

trends and sources

report on zoonotic agents in belgium

2008-2009



working group on foodborne infections and intoxications

.be



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

Bacterial diseases

Brucellosis
 Campylobacteriosis
 Escherichia coli (VTEC)
 Leptospirosis
 Listeriosis
 MRSA
 Q-fever
 Salmonellosis
 Tuberculosis
 Yersiniosis

Viral diseases

Avian influenza
 Hantaviruses
 Rabies
 West Nile virus

Parasitic diseases

Cysticercosis
 Echinococcosis
 Sarcosporidiosis
 Toxoplasmosis
 Trichinellosis

Prion diseases**Foodborne outbreaks**

Executive summary

Zoonoses are diseases or infections that are transmissible from animals to humans. The infection of humans can be acquired directly from animals or indirectly through the ingestion of contaminated foodstuffs. It's important that humans in contact with animals are aware of possible transmission of a zoonotic disease and that consumers are informed about potential zoonotic pathogens which can cause foodborne illness.

Surveillance of zoonoses remains an enormous task as well as an opportunity for all competent authorities. Measures and systems of disease surveillance, diagnosis and control must be implemented on a national level and have to be based on a suitable regulatory framework and an appropriate level of funding. Active collaboration between all actors of the food chain, stakeholders, industry, scientists, experts of the national reference laboratories and other laboratories, specialists of the competent authorities, technical committees have to bring together their expertise, experiences, methods and findings. Only a collaborative approach and effective partnership at all levels will achieve success to control zoonoses and to improve food safety.

The most commonly reported zoonotic infections in humans are those caused by bacterial zoonotic agents that can be shed by asymptomatic farm animals. Since 2005 Campylobacteriosis is the most frequently reported zoonotic disease in humans. Broiler and other poultry meat are an important source of foodborne Campylobacter infections. Salmonellosis is the second most frequently reported zoonotic disease in humans. The most important cause of foodborne outbreaks (FBO's) are Noroviruses followed by Campylobacter in 2008 and Salmonella in 2009 as the second most important cause of foodborne outbreaks. The major sources of Salmonella in foodborne outbreaks are table eggs, poultry meat and pig meat. Salmonella and Campylobacter reduction remain an important task.

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Preface

All European member states have the obligation to yearly submit an official report on the monitoring of zoonoses and zoonotic agents to the European Food Safety Authority (EFSA) based on article 9 of Directive 2003/99/EC of the European Parliament and the Council. In that report all the relevant official monitoring programmes on animals in primary production as well as on feed and food are presented. The report specifies all available data from monitoring and research activities, as well as laboratory findings from the previous year and includes results from antimicrobial susceptibility testing and FBOs.

Similarly, based on article 1 of Council Decision 2119/98/EC, data on zoonotic infections in humans are officially reported each year to the European Centre for Disease Prevention and Control (ECDC).

Based on these two official reports, the Federal Agency for the Safety of the Food Chain (FASFC), together with the federal scientific institutions Veterinary and Agrochemical Research Centre (VAR) and Scientific Institute of Public Health (IPH) agreed to publish a booklet which contains this same information combined with data of previous years to indicate some trends of diseases or sources of infection. This Trends and Sources report will focus especially on the data of 2008 and on the data of 2009 as this report is a bi-annual edition. The aim of this booklet is to inform professional readers as well as persons who have a general interest in animal and human infections and in the safety of our food and at last but not least to inform the consumer.

We hope that the reader will enjoy this seventh edition of the Belgian Trends and Sources report on zoonotic agents.

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Preface
Introduction
Belgian reference laboratories for zoonotic agents
Acronyms, abbreviations and special terms
General information

Introduction

This report compiles especially the data of 2008 and 2009 and compares these data to some data of previous years on zoonoses and zoonotic agents, and is derived from the official documents reported to EFSA and ECDC. For this reason, it is a unique document in which laboratory results from the primary production, from food, from feed and from clinical public health sources are combined. In addition to the compulsory reporting on zoonoses and zoonotic agents as listed in the European Directive 2003/99/EC, this document contains data on other foodborne agents that may be of interest to the reader, e.g. on avian influenza, transmissible spongiform encephalopathies (TSE, e.g. mad cow disease) or Norovirus infections. For the second time, the parasitic infection cryptosporidiosis and the emerging disease West Nile Fever are described.

Together with the general descriptive information on the diseases or the infections themselves, their evolution over time, some recommendations on prevention of the infection are provided. This booklet should meet the expectations of those concerned with the possible (micro)biological contamination of our food.

The FASFC organises diverse monitoring and eradication programmes in, among others, the primary production and in the transformation and distribution sectors. From their description follows that much effort is being paid to control the contamination of foodstuffs with pathogens. Some infectious diseases have successfully been reduced or even eliminated (for instance brucellosis, mad cow disease) and for others (for instance campy-

lobacteriosis) further programmes should be developed. In addition to the continuous effort from the authorities, the consumer plays also an important role. Indeed, respect for the cold chain and simple hygiene measures in the kitchen may be very efficient in preventing foodborne contaminations and illness.

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

- The Federal Agency for the Safety of the Food Chain (FASFC – FAWV - AFSCA);
- The Scientific Institute of Public Health (IPH – WIV - ISP);
- The Veterinary and Agrochemical Research Centre (VAR – CODA - CERVA).

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	D. Fretin	CODA-CERVA Groeselenberg, 99 1180 Brussels	dafre@var.fgov.be http://www.var.fgov.be/
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Salmonella (public health)	S. Bertrand	WIV-ISP, Bacterial diseases Rue J. Wytsman, 14 1050 Brussels	sophie.bertrand@wiv-isp.be http://www.wiv-isp.be/
Salmonella (animal health)	H. Imberechts	CODA-CERVA Groeselenberg, 99 1180 Brussels	heimb@var.fgov.be http://www.var.fgov.be/

Zoonotic agent or domain	Contact	Address	E-mail address / Web site
Salmonella (food)	K. Dierick	WIV-ISP, Food Pathogens J. Wytsmanstraat, 14 1050 Brussels	Katelijne.Dierick@wiv-isp.be http://www.wiv-isp.be/
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Acronyms, abbreviations and special terms

ADNS	Animal Disease Notification System
ARSIA	Association Régionale de Santé et d'Identification Animales, Walloon Regional Animal Health Association
BAPCOC	Belgian Antibiotic Policy Coordination Committee
CFT	Complement Fixation Test
CLSI	Clinical and Laboratory Standards Institute
CODA – CERVA - VAR	Veterinary and Agrochemical Research Centre Centrum voor Onderzoek in Diergeneeskunde en Agrochemie Centre d'Étude et de Recherches Vétérinaires et Agrochimiques
CRL	Community Reference Laboratory
DGZ Vlaanderen	Dierengezondheidszorg Vlaanderen, Flanders Regional Animal Health Association
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immuno Sorbent Assay
FASFC - FAVV – AFSCA	Federal Agency for the Safety of the Food Chain Federaal Agentschap voor de Veiligheid van de Voedselketen Agence Fédérale pour la Sécurité de la Chaîne alimentaire
FBO	Foodborne outbreak
FPS	Federal Public Service (Ministry) – Public Health, Food Chain Security and Environment

IFA	Indirect Fluorescent Antibody assay
IFN	Interferon
IPH - WIV - ISP	Scientific Institute of Public Health Wetenschappelijk Instituut Volksgezondheid Institut Scientifique de Santé Publique
ITM - ITG – IMT	Institute of Tropical Medicine Instituut voor Tropische Geneeskunde Institut de Médecine Tropicale
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats
NRCSS	National Reference Centre for Salmonella and Shigella, IPH
NRL	National Reference Laboratory
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
RBT	Rose Bengal Test
RFLP	Restriction Fragment Length Polymorphism
RT PCR	Reverse Transcriptase Polymerase Chain Reaction
Sanitel	Registration and Identification Database of farm animals
SAT	Slow Agglutination Test
SFM	French Society for Microbiology
WHO	World Health Organization

General information

Susceptible human population

The evolution of the total human population in Belgium from 2004 to 2009 is shown in table 1

Table 1. Evolution in the total human population 2005-2009

Source: National statistical authority, <http://statbel.fgov.be/>

	2005	2006	2007	2008	2009
Total	10.445.852	10.511.382	10.584.534	10.666.866	10.750.000
Male	5.111.325	5.143.821	5.181.408	5.224.309	
Female	5.334.527	5.367.561	5.403.126	5.442.557	
Brussels	1.006.749	1.018.804	1.031.215	1.048.491	
Flanders	6.043.161	6.078.600	6.117.440	6.161.600	
Wallonia	3.395.942	3.413.978	3.435.879	3.456.775	
Foreigners	870.862	900.473	932.161	971.448	

All figures on human population 2009 are not available at the finalisation date of this report due to the implementation of a new internal data exchange procedure and the use of a new IT application for data exchange of the statistical authority of FPS Economy, S.M.E.'s, Self-employed and Energy. Only a global total figure for 2009 is available.

The total number of immigrants in Belgium is important. Foreigners are found in four typical areas: former coal basins, cities, border regions and some concentrations in the triangle between Brussels, Antwerp and Ghent, or in the triangle between Brussels, Mons and Namur. Immigration and tourism may play an important role in the introduction and the transmission of zoonotic or other infectious diseases.

Susceptible animal populations

The figures on susceptible animal populations indicate the number of animals at a certain time point of the year.

Ruminants and pigs

The figures in the table 2 are originating from SANITEL, the computerised registration and identification database of farm animals of the FASFC.

Table 2. Total number of herds and animals in the period 2005 – 2009

	2005		2006		2007		2008		2009	
	herds	animals	herds	animals	herds	animals	herds	animals	herds	animals
Cattle	42.204	2.492.757	40.640	2.697.824	38.690	2.699.258	36.423	2.618.040	36.064	2.594.358
Pigs	10.792		10.631		9.950		9.419		9.243	
Breeding sows		657.998		653.358		632.360		615.298		598.857
Fattening pigs		4.989.016		4.850.501		5.007.614		5.123.189		5.113.202
Sheep	32.323	219.274	30.924	220.600	31.523	220.611	31.037	205.624	30.626	215.262
Goats	14.247	43.727	13.025	46.950	13.381	46.950	12.692	48.379	12.530	57.371
Deer	3.093	14.655	2.021	12.805	2.907	12.648	2.825	10.834	2.810	9.502

Next figures represent the evolution of total number of respectively porcine and bovine herds and animals over last years.

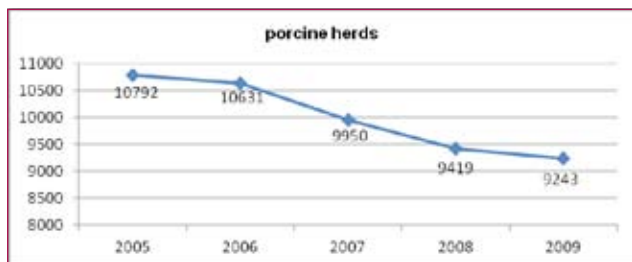


Figure 1. Evolution of the total number of pig herds, period 2005–2009

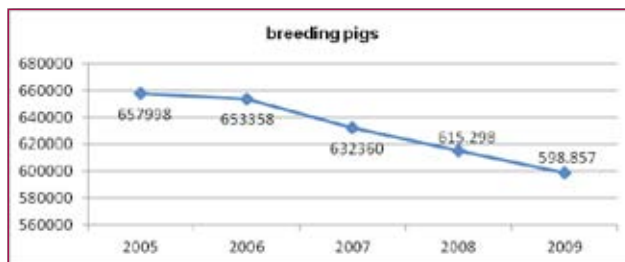


Figure 2. Evolution of the total number of breeding pigs, period 2005–2009

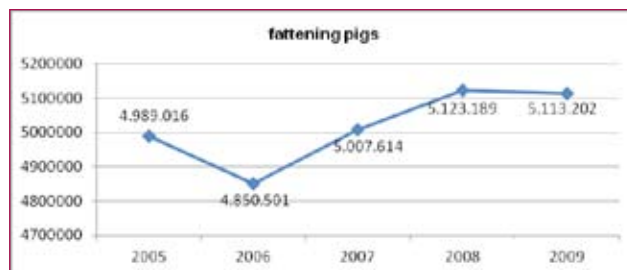


Figure 3. Evolution of the total number of fattening pigs, period 2005–2009

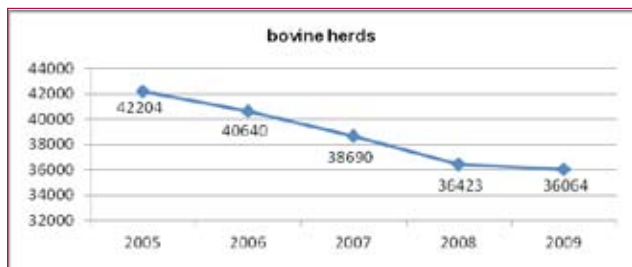


Figure 4. Evolution of the total number of cattle herds, period 2005–2009

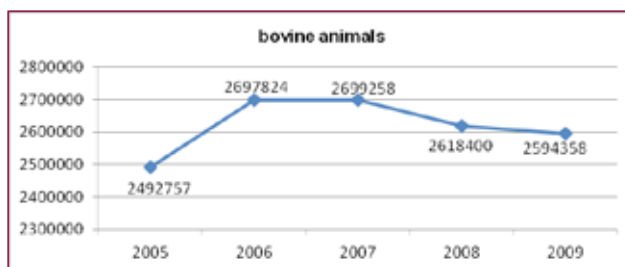


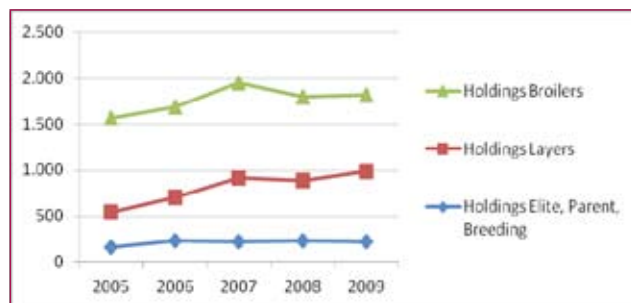
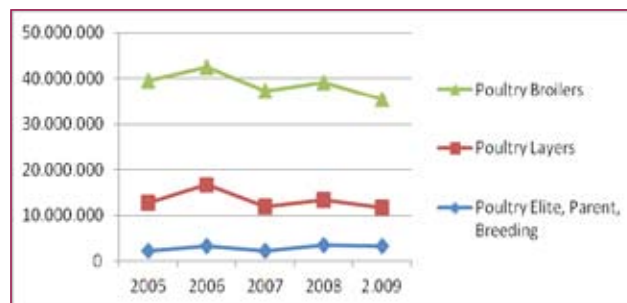
Figure 5. Evolution of the total number of bovines, period 2005–2009

Poultry

Table 3. Total number of herds and animals of poultry in the period 2005–2009

	2005		2006		2007		2008		2009	
	holdings	animals	holdings	animals	holdings	animals	holdings	animals	holdings	animals
Gallus gallus										
Layers	386	10.562.160	472	13.377.548	472	9.878.202	444	9.841.759	421	8.449.074
Broilers	1.024	26.754.817	978	25.894.597	1.036	25.311.775	911	25.700.000	838	23.718.984
Elite, Parent, Breeding	156	2.144.874	232	3.170.815	221	2.089.933	233	3.509.618	216	3.298.029
Total	1.566	39.461.851	1.682	42.442.960	1.951	37.279.910	1.793	39.051.377	1.817	35.466.087
Ducks	17	45.140	27	77.140	17	37.880	23	63.845	17	42.040
Geese	5	3.800	6	4.900	3	1.800	12	91.238	1	400
Turkeys	37	246.076	41	248.006	46	267.855	57	533.151	37	272.705

Figures 6 and 7 represent the evolution of the total number and the number per category of poultry herds and animals over the last years.

**Figure 6.** Gallus gallus, evolution number of poultry herds, period 2005–2009**Figure 7.** Gallus gallus, evolution total number of poultry, period 2005–2009

Animals slaughtered 2005 – 2009

Table 4. Number of slaughtered animals in the period 2005–2009

	2005	2006	2007	2008	2009
Cattle	523.795	496.181	495.492	522.557	480.068
Calves	313.115	327.467	306.961	301.102	319.188
Pigs	10.861.234	10.794.757	11.536.172	11.588.072	11.677.883
Solipeds	11.542	10.728	10.064	9.173	8.910
Sheep	112.771	148.767		133.192	135.071
Goats	2.585	3.036		6363	6143
	115.356	151.803	137.492	139.555	141.214
Broiler	237.670.666	247.721.072	0	242231046	262935369
Layer	29.907.674	32.265.603	0	32.196.678	27.621.546
	267.578.340	279.986.675	274505734	274427724	290556915

Figures 8 to 12 represent the evolution of the total number of slaughtered bovines, porcines, solipeds, sheep & goats and broilers & layers over the last years.

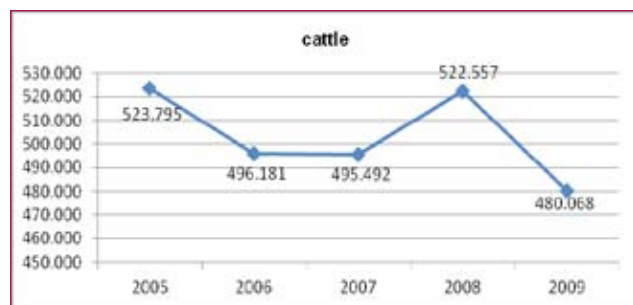


Figure 8. Evolution of slaughtered cattle 2005–2009

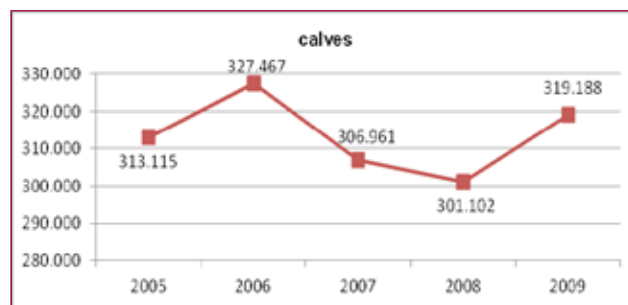


Figure 9. Evolution of slaughtered calves 2005–2009

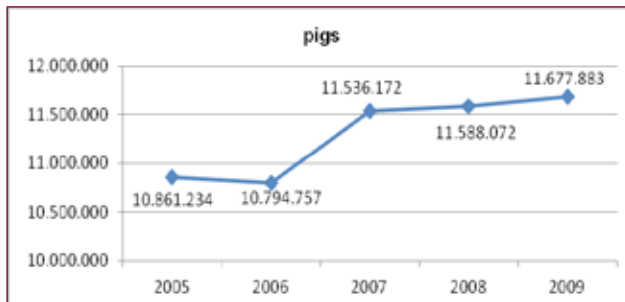


Figure 10. Evolution of slaughtered pigs 2005–2009

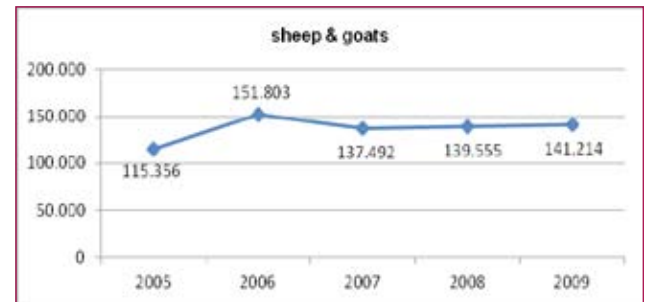


Figure 11. Evolution of slaughtered sheep & goats 2005–2009

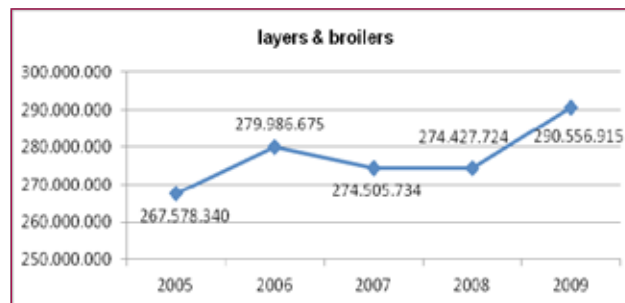
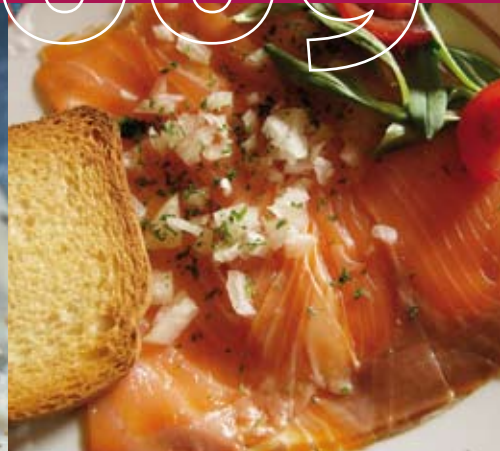


Figure 12. Evolution of slaughtered broilers and layers 2005–2009

trends and sources

2008-2009



bacterial diseases

Brucellosis

David Fretin, Luc Vanholme

Zoonotic brucellosis

Brucellosis is an infectious disease caused by bacterial species of the genus *Brucella*. Most species have a specific animal reservoir that can cause human disease: *B. abortus* in cattle, *B. melitensis* in sheep and goats, *B. suis* in pigs and *B. canis* in dogs. Transmission occurs through contact with infected animals, contaminated animal tissue or through ingestion of contaminated products.

In people brucellosis is characterised by flu-like symptoms such as fever, headache, back pain and physical weakness. Nocturnal sweating is frequently observed. Infections of the central nervous system 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 (*B. melitensis*).
- In Northern Europe, besides some occupational human cases of *B. abortus* infections, the majority of brucellosis cases are imported and are mainly caused by *B. melitensis*.

Zoonotic brucellosis

Brucellosis in cattle

Brucellosis in sheep and goats

Brucellosis in pigs

Brucellosis in humans

Brucellosis in cattle

Belgium is officially free from bovine brucellosis since the 25th of June 2003 (Commission Decision 2003/467/EC establishing the official tuberculosis, brucellosis and enzootic-bovine-leucosis-free status of certain Member states and regions of Member states as regards bovine herds).

Vaccination has been prohibited in Belgium since 1992.

An intensified bovine brucellosis eradication 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), the slaughtered animals were compensated based on the replacement value.

