main Salmonella serotypes in poultry since 1992. The evolution of serotypes among poultry isolates probably correctly represents the incidence of Salmonella infections in the sector due to the official monitoring programmes. Serotype Enteritidis has increased somewhat as compared to 2002, whereas Typhimurium remained at the same level, i.e. about 9%. Serotype Hadar is clearly of less importance among poultry, whereas the rise of serotype Virchow continues steadily. The significant increase of Serotype Paratyphi B is noteworthy.



**Figure A.** Evolution of the percentages of the principal *Salmonella* serotypes isolated from poultry between 1992 and 2003.

The bars represent the total number of poultry isolates per year, and refer to the right axis; the lines represent the percentage of each serotype per year and refer to the left axis.

## Salmonella in breeders and hatcheries

From 1993, all breeder flocks are examined once at the age of 16 weeks. For this purpose, the federation's technician who is affiliated to an official laboratory takes litter samples. All laboratories agreed on uniform methods of sampling, as well as on the bacteriological technique for isolation of *Salmonella*. Additional bacteriological examinations of the breeder flocks are done from 22 weeks on and each subsequent 6 weeks during the production period. Since all hatcheries and breeder flocks are regularly examined by the federations, the results are representative for the population under investigation.

Since 1998 all breeder flocks are routinely examined for *Salmonella* at delivery as day-old birds (imported and domestic flocks), or at the age of about 16 weeks for birds that were raised abroad. The delivery boxes of the day-old chickens are checked (bacteriology) as well as living animals (serology). Imported reared hens and cocks are examined by means of bacteriology on pooled faecal samples. Cocks added to a breeding flock during laying to improve production are checked on arrival at the farm in the same way.

The programme also examines the hygiene level of hatcheries by performing 4 controls a year. These are done during visits of the technician at non-hatching days, and comprise various sites of the hatchery, including hatching drawers. Rodac swabs are taken, and both total bacteria and moulds are counted. After appropriate incubation, an index or code is given to the number of colonies per surface of approximately 16 cm<sup>2</sup> in order to facilitate comparisons. In addition, a specific Salmonella control is done 4 times a year, on pooled samples from dead-in-shell chicks, and on fluff and meconium. The hatchery's owner sends these samples to the laboratory, and therefore the success of these controls depends on his active collaboration.

In 1999 the royal and ministerial decrees concerning the sanitary qualification (Gezondheidskwalificatie - Qualification sanitaire) came into force that prescribe both general hygienic measures and specific sampling for Salmonella detection on the farms. Thus, all poultry flocks before arrival at the slaughterhouse (i.e. breeders, layers and broilers) are controlled by bacteriological examination. A poultry flock is regarded as positive if Salmonella was isolated. A herd is considered as positive if at least one flock is positive. Both inactivated and live attenuated *S. Enteritidis* vaccines are available.

All laboratory results are submitted to the Federal Agency for the Safety of the Food Chain that can take measures on the affected herds.

A total of 258 breeder herds with 746 flocks were visited in 2003. Three *S. Enteritidis* positive broiler breeder herds were identified. In addition, 5 breeder broiler herds were found infected with *S. Typhimurium*. All breeder layers were found negative for *S. Enteritidis* or *S. Typhimurium*. (Table E. Evolution of the prevalence of Salmonella in poultry breeders).

Year	Number of holdings	Number of flocks	S. Enteritidis		S. Typhimurium	
			% pos herds	% pos flocks	% pos herds	% pos flocks
1993	255	497	4,3	3,0	7,1	5,6
1994	236	523	5,5	3,8	5,1	2,9
1995	254	540	10,6	7,7	4,3	2,4
1996	233	559	11,6	6,3	6,0	3,4
1997	227	588	13,7	7,7	7,5	4,3
1998	233	621	9,4	6,3	5,2	2,9
1999	247	634	6,9	3,0	3,2	2,1
2000	235	647	3,0	1,7	1,7	0,9
2001	225	621	2,2	1,3	2,2	1,1
2002	234	751	0,4	0,1	3,0	1,1
2003	225	703	1,3	0,6	1,8	0,7

**Table E:** Evolution of the prevalence of Salmonella in poultry breeders.

Data collected by the laboratory Dierengezondheidszorg Vlaanderen involved in the sanitary program Salmonella in layers and broilers

There was no official surveillance system for layers. However, the industry is responsible for sampling at entrance (voluntary) and before slaughter (compulsory). A poultry farm was regarded as positive if Salmonella was identified by bacteriology (layers and broilers) or by serology (layers only).

Three vaccines were available, one inactivated and one attenuated *S. Enteritidis* vaccine and one attenuated *S. Gallinarum* vaccine only for layers.

Almost 63% of layer isolates were serotype Enteritidis, while also Typhimurium (10,4%) and Virchow and Senftenberg (both 6,0%) were frequently found. Two *S. Gallinarum* strains were identified on one of the herds that were found infected in 2002.

As for broilers, especially Virchow (25,3%) but also Typhimurium (9,6%), Agona and Paratyphi B (both 8,9%) and Enteritidis (6,8%) represented a large number of isolates.

## Salmonella in pigeons

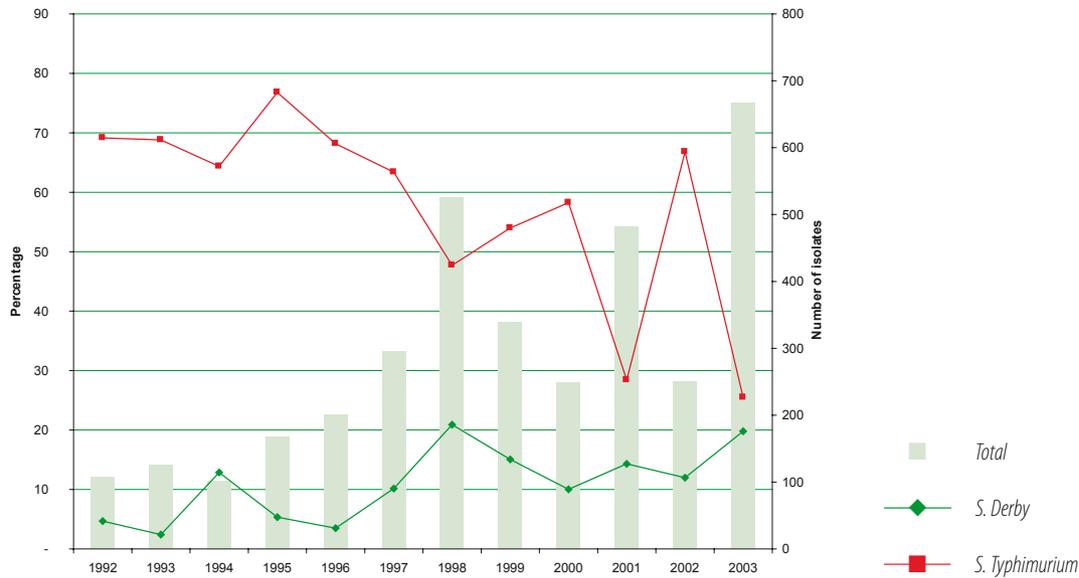
The National Reference Laboratory Salmonella, animal health received 22 isolates from pigeons. All these isolates were *S. Typhimurium* (all variant Copenhagen O5<sup>-</sup>).

## Salmonella in pigs

There was no surveillance system for Salmonella in pigs. A guidance programme (coordinated by Dierengezondheidszorg) started in 2002. Only laboratory findings from the National Reference Laboratory were available. In Belgium no vaccine was registered against salmonellosis in pigs.

Salmonellosis in pigs was not reported to the Federal Agency for the Safety of the Food Chain.

The evolution of the main Salmonella serotypes found among pigs is shown in Figure B. The number of pig isolates more than doubled in 2003 (n=667) as compared to 2002, due to the increased number of samples in the guidance programme. Among these, serotype Typhimurium (25,5%) [58,8% belong to Classic variant O5<sup>+</sup>] was the most prominent one. In addition, *S. Derby* (19,8%), *S. Panama* (10,5%) and *Goldcoast* (10,3%) and to a lesser extent *S. Livingstone* (7,0%) were identified.



**Figure B.** Evolution of the percentage of the principal *Salmonella* serotypes isolated from pigs between 1992 and 2003.

The bars represent the total number of pig isolates per year, and refer to the right axis; the lines represent the percentage of each serotype per year and refer to the left axis.

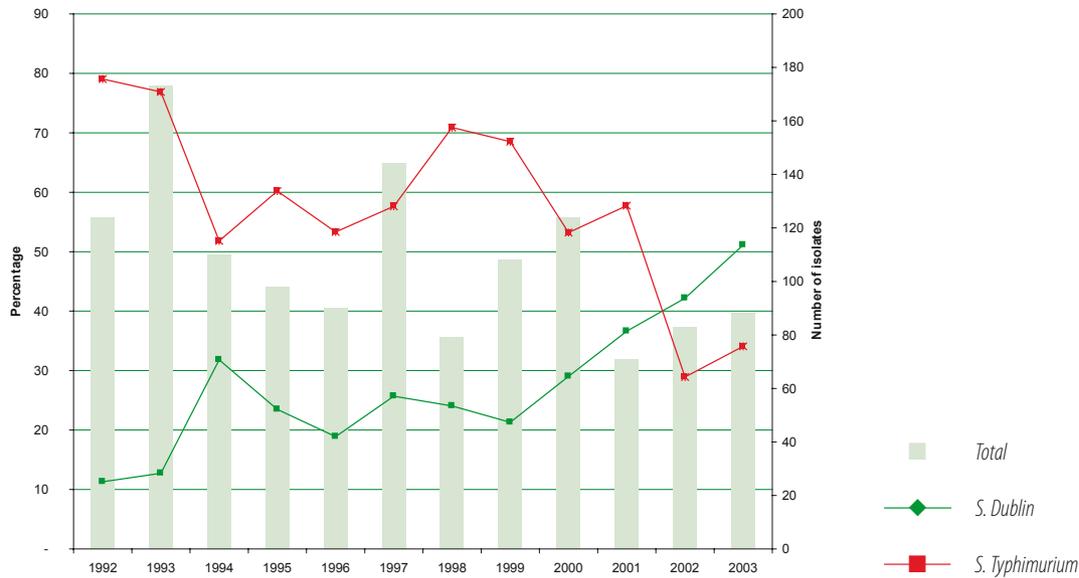
## Salmonella in cattle

There was no surveillance system for *Salmonella* in cattle. Only laboratory findings from the National Reference Laboratory are available.

In Belgium no vaccine was registered against salmonellosis in cattle.

Salmonellosis in cattle is not reported to the Federal Agency for the Safety of the Food Chain.

The evolution of the main *Salmonella* serotypes found among cattle is shown in Figure C. The number of cattle *Salmonella* isolates analysed was limited (n=88). Most frequently found serotypes were Dublin (51,1%) and Typhimurium (34,1%). Classic type *S.* Typhimurium (O<sub>5</sub><sup>+</sup>, 73,3%) outnumbered Copenhagen type O<sub>5</sub><sup>-</sup>. Two *S.* Enteritidis were detected in cattle in 2003.



**Figure C.** Evolution of the percentage of the principal *Salmonella* serotypes isolated from cattle between 1992 and 2003.

The bars represent the total number of cattle isolates per year, and refer to the right axis; the lines represent the percentage of each serotype per year and refer to the left axis.

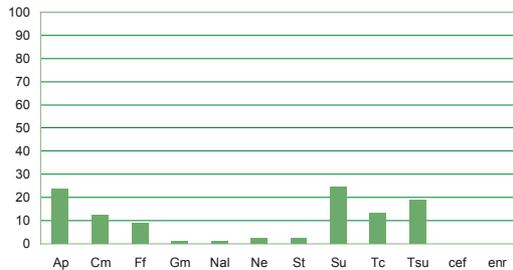
## Antimicrobial resistance in strains isolated from living animals

Data on antibiotic resistance of *Salmonella* strains from livestock came from the National Reference Laboratory for *Salmonella*, animal health. A selection of *Salmonella* isolates sent to the National Reference Laboratory was also routinely analysed for their resistance against antibiotic drugs by means of agar diffusion disks. The antimicrobial drugs tested are the beta-lactam ampicillin (Ap), the cephalosporin ceftiofur, the aminoglycosides streptomycin (Sm), gentamicin and neomycin, tetracycline (Tc), trimethoprim + sulfonamides (TSu) and sulfonamides (Su), the quinolones nalidixic acid (Nal) and enrofloxacin, and chloramphenicol (Cm) and Florfenicol (Ff). In 2003 only selected serotypes were tested for antibiotic susceptibility, i.e. strains from serotypes Agona, Dublin, Enteritidis, Hadar, Paratyphi B, Typhimurium and Virchow. Data refer to samples analysed in 2003.

Susceptibility tests were performed by the disk diffusion test, using Neo-Sensitabs (Results were accepted when results with the QC strain were within the limits as proposed by Rosco).

## S. Agona

A total of 89 *S. Agona* strains were tested for susceptibility. In this serotype, multiresistance was reported frequently last years. However, 75% of the 2003 strains were fully susceptible while only 12% of the strains showed multiresistance. Most resistance was found against sulfonamides and ampicillin. Resistant strains originated mainly from poultry and pigs. No resistance was found against the antibiotics ceftiofur and enrofloxacin (Figure D).

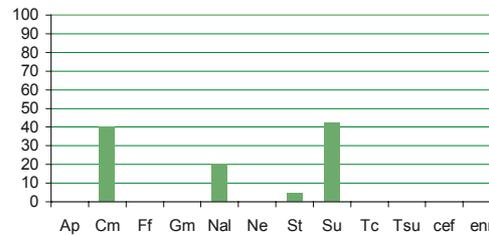


**Figure D.** Antibiotic resistance percentages in *S. Agona*.

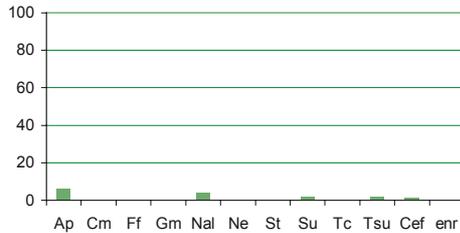
Ap : ampicillin, Cm : chloramphenicol, Ff : florfenicol, Gm : gentamicin, Nal : nalidixic acid, Ne : neomycin, St : streptomycin, Su : sulfonamides, Tc : tetracycline, Tsu : sulfonamide+trimethoprim, Cef: ceftiofur, Enr: enrofloxacin

## S. Dublin

Except of three strains, all *S. Dublin* originated from cattle, with a total of 49 strains tested. Forty-two percent of the strains were susceptible. The main resistance profile was Cm Su with 35,5% of the strains showing this profile and 61,5% of the resistant strains showing this profile. Note also that nalidixic acid resistance is 20% (Figure E)



**Figure E.** Antibiotic resistance percentages in *S. Dublin*



## S. Enteritidis

As usual *S. Enteritidis* is very susceptible with 90% of the strains (n=154) tested being susceptible. Only one remarkable evolution is the appearance of ceftiofur resistance in two strains (Figure F).

**Figure F.** Antibiotic resistance percentages in *S. Enteritidis*.

### S. Hadar

A total of 23 strains were tested. As usual, most strains are nalidixic acid resistant. No strain was fully susceptible and all but one strain had the profile Ap Nal Tc (Figure G)

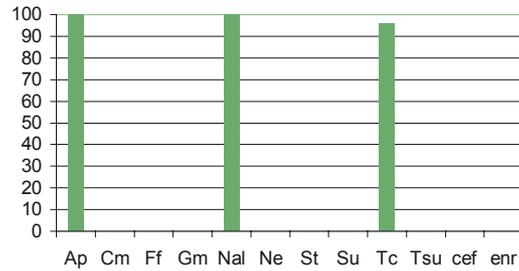


Figure G. Antibiotic resistance percentages in *S. Hadar*.

### S. Paratyphi B

All strains (n=29) originated from poultry. Except of two strains that were susceptible, all strains showed the resistance profile: Ap Nal Su Tsu. This may indicate a clonal spread of a resistant strain in Belgium.

### S. Typhimurium

A total of 147 *S. Typhimurium* O<sub>5</sub><sup>-</sup> (var Copenhagen) were tested. Most of them showed multiresistance with a main phenotype: Ap Cm Ff Su Tc and included also frequently St (approx. 50% of the strains). Also one fluoroquinolone resistant strain appeared. This strain originated from a chicken and was multiresistant. Only 26% of the strains were fully susceptible (Figure H).

A total of 199 *S. Typhimurium* O<sub>5</sub><sup>+</sup> (var Classic) were tested and 33% of them were fully susceptible. The main profile was as in the O<sub>5</sub><sup>-</sup> strains: Ap Cm Ff Su Tc. Total resistance percentages were slightly lower than for the O<sub>5</sub><sup>-</sup> strains (Figure I).