The annual herd prevalence notified was in 1988 was 1.13% and has fallen below 0.01% since 1998. On 27th March 2000, the last case of bovine brucellosis was identified. No infected herd was detected in Belgium since then.

Surveillance programme and methods used

Since Belgium obtained an official brucellosis free status, the eradication programme has been replaced by a surveillance programme.

Dairy cattle herds are checked at least 4 times a year via tank milk control. Tank milk is examined by means of the milk ring test. If tank milk is positive, all individual animals of the herd older than 2 years are tested serologically. Beef cattle older than 2 years are serologically monitored once every three years.

The herds are selected on the basis of geographical localisation. Furthermore, all female animals older than 1 year and breeding bulls are serologically tested at purchase. Each abortion or premature birth in animals at risk is subject to compulsory notification to the FASFC and testing for brucellosis is obligatory. Aborting females should be kept in isolation until the results of the investigation exclude *Brucella* infections.

Blood sera are analysed by micro-agglutination as screening test; in case of a positive result, an indirect ELISA test is performed as confirmatory test. Bacteriological examination is done in case of serological and/or epidemiological suspicion. An animal is legally suspected of brucellosis in case of a positive ELISA. If, according to the epidemiology 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 a cattle herd is considered as infected if one of its animals is positive for brucellosis by culture.

Table 5 indicates the evolution of the total individual serological tests related to the monitoring programme of beef cattle and the mandatory examination at purchase. The evolution of the total number of bulk milk tests is in line with the continuous decrease in number of dairy herds over the last years.

Table 5. Evolution of individual serological tests and testing of pools of bulk milk samples

	individual serological tests	pools of bulk milk tests
2005	579.390	80.025
2006	500.766	73.482
2007	563.948	70.067
2008	544.135	65.572
2009	452.016	60.031

Figures 13 and 14 represent the evolution of individual serological tests and testing of pools of bulk milk samples.

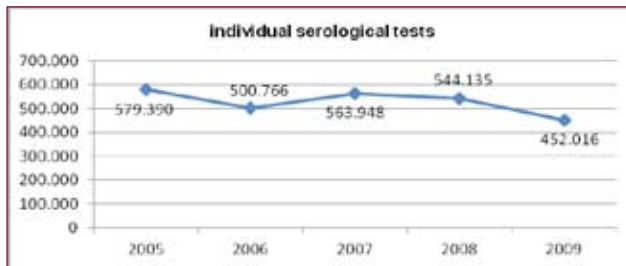


Figure 13. Evolution of individual serological tests for bovine brucellosis, 2005–2009

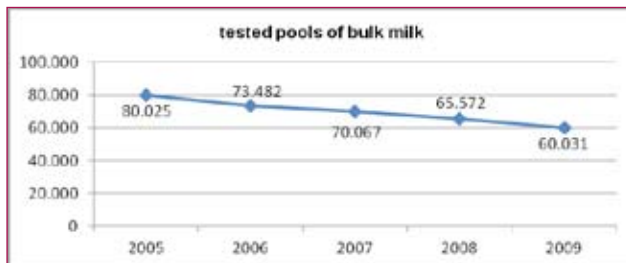


Figure 14. Evolution of pools of bulk milk tests for bovine brucellosis, 2005–2009

Epidemiological investigations and results of 2008 and 2009 surveillance

In the surveillance programme, animals are slaughtered for additional testing in case of serological and/or epidemiological suspicion. In 2008 and 2009, the FASFC instructed only a few test slaughters of animals in consequence of positive serological tests. In all cases, additional analyses could not detect a brucellosis infection.

New sanitary policy.

In 2009, a study was realized to evaluate the current national surveillance program of bovine brucellosis. If a Member State has maintained the officially free status of brucellosis for at least 5 consecutive years, the existing surveillance program can be re-evaluated and some modifications on the sampling design are allowed on condition of further proof of freedom of disease (Council Directive 64/432/EEC). The scientific veterinary experts used 'risk-based' models to evaluate different scenarios within the current surveillance program and the study was also based on a statistical confidence level approach. This methodology has underlined a few important features of the current brucellosis surveillance program. The study showed that in order to obtain a 99% confidence level to prove freedom of disease consistently an important decrease in total number of tested animals can be realised (600.000 to 30.000 individual serological tests a year, no further testing of bulk milk samples). The study also clearly indicated that the best approach is to test bovines imported from officially free and specially from non-officially free Member States of *Brucella* spp., to test animals at purchase in consequence of national trade as well as to analyse aborting animals in order to

early detect (re-)infection. Regarding the passive surveillance (abortions), the study indicated that there is a need to increase the number of analysed abortions. In consequence of this study and an opinion of the Scientific Committee of the FASFC, a new surveillance program will be applied for winter screening 2009–2010 starting mid November 2009.

For more details on the study and the scientific advices see:

- CCVD report "Evaluation of the Belgian surveillance program for bovine brucellosis and leucosis". S. Welby, Y. Van der Stede, C. Letellier, D. Fretin, J.-Y. Houtain, M. Lomba, S. Stoop, M. Van-robaeys, L. Van Schoubroeck, J. Hooyberghs, L. Vanholme, L. Lengelé, G. Lamsens, E. Pottie.
- Advice 26-2009 of the Scientific Committee of the FASFC on the evaluation of an alleviated brucellosis and leucosis surveillance program and on propositions for a new surveillance program of other bovine diseases.
- Addendum of Advice 26-2009 of the Scientific Committee of the FASFC
- Advice 05-2010 of the Scientific Committee of the FASFC on the new surveillance policy of animal diseases – part cattle (other matrices), small ruminants and pigs.
- Advice 10-2010 of the Scientific Committee of the FASFC on the new surveillance policy of animal diseases – part poultry and solipeds.

Regarding the passive surveillance by examination of abortions, next figure 15 represents the total number of notified abortions in the period 2005 – 2009.

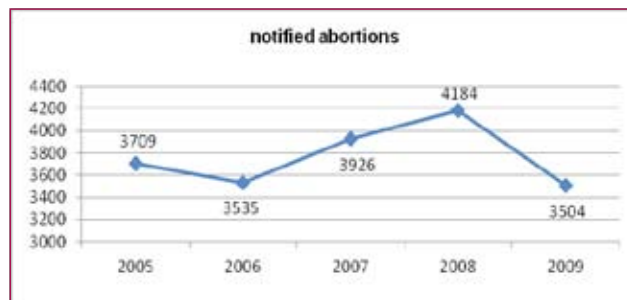


Figure 15. Number of notified abortions, 2005–2009

Brucellosis in sheep and goats

Belgium is official free for sheep and goat brucellosis (*B. melitensis*) since 29 March 2001 (Commission Decision 2001/292/EC amending Decision 93/52/EEC recording the compliance by certain Member States or regions with the requirements relating to brucellosis (*Brucella melitensis*) and according them the status of a Member State or region officially free of the disease).

Surveillance programme

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 (about 5% of the sheep and goats population should be tested by legal provisions). Positive samples were subsequently tested with Rose Bengal test (RBT) and Complement Fixation test (CFT). A sample is classified as positive for brucellosis only if it is positive in all three tests.

Since 2001, yearly serum samples from sheep and goats were tested at the NRL. In addition, serum samples from sheep for export were analysed. In 2008 and 2009, respectively 3,375 and 2,321 samples were tested. Serological positive reacting animals after serial and repeated testing were finally negative. The NRL has confirmed infections of *Yersinia enterocolitica* O:9 in sheep. Those infections are associated with false positive serology in the tests ELISA, RBT and possibly CFT of brucellosis. The phenomenon of FPSR (false positive serological reactors) as known for bovines is also observed in sheep.

Next figure 16 represents the total number of tests realised by the NRL for *Brucella melitensis*.

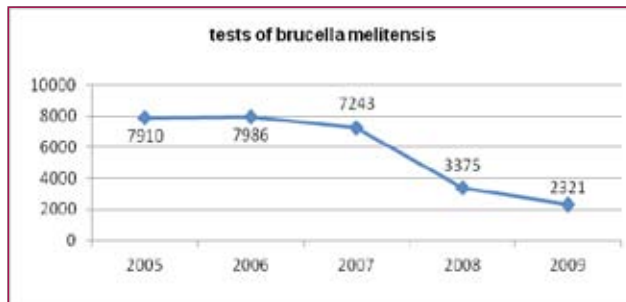


Figure 16. Number of tests of *Brucella melitensis*, 2005 – 2009

Brucellosis in pigs

Surveillance programme in pigs and epidemiological investigations

Serological screening for *Brucella* is done in breeding pigs that are brought together (e.g. at a fair), at artificial insemination centres or in animals intended for trade. The methods used are Rose Bengal test (RBT), Slow Agglutination test (SAT) according to Wright, CFT and ELISA. Bacteriological examination for *Brucella* and *Yersinia* is done in case of positive serology.

Sometimes, false positive serological reactions are reported. These are due to a *Yersinia enterocolitica* O:9 infection and are confirmed by *Yersinia* spp. isolation in the absence of *Brucella* spp. isolation.

The domestic pig population is free of brucellosis (last *Brucella* isolation in pigs in Belgium was in 1969). In 2007, 2008 and 2009 all samples were negative.

Brucellosis in humans

The last indigenous case of *Brucella* was reported in 1997. It is helpful to note that *B. suis* biovar 2, the only known biovar circulating in Belgium among wild boars, shows only limited pathogenicity for humans, if pathogenic at all.

In 2007, the NRL confirmed two cases of *Brucella melitensis* biovar 2 and one case of *Brucella melitensis* biovar 3. The country of origin of these three imported cases was not known.

In 2008, a suspected case was finally negative by culture.

In 2009, the NRL confirmed one imported case of *Brucella melitensis* biovar 1 on a patient from Lebanon where brucellosis is endemic.

Campylobacteriosis

Katrien Beullens, Katelijne Dierick, Geneviève Ducoffre, Olivier Vandenberg, Luc Vanholme

Campylobacteriosis

Campylobacteriosis continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in Belgium since 2005. Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp. Typically, the infective dose of these bacteria is low. The species most commonly associated with human infection are *C. jejuni* followed by *C. coli* and *C. lari*, but other *Campylobacter* species are also known to cause human infections.

The incubation period in humans averages from two to five days. Patients may experience mild to severe illness, with general clinical symptoms including watery, often bloody diarrhea, abdominal pain, fever, headache and nausea. Usually, infections are self-limiting and last only a few days. Infrequently, complications as reactive arthritis and neurological disorders occur. *C. jejuni* has become the most recognised cause of Guillain-Barré syndrome, a polio-like form of paralysis that can result in respiratory and severe neurological dysfunction and even death.

Campylobacteriosis

Campylobacter in food

Antimicrobial resistance in strains
isolated from meat and meat products

Campylobacter in humans

Thermotolerant *Campylobacter* spp. are widespread in nature. The principal reservoir is the alimentary tract of wild and domesticated birds and mammals. Thermotolerant *Campylobacter* spp. are prevalent in food animals such as poultry, cattle, pigs and sheep; in pets, including cats and dogs; in wild birds and in environmental water sources. Animals are mostly asymptomatic carriers.

The bacteria can contaminate various foodstuffs, including meat, raw milk and dairy products, and less frequently fish, fishery products and fresh vegetables. Contact with live poultry, consumption of undercooked poultry meat, drinking water from untreated water sources, and contact with pets have been identified as important sources of infection.

The contamination of poultry carcasses and meat with *Campylobacter* is monitored by the FASFC since 2000. The incidence of positive poultry samples is high and remains stable. Poultry meat has to be well cooked before consumption and cross-contamination should be avoided during preparation.

Campylobacter in food

Monitoring programme

In 2008 and 2009, a monitoring programme in Belgian slaughterhouses, meat cutting plants, processing plants and retail trades representative of the Belgian production of poultry carcasses and meat, pork carcasses and minced meat of all species was realised by the FASFC. In addition, samples from raw milk cheese and live bivalve molluscs were also analysed.

Specially trained staff of the FASFC performed the sampling. Different sample sizes (25g, 0.01g and 600 cm²) were analysed and in some cases an enumeration was performed. For broiler carcasses at slaughter and cutting meat at processing plants, independent samples were taken per matrix in order to estimate prevalence of the contamination at 95% confidence level.

Results of the 2008-2009 monitoring

The results of the monitoring of the FASFC are shown in the next table 6.

Table 6. Zoonosis monitoring programme – *Campylobacter* in food

Sample	Sample size	Percentage of positive samples 2008	Percentage of positive samples 2009
Broiler			
Carcasses at slaughter	25g (caeca)	53.8% (n=292)	31% (n=337)
Carcasses at slaughter	1g	33% (n=185)	32% (n=261)
Carcasses at retail	0.01g	19.1% (n=115)	25.4% (n=118)
Meat cuts (skinned or with skin) at processing plant	1g	7.3% (n=523)	8.6% (n=513)
Layer			
Carcasses at slaughter	25g (caeca)	96% (n=76)	-
Carcasses at slaughter	1g	27% (n=37)	37% (n=317)
Carcasses at retail	0.01g	-	15% (n=60)
Poultry			
Meat cuts (with skin) at retail	0.01g	7.0% (n=57)	21.7% (n=92)
Meat cuts (without skin) at retail	0.01g	0.0% (n=69)	0.0% (n=91)
Minced meat at retail	Enumeration (M=100 cfu/g)	2.2% (n=183)	0.0% (n=71)
Meat preparation at processing plant	0.01g	1.6% (n=123)	0.0% (n=112)
Meat preparation at retail	Enumeration (M=100 cfu/g)	0.0% (n=94)	0.0% (n=100)
Pork			
Carcasses at slaughter	600 cm ²	16.6% (n=500)	14% (n=656)
Minced meat (intended to be eaten raw) at retail (all species)	Enumeration (M=10 cfu/g)	0.0% (n=129)	0.0% (n=184)
Minced meat (intended to be eaten cooked) at retail (all species)	Enumeration (M=100 cfu/g)	0.0% (n=125)	0.0% (n=183)
Raw milk cheese at retail	25g	0.0% (n=46)	0.0% (n=49)
Raw milk cheese at farm	25g	0.0% (n=45)	0.0% (n=40)
Live bivalve molluscs at retail	25g	0.0% (n=61)	0.0% (n=94)

The contamination rate of pork carcasses raised until 2006 and remained stable later on. In 2007, the contamination rate decreased a bit to increase again in 2008 and to decrease in 2009. From 2008 on, the *Campylobacter* spp. contamination has been enumerated. The results show a low level of contamination.

Table 7. Evolution of the *Campylobacter* prevalence of pork carcasses 2005-2009

		Sampling level	2004	2005	2006	2007	2008	2009
Pork	Carcasses	600 cm ²	4.9%	7.2%	13.4%	12.2%	16.6%	14.0%

Antimicrobial resistance in strains isolated from meat and meat products

Surveillance programme and method used

In 2008 and 2009, respectively 484 and 461 *Campylobacter* strains, were isolated in the zoonoses monitoring program. Isolates originating from poultry (carcasses of broilers, filets, meat preparations, turkey and carcasses of spent hens) and pork were examined for antimicrobial susceptibility by the NRL. Compared to 2007, the number of analysed isolates has more than doubled.

Seventy-five and 77 strains were respectively isolated from pork meat or carcasses in 2008 and 2009, while 402 and 407 poultry-derived isolates (broiler meat or carcasses, spent hens and turkey) were analysed. *C. coli* was the most prevalent strain isolated from pork carcasses (90%/71% in 2008/2009), while for poultry meat *C. jejuni* was the most isolated *Campylobacter* strain (78%/72% in 2008/2009), followed by *C. coli* (21%/28% in 2008/2009).

Minimum Inhibitory Concentrations (MIC) were determined by using E⁹-test on blood agar plates. The antimicrobials tested and the breakpoints (following the CLSI standards) used are listed in the following table 8.

Table 8. *Campylobacter* in meat and meat products: list of antimicrobials tested and breakpoints used

Antimicrobial	Breakpoints (µg / ml)	
	<i>C. jejuni</i>	<i>C. coli</i>
Ampicillin	16	16
Tetracycline	2	2
Nalidixic acid	16	32
Ciprofloxacin	1	1
Erythromycin	4	16
Gentamicin	1	2

The percentage of resistant strains of *Campylobacter* in meat is reported in the next table 9.

Table 9. Antimicrobial susceptibility testing of *Campylobacter* in food: percentage of resistant strains

	Poultry meat				Pork	
	<i>C. jejuni</i>		<i>C. coli</i>		<i>C. coli</i>	
	2008 (n=313)	2009 (n=292)	2008 (n=86)	2009 (n=115)	2008 (n=70)	2009 (n=55)
Tetracycline	44%	45%	87%	64%	86%	73%
Ciprofloxacin	43%	41%	81%	71%	31%	36%
Nalidixic acid	46%	45%	86%	69%	31%	36%
Gentamicin	20%	25%	10%	23%	23%	26%
Erythromycin	8%	12%	14%	20%	21%	20%
Ampicillin	29%	46%	29%	30%	13%	18%

Antimicrobial resistance in *Campylobacter* from poultry meat

In total, 402 and 407 *Campylobacter* strains were isolated from poultry in 2008 and 2009, respectively, and tested for antimicrobial susceptibility (313/86-*C. jejuni*/*C. coli* in 2008 and 292/115-*C. jejuni*/*C. coli* in 2009). In 2008 and 2009, 20% and 17% of all *Campylobacter* strains, respectively, were sensitive to all tested antibiotics.

In 2008, tetracycline and nalidixic acid resistance were most dominantly present (54%), followed closely by resistance to ciprofloxacin (52%). When comparing different food matrices investigated, it is clear that *Campylobacter* isolates from the more processed meat matrices showed higher antibiotic resistance. For example, isolates from minced poultry meat showed a high ampicillin resistance (62.5%), and isolates from poultry meat preparations had high resistance to tetracycline (92%), while both matrices contained little or no isolates sensitive to all antibiotics tested. On the other hand, 40% of all isolates from spent hens were sensitive to all antibiotics tested. In 2009, resistance profiles were similar: respectively 52%, 50% and 50% of the tested strains were resistant to nalidixic acid, ciprofloxacin and tetracycline.

Overall, antibiotic resistance was more prevalent in *C. coli* than in *C. jejuni* (see graph), with in 2008 and 2009 only 3 and 8 strains sensitive to all antibiotics, respectively. The number of multiresistant strains, resistant to three or more antibiotics, significantly decreased from 80% in 2008 to 61% in 2009. This trend was also observed for resistance to ciprofloxacin (81%/71%-2008/2009), nalidixic acid (86%/69%-2008/2009) and tetracycline (87%/64%-2008/2009), but remains high. For *C. jejuni*, 25% and 21% of all

strains were sensitive to all antibiotics tested, while 38% and 41% were resistant to three or more antibiotics in 2008 and 2009, respectively. High prevalence of resistance was observed for nalidixic acid (46%/45%-2008/2009), tetracycline (44%/45%-2008/2009) and ciprofloxacin (43%/41%-2008/2009). Resistance of *C. jejuni* to ampicillin increased from 36% in 2008 to 46% in 2009.

Compared to previous years, general resistance has increased (see figures 17 and 18), mostly due to adaptation of the breakpoint values used to assess resistance. This is most obvious for gentamicin.

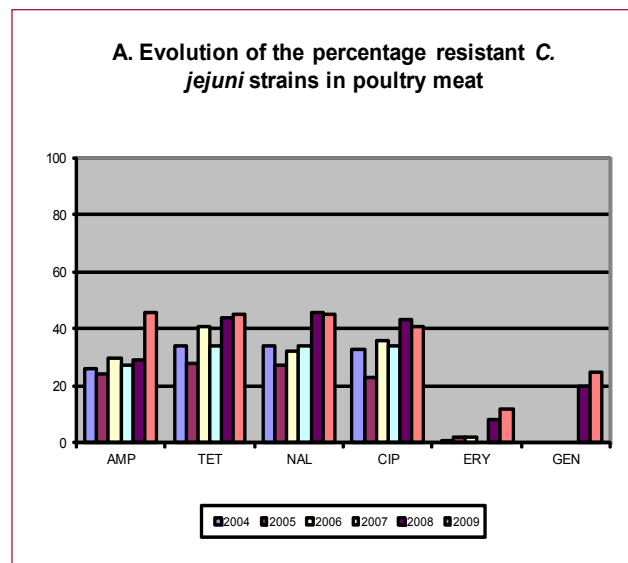


Figure 17. Evolution of the percentage resistant *Campylobacter jejuni* strains in poultry

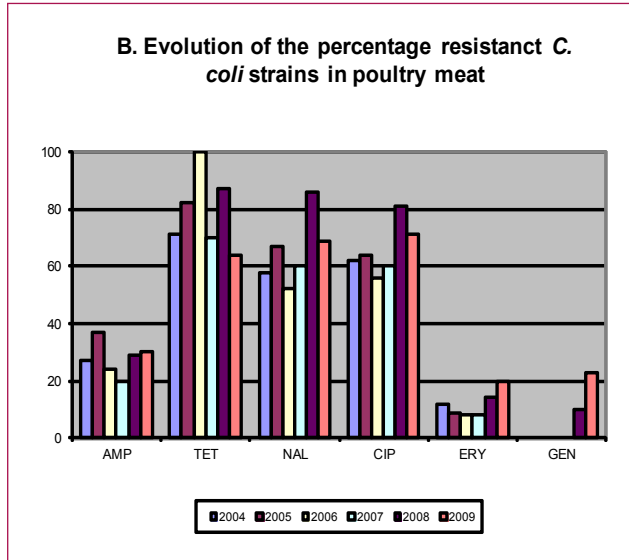


Figure 18. Evolution of the percentage resistant *Campylobacter coli* strains in poultry

Antimicrobial resistance in *Campylobacter* from pork

In total, respectively 75 and 59 *Campylobacter* isolates from pork were analysed in 2008 and 2009 (5/70-*C. jejuni*/*C. coli* in 2008 and 4/55-*C. jejuni*/*C. coli* in 2009). The number of isolates that were sensitive to all tested antibiotics decreased by half in 2009, to only 7%, compared to 2008. The resistance against tetracycline (82%/73%-2008/2009) was high, while 36% and 38% of all isolates showed resistance to three or more antibiotics tested in 2008 and 2009, respectively. Complete resistance was not observed.