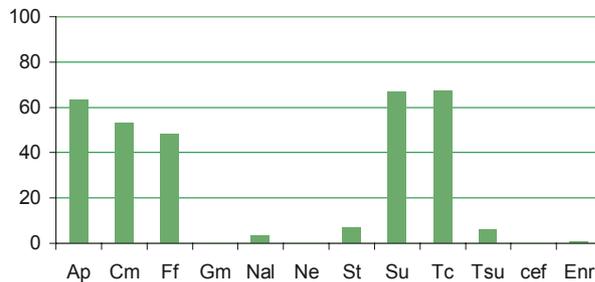


Figure H. Antibiotic resistance percentages in *S. Typhimurium* O<sub>5</sub><sup>-</sup>.

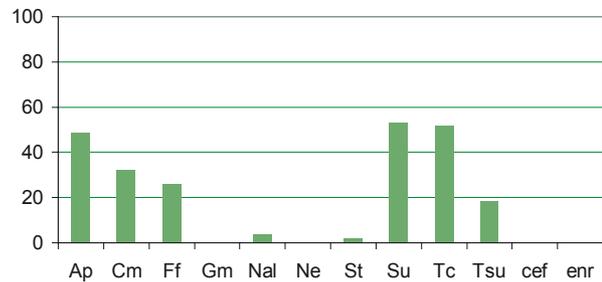


Figure I. Antibiotic resistance percentages in *S. Typhimurium* O<sub>5</sub><sup>+</sup>.

## S. Virchow

A huge amount of *S. Virchow* was isolated in 2003 and 290 strains were tested for susceptibility (Figure J). The further emergence of cephalosporin resistance in this serotype is worrisome, 30% of the strains tested being resistant against this antibiotic. All of them originated from poultry. It should be noted that this antibiotic is not registered for use in poultry in Belgium. The reason for this emergence is still unknown, but the trend in resistance needs special attention. Also the emergence of enrofloxacin resistance is worrying: resistance against nalidixic acid seems to be a nearly natural feature (more than 96% of the strains are resistant). Of special interest may be the frequently encountered intermediate resistance against enrofloxacin (approximately 19%, data not shown).

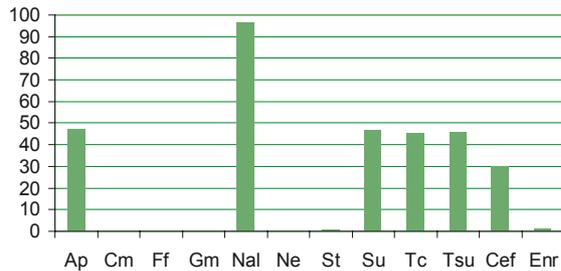


Figure J. Antibiotic resistance percentages in *S. Virchow*

## Salmonella in food (meat and meat products)

A monitoring programme was organised in 2003 by the Federal Agency for the Safety of the Food Chain. More than 200 Belgian slaughterhouses, more than 100 meat cutting plans and more than 200 retail trades representative of the Belgian production of carcasses and meat, were selected for this study. The matrixes were carcasses, cuts and minced meat of pork, cuts and minced meat of beef, carcasses, meat preparation and fillets of broilers, and layer carcasses. Sampling of pork carcasses was done by means of swabs. The carcass samples of broiler and fowl consisted of 10g of neck skin. The following contamination levels were analysed : 25g (cutting, minced meat of pork, chicken and beef), 600 cm<sup>2</sup> (pork carcasses), 1g (chicken carcasses) and 0,1g (layer carcasses). Sampling was done by a specially trained staff. For most matrixes, approximately 100 - 300 independent samples were taken per matrix in order to detect a minimal contamination rate of 1% with 95% confidence.

For the detection of *Salmonella*, the following method SP-VG-Moo2 was used: pre-enrichment in buffered peptone water at 37°C for 16 to 20 h, selective enrichment on Diassalm at 42°C for 24 h, isolation of positive colonies on XLD and confirmation on TSI and miniaturised biochemical tests.

At the Institute of Public Health, Brussels, all Salmonella isolates were serotyped and serotypes Typhimurium, Enteritidis, Virchow and Hadar were phage-typed.

The results of the monitoring of Federal Agency for the Safety of the Food Chain are shown in table F. The trends of Salmonella prevalence since 2000 are shown in table G.

Species			Prevalence	Predominant serotype	Other serotypes(in decreasing order)
Beef	Cutting meat	(n=100)	2,0%		
	Minced meat	(n=299)	0,3%	Derby	
Pork	Carcasses	(n=287)	14,6%	Typhimurium	Derby, Brandenburg, Anatum, Infantis, Give, Kedougou, Rissen
	Cutting meat	(n=278)	6,1%	Typhimurium	Derby, London
	Minced meat	(n=299)	6,4%	Typhimurium	Derby, Enteritidis, Livingstone, Agona, Brandenburg
Broilers	Carcasses	(n=290)	12,1%	Virchow	Hadar, Paratyphi B, , Agona, Barkley, Enteritidis, Kottbus, Livingstone
	Fillets	(n=247)	11,7%	Virchow	Enteritidis, Typhimurium, Agona, Bredeney, Hadar
	Meat preparation	(n=99)	29,3%	Virchow	Hadar, Paratyphi B, Blockley, Bredeney, Enteritidis, Infantis, Typhimurium
Layers	Carcasses	(n=102)	18,6%	Enteritidis	

**Table F:** The results of the monitoring – Salmonella in meat and meat products

	Samples	Sampling level	2000	2001	2002	2003
Pork	Carcasses	600cm2	24,1%	20,8%	15,4%	14,6%
	Retail cuts	25g	32,3%	17,7%	11,2%	6,1%
	Minced meat	25g	16,6%	10,3%	11,0%	6,4%
	Salami	25g	0,7%			
Broilers	Carcasses	1g	6,6%	11,4%	7,0%	12,1%
	Minced meat	25g			21,0%	29,3%
	Fillets	25g	12,7%	15,1%	12,6%	11,7%
Layers	Carcasses	0,1g	26,7%	21,9%	20,3%	18,6%
Beef	Carcasses	1600 cm2		2,7%	0,0%	
	Retail cuts	25g			0,9%	2,0%
	Minced meat	25g	6,1%	2,7%	3,3%	0,3%

**Table G:** Evolution of the food Salmonella prevalences 2000–2003

## Antibiotic resistance in strains isolated from meat and meat products

Data on antibiotic resistance of the 178 *Salmonella enterica* strains isolated from food were tested for their antimicrobial susceptibility. The disk diffusion method (Kirby-Bauer) was used following NCCLS recommendations at the Institute of Public Health, (IPH) Food Section. The following antibiotics were tested: ampicillin (AMP), ceftriaxone (CTRX), chloramphenicol (CHL), ciprofloxacin (CIP), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfonamides (SUL), tetracycline (TET), trimethoprim (TMP) and trimethoprim+sulfonamides (SXT). The results are shown in table H.

The level of resistance of *Salmonella* from broilers, spent hens, beef and pork is influenced by the serotype distribution in the respective meat species. The presence of highly resistant serotypes as Hadar, Virchow, Paratyphi B and Typhimurium contributes mainly to the high resistance levels in some matrices.

In general, the highest resistance was found against ampicillin (29%), tetracycline (25%), sulfonamides (23%), trimethoprim-sulfonamides (18%) and nalidixic acid (24%). No resistance was found against ciprofloxacin and kanamycin. Some isolates were found resistant against ceftriaxone (2%), all from broilers. Overall resistance remained at the same level as in 2002 and 2001 except for nalidixic acid where an increase of resistance was noticed. No strains were found resistant to ciprofloxacin. The appearance of ceftriaxone resistant strains in 2002, was confirmed in 2003.

### Resistance data according to meat species

The highest resistance was found in *Salmonellae* isolated from broilers and meat preparations of chicken (n=58) (predominant serotypes Virchow, Hadar, and Paratyphi B): ampicillin (43% and 52%), nalidixic acid (45% and 52%), sulfonamides (31% and 33%) and trimethoprim (33% and 30%). 28% of the *Salmonellae* isolated from broilers and 23% isolated from chicken meat preparation were resistant to 5 antibiotics or more.

Pork isolates (n=78) (mainly *S. Typhimurium*, *S. Derby* and *S. Brandenburg*) showed a high resistance against tetracycline (31%) and to a less extent to sulfonamides (18%), streptomycin (17%) and ampicillin (15%). *Salmonellae* isolated from spent hens belonged almost all to the serotype Enteritidis and showed very little resistance.

The few beef (n=4) isolates showed no resistance at all.

## Resistance data of the most prevalent *Salmonella* serotypes

In total 29 *S. Typhimurium* strains were tested for their susceptibility. The overall resistance was high: 52% for tetracycline, 41% for ampicillin: 34% for sulfonamides, 31% for streptomycin and 21% for chloramphenicol, trimethoprim and the combination trimethoprim-sulfonamide. 31% of the strains were resistant to 5 antibiotics or more. No resistance was noticed against ceftriaxone, ciprofloxacin or nalidixic acid.

All 18 *S. Virchow* strains were resistant against nalidixic acid (100%), and about 39% of the strains were resistant against ampicillin, 33% against tetracycline, sulfonamides or trimethoprim-sulfonamides. Four strains (22%) were resistant against ceftriaxone, all were isolated from broilers or chicken meat preparations. *S. Virchow* is the only serotype that presented resistant strains against this antibiotic agent.

The majority of the 21 *S. Enteritidis* isolates tested were susceptible to all antimicrobials. Only one strain from a chicken fillet was resistant to nalidixic acid, another strain from a spent hen was resistant to both nalidixic acid and ampicillin.

A total of 10 *S. Agona* isolates were tested: 70% were resistant to ampicillin, 60% to sulfonamides, 40% to trimethoprim and to trimethoprim-sulfonamides, 30% to tetracycline and chloramphenicol and 20% to streptomycin. No resistance was observed against ceftriaxone, nalidixic acid and ciprofloxacin.

*S. Derby* (n=24) showed a resistance of 25% against tetracycline, 17% against sulfonamides and streptomycin, and 8% against trimethoprim and the combination trimethoprim-sulfonamides.

*S. Paratyphi B* (n=9) was 100% resistant to ampicillin and trimethoprim and 89 % to nalidixic acid.

No resistance was detected in the 16 strains of *S. Bredeney* that were tested.

Only 8 strains of *S. Hadar* were isolated in 2003. However all were resistant to ampicillin, nalidixic acid and tetracycline. Two strains presented an additional resistance to streptomycin.

## Salmonella in humans

Data were obtained by a weekly updated surveillance system. In 2003, the National Reference Centre for Salmonella and Shigella received human Salmonella isolates from 194 clinical laboratories. All isolates were serotyped by slide agglutination with commercial antisera following the Kauffmann-White scheme. When necessary, additional biochemical tests were realized to confirm the identification or to differentiate between the subspecies. Phage typing (Scientific Institute of Public Health – Pasteur Institute Dept)) and antimicrobial susceptibility testing were realized on isolates randomly sampled from the four serotypes Enteritidis, Typhimurium, Hadar and Virchow. Two additional serotypes (Brandenburg and Derby) were also randomly sampled and only tested for their antimicrobial susceptibility.

The aim of our national surveillance program (in collaboration with the Epidemiology Section of the Scientific Institute of Public Health) is to document the occurrence and trends of serovars, to detect local, regional, national or even international outbreaks (in collaboration with the Enter-net network), to find and eliminate the source, and to suggest preventive actions to the Federal Agency for the Safety of the Food Chain. Since 1987 a remarkable increase in the number of registered human salmonellosis was monitored by the National Reference Centre, with a peak of 15,774 cases in 1999. This situation was chiefly linked to the increase of Salmonella Enteritidis, the most important serotype in Belgium. From 1987 to 1999, the incidence of laboratory-confirmed cases doubled to reach a value of 160/100.000 inhabitants in 1999.

Since then the total number of laboratory-confirmed cases fell to 14.088, 10.783 and 10.075 reports in 2000, 2001 and 2002, respectively (Table H). In 2003, an increase in the total number of human salmonellosis was again recorded (28% more than in 2002). This resulted from the spectacular increase of the serotype Enteritidis in 2003 which exceeded for the first time 70% of the total representativeness.

Typhimurium, the second serotype in importance, declined from 1999 until 2001 and then remained stable in 'number of isolates' (although its representativeness decreased in 2003 due to the increase of Enteritidis).

	1998		1999		2000		2001		2002		2003	
S. Enteritidis	9003	62,0%	10492	66,5%	9503	67,8%	7104	64,3%	6398	63,5%	9201	71,4%
S. Typhimurium	3221	22,2%	3348	21,2%	2799	20,0%	2370	21,4%	2438	24,2%	2512	19,5%
S. Virchow	115	0,8%	86	0,5%	147	1,1%	142	1,3%	132	1,3%	152	1,2%
S. Derby	162	1,1%	138	0,9%	169	1,2%	158	1,4%	92	0,9%	100	0,8%
S. Brandenburg	274	1,9%	279	1,8%	322	2,3%	200	1,8%	148	1,5%	66	0,5%
S. Hadar	459	3,2%	237	1,5%	178	1,3%	143	1,3%	74	0,7%	60	0,5%
Others	1280	8,8%	1194	7,6%	970	6,4%	948	8,5%	793	7,9%	803	6,1%
Total number	14514	100%	15774	100%	14088	100%	11065	100%	10075	100%	12894	100%

**Table H:** Trends for the most prevalent *Salmonella* serotypes from 1998 to 2003.

Other important *Salmonella* serotypes in 2003 with 50 isolates or more were Virchow (n=152), Derby (n=100), Brandenburg (n=66), Hadar (n=60), 4:i:- (n=59), Goldcast (n=55), and Infantis (n=52).

It is noteworthy that the number of *S. Virchow* isolates increased from 1999 and then remained stable (between 132 and 162 isolates a year). This serotype represents now the third serotype in importance. This augmentation reveals an unfavourable evolution in view of the high antimicrobial resistance level of that serotype (+ ESBL production and reduced susceptibility to ciprofloxacin in some isolates).

A significant drop of *S. Hadar* (459 in 1998 vs 60 in 2003) and *S. Brandenburg* (322 in 2000 vs 66 in 2003) cases was also noted over the last years. 301 cases of bacteraemia were reported in 2003. Most bacteraemia cases were due to serovars Enteritidis and Typhimurium (86.7%). *Salmonella* Paratyphi A, 9(Vi+):-:-, Typhi, Paratyphi B, and Dublin were found as the most invasive serovars.

## Antimicrobial resistance and phage typing of human isolates

A total of 523 human *Salmonella* isolates randomly collected in 2003 from the most important serotypes were examined for their resistance by disk diffusion to thirteen antibiotics which are of therapeutic or epidemiological interest. Antimicrobial susceptibility was determined by the disk diffusion method according to the NCCLS recommendations. The following antibiotics were tested: ampicillin (AMP), amoxicillin + clavulanic acid (AMX), cefotaxime (CTX), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfonamides (SUL), tetracycline (TET), trimethoprim (TMP), trimethoprim + sulfamethoxazole (SXT).

Resistance was mostly found against tetracycline (31.3%), ampicillin (29.9%), nalidixic acid (29.5%), and to a lesser extent against streptomycin (20.6%), sulfonamides (17.2%) and trimethoprim + sulfamethoxazole (11.6%).

All *S. Hadar* isolates (n=42) were resistant to at least two antibiotics. The highest antibiotic resistance levels were observed for this serotype. Resistance to tetracycline, nalidixic acid, ampicillin and streptomycin reached values from 71.4 up to 90.5% (Table I). Simultaneous resistance to these four antibiotics was observed in 57% of these isolates. However, isolates from this serotype remained fully sensitive to cefotaxime, chloramphenicol, and gentamicin.

*S. Typhimurium* (n=314) also showed a high level of antibiotic resistance with 29% of isolates resistant to four or more antimicrobial agents (defined as multiresistance). Eighteen percent of the isolates were shown resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (R-type ACSSuT with or without additional resistances), of which 73% were of definitive phage type (DT)<sub>104</sub>. Three full resistances against ciprofloxacin were also detected in multiresistant *S. Typhimurium* isolates (all three from phagetype 12/ad).

Multiresistance was also common in *S. Virchow* (n=44; 60% of the isolates in place of 29.7% in 2002). The highest incidence of resistance was observed for nalidixic acid (86.4%). Resistances to ampicillin, tetracycline and to trimethoprim + sulfamethoxazole were common (> 50%). Resistance to cefotaxime was found in 14% of the *S. Virchow* multiresistant isolates.

In contrast, the vast majority of *S. Enteritidis* (95.9%), *S. Brandenburg* (93.5%) and *S. Derby* (93%) isolates remained sensitive to all tested antibiotics.

Resistance patterns and levels in 2003 were generally the same than those in 2002 and 2001, except for the serotype *Virchow* for which a significant increase of resistance against cefotaxime, tetracycline, sulfonamides, and trimethoprim + sulfamethoxazole was observed (Table I). Co-trimoxazole resistance (trimethoprim + sulfamethoxazole) is also appearing in the serotype *Hadar*.