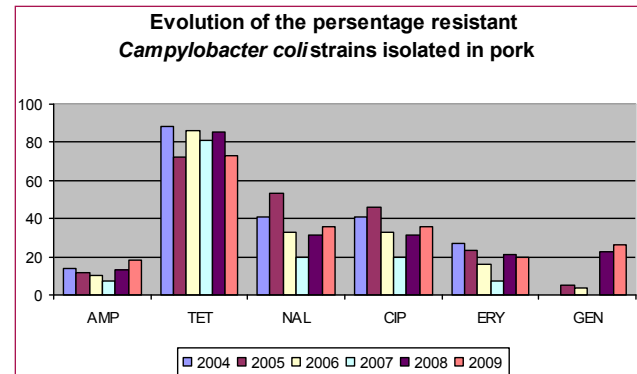


Figure 19. Evolution of the percentage resistant *Campylobacter coli* strains isolated in pork

Campylobacter in humans

In 2008 and 2009, the Sentinel Laboratory Network consisted of 103 and 102 laboratories respectively; 93 and 90 of them reported Campylobacter. 5,111 and 5,635 strains of Campylobacter were isolated respectively, which represent at country level isolation rates of 48 and 53 per 100,000 inhabitants. The number of Campylobacter infections shows a significant decreasing trend since 2000 ($p < 0.05$; figure 20). Since 2005, Campylobacteriosis remains the most frequently reported zoonosis in humans.

Cases are reported during the entire year, with a peak in the summertime (Figure 21).

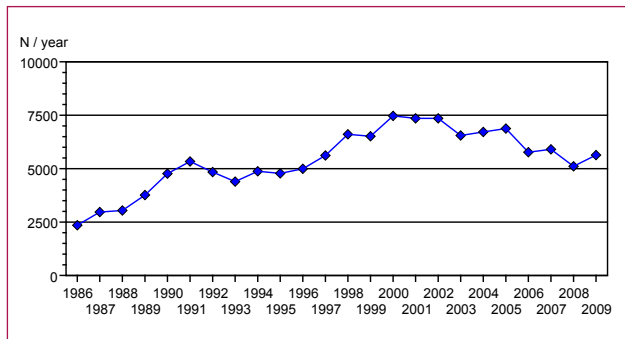


Figure 20. Total number of Campylobacter infections in humans by year (1986-2009) Source: Sentinel Laboratory Network

Campylobacter isolation rates are higher in men (52%) than in women (48%); 21% of cases are diagnosed in children of 1-4 year old and 20% in adults of 25-44 year old. This distribution is observed since many years. There is no explanation for this observation (Table 10).

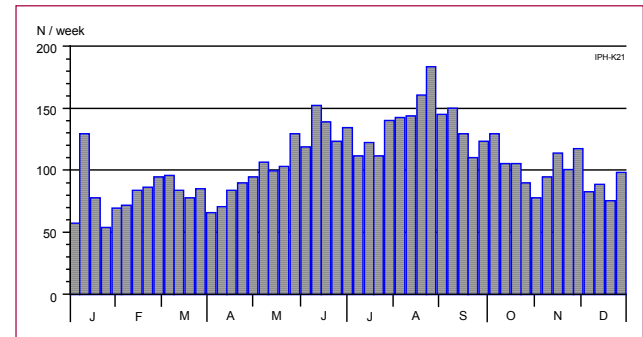


Figure 21. Weekly number of Campylobacter infections in humans, 2009 Source: Sentinel Laboratory Network

Table 10. Number of Campylobacter infections in humans by sex and by age groups, 2009 Source: Sentinel Laboratory Network

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	175	6,3	130	5,3	305	5,8
1 - 4	634	22,3	496	20,2	1130	21,3
5 -14	476	15,1	339	12,5	815	13,9
15 -24	295	10,1	340	13,4	635	11,7
25 -44	528	19,1	566	21,4	1094	20,2
45 -64	436	16,3	385	14,7	821	15,5
≥ 65	330	11,0	354	12,5	684	11,7
Total	2874	100,0	2610	100,0	5484	100,0

Since the beginning of the registration (1983), the incidence in Flanders is higher than in Wallonia. This was confirmed in 2009 with an estimated incidence of 61/100,000 inhabitants in Flanders, 42/100,000 inhabitants in Wallonia and 34/100,000 inhabitants in Brussels-Capital Region. The incidence is very high in a few districts since many years and also in 2009:

134/100,000 inhabitants in Mouscron, 122/100,000 inhabitants in Eeklo, 109/100,000 inhabitants in Mechelen and 109/100,000 inhabitants in Leuven (Figure xx). It would be useful to make a study to further explore the reasons for the observed difference in occurrence of Campylobacteriose between districts in Belgium.

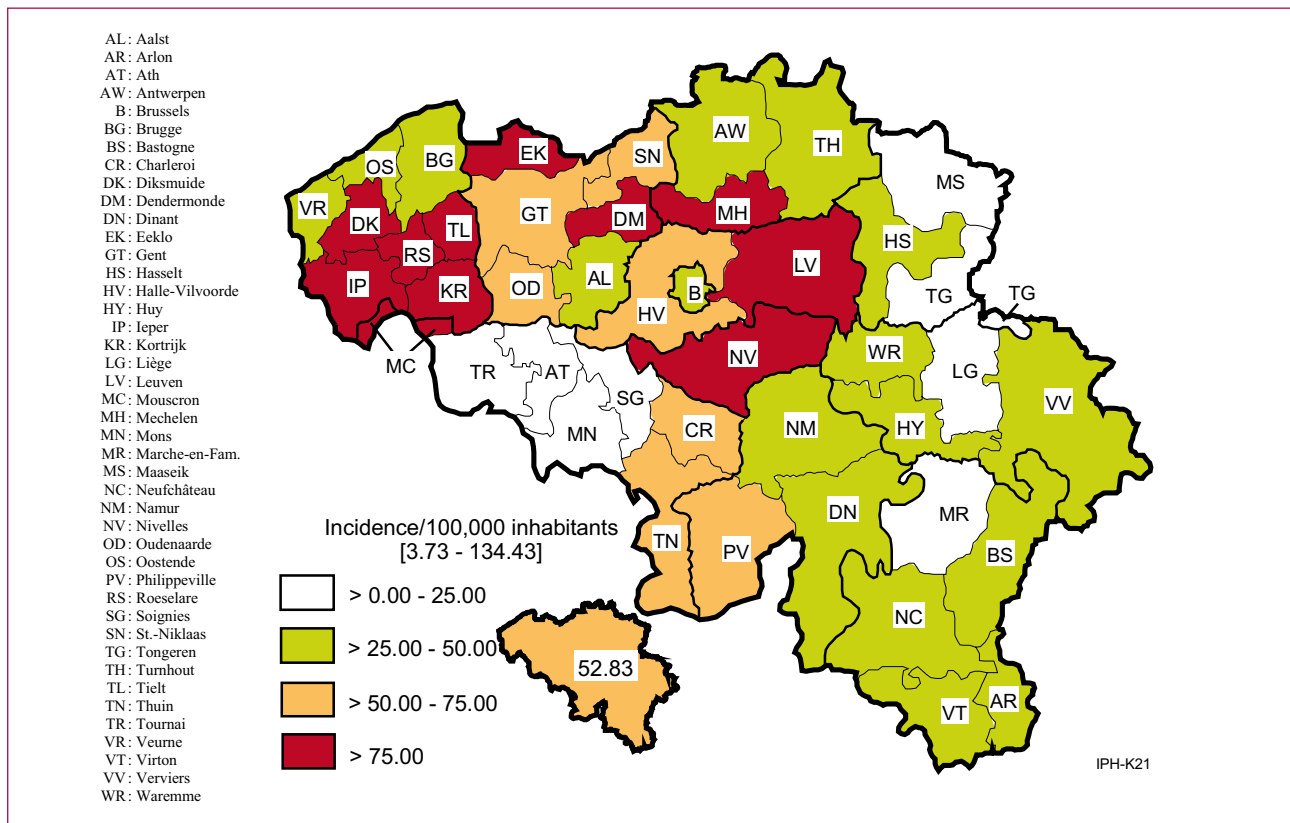


Figure 22. Incidence of *Campylobacter* infections in humans by district (N/105 inhab., 2009)

Source: Sentinel Laboratory Network

Epidemiology Enteric Campylobacter

Data were obtained from the NRL for human Enteric Campylobacter. Since there is not obligation for clinical laboratories to send human isolates to the NRL to confirm the presence of Campylobacter, a correct epidemiological situation of Campylobacter in human populations cannot be made.

Data about human Campylobacter cases were only obtained from two laboratories deservng the Brugmann, Queen Fabiola, Bordet and St.-Peter University Hospitals located in Brussels.

Methods used

From January 2008 to December 2009, a total of 18,737 stool specimens from 12,084 patients submitted to the NRL were routinely examined for Campylobacter spp. using one selective medium and a filtration method.

Results of surveillance

By the application of this comprehensive isolation procedure, enteric Campylobacter was isolated in 631 patients, giving a prevalence of Campylobacter infection of 5.2%. Similar reported prevalence has been reported from other European countries.

Antimicrobial resistance of human isolates

Methods used

A total of 2447 human Campylobacter strains isolated in two laboratories serving the Brugmann, Queen Fabiola, Bordet and St-Peter University Hospitals located in Brussels, Belgium from January 2001 to 2009 were examined for their resistance.

Three antibiotics of therapeutic interest were tested in disk diffusion according to Kirby-Bauer, following SFM recommendations.

Results of 2009 surveillance

In 2009, resistance was mostly found to ampicillin (53.6%), and to ciprofloxacin (39.7%). Most Campylobacter were susceptible to erythromycin (87.1%). These results point out the need to monitor antibiotic resistance in Campylobacter from human and food (Table 11).

Table 11. Resistance of Campylobacter in Belgium fecal isolates, trend from 2001 till 2009, based on data from the NRL (Laboratory for Microbiology, St.-Peter University Hospital, Brussels)

Antimicrobial Agent	2001	2002	2003	2004	2005	2006	2007	2008	2009
	N = 280	N = 266	N = 212	N = 291	N = 260	N = 246	N = 263	N = 284	N = 345
	% Resistant	% Resistant	% Resistant	% Resistant	% Resistant	% Resistant	% Resistant	% Resistant	% Resistant
Ampicillin	13,9	15,8	16,0	29,2	32,3	51,6	57,4	50,7	53,6
Erythromycin	3,2	0,0	9,4	6,5	6,9	8,1	5,2	3,5	2,9
Ciprofloxacin	18,9	22,6	24,5	28,1	33,1	50,4	49,8	40,8	39,7

(Sources: Trends of antimicrobial resistance of Campylobacter strains isolated in two laboratories serving the Brugmann, Queen Fabiola, Bordet and St.-Peter University Hospitals located in Brussels, Belgium from January 2001 to 2009. Antimicrobial susceptibility testing was realized by disk diffusion methods following the SFM recommendations).

Invasive *Campylobacter* in humans

In 2009, the NRL for *Campylobacter* confirmed 28 invasive *Campylobacter* isolates. Among these, *C. fetus* was recovered in 15 patients. The other strains were *C. jejuni* and *C. coli* in 11 and 2 cases respectively (Table 12).

Table 12. Repartition by biotype of invasive *Campylobacter* in humans, 2009

Source: NRL

N	<i>Campylobacter jejuni</i>				<i>C. coli</i>		<i>C. fetus</i>
	I	II	III	IV	I	II	subsp.fetus
28	10	1			1	1	15

Escherichia coli (VTEC) infections

Katrien Beullens, Hein Imberechts, Denis Pierard, Luc Vanholme

Verotoxin producing Escherichia coli

Verotoxigenic *Escherichia coli* (VTEC) is a group of *E. coli* characterised by the ability to produce 'verocytotoxins' or 'shiga like toxins'. Human pathogenic VTEC usually have additional virulence factors that are important for the development of disease in man and are called EHEC (enterohemorrhagic *E. coli*). EHEC infections in man are usually associated with a minor number of O:H serogroups. Of these, the O157:H7 or the O157:H- serogroup (EHEC O157) are the ones most frequently reported to be associated with the human disease. Other pathogenic serotypes of *E. coli*, e.g. O26, O91, O103, O111 and O145 may also be involved.

Human EHEC infections are mostly sporadic. Human infection may occur after consumption of contaminated food or water, after contact with contaminated water, or by direct transmission from person to person or through contact with infected animals.

The clinical symptoms range from mild to bloody diarrhoea through haemorrhagic colitis, which is often accompanied by abdominal cramps, usually without fever. VTEC infections can result in haemolytic uremic syndrome (HUS), characterised by acute renal failure, anaemia and lowered platelet counts. HUS develops in up to 10% of patients infected with *E. coli* O157 and is the leading cause of acute renal failure in young children.

Verotoxin producing *Escherichia coli*

Verotoxin producing *Escherichia coli* in cattle

Escherichia coli O157 in food

Verotoxinogenic *Escherichia coli* in humans

Animals are a reservoir for VTEC, and VTEC have been isolated from many different animal species. The gastrointestinal tract of healthy ruminants seems to be the foremost important reservoir for VTEC. Cattle are the principal reservoir of VTEC. The organism is excreted in the faeces. Food of bovine origin is frequently reported as a source for human VTEC infections. Other important food sources include faecally contaminated vegetables and drinking water.

Prevention mainly relies on bio-security measures at farm-level and hygienic measures at the level of the slaughterhouses. Since August 2005, the sampling of cattle at farms that had sent *E. coli* O157 positive animals to the abattoir is not compulsory any more. Since then, epidemiological investigations and additional sampling at the farm of origin is only done following EHEC infections in humans.

In Belgium, approximately 50 to 100 sporadic human cases are registered per year.

Verotoxin producing *Escherichia coli* in cattle

Surveillance programme, measures and methods used

No surveillance programme was in place in 2008 or 2009. In case a cattle farm is suspected to be at the origin of an EHEC outbreak in humans, a representative number of animals this farm is sampled (faecal, feed, environmental samples). FASFC officials inform the owner that EHEC circulate on his farm and encourage the implementation of hygienic measures, i.e. cleaning and disinfection

of milk reservoirs and milking equipment, and cleaning of animals before transport to the slaughterhouse.

The ISO 16654:2001 method was used for isolation of *E. coli* O157. Briefly, the samples are enriched in mTSB with novobiocin and treated by immunomagnetic separation. Subsequently, the suspected colonies on CT-SMAC are latex agglutinated for the detection of *E. coli* O157. Confirmation of serotype (O group) is done by means of slow tube agglutination after heating of the bacterial cultures. Virulence factors are determined by PCR for toxin genes *stx1* and *stx2* and for *eae* (intimin). Enterohemolysis is done on appropriate culture media.

Epidemiological investigations and results of 2008-2009 surveillance

In 2008 and 2009, no cattle farms were sampled to identify the source of an *E. coli* O157 outbreak. Only one bovine VTEC strain was sent to the NRL (animal health) for typing, with pathotype *stx1 eae*.

Escherichia coli O157 in food

Monitoring programme

E. coli O157 was analysed in diverse beef and dairy products.

Notification is mandatory since March 2004 (Ministerial Decree on mandatory notification in the food chain). For enterohemorrhagic *E. coli*, absence in 25g in ready-to-eat food products put on the market is mandatory.

Results of the 2008-2009 monitoring

The results of the monitoring by the FASFC are shown in the following table 13.

Table 13. Zoonosis monitoring programme - *E. coli* O157, 2008 -2009

	Sample	Prevalence 2008	Prevalence 2009
Beef	Carcasses	0.7% (n=1353)	1.0% (n=995)
	Fresh meat at cutting plant	0.0% (n=766)	0.0% (n=291)
	Minced meat (steak tartare) at processing	-	0.0% (n=294)
	Minced meat (steak tartare) at retail	0.6% (n=159)	0.0% (n=147)
	Meat preparations (steak tartare with herbs and sauce) at retail	0.0% (n=155)	1.0% (n=147)
Cheese	From raw cow's milk, at farm	0.0% (n=35)	0.0% (n=59)
	From raw cow's milk, at retail	0.0% (n=95)	0.0% (n=106)
	From raw milk, at processing	0.0% (n=21)	0.0% (n=40)
	From raw sheep's milk, at farm	0.0% (n=18)	0.0% (n=15)
	From raw sheep's milk, at retail	0.0% (n=44)	0.0% (n=63)
	From raw goat's milk, at farm	0.0% (n=18)	4.0% (n=25)
	From raw goat's milk, at retail	0.0% (n=50)	0.0% (n=64)
Butter	At farm	0.0% (n=121)	0.0% (n=132)
	From raw milk, at retail	0.0% (n=48)	0.0% (n=24)
Cream	At farm	0.0% (n=51)	0.0% (n=46)

Verotoxinogenic *Escherichia coli* in humans

Only few clinical laboratories examine human stools for the presence of *E. coli* O157. Therefore, a correct incidence of VTEC in human populations cannot be given.

In 2008, the NRL confirmed 103 verotoxinogenic *E. coli* isolated from 102 patients. Among these:

- 73 typical VTEC isolates, positive for two factors of additional virulence: the presence of the gene *eae* (intimin) gene and enterohemolysin (EHEC virulence plasmid) gene.
- 30 atypical VTEC isolates, negative for intimin and/or enterohemolysin.

In 2009, the NRL confirmed 112 verotoxinogenic *E. coli* isolated from 110 patients. Among these:

- 86 typical VTEC isolates, positive for two factors of additional virulence: the presence of the gene *eae* (intimin) gene and enterohemolysin (EHEC virulence plasmid) gene.
- 26 atypical VTEC isolates, negative for intimin and/or enterohemolysin.

The number of isolates confirmed annually by the NRL is increasing but probably still corresponds to a large rate of underdiagnosis (Table 14 on next page).

Table 14. *E. coli*: evolution in number of isolates in humans, 1998-2009.

Source: NRL

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Number of isolates	48	53	47	46	46	47	45	47	46	47	103	112
Number of typical isolates	38	46	33	36	37	40	36	36	36	40	73	86
Number of O157 isolates	25	33	26	29	26	21	29	27	21	25	55	61

In 2008, 11 strains (all from serotype O157:H7) were associated with haemolytic uremic syndrome (HUS). Six patients were less than 5 years old (of which one was infected with two strains, see below), three were older children (respectively 6, 7 and 11 years old) and two were adults (55 and 59 years old). These last patients were involved in a small outbreak of bloody diarrhea in a psychiatric hospital, related to ground pork meat (that was later shown by PCR to contain also ground beef). These isolates were atypical since they were urease positive; PFGE analysis showed that 9 other urease positive O157 isolated from the same province in the same period as well as two other provinces were indistinguishable, but no common source could be identified. According to the information available at the NRL, all other cases were not related.

In 2009, 15 strains from 14 patients – 9 of serotype O157:H7, 3 O157:H-, 1 O103, 1 O111 and 1 not typable - were associated with haemolytic uremic syndrome (HUS), comprising 7 children less than 6 years old, 6 older children (6 to 13 years old), one 48 years old adult and one patient with unknown age. According to the information available at the NRL, all these cases were unrelated.

In conclusion, the number of O157 isolates has more than doubled in 2008 and increased slightly in 2009. No explanation was found for this fact.

Leptospirosis

Jean-Jacques Dubois, Geneviève Ducoffre, Els Goossens, Marjan Van Esbroeck

Leptospirosis

Leptospirosis or Weill's disease is a disease caused by *Leptospira interrogans* sensu lato, which is divided into more than 26 serogroups and more than 230 serovars. This aerobic mobile spirochete is able to survive for short times outside the host in a warm and humid environment (stagnant water, muddy soils). It endures mostly and for longer periods in host reservoirs, mainly rodents. After infection, a short bacteraemia is followed by the invasion of mainly kidneys and liver, in which leptospires can survive for years and can be intermittently excreted. Accidental hosts, infected through contact with contaminated water or soil (or by contact with infected animals) can develop mild flu-like symptoms. Fatal subacute kidney and or liver failure can also occur.

Leptospirosis

Leptospirosis in animals

Leptospirosis in humans

Laboratory tests

The standard serological test to detect leptospirosis is the microscopic agglutination test, which is sensitive and specific and allows a first identification at serogroup level. This test requires however the maintenance of a panel of reference strains, so only a limited number of laboratories are able to perform this assay. Other serological tests used are rapid agglutination tests and ELISAs, which perform well for a rapid detection, but are less specific. Isolation of the antigen is very difficult and laborious. Antigen detection is possible by immunofluorescence techniques and molecular techniques, but do not allow a typing at serogroup or serovar level.

Leptospirosis in animals

Data from the NRL indicate that in 2008-2009 *Leptospira* infections are only seldom diagnosed. The microscopic agglutination test of 429 cattle and 606 pig sera demonstrated about 10 samples positive in each animal species. However, in 371 horse sera tested, antibodies against serovars Australis, Pyrogenes and Grippotyphosa were found in 10,0%, 4,6% and 4,0% of the cases, respectively.

Leptospirosis in humans

Leptospirosis occurs worldwide but is most common in tropical and subtropical areas with high rainfall. The disease is found mainly wherever humans come into contact with the urine of infected animals or a urine-polluted environment.

Animal-human transmission occurs through direct contact with urine of a natural host via a wound, the mucous membrane of the mouth, the nose or the eyes or indirectly by contaminated water or food. The longer the exposure the higher the risk of infection. Human to human transmission is possible but extremely rare. The incubation period is usually 6 to 12 days with a range of 2 to 30 days.

The professions at highest risk of acquiring the disease are sewage workers, but also farmers, veterinarians, slaughterhouse staff, garbage collectors, etc. Certain hobbies can also lead to contamination: persons involved in water sports such as swimming, kayaking, diving, surfing, fishing and (wind)surfing are at risk. This disease is mainly observed at the end of summer and during autumn.

In Belgium, the number of human cases remain so far limited.

Clinical manifestations

Leptospirosis may induce a wide variety of clinical manifestations. Symptoms are divided into 4 main clinical categories:

- moderate influenza-like complaints
- Weil syndrome with jaundice and renal failure
- meningitis, encephalitis
- difficult breathing, including coughs and breathlessness.

Classically this disease has two phases with an abrupt beginning marked by a high fever ($\geq 40^{\circ}\text{C}$), shivers and muscle ache for approximately one week.

After a recovery period of one to three days without symptoms a second phase follows with multiple problems of internal organs (e.g. kidney, liver). Generally the disease has a favourable outcome if treatment is started quickly after the onset of disease.