Serotype	No. of isol.	% of resistant strains												
		AMP	AMX	CTX	NAL	CIP	TET	CHL	GEN	KAN	STR	TMP	SUL	SXT
<b>Enteritidis</b>														
2003	49	4.1	0	0	0	0	0	0	0	0	0	0	0	0
2002	203	1.5	1.0	0	1.0	0	0.5	0.5	0	0	0.5	0	0	0
2001	197	2.5	-	0	1.0	0	1.0	0	0.5	0	0.5	1.5	2.5	1.5
<b>Typhimurium</b>														
2003	314	43.6	6.1	0	2.5	1.0	41.7	20.5	1.6	2.2	34.1	9.6	40.4	9.9
2002	319	39.0	14.0	0	1.6	0.3	52.0	26	0.9	0.6	39.0	9.1	41.0	8.8
2001	308	50.0	-	0	3.2	0.6	59.1	39	0.6	1.3	46.8	12.0	52.3	12.3
<b>Brandenburg</b>														
2003	31	3.2	0	0	0	0	3.2	3.2	0	0	6.5	0	3.2	0
2002	34	0	0	0	0	0	8.8	0	0	0	2.9	0	0	0
2001	38	5.3	-	0	0	0	18.4	2.6	2.6	0	5.3	5.3	7.9	2.6
<b>Virchow</b>														
2003	44	52.3	15.9	13.6	86.4	0	50	0	2.3	4.5	9.1	52.3	52.3	52.3
2002	47	40.0	19.1	6.4	80.9	0	25.5	2.1	0	0	10.6	31.9	34.0	29.8
2001	51	19.6	-	3.9	47.1	0	15.7	3.9	0	0	11.8	15.7	15.7	8.0
<b>Derby</b>														
2003	43	0	0	0	0	0	2.3	0	2.3	0	2.3	2.3	2.3	2.3
2002	34	0	0	0	0	0	2.9	2.9	0	0	17.6	2.9	2.9	2.9
2001	37	2.7	-	0	0	0	5.4	0	0	0	2.7	5.4	8.1	5.4
<b>Hadar</b>														
2003	42	76.2	28.6	0	88.1	0	90.5	0	0	7.1	71.4	4.8	4.8	4.8
2002	44	80.4	56.5	0	93.5	0	97.8	0	0	2.2	95.7	2.2	2.2	0
2001	51	66.0	-	0	92.0	0	94.0	0	0	2	94.0	0	0	0

**Table I** : Antimicrobial resistance in human *Salmonella* of serotypes Enteritidis, Typhimurium, Brandenburg, Derby, Hadar and Virchow isolated in 2003 (N=523), 2002 (N=681) and in 2001 (N=682)



## trichinellosis

# Trichinella

Trichinella is an intestinal parasite whose larvae can be present in the muscles of different animal species, and is transferred to humans by the consumption of contaminated raw or rare meat. Therefore, pork, wild boar and horsemeat are examined before marketing, except when appropriately frozen.

Carcasses found positive for the presence of Trichinella are declared unfit for consumption. It is recommended to travellers not to import raw meats of susceptible animals, e.g. sausages, bear; and not to consume meats of unknown quality abroad.

Human pathology: after a 1 to 4 weeks incubation, trichinellosis can cause myalgia, fever, eosinophilia, facial oedema, myocarditis. Trichinella has not been detected in carcasses of animal species produced for human consumption in Belgium for years.

- Trichinella in food animals

# Trichinella in food animals

## Surveillance system and methods used

Pig carcasses intended for export and all locally slaughtered horses and wild boars marketed were checked for Trichinella.

The examination is done by magnetic stirrer digestion of pooled 100 gram samples (1 g in case of pig, 5 g in case of boar and horse) as described in Directive 77/96/EEC. Serology may be done in live pigs and in epidemiological studies on wildlife.

Notification to the Federal Agency for the Safety of the Food Chain is compulsory.

## Results of the investigations in 2003

A total of 10.226.408 pigs, 12.304 solipeds (mainly horses) and 8.834 wild boars were examined. Not one sample was found positive for Trichinella.





rabies

# Rabies

Rabies is an acute viral encephalomyelitis of warm blooded animals (foxes, dogs, cats, bats ...) including human beings. The disease is caused by a Lyssavirus (8 genotypes), which is spread in the saliva of infected animals. In humans, the inability to swallow liquids has given the disease the name of hydrophobia.

Infected animals pass on the infection especially through bites or scratches, or less frequently via the injured skin or mucous membranes. The incubation period is usually from 4 to 8 weeks, but may range from 10 days to as long as one year or more. If not treated, human rabies is almost always fatal. Administration of rabies post-exposure prophylaxis combining wound treatment, passive immunization and vaccination are effective when appropriately applied. Pre-exposure vaccination should be offered to persons at risk, such as laboratory workers, veterinarians, animal handlers, international travellers.

In July 2001, Belgium has obtained the official status of rabies-free country according to the WHO recommendations. No indigenous cases of human rabies have been reported since 1923 although cases imported from Africa are diagnosed from time to time.

- Rabies in animals

# Rabies in animals

## Surveillance system and methods used

Food animals with nervous symptoms are suspect for rabies and therefore these cases have to be notified to the veterinary officer. Wildlife found dead or shot should also be transferred to the Federal Agency for the Safety of the Food Chain.

Affected animals are killed, and their brain is examined by immunofluorescence and virus cultivation in neuroblasts at the National Reference Laboratory.

## Vaccination policy

Vaccine baits (Raboral, Rhône-Mérieux) were dispersed for the vaccination of foxes. In April and October 2003, a zone of approximately 1 800 km<sup>2</sup> along the German border was covered by spreading 32 000 baits by means of a helicopter (17.78 baits per km<sup>2</sup>). Since there were no more cases of rabies for the last years, vaccination of foxes by baits will be stopped in 2004.

In the south of the country, below the rivers Sambre and Meuse, vaccination of dogs and cats is compulsory. In addition, all pets staying on any Belgian public camping must be vaccinated.

## Results of the investigations in 2003

A total of 615 analyses were done at the National Reference Laboratory. The majority of samples originated from foxes (44%) and cattle (41%). The high percentage for cattle is the consequence of the surveillance system for TSE in cattle: all suspected cases were first examined for rabies. Rabies must be considered in the differential diagnosis of BSE, although the course of the disease is usually shorter.

None of the samples was found positive. Since the last indigenously acquired case of rabies occurred in Belgium in a bovine in July 1999, Belgium obtained the official status of rabies-free country in July 2001 according to the WHO recommendations.





## campylobacteriosis

# Campylobacteriosis

Campylobacter is a leading source of bacterial foodborne gastrointestinal diseases in humans in all parts of the world. It can also cause postinfectious complications as Guillain-Barré syndrome.

In 80% of the cases, the infection route of campylobacteriosis is food, but domestic animals including pets are also involved. The transmission of this pathogen to humans is mostly due to consumption of undercooked poultry, pork and beef, unpasteurized milk, contaminated drinking water, or contacts with the faeces of infected pets. This chapter will focus on *Campylobacter jejuni* and *Campylobacter coli* that are the main causes of enteritis in humans<sup>1</sup>.

The contamination of poultry carcasses and meat with *Campylobacter* are monitored since 2000 by the Federal Agency for the Safety of the Food Chain. The rate of positive poultry samples is stable, but high. Chicken and layer meat have to be well cooked and cross-contamination should be avoided during preparation.

- Campylobacter in food
- Campylobacter in humans

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<sup>1</sup> J.Hu and D.J. Kopecko. *Campylobacter* species. In: International handbook of foodborne pathogens. p181-198 Ed. M.D. Milliotis and J.W. Bier. Marcel Dekker, New York, 2003.

## Campylobacter in food

In 2003, the zoonosis monitoring programme was putted in place by Federal Agency for the Safety of the Food Chain. More than 200 Belgian slaughterhouses, more than 100 meat cutting plans and more than 100 retail trades representative of the Belgian production of carcasses and meat, were selected for this study.

The matrixes were carcasses, meat preparations and boneless breast of broilers, and carcasses of layers. Three contamination levels, 25g, 1g and 0,01g were analysed. For chicken carcasses and fillets, approximately 300 independent samples were taken per matrix in order to detect a minimal contamination rate of 1% with 95% confidence.