Diagnosis

Diagnosis is based on clinical symptoms, risk factors and laboratory analyses. Bacterial culture is difficult and takes a long time. Serology is done for diagnosis. Five to ten days after onset of symptoms, antibodies against the leptospire can be detected in the blood. A negative result at the beginning of an infection does not exclude a diagnosis of leptospirosis.

Requests for analysis or confirmation of a screening result can be sent to the NRL. It is recommended to analyse paired samples taken with an interval of one or two weeks.

Treatment

Leptospirosis is treated by antibiotics. The earlier the treatment, the fewer symptoms and complications the patient will have. Therefore, it is recommended not to wait for laboratory results before starting treatment. With appropriate antibiotics a full recovery can be expected about a month after the start of treatment.

Prevention

There is no vaccine against leptospirosis available. Professionals with risk of leptospirosis are recommended to wear watertight glasses, gloves, boots and clothing.

The following measures could be used in the prevention and control of leptospirosis:

- to increase awareness of the disease among the population, risk groups and health care providers;
- to avoid contact with animal urine, infected animals or an infected environment;
- to wear protective clothing;
- to wash the hands after any contact with a contaminated animal or object;
- to cover all injuries and wounds with waterproof dressings before contact with contaminated freshwater or humid environment;
- not to swim or do any water sports in contaminated water;
- to stop access of rodents into housing by obstructing possible entrances;
- to remove all rubbish and to keep areas around human habitations clean;
- not to leave food around, especially in recreational areas where rats may be present.

Results of the 2008 surveillance

In the Institute of Tropical Medicine (NRL), a total of 596 human sera have been examined for the presence of antibodies to *Leptospira* by the microscopic agglutination technique (MAT).

Five confirmed cases have been diagnosed. All patients were men. The age of the patients ranged from 21 to 66 years with a median age of 42 years. Four patients were exposed in Belgium (3) or France (1), one patient contracted the disease during or

after a stay in a (sub)tropical region (Kenia). Three patients were exposed to water and/or rats, one patient was exposed to a horse with leptospirosis. This infection goes back to October 2007 but the diagnosis was made one year later. The way of contracting the disease is not known for one patient. All infections were diagnosed between June and November.

Results of the 2009 surveillance

In 2009, a total of 579 human sera have been examined.

Eight confirmed cases have been diagnosed. All patients were men. The age of the patients ranged from 25 to 66 years with a median age of 46 years. Five patients were exposed in Belgium (4) or France (1), 3 patients contracted the disease during or after a stay in a (sub)tropical region (Indonesia, Thailand, Congo). Seven patients were exposed to water and/or rats. The exposition of the patient who came back from Indonesia is not known. All but one infections were diagnosed between August and December.

Listeriosis

Katrien Beullens, Geneviève Ducoffre, Marc Yde

Listeriosis

The bacterial genus *Listeria* currently comprises six species, but human cases of listeriosis are almost exclusively caused by the species *Listeria monocytogenes*. Listeriae are ubiquitous organisms that are widely distributed in the environment, especially in plant matter and soil. The principal reservoirs of *Listeria* are soil, forage and water. Other reservoirs include infected domestic and wild animals. The main route of transmission to both humans and animals is believed to be through consumption of contaminated food or feed. However, infection can also be transmitted directly from infected animals to humans as well as between humans. Cooking kills *Listeria*, but the bacteria are known to multiply at temperatures down to 4°C, which makes the occurrence in ready-to-eat foods with a relatively long shelf life of particular concern.

In humans severe illness mainly occurs in the unborn child, infants, the elderly and those with compromised immune systems. Symptoms vary, ranging from mild flu-like symptoms and diarrhea to life threatening infections characterised by septicemia and meningoencephalitis. In pregnant women the infection can spread to the foetus resulting in foetal death with abortion or neonatal infection. Illness is often severe and mortality is high. Human infections are rare yet important given the high mortality rate associated with them. These organisms are among the most important causes of death from foodborne infections in industrialized countries.

Listeriosis

Listeria monocytogenes in food

Listeria monocytogenes in humans

In domestic animals, especially cattle, sheep and goats, clinical symptoms of listeriosis are usually encephalitis, abortion, mastitis or septicaemia. However, animals may also be asymptomatic intestinal carriers and shed the organism in significant numbers, contaminating the environment.

General food hygiene rules are essential for the prevention of human listeriosis. As some persons are at high risk (pregnant women, the elderly, immuno-compromised people), they are advised not to eat certain categories of food with proven elevated risk of *Li. 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, 'rillettes', 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, and unpeeled fruit. People should be made aware of the considerable risk of infection by consuming ready-to-eat food products.

Listeria monocytogenes in food

Monitoring programme

The matrices for *Listeria monocytogenes* analyses were diverse products of beef, pork, dairy products, fish and ready-to-eat products. Notification is mandatory since March 2004 (Ministerial Decree on mandatory notification in the food chain). For *Listeria monocytogenes* in ready-to-eat products put on the market, a maximum limit of 100 cfu/g is set.

Results of the 2008-2009 monitoring

Table 15. Zoonosis monitoring programme - *Listeria monocytogenes* in food, 2008-2009

Sample	Quantity analysed	Percentage of positive samples 2008	Percentage of positive samples 2009	
Beef	Minced meat at retail intended to be eaten raw (steak tartare)	Enumeration (M=100 cfu/g)	0.0% (n=158)	0.0% (n=98)
	Meat preparation at retail intended to be eaten raw (steak tartare with herbs and sauce)	Enumeration (M=100 cfu/g)	0.0% (n=160)	0.0% (n=98)
Pork	Cooked ham at processing plant	25g	7.4% (n=54)	2.7% (n=37)
	Cooked ham at retail	Enumeration (M=100 cfu/g)	0.0% (n=59)	0.0% (n=76)
	Raw ham at processing plant	25g or enumeration (M=100 cfu/g) (*)	4.4% (n=159)	3.1% (n=96)
	Raw ham at retail	Enumeration (M=100 cfu/g)	0.0% (n=33)	0.0% (n=34)
	Pâté at processing plant	25g	1.7% (n=58)	0.0% (n=38)
	Pâté at retail	Enumeration (M=100 cfu/g)	0.0% (n=57)	0.0% (n=84)
	White pudding at processing plant	25g	5.3% (n=38)	2.4% (n=41)
	White pudding at retail	Enumeration (M=100 cfu/g)	0.0% (n=77)	0.0% (n=76)
	Sausages at processing plant	25g or enumeration (M=100 cfu/g) (*)	7.0% (n=157)	10.3% (n=185)
	Sausages at retail	Enumeration (M=100 cfu/g)	0.0% (n=34)	0.0% (n=69)
Meat (unspecified)	Minced meat intended to be eaten raw, at retail	Enumeration (M=100 cfu/g)	0.0% (n=161)	0.0% (n=200)
	Minced meat intended to be eaten raw, at processing plant	25g or enumeration (M=100 cfu/g) (*)	-	13.8% (n=72)

Sample	Quantity analysed	Percentage of positive samples 2008	Percentage of positive samples 2009	
Cheeses	Cheeses made from raw milk at retail	Enumeration (M=100 cfu/g)	0.0% (n=93)	0.0% (n=106)
	Cheeses made from pasteurised milk at retail	Enumeration (M=100 cfu/g)	0.0% (n=136)	0.0% (n=120)
	Cheeses made from raw milk at farm	Enumeration (M=100 cfu/g)	2.8% (n=35)	0.0% (n=58)
	Cheeses made from raw milk at processing plant	25g or enumeration (M=100 cfu/g) (*)	6.0% (n=33)	1.6% (n=63)
	Cheeses made from pasteurised milk at processing plant	25g or enumeration (M=100 cfu/g) (*)	1.0% (n=190)	0.0% (n=162)
Dairy products	Butter at farm	Enumeration (M=100 cfu/g)	0.0% (n=146)	0.0% (n=161)
	Butter made from raw milk at retail	Enumeration (M=100 cfu/g)	0.0% (n=51)	0.0% (n=25)
	Cream at farm	Enumeration (M=100 cfu/g)	0.0% (n=20)	0.0% (n=20)
	Ice cream at farm	Enumeration (M=100 cfu/g)	0.0% (n=47)	-
	Ice cream at retail	Enumeration (M=100 cfu/g)	0.0% (n=81)	0.0% (n=80)
	Milk desserts at processing plant	25g	-	0.0% (n=94)
	Milk desserts at retail	Enumeration (M=100 cfu/g)	0.0% (n=161)	0.0% (n=160)
	Yoghurt at retail	Enumeration (M=100 cfu/g)	0.0% (n=50)	0.0% (n=60)
	Yoghurt at farm	Enumeration (M=100 cfu/g)	-	0.0% (n=60)
	Milk powder at processing plant	Enumeration (M=100 cfu/g)	0.0% (n=29)	0.0% (n=56)
Fish	Smoked salmon at retail	Enumeration (M=100 cfu/g)	0.5% (n=402)	1.0% (n=398)
	Fresh fish at processing plant	25g or enumeration (M=100 cfu/g) (*)	2.5% (n=39)	-
	Fresh fish at retail	Enumeration (M=100 cfu/g)	0.0% (n=82)	1.6% (n=62)

(*) Depending on the values of the pH and the water activity (Confer Regulation (EC) Nr 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs)

The national monitoring program for *Listeria monocytogenes* in other foods of animal origin in 2008 consisted of the following samples:

- at retail: meat salad (n=51), crustacean salad (n=48), chicken salad (n=50), nursing bottles (n=117), prepared food for babies (n=144), bakery products with cream (n=150), desserts based on raw eggs (n=214)
- at processing plant: sandwich spreads (meat, chicken, crustacean) (n=287), bakery products with cream (n=76).

All results were negative, except for prepared food for babies at retail (0.7% positive), desserts based on raw eggs at retail (0.5% positive), sandwich spreads at processing plants (5.9% positive) and bakery products with cream at processing plant (2.6% positive).

The national monitoring program for *Listeria monocytogenes* in other foods of animal origin in 2009 consisted of the following samples:

- at retail: meat salad (n=49), crustacean salad (n=101), chicken salad (n=49), nursing bottles (n=95), prepared food for babies (n=147), bakery products with cream (n=152), desserts based on raw eggs (n=169)
- at processing plant: sandwich spreads (meat, chicken, crustacean) (n=162), bakery products with cream (n=75).

All results were negative, except for meat salads at retail (1.9% positive), crustacean salads at retail (2.9% positive), desserts based on raw eggs at retail (0.5% positive), sandwich spreads at processing plants (4.9% positive) and bakery products with cream at processing plant (3.8% positive).

Listeria monocytogenes in humans

In 2008 and 2009, the Sentinel Laboratory Network and the NRL reported 64 and 81 cases of listeriosis respectively. This number is less than in 2004 (N=89), when particularly high numbers of listeriosis cases were recorded.

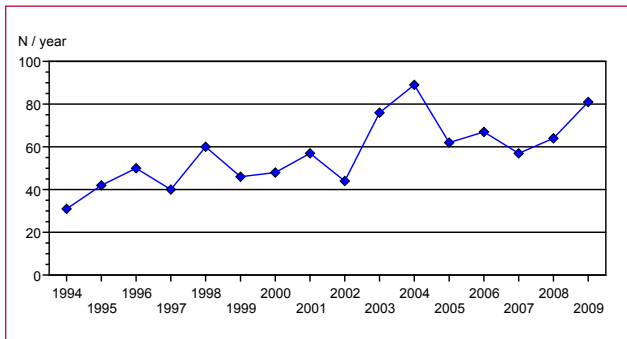


Figure 23. Number of *Listeria monocytogenes* infections in humans by year (1994-2009) Sources: Sentinel Laboratory Network and NRL

Listeria monocytogenes isolation rates are higher in men (64%) than in women (36%); 68% of cases are diagnosed in adults older than 64 year (Table xx). Elderly people and those with a low immunity are more at risk to contract this disease. *Listeria monocytogenes* can cause a transplacental infection or an infection during childbirth resulting in miscarriage, premature delivery or infection of the newborn. The newborn can develop septicaemia after birth or meningitis after the first week of live.

Geographic distribution of the cases in 2009 is as follows: 3 cases were reported in Brussels, 59 cases were reported in Flanders and 17 in Wallonia (2 from unknown geographic origin) (Figure 25).

For the period 1994-2009, the annual number of cases reported is depicted in Figure 23, corresponding to an annual mean number of 57 cases. Cases are reported all over the year (Figure 24).

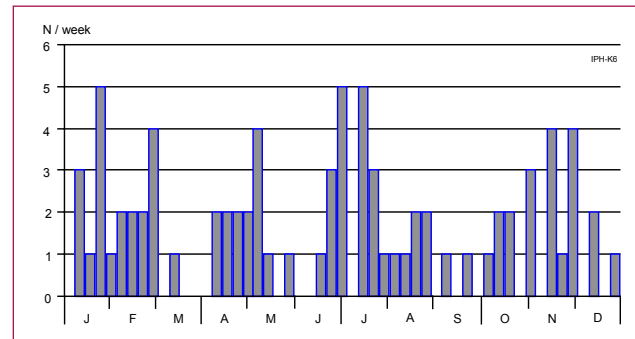


Figure 24. Weekly number of *Listeria monocytogenes* infections in humans, 2009 Sources: Sentinel Laboratory Network and NRL

Table 16. Number of *Listeria monocytogenes* infections in humans by sex and by age group, 2009 Sources: Sentinel Laboratory Network and NRL

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	0	0,0	1	4,0	1	1,4
1 - 4	0	0,0	0	0,0	0	0,0
5 -14	1	2,3	0	0,0	1	1,4
15 -24	0	0,0	2	8,0	2	2,9
25 -44	4	9,1	2	8,0	6	8,7
45 -64	7	15,9	5	20,0	12	17,4
≥ 65	32	72,7	15	60,0	47	68,1
Total	44	100,0	25	100,0	69	100,0

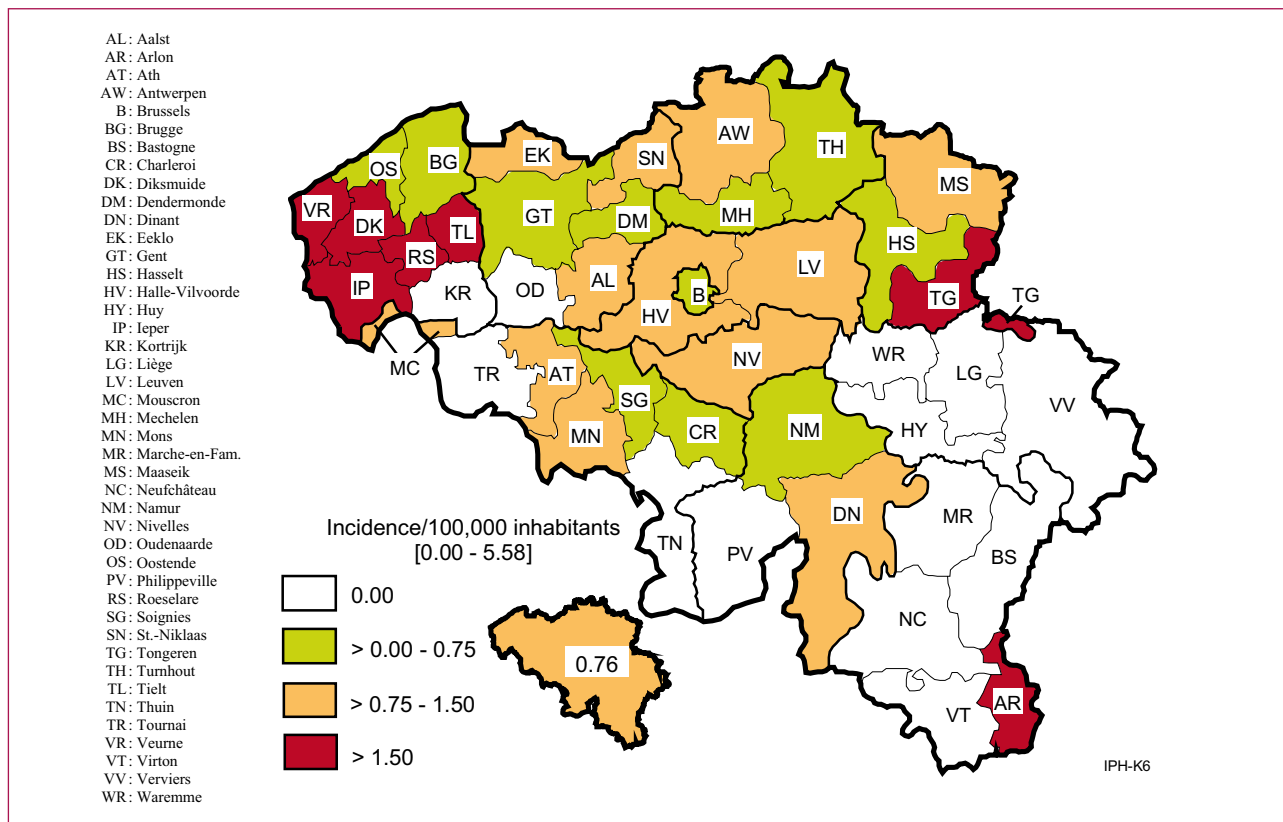


Figure 25. Incidence of *Listeria monocytogenes* infections in humans by district (N/105 inhab., 2009)

Sources: Sentinel Laboratory Network and NRL

In 2008, the NRL received 53 strains of *Listeria monocytogenes* of clinical human origin, representing a small increase of 1.9% with respect to 2007. Distribution of the serovars revealed the dominating position of 1/2a with 41.5% of the isolates and 4b (33.9%), followed by 1/2b (18.9%) and 1/2c (1.9%). Two strains (3.8%) were defined as belonging to serogroup 4. Five strains were related to

pregnancy (2 strains isolated from the mother and 2 strains from the newborn, 1 strain from the mother and the newborn). Eight strains were isolated from patients with a meningo-encephalitis form of listeriosis; 36 strains were isolated from blood and 1 strain from pus, 1 strain from ascite fluid and 1 strain from bile.

In 2009, the NRL received 64 strains of *Listeria monocytogenes* of clinical human origin, representing a large increase of 20.8% with respect to 2008. Distribution of the serovars revealed the dominating position of 1/2a with 62.5% of the isolates; the other serovars were represented with 4b (26.5%), 1/2b (9.4%) and 1/2c (1.6%). Two strains were related to pregnancy (1 strain isolated from the mother and newborn and 1 strain from the newborn). Eight strains were isolated from patients with a meningo-encephalitis form of listeriosis; 50 strains were isolated from blood, 1 strain from pus and 2 strains from synovial fluid.

An episode of listeriosis was observed in the period October 2008 – January 2009; 8 patients were infected with *Listeria monocytogenes* (serovar 1/2a, identical pulsotype). The source of contamination could not be determined.

MRSA

Patrick Butaye, Olivier Denis, Luc Vanholme

MRSA

Staphylococcus aureus is an important pathogen both for humans and for animals. *S. aureus* are bacteria commonly carried on the skin or in the nose of healthy people. Last decades, resistance of this pathogen to methicillin and all other beta-lactam antimicrobial agents has been of special concern, especially since these so-called methicillin-resistant *Staphylococcus aureus* (MRSA) also tend to accumulate resistance genes to most other (broad-spectrum) antibiotics as well, making it difficult to treat infections by these strains.

MRSA is a well recognised pathogen in humans, causing mainly septicaemia, pneumonia and skin infections. Some clones of these MRSA strains have been spread among hospitals and rest homes (HA [Hospital acquired] MRSA) causing major outbreaks. In addition, MRSA clones related to communities were identified (CA [Community Acquired] MRSA). These latter strains were found different from the nosocomial isolates and are generally less resistant to antimicrobial agents other than beta-lactams.

MRSA

MRSA in animals

MRSA in humans

MRSA have also been identified in association with infection in horses, cattle and dogs. Transfer of these bacteria from animals to man through direct contact have been demonstrated. Recently, a new type of MRSA (i.e. ST398) was found in pigs. The strain was also isolated from the pig owner, his family, a patient and the nurse in the hospital. Further studies confirmed that MRSA ST398 was present in various countries in pigs, but also in other species, including dogs.

MRSA in pigs

A survey was performed in 2007 to determine the prevalence of MRSA colonization among the pig farms and in the individual pigs.

In 2008 and 2009 no official monitoring programme was in place.

MRSA in humans

The NRL of Staphylococci – MRSA of the Université Libre de Bruxelles provided specialized analysis:

- Identification, characterisation and antimicrobial resistance pattern of atypical Staphylococci strains by means of
 - Phenotypic methods such as colony morphology, biochemical tests, minimal inhibiting concentrations (MIC determination), glycopeptide susceptibility (population analysis)
 - Genotypic methods: identification of different Staphylococci species by sequence analysis of gene *rpoB*, detection by PCRs for *nuc* gene (for *S. aureus* identification), *mecA* gene

(encoding oxacillin resistance), *mupA* gene (encoding mupirocin resistance) and of resistance genes to macrolides-lincosamides-streptogramins (MLS), tetracyclines and aminoglycosides.

- Detection of toxin genes: PCRs for exfoliative toxins A, B and D, Panton Valentine Leucocidin (PVL), Toxic Shock Syndrome Toxin-1 (TSST-1) and staphylococcal enterotoxins including *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq*, *selr*.
- Molecular typing, by Pulsed Field Gel Electrophoresis (PFGE) after *Sma*I macrorestriction, by *spa* sequence and typing of the Staphylococcal Chromosome Cassette *mec* (SCC*mec* typing), detection of the *agr* group, detection of the presence of the *arcA* gene as marker of the pathogenic locus of the Arginine Catabolic Mobile Element (ACME).

The NRL especially analysed clinical strains for additional susceptibility testing or for searching virulence genes, and collections of staphylococcal strains isolated during local outbreaks for epidemiological studies.

Since 1992, the NRL organises epidemiological surveillance of MRSA by means of biannual surveys in collaboration with the IPH and the Belgian Infection Control Society (BICS). The objectives are to follow the evolution of genotypes and of antimicrobial resistance profiles of MRSA isolates from patients admitted to Belgian acute-care hospitals. Detailed information on the results of the last survey conducted in 2008 is available on the website <http://www.mrsa.be>.

Since 1999, the NRL participated in the EARSS programme (European Antimicrobial Surveillance System) in collaboration with the section of epidemiology of IPH. Information about the protocol and the data of the EARSS programme is available at the address [http:// www.rivm.nl/earss/](http://www.rivm.nl/earss/).