The detection consisted of a selective enrichment in Preston at 42°C for 48h, followed by the isolation on mCCDA at 42°C for 24h-120h. Confirmation of minimum 1 colony was done by miniaturised biochemical tests (API Campy, BioMérieux, France) and by PCR typing. The results of the monitoring of Federal Agency for the Safety of the Food Chain are shown in table J. The trends of Campylobacter prevalence since 2000 are shown in table K.

Sample	Quantity of s ample analysed	Percentage of positive samples	Predominant Campylobacter species	Other species
<b>Broiler</b>				
Carcasses(n=286)	0,01g	28,0%	jejuni	coli
Minced meat(n=98)	1g	44,9%	jejuni	coli, lari
Fillets(n=241)	25g	17,8%	jejuni	coli
<b>Layer</b>				
Carcasses(n=102)	0,01g	12,8%	jejuni	

**Table J:** Zoonosis monitoring programme – Campylobacter in food

		Sampling level	2000	2001	2002	2003
Broilers	Carcasses	0,01g	33,9%	27,1%	34,9%	28,0%
	Fillets	1g	22,5%	15,3%	18,3%	17,8%
	Minced meat	25g			49,4%	44,9%
Layers	Carcasses	0,01g	23,0%	19,3%	20,5%	12,8%

**Table K:** Evolution of the food *Campylobacter* prevalences 2000-2003

## Campylobacter in humans

Data were obtained from passive surveillance through sentinel laboratory results. All cases were updated weekly.

From 2000 to 2002, the number of diagnosed cases was stable and 71% of the cases were located in Flanders. In 2003, the number of cases decreased by 11% and this reduction was observed in most districts of Flanders.

	1996	1997	1998	1999	2000	2001	2002	2003
Number of isolates	4991	5465	6610	6514	7473	7357	7354	6556

**Table L:** *Campylobacter* in humans



## echinococcosis

# Echinococcosis

Echinococcosis is caused either by *Echinococcus granulosus* or *Echinococcus multilocularis*.

*Echinococcus granulosus* produces unilocular human hydatidosis. It is a small tapeworm (6 mm) that lives in the small intestine of domestic and wild canids. Sheep and cattle serve as intermediate hosts for the infection. Humans acquire infection by ingestion of typical taeniid eggs, which are excreted in the faeces of infected dogs. When eggs are ingested by the intermediate hosts or by humans, the oncospheres liberated from the eggs migrate via the bloodstream to the liver, lungs and other tissues to develop in hydatid cysts. Within the cyst brood capsules and protoscoleces develop. Each protoscolex is a potentially infective organism for canids. Indigenous unilocular hydatidosis in man has been reported in Belgium. *Echinococcus multilocularis* causes alveolar (multilocular) echinococcosis in humans. Foxes and dogs are the definitive hosts of this parasite and small rodents the intermediate hosts. In the liver of rodents the invasive larval stage has a multi-compartmented appearance containing many protoscoleces. Ingestion of the eggs by humans can result in the development of invasive cysts in the liver.

In Belgium, the percentage of infected foxes varies with the region, with a decreasing rate from the South-East to the North-West: e.g 33% in the Ardennes, 13% in the Condroz region and 2% in Flanders. The endemic region is situated under the river Meuse, on the heights of the Ardennes.

Only six human cases of alveolar echinococcosis have been detected in Belgium since 1999, thanks to an efficient information campaign in wooded areas.

## Surveillance system and results:

Post-mortem macroscopic examination is performed at the slaughterhouse in the domestic intermediate hosts: cattle, sheep, horses and pigs. Whole carcasses or parts are rejected in case *Echinococcus granulosus* cysts were

found.

The following partial rejections were noted by the Federal Agency for the Safety of the Food Chain in 2003: 200 cases of adult cattle and 3 of sheep. *Echinococcus granulosus* was not detected in calves, pigs, goats or wild boars.



## listeriosis

# Listeriosis

*Listeria monocytogenes* has become a major concern for the food industry and public health authorities. Ingestion of food contaminated with *Listeria monocytogenes* may cause either a serious invasive illness affecting people with altered or deficient immune responses, or a non-invasive febrile gastro-enteritidis. Although the incidence of listeriosis is low, the high mortality rate, which often reaches as high as 30-40%, requires early diagnosis and appropriate antimicrobial therapy.

Listeriosis is transmitted to humans via contact with animals, cross-infection of foetus or newborn babies and foodborne infection<sup>2</sup>. *Listeria* is ubiquitous and widely distributed in the environment (soil, vegetables, meat, milk, fish). All food associated with *Listeria monocytogenes* outbreaks were consumed without further processing or after minimal heat treatment, and many of them had a suitable environment for growth<sup>3</sup>.

The contamination of food in the Belgian surveillance plan of the Federal Agency for the Safety of the Food Chain is stable, except for the chicken meat preparation (containing raw minced meat) that is very high in 2003. The number of human cases has almost doubled in comparison with 2002, but no large-scale listeriosis outbreak was reported.

General food hygiene rules are essential for the prevention of human listeriosis. As some persons are at high risk (pregnant women, immunocompromised people), they are advised not to eat certain categories of food with proven elevated risk of *Listeria monocytogenes* contamination, such as unpasteurized milk and butter, soft cheeses and ice cream made from unpasteurized milk, any soft cheese crust, smoked fish, pâté, cooked ham, salami, cooked meat in jelly, raw minced meat from beef, pork and poultry, steak tartar, raw fish and shellfish (oysters, mussels, shrimps), fish, meat and surimi salads, insufficiently rinsed raw vegetables, unpeeled fruit.

- *Listeria monocytogenes* in food
- *Listeria monocytogenes* in humans

<sup>2</sup> C. Bell and A. Kyriakides. *Listeria*. A practical approach to the organism and its control in foods. Blackie academic & professional, London, 1998.

<sup>3</sup> A.R. Datta. *Listeria monocytogenes*. In: International handbook of foodborne pathogens. p105-121 Ed. M.D. Milliotis and J.W. Bier. Marcel Dekker, New York, 2003.

# Listeria monocytogenes in food

In 2003, the zoonosis monitoring programme was put in place by Federal Agency for the Safety of the Food Chain. More than 100 meat cutting plants and more than 200 retail trades representative of the Belgian production of carcasses and meat, were selected for this study.

The matrixes were minced meat of pork, beef and poultry, cooked ham, pâté, salami and smoked salmon. Two contamination levels, 25g (cooked products and fish) and 1g (raw meat and raw products) were analysed. Approximately 300 independent samples were taken for pork and beef products in order to detect a minimal contamination rate of 1% with 95% confidence.

For detection of *Listeria monocytogenes* in meat samples, the validated method AFNOR BIO-12/9-07/02 VIDAS LMO2 followed by a validated chromogenic confirmation (AFNOR SDP-07/4-09/98 Rapid'L.mono or AFNOR AES-10/3-09/00 ALOA ONE DAY) was used. Briefly, the method consisted in a pre-enrichment on half-Fraser broth at 30°C for 24 h, followed by an enrichment on Fraser broth at 30°C for 24 h, the immunoassay (VIDAS LMO2) and isolation on of minimum 1 colony with Rapid'L.mono or ALOA (24-48h at 37°C).

The results of the monitoring of Federal Agency for the Safety of the Food Chain are shown in table M. The trends of *Listeria monocytogenes* prevalence since 2000 are shown in table N.

Sample		Quantity analysed	Percentage of positive samples
Beef	Minced meat (n=299)	1g	10,7%
Pork	Minced meat (n=298)	1g	21,5%
	Cooked ham (n=395)	25g	2,5%
	Pâté (n=402)	25g	4,0%
	Salami (n= 397)	1g	9,8%
Poultry	Meat preparation (n=95)	1g	60,0%
Fish	Smoked salmon (n=86)	25g	22,1%

**Table M** : Zoonosis monitoring programme - *Listeria monocytogenes* in food

		Sampling level	2000	2001	2002	2003
Pork	Minced meat	1g	25,0%	18,3%	20,7%	21,5%
	Cooked ham	25g	6,0%	4,6%	3,0%	2,5%
	Pâté	25g	4,3%	4,9%	5,4%	4,0%
	Salami	25g	16,0%			
	Salami	1g		8,6%		9,8%
Beef	Minced meat	1g	16,0%	14,8%	13,7%	10,7%
Chicken	Meat preparation	1g			33,8%	60,0%
Fish	Smoked salmon	25g			23,1%	22,1%

**Table N:** Evolution of the food *Listeria monocytogenes* prevalences 2000-2003

## Listeria monocytogenes in humans

Data were obtained from passive surveillance through sentinel laboratory results and from the National Reference Laboratory. All cases were updated weekly.

In 2003, 76 cases of listeriosis were reported. The number of diagnosed cases in 2003 has almost doubled in comparison with 2002. This increase has been observed in Flanders as well as in Brussels and Wallonia.

	1997	1998	1999	2000	2001	2002	2003
Number of isolates	45	60	64	48	57	44	76

**Table O:** *Listeria monocytogenes* in humans



## yersiniosis

## Yersinia enterocolitica

*Y. enterocolitica* is a relatively infrequent cause of diarrhea and abdominal pain. Infection with *Y. enterocolitica* occurs most often in young children. Common symptoms in children are fever, abdominal pain, and diarrhea, which is often bloody. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In older children and adults, right-sided abdominal pain and fever may be the predominant symptoms, and may be confused with appendicitis. In a small proportion of cases, complications such as skin rash, joint pains, or spread of bacteria to the bloodstream can occur.

Only a few strains of *Y. enterocolitica* cause illness in humans. The major animal reservoir for *Y. enterocolitica* strains that cause human illness is pigs, but other strains are also found in many other animals including rodents, rabbits, sheep, cattle, horses, dogs, and cats. In pigs, the bacteria are most likely to be found on the tonsils. Infection is most often acquired by eating contaminated food, especially raw or undercooked pork products. Drinking contaminated unpasteurized milk or untreated water can also transmit the infection<sup>4</sup>.

- Yersinia enterocolitica in food
- Yersinia enterocolitica in humans

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<sup>4</sup> U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, <http://www.cdc.gov/az.do>

# Yersinia enterocolitica in food

## Surveillance system

The FASFC organised a food surveillance of meat and meat products in 1997, which showed a very low prevalence of meat and meat products from pork, beef and poultry.

No food surveillance programme was organised in 2003.

# Yersiniosis in humans

## Surveillance system

Data were obtained from passive surveillance through sentinel laboratory findings. All cases were updated on a weekly base.

## Results of the investigations in 2003 and epidemiological evolution

The number of cases reported for human yersiniosis was 338 in 2003. As compared to 330 in 2002, 375 in 2001 and 507 cases in 2000, there is a clear stabilization in the number of infections reported in humans in Belgium.





## verotoxin producing escherichia coli (vtec)

## Verotoxin producing *Escherichia coli*

Zoonotic verotoxin producing *E. coli* may cause life-threatening diseases in young children or in immunocompromised or elderly people, i.e. hemorrhagic colitis, hemorrhagic uremic syndrome (HUS) and even death. *E. coli* O157 is the best known and most studied VTEC. Cattle are often indicated as the principal reservoir of VTEC, but are not clinically affected by zoonotic VTEC infection. Infection of humans takes place via consumption of contaminated food, through contact with contaminated water, or by direct transmission of VTEC from infected humans or animals. Therefore, prevention mainly relies on hygienic measures.

In 2003, typical *E. coli* O157 (i.e. verotoxigenic, *eaeA*-positive) were found on cattle carcasses, in sliced and minced meat in 0.7%, 0.7% and 1.7% of the cases, respectively. Living cattle were not analysed in this official monitoring programme.

As for VTEC infections in humans, approximately 40 verotoxin producing *E. coli* strains are annually analysed at the National Reference Laboratory. About half of these belong to serogroup O157. Eight typical EHEC isolates were from children between 10 months and 11 years old suffering from HUS: 5 belonged to serotype O157:H7, 2 to O157:H- and one to O145:H-. Since only few clinical laboratories examine human stools for the presence of *E. coli* O157, the incidence of VTEC among humans cannot be correctly estimated.

- Verotoxin producing *Escherichia coli* in cattle
- *Escherichia coli* O157 in food
- Verotoxinogenic *Escherichia coli* in humans

# Verotoxin producing *Escherichia coli* in cattle

## Surveillance systems and methods used

In case *E. coli* O157 was isolated from a carcass at the slaughterhouse (official zoonosis programme), the farm of origin was traced back. Faecal samples were taken from twenty percent of the animals aged between 6 months and 2 years. In addition, samples of the available feed and of dust were collected. Hygienic and management measures were imposed on these farms during the period that the samples were analysed in the laboratory. The sale of not heat-treated milk or milk products was forbidden.

The faecal, feed and dust samples were enriched in mTSB and treated by immunomagnetic separation. Subsequently, the suspected colonies on CT-SMAC were latex agglutinated for the detection of *E. coli* O157. Confirmation of serotype (O group) was done by means of slow tube agglutination after heating of the bacterial cultures. Virulence factors were determined by PCR for toxin genes vt1 and vt2, and for eae (intimin) specific sequences. A typical *E. coli* O157 isolate is defined as a strain isolated by immunomagnetic separation and O157 specific agglutination and confirmed by PCR as vt2 and eae positive. An atypical *E. coli* O157 had either no eae or vt gene. Laboratory findings are available on clinical *E. coli* strains sent to the National Reference Laboratory for VTEC, animal health for analysis. A VTEC strain was identified as a VT1 or VT2 positive *E. coli* strain.

## Results of the investigations in 2003

Monitoring. In 2003 only 4 herds were sampled following identification of *E. coli* O157 on carcasses in the slaughterhouse. On three herds *E. coli* O157 VT2 eae was isolated and on one herd *E. coli* O157 without vt (atypical EHEC). Of the 184 bovine *E. coli* strains from clinical cases analysed in 2003 at the National Reference Laboratory, only 6 were VTEC. Of these, 5 were of patho-type VT1 eae (known to be associated with diarrhea), and 1 was VT1.

# *Escherichia coli* O157 in food

## Surveillance system and method used

In 2003, the zoonosis monitoring programme developed by the Federal Agency for the Safety of the Food Chain (FASFC) was implemented in over 200 Belgian abattoirs, over 100 meat cutting plants and over 100 retail outlets representative of the Belgian production of carcasses and meat. The samples tested were carcasses (1600cm<sup>2</sup>), cutting meat (25g) and minced meat of beef (25g). Staff of the FASFC was specifically trained to obtain uniform results.

The detection method (SP-VG-MOO1) consisted of a pre-enrichment in mTSB + novobiocin at 42°C for 6 hours. After that, enrichment was done in CT-Mac Conkey at 37°C for 16-18 hours and subsequent testing in the immunoassay O157 (VIDAS ECO, bioMérieux). Further on followed a selective immunomagnetic enrichment (Dynabeads, Dynal or VIDAS ICE, bioMérieux) and an isolation on sorbitol-Mac Conkey that was incubated at 42°C for 18 h. Isolation and confirmation were by means of agglutination of latex particles (Oxoid). Suspected isolates were sent to the National Reference Laboratory for detection of genes encoding virulence factors.

### Results of the investigations in 2003

The results of the monitoring of Federal Agency for the Safety of the Food Chain are shown in table P.

Sample		Prevalence(95% confidence interval)
Beef	Carcasses (n=1479)	0,7% [0,3 – 1,2%]
	Cutting meat (n=285)	0,7% [0,08 – 0,7%]
	Minced meat (n=298)	1,7% [0,5 – 3,9%]

**Table P:** Zoonosis monitoring programme - *E. coli* O157.

## Verotoxinogenic *Escherichia coli* in humans

Data were obtained from the National Reference Laboratory. In 2003, 41 typical EHEC isolates were identified, that is VTEC with virulence gene *eae* (intimin) and with the gene encoding enterohemolysin (EHEC virulence plasmid). The majority of these isolates (n=21) belonged to serogroup O157, and 18 were O157:H7. Eight typical EHEC isolates were from children between 10 months and 11 years old suffering from HUS: 5 belonged to serotype O157:H7, 2 to O157:H- and one to O145:H-. In addition, 6 atypical EHEC isolates (without intimin or enterohemolysin genes) were characterised.

Fifteen of the 21 *E. coli* O157 strains produced only VT2 and 6 strains both VT1 and VT2. Fifteen of the 20 typical non-O157 EHEC produced only VT1 and 3 only VT2 and 2 produced both VT1 and VT2. Three of the 6 atypical non-O157 isolates produced VT1, one isolate VT2 and 2 produced both VT1 and VT2. During the last seven years, the number of isolates analysed annually by the Reference Laboratory has been rather constant.



## cysticercosis

# Cysticercosis

*Cysticercus bovis* is the larval stage of the tapeworm, *Taenia saginata*, a parasitic cestode of the human gut (taeniasis).

The adult tapeworm consists of a chain of segments called proglottids, which produce eggs and contaminate the environment directly or through surface waters.

Cattle are the only intermediate hosts and develop the larval forms (cysticerci), after ingestion of eggs while grazing or feeding. The cysticerci survive in muscle tissue, diaphragm, heart, tongue or masseter, which are predilection sites.