Conclusions

The number of PVL-positive MRSA cases reported in 2008 increased compared to the previous years (43 cases in 2008 versus 25-30 recorded from 2005 to 2007). These PVL-positive strains caused mainly skin and soft-tissue infections. By molecular typing, the ST80-SCCmec IV clone, which is widely disseminated in Europe, was still predominant. However, MRSA strains belonging to ST8-SCCmec IV USA-300 clone were more frequently isolated. The emergence of USA300 MRSA is of increasing concern in view of severe and fatal infections recently reported from Belgium.

Since 2003, MRSA clone ST398 strains of animal origin (livestock-associated MRSA, LA-MRSA) are regularly isolated in humans in contact with livestock animals, including veterinarians and swine farmers. The swine population appeared to be highly colonized by ST398 LA-MRSA clone. This clone was also recovered from a number of other animal species including bovine, equine, poultry and pets. These LA-MRSA strains were infrequently associated with active disease in humans.

The proportion of MRSA among patients with *S. aureus* bacteraemia in acute-care hospitals decreased continuously after a peak (33%) in 2004 to stabilize at 23% in 2008 (EARSS data). This observation corresponded to the results of the national surveillance

programme NSIH conducted by the IPH. Since 2005, a dramatic decrease has halved the incidence of nosocomial MRSA acquisition to 1.7 cases per 1000 admissions in 2008 (data available at <http://www.nsih.be/>).

The last microbiological survey conducted in 2008 showed that the majority (86%) of MRSA strains belonged to 4 epidemic clones: spa CC38 ST45-SCCmec IV (formerly PFGE type B2), spa CC8 ST8-SCCmec IV (PFGE type A20), spa CC2 ST5-SCCmec II (PFGE type G10) and spa CC2 ST5-SCCmec IV (PFGE type C3). These MRSA clones are still widely disseminated in Belgian acute-care hospitals causing local outbreaks as confirmed by the NLR in 2008-2009. LA-MRSA and PVL-positive MRSA strains only represent a small proportion (<3%) of the burden of MRSA strains in the population of patients admitted in acute-care hospitals. Their frequency has remained stable since their first detection in 2003.

Q-fever

David Fretin, Marjan Van Esbroeck, Luc Vanholme, Katie Vermeersch

Coxiella burnetii

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Q-fever (Q for query) is a systemic disease caused by an obligate intracellular bacterium *Coxiella burnetii* that is highly resistant to heat, drying and many common chemical and physical agents. This resistance enables the bacteria to survive for a long period in the environment. *Coxiella burnetii* occurs worldwide except in New Zealand.

Natural reservoirs are more than 40 species of ticks and free-living vertebrates, primarily rodents. Ticks or their excreta may spread *Coxiella burnetii* to domestic animals, e.g. sheep, goats, cattle and dogs. These animals may display a cycle that does not involve ticks since Coxiellae can multiply in the trophoblast of the placenta. The placentas and amniotic fluids of these animals contain large numbers of bacteria which contaminate pastures and soil. Once animal secretions or excreta have dried, infectious dust is created.

Coxiella burnetii

Q-fever in animals

Q-fever in humans

Q-fever in animals

Cattle, sheep, and goats are the main reservoirs but a wide variety of other animals can be contaminated, including domesticated pets. *Coxiella burnetii* usually does not cause clinical disease in these animals, although an increased abortion rate and fertility problems in cattle, sheep and goats are observed. The emergence of these common symptoms over a longer period of time leads finally to the suspicion of Q-fever.

Organisms are excreted in milk, urine and faeces by infected animals. Animals shed the organisms especially during parturition within the amniotic fluids and the placenta. Airborne transmission can occur in premises contaminated by placental material, birth fluids or excreta from infected animals. Airborne inhalation is the most important transmission route of infection.

In 2008 and 2009, only limited testing by the NRL was performed on individual animal level of breeding bulls at the Artificial Insemination Centers and for confirmation of laboratory diagnosis in case of a clinical suspicion in case of an increased number of abortions of ruminants.

In 2008, the NRL analysed 314 sera of bovine animals. In total 25 bovine sera were positive.

In 2009, the NRL analysed 119 sera of breeding bulls at the Artificial Insemination centers, all sera were negative by RT-PCR. Individual analysis of 117 abortions of cattle resulted in 8 positive cases by RT-PCR. In case of RT-PCR tests for diagnosis, 69 PCR's were positive on a total of 208 analyses. In case of serology by ELISA for diagnosis, 40 ELISA's were positive on a total of 165 analyses. Screening of goats by PCR, 9 tests were positive on a

total of 56 and 15 sera were positive. 6 sera analysed of sheep were negative.

The NRL tested 44 tank milk samples of dairy cattle by PCR, 4 sera were positive. Tank milk samples of goats were tested by RT-PCR and ELISA. Respectively 9 and 15 samples were positive on a total of 60 and 56 tankmilk samples. Only one tankmilk sample of sheep was tested by PCR and ELISA with a negative result of both tests (table 17).

Table 17. NRL analyses for Q-fever

		test	animals	animals	
			tested	positive	
NRL	cattle	artificial insemination center	PCR	119	0
	cattle	clinical investigation abortion	PCR	117	8
	cattle	diagnosis	PCR	208	69
	cattle	diagnosis	ELISA	165	40
			test	herds	herds
				tested	positive
	cattle	monitoring tankmilk	PCR	44	4
	goats	monitoring tankmilk	PCR	60	9
	goats	monitoring tankmilk	ELISA	56	15
	sheep	monitoring tankmilk	PCR	1	0
sheep	monitoring tankmilk	ELISA	1	0	

In 2009, also ARSIA and DGZ (Regional veterinary health laboratories) did analyses for Q-fever (table 18).

Table 18. DGZ/ARSIA analyses for Q-fever

			test	animals tested	animals positive
ARSIA	cattle	clinical investigation abortion	ELISA	680	58
ARSIA	cattle	clinical investigation abortion	PCR	122	23
ARSIA	sheep	clinical investigation abortion	PCR	1	0
DGZ	cattle	Monitoring tankmilk	ELISA	871	214
			test	herds tested	herds positive
ARSIA	cattle	monitoring tankmilk	ELISA	1043	727
ARSIA	cattle	monitoring tankmilk	PCR	159	37
DGZ	cattle	monitoring tankmilk	ELISA	364	270

Recommendations for prevention and control in case of detection of Q-fever:

- Inform the farmer and his family on Q-fever disease, sources of infection, transmission, symptoms of infected people, ...
- Give special advice to persons 'at risk', especially persons with pre-existing cardiac valvular disease or individuals with vascular grafts and pregnant women
- Restrict access to barns used in housing potentially infected animals
- 'Good-practice' recommendations in particular for dealing with animal birth
 - Stimulate the mandatory declaration of abortions
 - Put aborted animals in quarantine
 - Analyse placenta and aborted foetuses in case of any abortion
 - Do appropriate disposal of placenta, birth products, foetal membranes and aborted foetuses
 - Regular cleaning and disinfection of residences of animals and materials
 - Use only pasteurised milk and pasteurised milk products of infected farms
 - 'Good-practice' recommendations in particular for manure:
 - Store manure a minimum distance from human dwellings
 - Compost manure of infected animals for a period of time with or without covering
 - Implement measures to prevent dust and airflow to occupied areas when spreading manure
 - Plough manure in immediately after spreading
 - Respect sanitary measures regarding animal movements, control of animal gathering, no participation in exhibitions of infected farms
 - Preventive vaccination of non-infected animals on seropositive farms with a phase-I vaccine containing inactivated *C. burnetii*
 - Public education and general information on the disease.

Q-fever in humans

Transmission in people is either airborne or results from direct or indirect contact with infected animals or their dried excreta. Consumption of infected food such as unpasteurised contaminated milk or unpasteurised contaminated dairy products may constitute a means of transmission of *Coxiella burnetii* to humans and can probably lead to infection and seroconversion but rarely to clinical symptoms. The significance of infection acquired via the oral route remains a subject of discussion and the role of drinking unpasteurised milk in a *Coxiella burnetii* infection of humans remains controversial.

Infection with *Coxiella burnetii* is either inapparent, acute, or chronic. The incubation period of acute Q-fever ranges from 2 to 4 weeks. The infection has an abrupt onset and patients present usually with high fever, hepatitis or pneumonia. A complete recovery can be observed most of the times, but in immunocompromised hosts a chronic infection with endocarditis may develop.

Consumption of pasteurised milk, limiting contact with infected animals or farms and applying proper hygiene when in contact with infected animals are the best preventive measures.

In the Institute of Tropical Medicine (NRL), a total of 1977 and 2186 human sera have been examined for the presence of phase I and II IgM and IgG antibodies to *Coxiella burnetii* in 2008 and 2009 respectively.

In 2008, 27 probable cases have been detected. The age of the patients ranged from 24 to 78 years with a median age of 43.3 years. Seventeen patients (63%) were male.

Ten patients stayed abroad before the start of their illness. The travel history of the other patients is not known.

In 2009, 33 probable cases have been detected, 32 with serology and one with PCR. The age of the patients ranged from 21 to 80 years with a median age of 50 years. The age of one patient is unknown. Twenty-one patients (64%) were male. Seven patients stayed abroad before the start of their illness. The travel history of the other patients is not known.

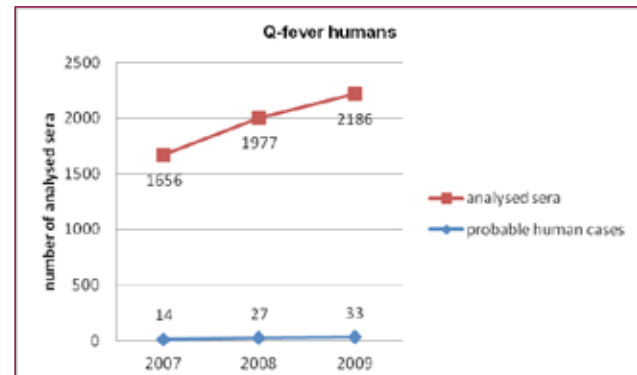


Figure 26. Number of analysed sera and probable human cases, 2007 – 2009



Salmonellosis

Katrien Beullens, Sophie Bertrand, Patrick Butaye, Katelijne Dierick, Hein Imberechts, Christophe Keppens, Luc Vanholme, Katie Vermeersch

Salmonella

Salmonella is an important zoonotic pathogen and a major cause of registered bacterial foodborne infections, both in individuals and in communities. The genus Salmonella is currently divided into two species: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six sub-species and most Salmonella belong to the subspecies *S. enterica* subsp. *enterica*. Members of this subspecies have usually been named based on where the serovar or serotype was first isolated. Mostly, the organisms are identified by genus followed by serovar, e.g. *S. Virchow*. More than 2,500 serovars of zoonotic Salmonella exist and the prevalence of the different serovars change over time.

Human salmonellosis is usually characterised by the acute onset of fever, abdominal pain, nausea, and sometimes vomiting. Symptoms are often mild and most infections are self-limiting, lasting a few days. However, in some patients, the infection may be more serious and the associated dehydration can be life threatening. In these cases, as well as when Salmonella causes septicaemia, effective antimicrobials are essential for treatment. Salmonellosis has also been associated with long-term and sometimes chronic sequelae e.g. reactive arthritis.

- Salmonellosis
- Salmonella in animal feed
- Salmonella in poultry
- Salmonella in pigs
- Salmonella in cattle
- Salmonella in food
- Salmonella in humans
- Antimicrobial resistance

The common reservoir of *Salmonella* is the intestinal tract of a wide range of domestic and wild animals which result in a variety of foodstuffs covering both food of animal and plant origin as sources of infections. Transmission often occurs when organisms are introduced in food preparation areas and are allowed to multiply in food, e.g. due to inadequate storage temperatures, inadequate cooking or cross contamination of ready-to-eat food. The organism may also be transmitted through direct contact with infected animals or faecally contaminated environments and humans.

Food prepared with contaminated raw eggs, egg products or insufficiently heated poultry meat or pork is the source of the human *Salmonella* infection. Therefore, surveillance programmes that in time detect *Salmonella* contaminations early in the whole food chain (feed, living animals, slaughterhouses, cutting plants, retail sector, restaurants) together with sanitary measures to reduce contamination are essential. In addition, good hygiene practices during food preparation in the kitchen, adequate refrigeration and adequate heating also help to prevent *Salmonella* infections.

In animals, sub-clinical infections are common. The organism may rapidly spread between animals in a herd or flock without detection and animals may become intermittent or persistent carriers. Infected cows may succumb to fever, diarrhea and abortion. Within calf herds, *Salmonella* may cause outbreaks of diarrhea with high mortality. Fever and diarrhea are less common in pigs than in cattle. Sheep, goats and poultry usually show no signs of infection.

Salmonella in animal feed

In June 2008 the Panel on Biological Hazards of EFSA identified *Salmonella* spp. as the major hazard for microbial contamination of animal feed. For other microbiological hazards feed was regarded a far less important source of contamination. This opinion confirmed the strategy of the FASFC to focus its efforts on microbiological contamination in feed on *Salmonella*. Special attention is given to the sampling procedure using $n=5$ and taking into account the heterogenic nature of a possible *Salmonella* contamination of feed. Feed materials of animal origin, oilseeds and wheat bran are considered as 'at-risk' products.

Using a statistically substantiated risk evaluation the FASFC re-evaluates and performs an official control program every year. Compound feed and feed materials are sampled and analysed for absence of *Salmonella* in 25g. Every detection of *Salmonella* is treated as a non-conformity, but the actions taken are depending on the serotype detected and the type of feed. In 2008 and 2009, more stringent actions were taken if the contamination concerned 5 critical serotypes in poultry feed and finishing feed. Those critical serotypes were *S. Typhimurium*, *S. Enteritidis*, *S. Virchow*, *S. Hadar* and *S. Infantis*. For 2010 a new strategy will be implemented determining more critical serotypes and fine-tuning the actions depending on the type of feed and the place in the feed chain where the contamination is detected.

In 2008 a total of 709 samples of feed were analysed for the detection of *Salmonella*. Out of those, 103 were feed materials of animal origin. 7 were found positive (serotypes detected: *S. Infantis*, *S. Jerusalem*, *S. Livingstone*, *S. Ohio*, *S. Senftenberg*, *S. Yoruba*, *S. 6,7:-:-*, *S. 6,7:z10:-* and *S. Paratyphi B* var. Java).

101 were other feed materials. 10 were found positive (serotypes detected: *S. Banana*, *S. Cubana*, *S. Kentucky*, *S. Lexington*, *S. Mbandaka*, *S. Rissen*, *S. Senftenberg*, *S. Worthington*, *S. Yoruba*, *S. 3,10:-:1,5* and *S. 3,19:-:1*). 505 samples were from compound feeding-stuffs. 17 were found positive (serotypes detected: *S. Brandenburg*, *S. Enteritidis*, *S. Havana*, *S. Jeruzalem*, *S. Lexington*, *S. Livingstone*, *S. Minnesota*, *S. Orion*, *S. Senftenberg*, *S. Worthington*, *S. 6,7:-:1* and *S. 3,19:-:1*).

In 2009 a total of 714 samples of feed were analysed for the detection of Salmonella. Out of those 65 were feed materials of animal origin. 1 was found positive (2 serotypes detected: *S. Bredeney* and *S. Montevideo*). 112 were other feed materials. 3 samples were found positive (serotypes detected: *S. Emek*, *S. Infantis*, *S. Rissen* and *S. Lexington*). 537 samples were from compound feeding-stuffs. 10 were found positive (serotypes detected: *S. Anatum*, *S. Cerro*, *S. Jeruzalem*, *S. Livingstone*, *S. Minnesota*, *S. Oranienburg*, *S. Rissen*, *S. Schwarzengrund* and *S. Senftenberg*).

Salmonella in poultry

Salmonella in breeders and hatcheries

Control programme in breeders

The regional animal health associations (i.e. "Association Régionale de Santé et d'Identification Animales" [ARSIA (<http://www.arsia.be/>)] and Dierengezondheidszorg Vlaanderen [DGZ Vlaanderen (<http://www.dgz.be/>)])) organise the official sampling in the framework of the Belgian Salmonella control programme in breeders.

All breeder flocks are routinely sampled for Salmonella at delivery as day-old chicks (imported and domestic flocks). At the farm, pieces (5 by 5 cm) of the inner linings of the delivery boxes of the day-old chicks are taken by the owner, i.e. one sample for the hen-chicks and one for the cock-chicks. Each sample consists of 20 pieces of inner linings. The two samples are analysed separately. In addition, 20 living hen-chicks and 20 living cock-chicks are tested serologically. The samples have to be taken the day of the delivery and have to reach the lab within 24h of sampling. Official samples of breeding flocks are taken at the age of 16 weeks, 22 weeks, 46 and 56 weeks by technicians of DGZ or ARSIA. Routine sampling is performed by the operator at the age of 4 weeks and every two weeks during production. The sampling is conform Regulation (EC) No 1168/2005 and consist of 5 pair of overshoes pooled in two samples. All samples are immediately analysed in the laboratories of DGZ or ARSIA according to ISO 6579:2002 Amd 1:2007.

The official programme also controls the hygiene level of hatcheries 4 times a year. These are done during visits of the technician at non-hatching days and include various sites of the hatchery, including hatching drawers. Rodac samples 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 22 cm² 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. These samples are sent to the laboratory by the owner.

Case definition, notification, sanitary measures and vaccination

A poultry breeding flock is considered Salmonella positive when *S. Enteritidis*, *S. Typhimurium*, *S. Virchow*, *S. Hadar* or *S. Infantis* is isolated from at least one sample. Confirmatory samples (5 faeces samples and 2 dust samples) during rearing or production may be requested by the farmer and are taken by or under the supervision of the competent authority. The results of these analyses are binding. Provisional measures are taken during the course of analysis.

The isolation of zoonotic Salmonella is notifiable to the FASFC by the approved laboratories since January 2004.

Several measures are taken in case of positive breeding flocks: prohibition of incubation of hatching eggs, removal and destruction of incubated hatching eggs and canalisation of not yet incubated hatching eggs for pasteurisation. In addition, positive flocks are logistically slaughtered within the month, after removal of the positive flock, the house is thoroughly cleaned and disinfected and a Salmonella control is performed of the house using 2 samples each consisting of 25 swabs. Cleaning and disinfection is repeated until the Salmonella control is negative.

Vaccination against *S. Enteritidis* is mandatory; vaccination against *S. Typhimurium* is strongly recommended for parent flocks. Both attenuated and inactivated vaccines are available.

Epidemiological investigations and results of 2008-2009 surveillance

In 2008, 224 rearing flocks and 550 flocks during production were tested. Six rearing flocks were positive for Salmonella (*S. Typhimurium*, *S. Infantis*, *S. Mbandaka*, *S. Montevideo*, *S. Senftenberg* and *S. O4*) and 45 flocks in production of which 3 for *S. Enteritidis*, 2 for *S. Typhimurium*, 9 for *S. Senftenberg*, 8 for *S. Agona*, 3 each for *S. Mbandaka*, *S. Montevideo* and *S. Cochise*, 2 each for *S. Anatum*, *S. Lexington* and *S. Rissen* and 1 each for *S. Cubana*, *S. Livingstone*, *S. Muenchen*, *S. Orion*, *S. Ruri*, *S. Tennessee*, *S. O4* and *S. O3,19:-:-*.

In 2009, 302 rearing flocks and 526 flocks during production were tested. Three rearing flocks were positive for Salmonella (1 *S. Hadar*, 1 *S. Cubana* and 1 *S. O3,19:-:-*) and 16 flocks in production of which 6 for *S. Senftenberg*, 3 for *S. Mbandaka*, 2 each for *S. Lexington* and *S. Minnesota* and 1 each for *S. Corvallis*, *S. Livingstone* and *S. O9,46:-:-*.

Salmonella in layers

Surveillance programme in commercial laying hen flocks

All laying hen flocks on farms with at least 200 laying hens must follow the national Salmonella control programme in layers. Flocks are sampled by the owner at the age of one day (at arrival) 16 weeks, 24, 39 and 54 weeks and in the last three weeks of production.

The day-old-chicks are sampled in the same way as the breeders. For all other sampling, two samples are taken each consisting of, depending on the housing system, either one pair of overshoes or in case of cages, 150g naturally pooled faeces or mixed fresh faeces.

The two samples are pooled to one sample at the laboratory. One flock of all farms with at least 200 laying hens is also sampled by the FASFC. This official sampling consists of two pair of overshoes pooled to one sample at the laboratory and 1 dust sample.

Case definition, notification, sanitary measures and vaccination

A laying hen flock is declared positive if *S. Enteritidis* or *S. Typhimurium* is isolated. Confirmatory samples (5 faeces samples and 2 dust samples) during rearing or production may be requested by the farmer. The results of these analyses are binding. Provisional measures are taken during the course of analysis.

The isolation of zoonotic Salmonella is notifiable to the FASFC since January 2004.

In case of positive findings in layers, eggs are placed on the market as B-eggs for heat treatment. The poultry house must be cleaned and disinfected after slaughter of the positive flock and a Salmonella control of the house is performed in the same way as for breeding flocks.

Vaccination against *S. Enteritidis* is mandatory for layers; vaccination against *S. Typhimurium* is strongly encouraged. Both attenuated and inactivated vaccines are available.

Epidemiological investigations and results of 2008-2009 surveillance

In 2008 of the 293 batches of day-old chicks, none were found positive for Salmonella. During rearing of the 293 flocks, 5 were positive for Salmonella (1 for *S. Enteritidis*, 3 for *S. Jerusalem* and 1 for *S. Lexington*). During production, of the 649 flocks, 76 flocks

were positive for Salmonella (23 for *S. Enteritidis*, 7 for *S. Mbandaka*, 6 for *S. Livingstone*, 4 each for *S. Infantis*, *S. Rissen* and *S. Senftenberg*, 3 each for the Salmonella group O4 and the Salmonella group O6, 2 each for *S. Hillingdon*, *S. Lexington*, *S. Virchow*, and the Salmonella group O9, 1 each for *S. Banana*, *S. Blegdam*, *S. Braenderup*, *S. Derby*, *S. Dublin*, *S. Gateshead*, *S. Grumpensis*, *S. Jerusalem*, *S. Orion*, *S. Typhimurium* and *S. Paratyphi B* var. Java.

In 2009 of the 283 batches of day-old-chicks, none were found positive for Salmonella. During rearing, 283 flocks were sampled of which two were positive for Salmonella (1 *S. Typhimurium* en 1 *S. O4: I*). During production, 763 flocks were sampled of which 54 were positive for Salmonella (26 for *S. Enteritidis*, 6 for *S. Infantis*, 3 each for *S. Typhimurium* and *S. Rissen*, 2 each for *S. Agona*, *S. Anatum*, *S. Livingstone*, *S. Mbandaka* and *S. Virchow*, 1 each for *S. O4:d:-*, *S. O6,7:-:-*, *S. Derby*, *S. Hadar*, *S. Idikan*, *S. Jerusalem*, *S. Minnesota* and *S. Yoruba*). There were 2 flocks positive for an untypable Salmonella spp. and 4 flocks were positive for 2 different serotypes.

Salmonella in broilers

Surveillance programme in commercial broiler flocks

Day-old-chicks are sampled on farms with the highest sanitary qualification, following the same procedure as for laying hens. All broiler flocks with a capacity of 200 or more broilers are sampled by the holder in the last three weeks before slaughter. The sample consists of two pair of overshoes. Ten percent of the holdings are also sampled by the FASFC.

Case definition, notification, sanitary measures

and vaccination

A flock is positive if zoonotic Salmonella is found. The isolation of zoonotic Salmonella is notifiable to the FASFC since January 2004. The positive Salmonella result is reported to the slaughterhouse by means of the food chain information system.

As of 2009, it is forbidden to use antimicrobials as treatment for Salmonella. Before restocking the house, the house is cleaned, disinfected and the presence of Salmonella is checked by means of two samples each consisting of 25 swabs taken throughout the house. A hygienogram is performed. If Salmonella is found or if the hygienogram indicates that too many germs are present, the Salmonella control and/or the hygienogram have to be repeated after the next round. In addition to the previous measures, the house has to be disinfected by an external company and thinning must be performed as last holding of the day if 2 consecutive flocks in 1 house are positive for the same serotype. If 3 consecutive flocks are positive for the same Salmonella serotype, an epidemiological investigation of the farm and optimizing the biosecurity and hygiene measures on the farm are added to the list of measures.

Epidemiological investigations and results of 2008-2009 surveillance

In 2008, 5,074 flocks were sampled as day-old chicks of which 4 were positive for Salmonella. 7,775 flocks were sampled within three weeks before slaughter. 234 were positive for Salmonella. Serotyping was not mandatory in 2008.

In 2009, 5,226 flocks were sampled as day-old-chicks of which 7

were positive for Salmonella (1 for *S. Enteritidis*, 1 for *S. Typhimurium*, 1 for *S. Paratyphi B* var. Java and 4 unspecified). 8,049 flocks were sampled in the last three weeks before slaughter. 247 flocks were positive for Salmonella. The most frequently found serotypes were: *S. Typhimurium* (26 flocks), *S. Paratyphi B* (22 flocks), *S. Paratyphi B* var. Java (18 flocks), *S. Enteritidis* (14 flocks), *S. Agona* (18 flocks), *S. Livingstone* (17 flocks), *S. Minnesota* (12 flocks), *S. Virchow* (12 flocks), *S. Rissen* (11 flocks) and *S. Hadar* (9 flocks).

Salmonella serotypes of the 2008–2009 surveillance in layers and broilers

Laboratory findings of the NRL show that in 2008 and 2009 a total 951 and 1142 Salmonella strains from poultry origin were analysed, respectively, which is considerably more than the 745 strains tested in 2007. As for the strains known to originate from the layer flocks, the majority (39.1% of 92 in 2008 and 59.0% of 122 in 2009) belonged to serotype Enteritidis. Other serotypes in 2008 were *Livingstone* (9.8%) and *Typhimurium* (7.6%), and in 2009 *Infantis* (8.2%), *Typhimurium* and *Livingstone* (both 4.1%). The majority of broiler isolates tested in 2008 (n=372) were *S. Enteritidis* (54.3%), and also serotypes *Typhimurium* (8.1%), *Paratyphi B* (4.8%), *Virchow* (4.0%) and *Infantis* (3.5%) were found. In 2009, the most common broiler isolates (n=192) were *S. Paratyphi B* (32.3%), but also *Hadar* (9.9%), *Agona* (7.8%), *Minnesota* (5.7%) and *Virchow* (5.7%) were frequently identified.

The number of poultry isolates yearly sent to the NRL varies between about 700 and almost 1,500. In 2009, almost one third of poultry strains belonged to serotype *Enteritidis*, which is less than in 2008 and 2007.

The proportion of *Typhimurium* strains is since 2001 less than 10% and reached 6.7% in 2009. The proportion of *Salmonella Paratyphi B* strains, after their peak in 2006, fell down in recent years, but reached again 20% in 2009. Serotypes *Hadar*, *Infantis* and *Virchow* represented about 2 to 3% of poultry isolates in 2009.

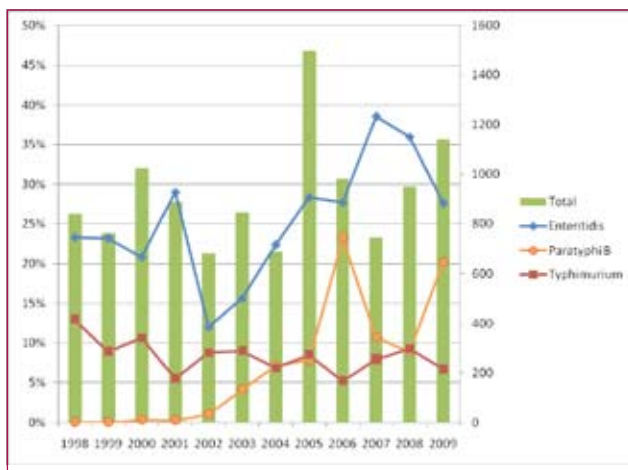


Figure 27. Evolution of the percentages of the principal *Salmonella* serotypes isolated from poultry between 1998 and 2009

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 turkeys, geese, ducks, pigeons and other poultry

Surveillance programme and sampling

The national control programme for *Salmonella* in turkeys, geese, ducks and guinea fowl is performed according to the sanitary qualification act. Sanitary Qualification A is mandatory for all commercial breeding flocks. Flocks are at least sampled as day-old chicks, at the age of 26 weeks when entering the production unit if this is on a different farm than the rearing unit, and within the last 3 weeks before slaughter. Meat production flocks are sampled within three weeks of slaughter if the holding has a capacity of more than 5,000 birds (Sanitary Qualification B). On a voluntary basis, one-day-old birds may also be sampled.

Samples for day-old-birds are taken at the farm, and consist of pieces (5 by 5 cm) of the inner linings of delivery boxes. Two samples, each composed of 20 pieces of inner linings, are taken for each flock, one for the hen chicks and one for the cock chicks. The two samples are analysed separately according to ISO 6579.

The owner of breeding flocks takes faeces samples from the delivery boxes at time of delivery. A sample consists of 60 x 5 to 10g sub-samples taken from every flock with different origin of rearing. The samples have to be examined by an accredited laboratory within 48 hours. At 26 weeks, 60 blood samples are taken of each breeding flock for the surveillance of *Salmonella*. If one or more blood samples are positive, faecal samples are taken to confirm the results.

Within 3 weeks before slaughter, the owner takes a pooled faecal sample consisting of 60 x 1g sub-samples of each flock.

Alternatively, the sampling may consist of a pooled faecal sample of 60 x 1g taken by hand, or recovered from two pair of overshoes that were pooled for analysis.

Case definition, sanitary measures and vaccination policy

A flock is considered positive if zoonotic Salmonella serotypes were isolated. Measures are taken only at time of slaughter: if the flock is Salmonella positive, at time of slaughter, it is slaughtered at the end of the day (logistic / sanitary slaughter).

There is no vaccination policy for breeding flocks, nor for meat production flocks. Notification of zoonotic Salmonella to the FASFC is compulsory since January 2004.

Results of the investigation in 2008-2009 surveillance

In 2008, 167 meat turkey flocks were tested of which 6 were positive for Salmonella. Serotyping was not performed.

For meat turkey flocks following results were found in 2009: 155 flocks were sampled at the end of production of which 6 were positive for Salmonella spp. (1 each for *S. Blockley*, *S. Indiana* and *S. Kottbus*, 1 isolate was not typable and 2 isolates were not serotyped).

2 flocks of guinea fowl were tested, both negative for Salmonella.

Data from the NRL Salmonella indicate that in 2008 eleven of the 13 pigeon strains analysed were *S. Typhimurium* var. Copenhagen, which is a common finding for these birds. In 2009, all 12 pigeon strains analysed were identified as *S. Typhimurium* var. Copenhagen. Two serotyped turkey strains were *S. Indiana* and *S. Kottbus*, and four pheasant isolates belonged to serotypes *Gallinarum*, *Indiana*, *Senftenberg* and *Typhimurium*.

Salmonella in pigs

Serology

Surveillance programme in fattening pigs

Similar to former years, in 2008-2009 the blood samples from fattening and growing pigs that were taken for the monitoring of Aujeszky's disease were also analysed for the surveillance of Salmonella. On each farm, which is visited every 4 months, 10 to 12 blood samples were taken. The analysis for Salmonella-specific antibodies was done in the veterinary laboratories ARSIA and DGZ by means of a commercially available ELISA kit, following the manufacturer's instructions.

Case definition, sanitary measures and vaccination policy

A herd is considered as a 'Salmonella risk herd' if 3 consecutive mean S/P ratio's were above 0,6. The S/P ratio is considered to be related to the presence of specific antibodies against Salmonella spp.

Following measures are taken on a Salmonella risk herd: completion of checklist on state of play on biosecurity, management and hygiene, formulating and implementing a herd specific Salmonella action plan, based on the results of the checklist and bacteriological evaluation of the farm.

Pigs were not vaccinated in 2008-2009, since no vaccine was authorised in Belgium.

Results in 2008-2009

In 2008, a total of 188.257 serological analyses were performed. Of these, 39.875 samples (21,2%) had a S/P ratio above 0,6. 40,2% of the 6.658 farms monitored had at least once a mean S/P ratio above 0,6.

In 2009, a total of 171.767 serological analyses were performed, 28.270 (16,5%) of the samples had a S/P ratio above 0,6; 32,6% of the 6.395 farms monitored had at least once a mean S/P ratio above 0,6. 231 farms were considered as Salmonella risk farms for the first time in 2009, 84 for a second time.

Bacteriology

One of the measures on 'Salmonella risk herds' is a bacteriological investigation of housing environment of the pigs. Four samples are taken by the responsible farm veterinarian. Samples were also taken for research activities.

Laboratory findings from the NRL showed that, as compared to 2007, the number of pig strains more than doubled in 2008 (n=481 in 2007 and n=1.017 in 2008) most likely due to the mandatory bacteriological investigation on Salmonella risk herds. Significantly less *S. Typhimurium* isolates were found (48,5%; 65,2% in 2007), but considerably more *S. Derby* (15,6%; 7,2% in 2007). In 2009, the number of pig strains tested resembled that of 2007 (n=536, 1 017). Again more *S. Typhimurium* isolates were found (63,8%) as compared to 2008), but an equal proportion of *S. Derby* (13,4%; 15,6% in 2008). Nine percent of pig strains were only partially typable, and belonged to group B Salmonella.

S. Typhimurium still is the most prevalent serotype among pig isolates, representing more than 60% of pig Salmonella. Serotype *Derby* is the second most important serotype, and represents about 13% of the strains.

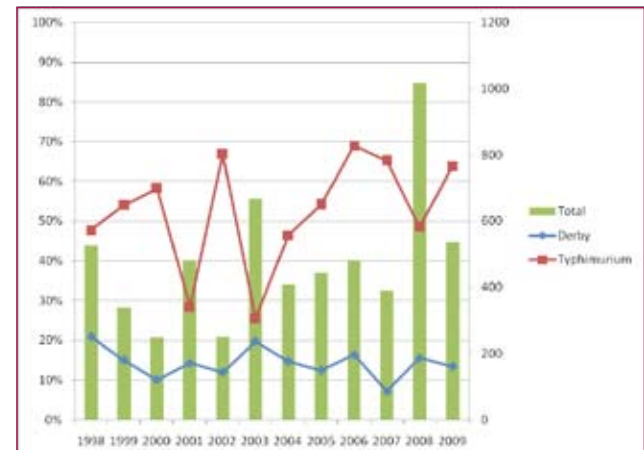


Figure 28. Evolution of the percentage of the principal Salmonella serotypes isolated from pigs between 1998 and 2009

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 food (meat and meat products)

Monitoring programme

In 2008 and 2009, sampling for Salmonella monitoring was done on the following matrices: carcasses, trimmings and raw ham of pork, minced meat and meat preparations of beef, carcasses, meat cuts and meat preparations of broilers, layer carcasses and minced meat and meat preparations of diverse species.

Sampling of pork carcasses was done by means of swabs. The carcass samples of broilers and layers at slaughter consisted of neck skin. Different contamination levels were analysed: 25g, 600 cm², 1g and 0.1g. Sampling was done by specially trained staff.

Notification is mandatory since March 2004 (Ministerial Decree on mandatory notification in the food chain). For Salmonella, absence in 25g in ready-to-eat food is requested.

Salmonella in meat and meat products.

Epidemiological investigations and results of 2008 surveillance

Table 19. The results of the monitoring – Salmonella in meat and meat products

Species	Sample size	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Beef				
Minced meat (steak tartare) at retail (n=127)	25g	1.6%		
Meat preparation (steak tartare with herbs and sauce) at retail (n=125)	25g	0.1%	Typhimurium	
Pork				
Carcasses at slaughter (n=281)	600cm ²	14.6%	Typhimurium	Derby, Brandenburg, Livingstone, Rissen, Ohio,
Meat cuts at processing plant (n=122)	25g	5.7%	Typhimurium	Paratyphi B, Infantis
Trimmings at processing plant (n=99)	25g	6.1%	Typhimurium	
Raw ham at retail (n=31)	25g	0.0%		
Raw ham at processing plant (n=57)	25g	1.8%	Typhimurium	
Broilers				
Carcasses at slaughter (n=157)	1g	7%	Paratyphi B	Typhimurium, Virchow, Infantis
Carcasses at slaughter (n=193)	25g (caeca)	9.8%	Paratyphi B	
Carcasses at retail (n=90)	25g	11.1%	Paratyphi B	Typhimurium, Agona, Hadar

Species	Sample size	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Meat cuts (skinned or with skin) at processing plant (n=516)	25g	7%	Paratyphi B	Typhimurium, Enteritidis, Mbandaka, Infantis, Virchow, Agona, Blockley, Bredeney, Montevideo
Meat cuts (skinned or with skin) at retail (n=74)	25g	0.0%		
Layers				
Carcasses at slaughter (n=128)	1g	23.4%	Enteritidis	Infantis, Bareilly, Braenderup, Agona, Virchow, Typhimurium
Carcasses at slaughter (n=200)	25g (caeca)	45.5%	Enteritidis	Typhimurium, Virchow, Infantis, Hilligdon, Bareilly, Braenderup
Poultry				
Meat cuts (with skin) at retail (n=56)	25g	1.8%		
Meat cuts (without skin) at retail (n=67)	25g	3.0%	Paratyphi B	
Minced meat at retail (n=240)	10g	13.8%	Paratyphi B	Typhimurium, Liningstone, Minnesota, Virchow
Meat preparation at retail (n=176)	10g	12.5%	Paratyphi B	Typhimurium, Livingston, Montevideo, Saintpaul
Meat preparation at processing plant (n=138)	10g	24.6%	Paratyphi B	Typhimurium, Saintpaul, Livingstone, Mbandaka
Meat products intended to be eaten cooked at processing plant (n=72)	10g	2.8%	Virchow	
Meat products intended to be eaten cooked at retail (n=117)	10g	1.7%	Virchow	
Meat and meat products				
Minced meat intended to be eaten raw (all species) at retail (n=129)	25g	2.3%	Brandenburg	Typhimurium
Minced meat intended to be eaten cooked at retail (all species) (n=126)	10g	3.2%	Typhimurium	Brandenburg, Bovismorbificans
Meat preparation intended to be eaten raw (all species) at retail (n=41)	25g	2.4%	Typhimurium	
Meat preparation intended to be eaten cooked (all species) at retail (n=42)	10g	2.4%	g1,v:-	

Species	Sample size	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Meat preparation intended to be eaten raw (all species) at processing plant (n=68)	25g	1.5%		
Meat preparation intended to be eaten cooked (all species) at processing plant (n=91)	10g	1.1%		
Mechanically separated meat (n=93)	10g	24.7%	Paratyphi B	Typhimurium, Enteritidis, Bareilly, Bredeney, Indiana, Infantis

Epidemiological investigations and results of 2009 surveillance

Table 20. The results of the monitoring – Salmonella in meat and meat products

Species	Sample size	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Beef				
Minced meat (steak tartare) at retail (n=118)	25g	0.0%		
Meat preparation (steak tartare with herbs and sauce) at retail (n=118)	25g	0.0%		
Pork				
Carcasses at slaughter (n=828)	600cm ²	13.4%	Typhimurium	Ohio, Brandenburg, Derby, Rissen, London
Meat cuts at processing plant (n=237)	25g	3.4%	Typhimurium	Enteritidis, Brandenburg, Rissen, Paratyphi B, Goldcoast
Trimmings at processing plant (n=243)	25g	4.1%	Typhimurium	Paratyphi B, Goldcoast, Rissen, Enteritidis, Brandenburg
Raw ham at retail (n=31)	25g	0.0%		
Raw ham at processing plant (n=58)	25g	1.7%	Typhimurium	
Broilers				
Carcasses at slaughter (n=432)	1g	6%	Paratyphi B	Typhimurium, Anatum
Carcasses at slaughter (n=178)	25g (caeca)	26%	Paratyphi B	Agona, Hadar, Infantis, Montevideo, Virchow, Newport, Brandenburg Mbandaka
Carcasses at retail (n=91)	25g	13.1%	Brandenburg	Paratyphi B, Livingstone, Muenchen, Indiana
Meat cuts (skinned or with skin) at processing plant (n=415)	25g	8%	Paratyphi B	Enteritidis, Agona, Virchow, Newport, Infantis

Species	Sample size	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Meat cuts (skinned or with skin) at retail (n=58)	25g	1.7%		
Layers				
Carcasses at slaughter (n=350)	1g	36%	Enteritidis	Virchow, Infantis, Typhimurium, Indiana, Paratyphi B
Carcasses at slaughter (n=171)	25g (caeca)	43%	Enteritidis	Paratyphi B, Typhimurium, Livingstone, Corvallis, Havana, Virchow
Carcasses at retail (n=60)	25g	11.6%	Typhimurium	Paratyphi B, Livingstone, Enteritidis
Poultry				
Meat cuts (with skin) at retail (n= 89)	25g	5.6%	Paratyphi B	Mbandaka
Meat cuts (without skin) at retail (n= 91)	25g	2.2%		
Minced meat at retail (n=66)	10g	12.1%	Paratyphi B	Agona
Meat preparation at retail (n=60)	10g	16.7%	Paratyphi B	Agona, Enteritidis, Typhimurium
Meat preparation at processing plant (n=58)	10g	15.5%	Typhimurium	Agona, Enteritidis, Paratyphi B, Virchow
Meat products intended to be eaten cooked at processing plant (n=37)	10g	0.0%		
Meat products intended to be eaten cooked at retail (n=55)	10g	1.8%	Paratyphi B	
Meat and meat products				
Minced meat intended to be eaten raw (all species) at retail (n=120)	25g	4.1%	Typhimurium	
Minced meat intended to be eaten cooked at retail (all species) (n=120)	10g	1.7%	Typhimurium	
Meat preparation intended to be eaten raw (all species) at retail (n=40)	25g	2.5%	Typhimurium	
Meat preparation intended to be eaten cooked (all species) at retail (n=79)	10g	1.3%	Typhimurium	
Meat preparation intended to be eaten raw (all species) at processing plant (n=77)	25g	1.3%	Typhimurium	

Species	Sample size	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Meat preparation intended to be eaten cooked (all species) at processing plant (n=77)	10g	3.9%	Typhimurium	Paratyphi B
Mechanically separated meat (n=116)	10g	15.5%	Typhimurium	Paratyphi B, Enteritidis, Infantis, Derby, Kentucky, Rissen, Virchow, Heidelberg

The contamination rate of different products of pork, broilers and layers is mentioned in the next table xx for the period 2005–2009. The Salmonella contamination of pig carcasses increased in 2007 and decreased slightly successively in 2008 and 2009.

Table 21. Evolution of the food Salmonella prevalence, 2005-2009

	Samples	Sampling level	2005	2006	2007	2008	2009
Pork	Carcasses	600cm ²	9.3%	7.1%	16%	14.6%	13.4%
	Cutting meats	25g	7.3%	2.4%	4.1%	5.7%	3.4%
	Trimming	25g	7.3%	2.4%	4.1%	4.1%	4.1%
Broilers	Carcasses	1g	5.7%	1.4%	10.3%	7%	6%
	Cutting meats	25g	14.2%	13.3%	7.4%	7.2%	8%
Layers	Carcasses	1g	22.6%	35.6%	45.4%	23.4%	36%

Salmonella in other food of animal origin

The results of the national monitoring program of milk and dairy products for 2008 are as follows:

- at retail: nursing bottles (n=117), cheese of pasteurised milk (n=137), cheese of raw milk (n=89), butter of raw milk (n=20), milk desserts (n=40), ice cream (n=62)

- at processing plant: cheese of raw milk (n=41), cheese of pasteurised milk (n=99), milk powder (n=21)
- at farm: cheese of raw milk (n=92), butter (n=49), cream (n=20), ice cream (n=30).

All results were negative.

The results of the national monitoring program in other foods of animal origin for 2008 are as follows:

- at retail: fresh fish (n=62), cooked molluscs (n=62), cooked crustaceans (n=31), meat salad (n=47), chicken salad (n=46), crustacean salad (n=45), bakery products with cream (n=76), live bivalve molluscs (n=61), raw crustaceans (n=54), chocolate (n=30), confectionary with chocolate (n=30), table eggs (n=129), liquid egg products (n=54), salty preparation based on raw eggs (n=105), dessert based on raw eggs (n=118)
- at processing plant: gelatine (n=10), cooked crustaceans and molluscs (n=108), sandwich spreads (crustacean, meat, chicken) (n=137), chocolate (n=15) and confectionery (n=16), bakery products with cream (n=40), egg products (n=125).

All results were negative, except for raw crustaceans at retail (14.8% positive) and egg products at processing plant (0.8% positive).