Beside the visual inspection of the lesions, confirmation by PCR and serological examination is possible.

Humans contaminate themselves by the ingestion of raw or undercooked beef containing the larval form. Usually the pathogenicity for humans is low.

Moreover, in other parts of the world man can be infected with *Taenia solium*, a more pathogenic tapeworm transmitted by pork.

## Cysticercosis in food animals

Post-mortem, macroscopic examination of carcasses is routinely done in the slaughterhouse.

Figures from the Federal Agency for the Safety of the Food Chain show that in 2003, 24 heavily contaminated

whole carcasses of adult cattle were rejected for cysticercosis. In addition, 3,849 carcasses of cattle (CI 95% : 0.7% [0.65 – 0.70%]) and 10 of calves (CI 95% : 0.003% [0.0015 – 0.0058%]) were slightly contaminated and were treated by freezing for 10 days before human consumption.



## sarcosporidiosis and toxoplasmosis

# Sarcosporidiosis and Toxoplasmosis

## **Sarcocystis hominis (bovine as intermediate host), Sarcocystis suihominis, Toxoplasma gondii**

Domestic carnivores are hosts of the adult stage of the protozoans Sarcocystis spp and Toxoplasma, with many species as intermediate hosts.

Human can be a definitive (sarcosporidiosis) or intermediate host (toxoplasmosis) by ingestion of infected meat or excreted oocysts and develop symptoms like diarrhoea, headache, eosinophilia, abortion, congenital disorder.

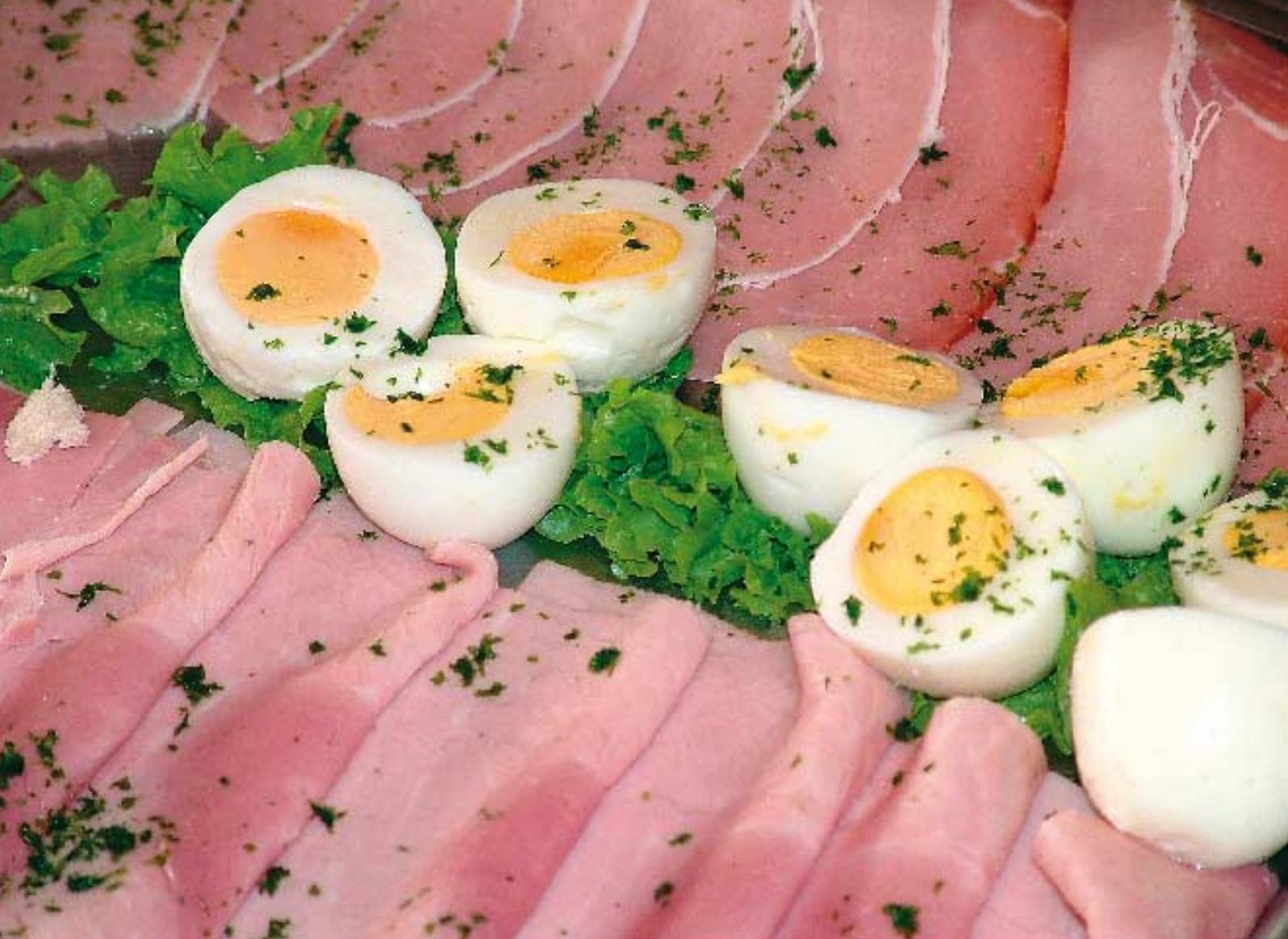
The majority of adult persons have acquired a degree of immunity to re-infection but can remain carrier for toxoplasmosis, while for human sarcosporidiosis there is no immunity development.

The majority of grazing animals are inapparent carriers of tissue cysts. There is a need for suitable microscopic, serological and molecular biological methods for the detection.

## **Surveillance system in food animals**

Carcasses are partially or enterily condemned when lesions of sarcosporidiosis or toxoplasmosis are apparent.

Number of partial rejections of cattle in 2003 because of sarcosporidiosis lesions: 14.



## foodborne outbreaks in humans

## Foodborne outbreaks in humans

During 2003, a total of 101 outbreaks of foodborne infections and intoxications were recorded in Belgium. Data came from the Flemish Community, the Federal Agency for the Safety of the Food Chain, the French Community, the Brussels Capital Region, the sentinel laboratories and the National Reference Laboratory for Salmonella and Shigella. More than 1293 people were ill, at least 142 persons were hospitalised and 1 child died. However not all outbreaks were notified and for many outbreaks data are incomplete.

- Causative agents
- Source of the foodborne outbreaks

## Causative agents

62% of the outbreaks were due to Salmonella, with Salmonella Enteritidis as predominant serotype. Other serotypes involved in the outbreaks were Dublin, Derby, Typhimurium, Virchow and 9: - -. Not in every outbreak of Salmonella the serotype was recorded.

B. cereus was the causative agent in 5% of the cases, twice in combination with Staphylococcus aureus and once in combination with Cl. perfringens. In one severe outbreak of B. cereus, 5 persons of a family had to be hospitalised with symptoms of toxic shock after a meal of pasta salad and one child died.

Staphylococcus aureus was demonstrated in 3% of the cases. Other confirmed agents were Giardia (n=3), Cl. perfringens (n=1), Campylobacter (n=1), Shigella (n=1). Canned apricots were responsible for a chemical intoxication (ethanol, acetone, hexanal).

In 22% of the outbreaks no causative agent could be identified.

## Source of the foodborne outbreaks

In 43% of the outbreaks, preparations with raw eggs (Tiramisu, chocolate mousse, mashed potatoes prepared with raw eggs, sabayon, mayonnaise, bavarois) were identified as the source of the illness. Meat or meat based products were responsible for 20% of the cases (beef, hamburger, chicken, pita and spaghetti sauce).

Causative agent	Outbreaks	Ill	Died	Hospitalised	Sources
Salmonella	63	696	-	83	Preparations with raw eggs, meat
B. cereus	2	43	1	5	Pasta salad, beef stew
S. aureus	3	15	-	10	Candy, milk
B. cereus and S. aureus	2	9	-	4	Pita, pasta salad
B. cereus and Cl. perfringens	1	61	-	2	Beef stew
Campylobacter	1	40	-	-	Barbecue meat
Other	7	39	-	1	Giardia, Shigella, chemical substances, toxins, heat strike
Unknown	22	390	-	37	
Total	101	1293	1	142	

**Table Q:** Foodborne outbreaks in humans in Belgium in 2003

Data from the Flemish Community, the Federal Agency for the Safety of the Food Chain, the French Community, the Brussels-Capital Region, the sentinel laboratories, and the National Reference Laboratory for Salmonella and Shigella.

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