The results of the national monitoring program of milk and dairy products for 2009 are as follows:

- at retail: dried follow-on formula (n=86), nursing bottles (n=115), cheese of pasteurised milk (n=116), cheese of raw milk (n=76), butter of raw milk (n=11), milk desserts (n=23), ice cream (n=61)
- at processing plant: cheese of raw milk (n=40), cheese of pasteurised milk (n=97), milk desserts (n=23), milk powder (n=45), ice cream (n=30)
- at farm: cheese of raw milk (n=94), butter (n=51), cream (n=20).

All results were negative, except for milk desserts at retail (4.3% positive).

The results of the national monitoring program in other foods of animal origin for 2009 are as follows:

- at retail: fresh fish (n=62), cooked molluscs (n=60), cooked crustaceans (n=22), meat salad (n=45), chicken salad (n=45), crustacean salad (n=59), bakery products with cream (n=59), live bivalve molluscs (n=95), raw crustaceans (n=131), chocolate (n=30), confectionary with chocolate (n=30), table eggs (n=118), liquid egg products (n=15), salty preparation based on raw eggs (n=21), dessert based on raw eggs (n=90)
- at processing plant: gelatine (n=4), cooked crustaceans and molluscs (n=154), sandwich spreads (crustacean, meat, chicken) (n=149), chocolate (n=15) and confectionery (n=16), bakery products with cream (n=31), egg products (n=76).

All results were negative, except for live bivalve molluscs at retail (0.6% positive) and egg products at processing plant (1.3% positive).

Salmonella in humans

Surveillance programme and methods used

Data about human salmonellosis cases and human isolates were obtained from 160 clinical laboratories. All isolates were serotyped by slide agglutination with commercial antisera following the Kauffmann-White scheme. When necessary, additional biochemical tests were performed to confirm the identification or to differentiate between the subspecies. Phage typing and antimicrobial susceptibility testing were performed on isolates randomly sampled from the four serotypes *Enteritidis*, *Typhimurium*, *Hadar* and. All isolates of *S. Brandenburg*, *S. Derby*, *S. Infantis*, *S. Newport*, *S. Typhi* and *S. Paratyphi* were selected and tested for their antimicrobial susceptibility.

The objective of the national surveillance programme is to document the occurrence and trends of serotypes, to detect local, regional, national or even international outbreaks, to find and eliminate the source and to suggest preventive actions to the FASFC. This national Salmonella surveillance also intended to rapidly interact at the international level via electronic communication (with the Food and Water Diseases international surveillance network, ECDC) and helped detecting outbreaks and targeting preventive strategies.

Epidemiological investigations and results of 2008-2009 surveillance

From 1987 on, a remarkable increase in the number of human salmonellosis cases was registered, consecutively to the rise of the serotype Enteritidis, leading to a peak of 15,774 cases in 1999 (Figure 29, Table 22). In that year, exceptionally high numbers of *S. Enteritidis*. Between 2000 and 2009, the total number of laboratory-confirmed cases varied between 14,088 and 3,208 (Table 22). In 2003, the high number of salmonellosis cases mainly resulted from the increase of the serotype Enteritidis. These isolates exceeded for the first time 70% of the total number of *Salmonella* strains analysed. From 2005 a substantial decrease of *S. Enteritidis* infections compared with the annual number of cases in the period 2000-2004 was recorded. This decrease persisted in 2008-9 where the total number of cases caused by *Salmonella* spp. and by *S. Enteritidis* decreased to 3693 and 824 in 2008 and 587 in 2009 cases, respectively.

In recent years, the number of *S. Typhimurium* isolates remained at a level of about 2 500 strains per year, but started to decrease from 2005 (Table 22). After decreasing over the last years, *S. Infantis* increased in 2004 up to more than 100 cases to become the third serotype in human cases in 2004, but decreased to 23 cases in 2009. Regarding *S. Virchow*, about 140 to 150 isolates were annually registered from 2000 to 2003, whereas from 2004 less than 100 strains were yearly reported. A remarkable drop of *S. Brandenburg* (322 in 2000 versus 8 in 2009) cases was noted over the last years. Similarly, the number of *S. Derby* cases is shrinking since the beginning of 2000.

Table 22. Trends for the most prevalent *Salmonella* serotypes from 1989 to 2009

	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Total	9752	11695	10891	10391	10840	11294	10754	12008	14239	14514	15774	14088	11065	10075	12792	9543	4916	3693	3975	3944	3208
Enteritidis	2236	3382	4721	4084	5260	5700	5138	6145	8284	9003	10492	9503	7112	6398	9118	6075	2226	1052	987	824	587
Typhimurium	4018	4756	3652	3835	3528	3418	3623	3522	3347	3221	3348	2799	2370	2438	2486	2459	1659	1826	2233	2279	1862
Autres	2498	2543	1760	1652	1369	1401	1226	1564	1778	1559	1262	1028	956	793	818	684	765	633	596	685	668
Derby	177	161	134	139	103	113	107	118	157	162	138	169	158	92	100	64	67	52	64	44	42
Brandenburg	255	302	176	161	147	204	241	214	296	274	279	322	200	148	66	63	76	47	29	36	8
Virchow	293	302	224	295	273	308	245	178	114	115	86	147	143	132	152	91	65	46	28	29	18
Infantis	275	249	224	225	160	150	174	267	263	180	169	120	126	74	52	107	58	37	38	47	23

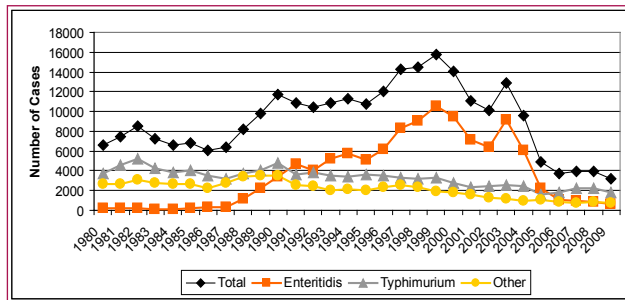


Figure 29. Trend of the human *Salmonella* isolates and of the two major serotypes Enteritidis and Typhimurium over the last twenty seven years in Belgium: number of laboratory confirmed cases

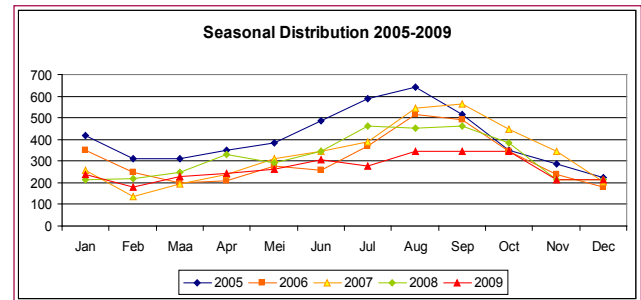


Figure 30. Seasonal distribution 2005 – 2009

Age and seasonal distribution

Most cases of salmonellosis were reported in children less than 5 years old (43,9% of cases), with no significant gender difference.

Table 23. Human cases of *Salmonella*: Age and gender distribution 2009. Note that the gender of all salmonellosis cases is not known.

M: male; F: female; SR: sex ratio

Age	Salmonella				Salmonella Enteritidis				Salmonella Typhimurium			
	Total	M	F	SR	Total	M	F	SR	Total	M	F	SR
< 1 year	271	156	114	1,36	36	23	13	1,76	111	60	50	1,2
1 to 4 years	1139	547	585	0,93	174	80	93	0,86	817	407	405	1
5 to 14 years	636	321	309	1,03	123	59	61	0,97	433	218	214	1,01
15 to 24 years	169	78	91	0,86	45	21	24	0,87	82	41	41	1
25 to 44 years	270	138	130	1,06	71	35	36	0,97	89	48	40	1,2
45 to 64 years	305	151	152	0,99	64	32	32	1	112	55	56	0,98
≥ 65 years	318	139	178	0,78	56	30	26	1,15	160	66	93	0,7
unknown	100	41	18	2,27	18	7	5	1,4	58	26	8	3,25
Total	3208	1571	1577	0,99	587	287	290	0,98	1862	921	907	1,01

Antimicrobial resistance

Antimicrobial resistance in isolates from living animals

Methods used

The NRL for Salmonella, Animal Health, performed the antibiotic susceptibility testing of Salmonella strains from livestock. Susceptibility tests were done by means of the disk diffusion test, using Neo-Sensitabs (Rosco). Tests and interpretation were done according to the manufacturers guidelines using the methodology as described by CLSI. Internal control was performed with quality control strain *E. coli* ATCC25922. Results were only accepted when results with the QC strain were within the limits as proposed by Rosco. In order to reduce bias due to multiple strains from the same origin at the same sampling time and belonging to the same serotype, only one isolate per serotype and per origin was selected for susceptibility testing. Therefore, strains were likely to be independent from each other.

Epidemiological investigations and results of 2008-2009 surveillance

The susceptibility of 1 125 and 1 128 Salmonella isolates was tested in 2008 and 2009, respectively. A total of 706 (62.6% in 2008) and 613 (54.3% in 2009) of Salmonella isolates was fully susceptible to all antimicrobial drugs tested. The apparent lower proportion of susceptible isolates may be due to the lower proportion of *S. Enteritidis* strains tested in 2009.

Table 24. *Animal Salmonella*: list of antimicrobials tested - For all susceptibility tests Neo-Sensitabs from Rosco were used according to the providers instructions

Antimicrobial	Amount of antimicrobial	Breakpoints (mm)
Ampicillin	33µg	17 – 19
Ceftiofur	30µg	20 – 22
Streptomycin	100µg	23 – 25
Neomycin	120µg	20 – 22
Gentamicin	40µg	20 – 22
Tetracycline	80µg	20 – 22
Sulphonamides	240µg	20 – 22
Trimethoprim - Sulphonamides	5.2µg + 240µg	27 – 31
Nalidixic acidid	130µg	21 – 24
Enrofloxacin	10µg	20 – 22
Chloramphenicol	60µg	21 – 24
Florfenicol	30µg	15 - 18

In 2008, 22.8% of *S. Paratyphi B* (n=31) strains were susceptible to the antimicrobials tested. Resistance was mainly observed against St (74.2%), Ap and Nal (both 54.8%) and against Su (48.4%). Also resistance against Cef was frequently observed (38.7%). However, 5.1% of 2009 *S. Paratyphi B* (n=78) isolates were fully sensitive. The most abundant profile was Ap Nal (69.2%). Almost half of the strains were found Cef resistant.

Only 22% to 23% of *S. Typhimurium* isolates (252 tested in 2008 and 289 in 2009) were found susceptible. Pentaresistance Ap St Tc Su Cm was encountered in 21.8% and 17.3% of the 2008 and 2009 isolates, respectively. Ff resistance was detected in 11.4% and 13.5% of the strains, whereas Cef resistance in 2.4% and 0.3%, respectively.

In 2008 and 2009, 22 and 13 *S. Virchow* isolates were tested, and about one quarter was found susceptible to the antimicrobials used. As in former years, most resistance was found against Ap (both 68.2% in 2008, 69.2% in 2009) and Nal (68.2% in 2008 and 53.8% in 2009). Cef resistance was remarkably high: 45.5% in 2008 and 38.5% in 2009.

In 2009, resistance was mainly found against Ap (37.6%), Su (32.1%), Tc (28.2%), St (23.9%), but also against TSu (21.8%), which are similar figures as for 2008. Noteworthy however, is the resistance against Nal (13.3%) and against Cef (7.1%), both considerably higher than in 2008 (8.7% and 3.0%, respectively). Resistance against Ne, Gm and Enr (all 0.4%), and against Cm and Ff (7.6% and 3.5%, respectively) was similar to that of 2008.

Eighty to ninety percent of *S. Agona* isolates were found fully susceptible for all antimicrobials tested: 92.9% of 42 strains in 2008 and 90.9% of 11 strains in 2009.

A limited number of *S. Blockley* isolates were tested (11 in 2008 and 7 in 2009). In 2008, all were resistant against Ap, Su and Nal, 90.9% against TSu and 81.8% against Tc. All 2009 isolates were from poultry and had the resistance profile Ap Tc Su TSu Nal.

Around 60.0% of *S. Derby* strains (50 isolates tested in 2008 and 26 in 2009) were sensitive, although 25 to 30% of strains were resistant against Tc and Su.

As for the 14 *S. Dublin* isolates tested in 2008, 28.6% were found completely susceptible. Resistance against Cm (57.13%), Su and Nal (both 50.0%) and St (14.3%) was noticed. In 2009, about half of 20 isolates were found completely susceptible.

Both in 2008 and 2009, about 95% of *S. Enteritidis* isolates (185 and 115 tested) were susceptible.

Only four *S. Hadar* strains were tested in 2008 and all were found resistant against St Tc Nal (100%). Two strains were Enr resistant. In 2009, 22 *S. Hadar* strains were tested and only one (from poultry) was found sensitive. Resistance profiles Ap Tc Nal and Tc Nal were most often demonstrated (45.5% and 40.9%, respectively).

All the *S. Indiana* strains (16 tested in 2008 and 17 in 2009) were multi-resistant and most showed profile Ap St Su TSu.

More than 80% of the *S. Infantis* isolates (n=42 in 2008 and 47 in 2009) were susceptible to the antibiotics tested. Two or three strains per year were found to be Cef resistant.

Antimicrobial resistance in strains isolated from meat and meat products

Surveillance programme and method used

During 2008 and 2009, respectively, 718 and 682 strains of *Salmonella enterica* isolated during the zoonosis monitoring program were sent to IPH for serotyping and determination of antimicrobial resistance. Different food matrices were sampled, mainly poultry (carcasses from broilers and spent hens, chicken parts and meat preparations) and pork (carcasses and cut meats). Other matrices where *Salmonella* was isolated were ready-to-eat meals, meat, meat preparations, bovine carcasses, frog's legs, pudding, liquid egg product, ham and dry sausage.

Minimum Inhibitory Concentrations (MIC) were determined by the broth dilution method using Sensititre, as recommended by the CRL antibiotic resistance. The antimicrobials reported are listed in the table below, as well as the breakpoints used for the interpretation of the results. Interpretation was according to CLSI and using epidemiological cut-off values from EUCAST. Quality control was performed by using an *Escherichia coli* ATCC 25922 strain.

Table 25. *Salmonella* from food, list of antimicrobials tested with their breakpoints

Antimicrobial	Breakpoints ($\mu\text{g} / \text{ml}$)
Ampicillin	4
Cefotaxim	0.5
Ceftazidim	2
Chloramphenicol	16
Ciprofloxacin	0.06
Colistin	16
Florfenicol	16
Gentamicin	2
Kanamycin	8
Nalidixic acid	16
Streptomycin	32
Sulphamethoxazole	256
Tetracycline	8
Trimethoprim	2

The level of resistance of *Salmonella* isolates from poultry and pork is influenced by the serotype distribution in the corresponding matrix; results are summarized in the next table. The presence of highly resistant serotypes as *Hadar*, *Virchow*, *Paratyphi B* and *Typhimurium* contributed mainly to the high resistance levels in some matrices. In 2008, 65 different serotypes were identified, whereas in 2009 only 48. *S. Typhimurium* was the most prevalent serotype on pork (44%/56%-2008/2009) while *S. Enteritidis* was dominantly found on carcasses of spent hens (57%/58%-

2008/2009). Broilers and poultry meat (products) contained a high diversity of serotypes, with *Paratyphi B* (26%/34%-2008/2009) as most prevalent.

Table 26. Antimicrobial susceptibility testing of *Salmonella* spp. isolated from different food matrices: percentage of resistant strains

Antimicrobial tested	Poultry		Pork	
	2008 (n=496)	2009 (n=399)	2008 (n=159)	2009 (n=200)
Ampicillin	46	43	53	44
Cefotaxim	21	16	2	4
Ceftazidim	20	13	2	3
Chloramphenicol	5	1	12	3
Ciprofloxacin	33	20	4	4
Colistin	0	0	0	0
Florfenicol	2	1	7	1
Gentamicin	1	1	0	1
Kanamycin	3	3	2	1
Nalidixic acid	31	19	3	0
Streptomycin	44	29	46	37
Sulphamethoxazole	37	37	49	44
Tetracycline	19	12	52	47
Trimethoprim	47	39	28	25

Antimicrobial resistance in strains isolated from poultry meat

In 2008 and 2009, 496 and 399 *Salmonella* isolates from poultry meats were tested for their antimicrobial susceptibility, respectively. Resistance to ampicillin (46%/43%-2008/2009), trimethoprim (47%/39%-2008/2009) and sulphamethoxazole

(37%/37%-2008/2009) were most prevalent. Between 2008 and 2009, resistance to streptomycin significantly decreased from 44% to 29% and resistance to trimethoprim from 47% to 39%. The resistance to ciprofloxacin was significantly decreased from 33% in 2008 to 20% in 2009, but remains high compared to previous years. The differences in ciprofloxacin and trimethoprim resistance compared to earlier years can be attributed to a serious lowering of the breakpoint values since 2008. Little or no resistance was found for colistin, gentamicin, florfenicol and kanamycin. The resistance to chloramphenicol decreased to 1%, compared to 5% in 2008.

The proportion of strains sensitive to all tested antibiotics slightly increased from 41% to 49% between 2008 and 2009. Multiresistance (resistance to more than four antibiotics) decreased, from 38% in 2008 to 22% in 2009.

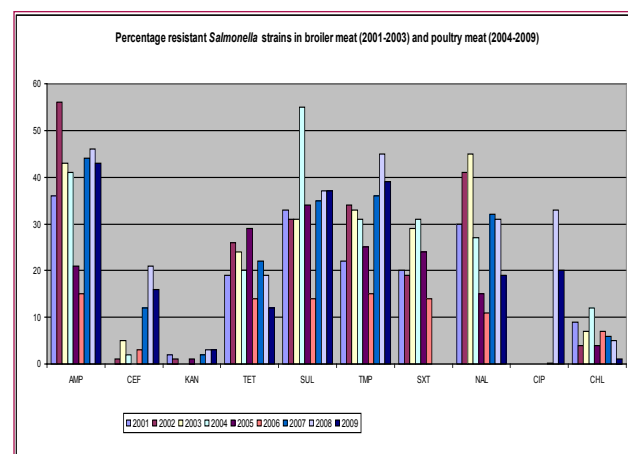


Figure 31. Evolution of the percentage resistant *Salmonella* strains in broiler meat (2001-2003) and poultry meat (2004-2009)

Compared to these general results, higher resistances were observed in chicken meat for cooked consumption and chicken parts (no carcasses) with, in 2009, 98% and 61% of the isolates resistant to trimethoprim, 83% and 53% to sulphamethoxazole, and 96% and 69% to ampicillin, respectively. Especially in chicken meat for cooked consumption the resistance to these three antibiotics drastically increased, compared to 2008 (78%, 62% and 70%, respectively). However, multiresistance significantly decreased among these isolates to 42% and 33%, respectively (compared to 68% and 65% in 2008). On the other hand, *Salmonella* isolates from spent hens showed little antibiotic resistance, with only 6% and 9% showing multiresistance in 2008 and 2009, respectively.

Antimicrobial resistance in strains isolated from pork

In total, 159 and 200 *Salmonella* strains from pork were tested for their antibiotic susceptibility in 2008 and 2009, respectively. This included strains from carcasses and cut meats. High resistance was observed to tetracycline (52%/47%-2008/2009), ampicillin (53%/44%-2008/2009), sulphamethoxazole (49%/44%-2008/2009) and streptomycin (46%/37%-2008/2009). Resistance to four or more antibiotics slightly decreased from 41% to 35% of the tested isolates, while sensitivity to all antibiotics increased from 28% to 39% between 2008 and 2009. All strains were sensitive to colistin and gentamicin. Low resistance was observed for cefotaxime (2%/4%-2008/2009), ceftazidim (1%/3%-2008/2009), kanamycin (3%/1%-2008/2009), ciprofloxacin (4%/4%-2008/2009) and florfenicol (7%/1%-2008/2009). Compared to 2008, overall resistance has slightly decreased in 2009.

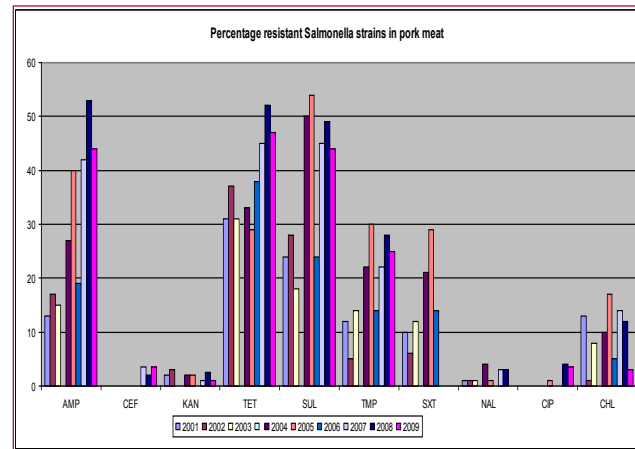


Figure 32. Evolution of the percentage resistant *Salmonella* strains in pork meat

S. Typhimurium was the most dominantly isolated serotype (70%/56%-2008/2009) from pork. The observed trends are similar as described above, with high resistance to ampicillin (70%/69%-2008/2009), tetracycline (63%/61%-2008/2009), sulphamethoxazole (70%/60%-2008/2009) and streptomycin (59%/54%-2008/2009). However, it is clear that *Typhimurium* strains are more resistant than other *Salmonella* strains found on pork, with only 9% and 22% of all *Typhimurium* strains sensitive to all antibiotics in 2008 and 2009, respectively.

Compared to previous years, ampicillin and tetracycline resistance has increased. This can be explained by the lowering of the breakpoint values used to assess resistance.

Antimicrobial resistance of human isolates

Methods used

Human *Salmonella* isolates randomly selected from the six most important serotypes in 2009 (*Enteritidis*, *Typhimurium*, *Hadar*, *Virchow*, *Brandenburg* and *Derby*), comprising as well all isolates of the serotypes *Infantis*, *Newport*, *Typhi* and *Paratyphi*, were examined for their resistance. Fourteen antibiotics of therapeutic or epidemiological interest were tested in disk diffusion according to Kirby-Bauer, following CLSI procedures (Table 27).

Table 27. List of antimicrobials used for susceptibility testing of *Salmonella*

Antimicrobial	Amount of antimicrobial	Breakpoints (mm)
Ampicillin	10 µg	14 - 16
Amoxicillin + clavulanic acid	20/10 µg	14 - 17
Cefotaxime	30 µg	15 - 22
Streptomycin	10 UI	12 - 14
Kanamycin	30 UI	14 - 17
Spectinomycin (excepted for Typhi, Paratyphi A and B)	100µg	19 - 25
Azithromycine (only for Typhi, Partyphi A and B)	15µg	ND
Gentamicin	10 µg	13 - 14
Tetracycline	30 µg	12 - 14
Sulfonamides	300 µg	16 - 13
Trimethoprim	5 µg	15 - 11
Trimethoprim + Sulfamethoxazole	1,25/ 23,75 µg	11 - 15
Nalidixic acid	30 µg	14 - 18
Ciprofloxacin	5 µg	16 - 20
Chloramphenicol	30 µg	13 - 17

Epidemiological history and results of 2009 surveillance

Resistance was mostly found to tetracycline (29.8%), sulfonamides (32.3%), ampicillin (38.7%), streptomycin (33.7%), and to a lesser extent to trimethoprim (13.2%).

The vast majority (83.0%) of human *S. Enteritidis* isolates (n=336) was fully sensitive to all antimicrobials tested.

S. Typhimurium (n=505) showed a high level of resistance; especially resistances to ampicillin (74.3%), sulfonamides (58.6%), tetracycline (54.9%) and streptomycin (53.3%) are striking. 51.48% of the isolates were found resistant to four or more antimicrobial agents. In addition, almost 17.4% of the isolates showed multi-resistance to at least ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. About 26.1% of these multi-resistant isolates (ACSSuT) were of phage type DT104.

Except one strain, all *S. Hadar* isolates (n=10) were resistant to at least one antibiotic. Resistance to tetracycline, nalidixic acid, ampicillin and streptomycin reached values from 60.0% up to 80.0%. Simultaneous resistance to these four antibiotics was observed in 50.0% of these isolates. The isolates from this serotype remained fully sensitive to cefotaxime, ciprofloxacin, chloramphenicol and gentamicin.

In *S. Virchow* (n=16), multi-resistance was less common as compared to 2003 (18.9% of the strains in 2009 instead of 60% of the 2003 isolates). The highest incidence of resistance was observed for nalidixic acid (55.2%). Resistances to tetracycline, sulfonamides, trimethoprim and trimethoprim+sulfonamides were common (approximately between 25 and 37.5%). In contrast, the

vast majority of *S. Brandenburg* (n=8) and *S. Derby* (n=42) isolates remained sensitive to the vast majority of tested antibiotics: 75.0% and 66.7% sensitive, respectively.

S. Infantis (N= 23) displayed in general a low level of multi-resistance.

The vast majority of *S. Paratyphi B* (n=73) and *S. Newport* (n=25) isolates remained sensitive to the vast majority of tested antibiotics: respectively 56.2% and 92% were fully sensitive to all antimicrobials tested. However, two isolates of *S. Newport* displayed resistance to at least 5 antibiotics but remained sensitive to cefotaxime and ciprofloxacin.

No tendency could be observed from the results on *S. Typhi*. That could be due to the fact that most of isolates are travel-associated and that the origins (country/region) of the isolates were different.

In general, resistance patterns and levels of Salmonella isolated in 2009 were comparable to those from 2002-2008.

Phagetyping of human isolates

In 2009, a total of 336 human *S. Enteritidis* isolates were phage typed. Of these, 16.0% were PT 21 and 19% were PT 4. In addition, 505 *S. Typhimurium* isolates were phage typed and most prevalent types were DT104 (5.5%), DT120 (32.5%), DT193 (15.8%), and U302 (13.4%).

Table 28. Antimicrobial resistance in human *Salmonella* of serotypes *Enteritidis*, *Typhimurium*, *Brandenburg*, *Derby*, *Hadar*, *Virchow*, *Infantis*, *Typhi*, *Newport*, *Paratyphi B* and *A* isolated in 2009

	Total	N	Amp	Amx	Ctx	Tet	Nal	Cip	Azy	Spe	Gen	Kan	Chl	Stp	Tmp	Sul	Stx
Enteritidis	587	336	6,0	0,6	0,9	2,1	12,5	0,0	ND	1,5	0,3	0,3	0,0	1,8	0,9	1,8	1,2
Typhimurium	1862	505	74,3	0,8	1,0	54,9	2,8	0,0	ND	23,4	1,4	2,6	13,1	53,3	20,2	58,6	20,4
Derby	42	42	14,3	0,0	0,0	21,4	4,8	0,0	ND	7,1	0,0	2,4	0,0	19,0	16,7	21,4	19,0
Hadar	12	10	80	10	0	80	60	0	ND	0	0	10	0	60	0	10	0
Infantis	23	23	4,3	0,0	0,0	21,7	30,4	0,0	ND	17,4	4,3	8,7	4,3	17,4	21,7	21,7	17,4
Virchow	18	16	12,5	0	0	25	43,75	0	ND	6,25	12,5	12,5	6,25	6,25	25	37,5	25
Brandenburg	8	8	0	0	0	25	0	0	ND	0	0	0	0	0	0	0	0
Newport	25	25	8	0	0	8	11,5	0	ND	0	0	0	4	4	4	8	4
Paratyphi B	73	73	24,7	2,7	11,0	16,4	15,1	0,0	11,0	ND	0,0	1,4	4,1	30,1	21,9	19,2	21,9
Typhi	26	25	24	0	0	12	56	16	8	ND	0	0	16	24	16	24	16
Dublin	8	8	0	0	0	0	12,5	0	ND	50	0	0	50	50	0	50	12,5
Paratyphi A	5	5	0	0	0	0	100	0	80	ND	0	0	0	0	0	0	0

Tuberculosis

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Zoonotic tuberculosis (*Mycobacterium bovis*)

Tuberculosis in humans caused by *Mycobacterium bovis* is rare.

In regions where *M. bovis* infections in cattle are largely eliminated, only few residual cases occur either 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 get infected by *M. bovis* by inhaling aerosols from coughing infected cattle and may subsequently develop typical pulmonary or genito-urinary tuberculosis. Such patients may infect cattle through cough or urine. Evidence for human-to-human transmission of zoonotic tuberculosis is only rarely reported.

In developing countries, where *M. bovis* is largely prevalent among cattle, some studies reported that 3-6% of all tuberculosis cases are due to *M. bovis* and that mostly young people get infected through the ingestion of contaminated raw milk. Also occupational contacts should be regarded as a risk factor for transmission to humans, although companion animals can provide a less common indirect route of infection.

Zoonotic tuberculosis
(*Mycobacterium bovis*)

Mycobacterium bovis in cattle

Mycobacterium bovis in wildlife

Mycobacterium bovis in humans

Human tuberculosis
(*Mycobacterium tuberculosis*)

In humans, the disease caused by *M. bovis* is clinically indistinguishable from that caused by *M. tuberculosis*. Pulmonary tuberculosis is frequently observed and cervical lymphadenopathy, intestinal lesions, chronic skin tuberculosis and other non-pulmonary forms are particularly common.

Mycobacterium bovis in cattle

Belgium is officially free from bovine tuberculosis (*Mycobacterium bovis*) since 25 June 2003 (Commission Decision 2003/467/EC establishing the official tuberculosis, brucellosis and enzootic bovine leucosis free status of certain Member States and regions of Member States as regards bovine herds).

Surveillance programme

The control of tuberculosis is based on Council Directive 64/432/EEC, which is implemented and adapted in the national legislation since 1963 and was last amended by Royal Decree of 17 October 2002.

The control implies:

- Skin testing of animals at purchase (mandatory),
- In case of a positive reactor, skin testing of all the animals of the holding and skin testing of all contact animals (tracing on and tracing back),
- Systematic post mortem examinations at the slaughterhouse. In case a suspected lesion is identified, a sample is sent to the NRL for analysis.

The FASFC 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, additional tests). If *M. bovis* is isolated as a consequence of post mortem examinations or of mandatory test-slaughter, all animals in the herd of origin are skin tested and a complete epidemiological investigation is performed.

An animal is defined as infected with bovine tuberculosis if the skin testing is positive or if *M. bovis* is isolated by culture or confirmed by laboratory testing (PCR). A holding is defined as infected if *M. bovis* was isolated or detected by PCR from an animal of the holding.

Isolation of *M. bovis* and biochemical testing is exclusively performed in the NRL where also IFN-gamma and molecular typing by means of IS6110 RFLP, spoligotyping and MIRU-VNTR are done.

In Belgium, vaccination against tuberculosis is prohibited.

Epidemiological investigations and results of 2008-2009 surveillance

At the slaughterhouse, tissue samples from individual animals were taken. The samples originated from animals suspected of being infected with *M. bovis*, i.e. skin test reactors, animals that had been in contact with *M. bovis* infected animals or animals that showed suspicious lesions at meat inspection. The samples were submitted to the NRL where culture, PCR and confirmatory tests were done. *M. bovis* was only detected in animals belonging to the outbreak herds.

The evolution of tuberculosis outbreaks in cattle herds over the last years is indicated in table 29 and figure 33.

Table 29. Evolution of bovine tuberculosis outbreaks in cattle herds in Belgium

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
TB breakdowns	24	23	13	7	8	5	8	5	12	2

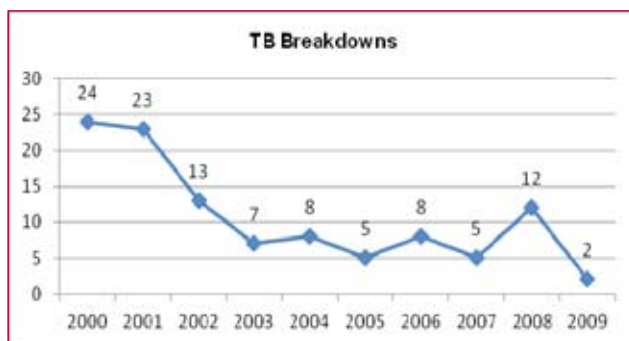


Figure 33. Evolution of bovine tuberculosis breakdowns in cattle herds in Belgium

The NRL performs routine IS6110 RFLP typing and spoligotyping of *M. bovis* field isolates. Since 1995, the strains of 96% of the outbreak herds are typed by both methods. More recently, all strains typed by RFLP and spoligotyping were additionally analysed by MIRU-VNTR, which is done in collaboration with IPH. As a consequence, a comprehensive database of the vast majority of *M. bovis* types isolated in Belgium since 1995 is maintained. Analysis of molecular profiles of all isolates obtained from Belgian TB breakdowns allowed the identification of a predominant spoligotype (SB0162).

Mycobacterium bovis in humans

Mycobacterium bovis can infect humans and cause a disease similar to infections with *M. tuberculosis*. The main transmission routes of *M. bovis* to humans are contaminated food (especially raw milk or milk products) or direct contact with animals.

In 2008, 7 human cases of bovine tuberculosis were identified and confirmed by culture followed by molecular identification in the NRL (IPH). No link between these patients and bovine tuberculosis in a Belgian herd could be detected. Five patients were coming from Morocco and 2 from Belgium. All patients had an extra pulmonary form of the disease (3 abdominal tuberculoses, 1 meningitis, 1 cervical lymph node, 1 tuberculosis of the tonsil, 1 cutaneous disease).

In 2009, 6 human cases of bovine tuberculosis were reported to the Belgian Register and confirmed by culture. Again, no link between these patients and bovine tuberculosis in a Belgian herd could be detected.

Five of the 6 patients (whom 4 from North Africa) had an extra-pulmonary form of the disease (mediastinal, abdominal, vertebral, neck lymph node); one of them had a pulmonary tuberculosis. The patient with Pott's disease (vertebra) was infected by an uncommon strain, whose genome included no IS6110 element. The patient had stayed for a long time in Asia where such strains were already described.

This report concerning the years 2008 and 2009 confirms that only a few cases of zoonoses caused by *M. bovis* are observed in Belgium. Most of them are identified in patients from North Africa and are extra pulmonary forms of the disease.

Human tuberculosis (*Mycobacterium tuberculosis*)

The incidence of human tuberculosis shows little variation over the last years. In 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008 and 2009 respectively 1321, 1309, 1128, 1226, 1144, 1127, 1028, 1006 and 1020 new notified cases of active human tuberculosis were detected. Over the 60% were male patients. In 2009, 49% of the tuberculosis cases were foreigners.

Groups at risk are persons with a marginal existence, asylum seekers and refugees. Alcoholism and a co-infection with HIV are known as specific risk factors. Human tuberculosis cases are mainly concentrated in urban populations.

Yersiniosis

Katrien Beullens, Michel Delmée, Lieven De Zutter, Geneviève Ducoffre, Luc Vanholme

Yersinia enterocolitica

The genus *Yersinia* comprises three main species that are known to cause human infections: *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* (plague which is believed to no longer exist in Europe). Specific types of *Y. enterocolitica* and *Y. pseudotuberculosis* cause foodborne infections. At the EU level Yersiniosis is the third common zoonotic pathogen with 8348 reported human cases in 2008. Since most human infections are caused by *Y. enterocolitica*, this chapter deals mainly with *Y. enterocolitica* infections.

Only certain biotypes of strains of *Y. enterocolitica* cause illness in humans. Infection with *Y. enterocolitica* most often causes diarrhoea and abdominal pain and occurs most often in young children. Common symptoms in children are fever, abdominal pain and diarrhoea, 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, and/or bacteraemia may occur.

Yersinia enterocolitica

Yersinia enterocolitica in food

Yersiniosis in humans

Pigs are the primary reservoir for the human pathogenic *Y. enterocolitica* types. In infected pigs the bacteria are most likely to be found in the tonsils. However other animal species, e.g. cattle, sheep, deer, small rodents, cats and dogs may also carry pathogenic biotypes. Clinical disease in animals is uncommon.

Infection is most often acquired by eating contaminated food, particularly raw or undercooked pork. Since the microorganism is able to grow at + 4°C the risk for the development of human infection may increase when contaminated refrigerated food with a relatively long shelf life is consumed. Drinking contaminated unpasteurised milk or untreated water can also transmit the organism. On rare occasions, transmission may occur by direct contact with infected animals or humans.

Within *Y. enterocolitica*, the majority of isolates from food and environmental sources belongs to non-pathogenic types. It is, therefore, most important to investigate which isolates are pathogenic for humans. Biotyping of the isolates is essential to determine whether or not isolates are pathogenic to humans, and this typing is ideally complemented by serotyping.

Infection caused by *Y. pseudotuberculosis* shows many similarities with the disease pattern of *Y. enterocolitica*. Infections are caused by the ingestion of the bacteria from raw vegetables, fruit, other foodstuffs or via contaminated water or by direct contact with infected animals.

Yersinia enterocolitica in food

Monitoring programme

The FASFC organised a monitoring of meat since 1997, which showed a very low prevalence of *Yersinia enterocolitica* in pork, beef and poultry. In 2008 and 2009, the monitoring programme concentrated on one matrix, i.e. minced meat containing pork meat.

Table 30. Monitoring *Yersinia enterocolitica* in pork meat

Sample	Quantity analysed	Percentage of positive samples 2008	Percentage of positive samples 2009
Minced meat (containing pig meat) intended to be eaten raw at retail	1g	0.0% (n=129)	0.0% (n=184)
Minced meat (containing pig meat) intended to be eaten cooked at retail	1g	0.0% (n=125)	0.0% (n=179)

Yersiniosis in humans

In 2008 and 2009, the Sentinel Laboratory Network registered 273 and 240 cases respectively, corresponding to a national incidence estimated at 2.6 and 2.2 per 100,000 inhabitants.

Since 1986, when 1514 cases were reported by this network, the number of human infections in Belgium significantly decreased (Figure 34).

Cases were observed all over the year (Figure 35)

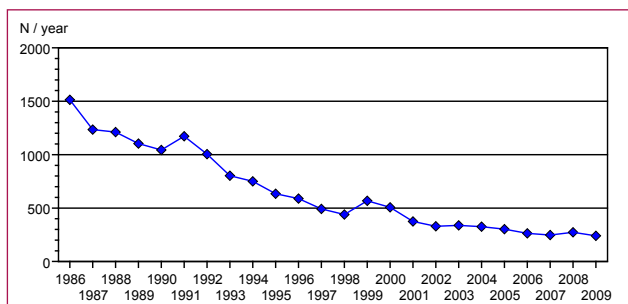


Figure 34. Total number of *Yersinia enterocolitica* infections in humans by year (1986-2009) Source: Sentinel Laboratory Network

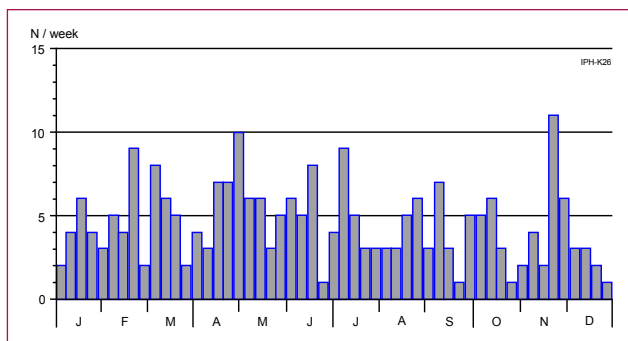


Figure 35. Weekly number of *Yersinia enterocolitica* infections in humans, 2009 Source: Sentinel Laboratory Network

Forty percent of cases were 1 to 4 year old children (Table 31).

Table 31. Number of *Yersinia enterocolitica* infections in humans by sex and by age groups, 2009 Source: Sentinel Laboratory Network

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	4	3,4	1	0,9	5	2,1
1 - 4	50	42,4	53	45,7	103	44,0
5 -14	43	36,4	23	19,8	66	28,2
15 -24	5	4,2	4	3,4	9	3,8
25 -44	8	6,8	11	9,5	19	8,1
45 -64	5	4,2	12	10,3	17	7,3
≥ 65	3	2,5	12	10,3	15	6,4
Total	118	100,0	116	100,0	234	100,0

As already reported in former years, the incidence in Flanders is higher than in Wallonia. In 2007, the incidence was 2.9 per 100,000 inhabitants in Flanders, 1.6 per 100,000 inhabitants in Wallonia and 0.6 per 100,000 inhabitants in Brussels-Capital Region (Figure 36 on the next page).

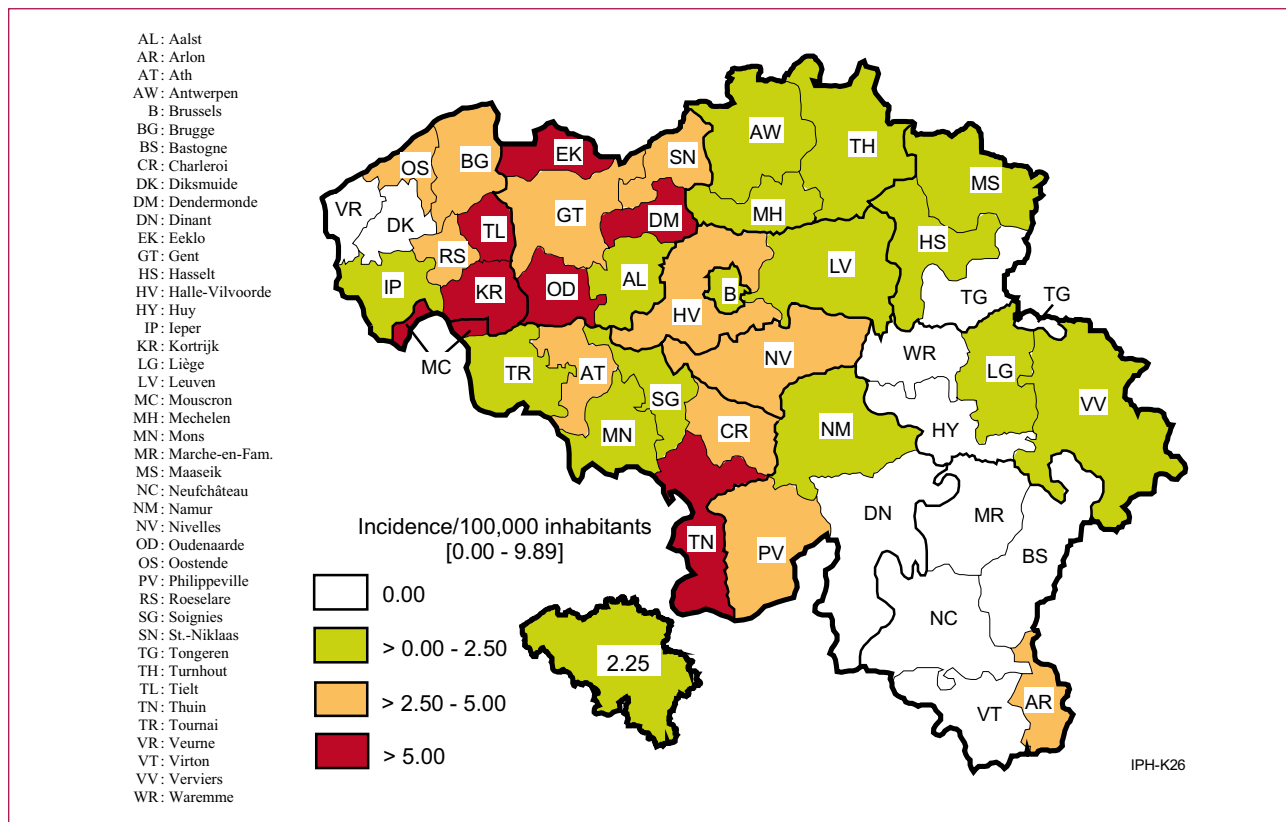


Figure 36. Incidence of *Yersinia enterocolitica* infections in humans by district (N/105 inhab., 2009)

Source: Sentinel Laboratory Network

Bio-serotyping was performed by the NRLs.

In 2008, 73.8% of the 397 isolates tested belonged to the pathogenic bio-serotypes (including 6 *Yersinia pseudotuberculosis*) with serotype O:3 / biotype 4 accounting for 68.7% of the total. The remaining 104 strains (26.2%) belonged to non-pathogenic *Yersinia enterocolitica* bio-serotypes or related species.

In 2009, 72% of the 359 isolates tested belonged to the pathogenic bio-serotypes (including 12 *Yersinia pseudotuberculosis*) with serotype O:3 / biotype 4 accounting for 66.3% of the total. The remaining 101 strains (28%) belonged to non-pathogenic *Yersinia enterocolitica* bio-serotypes or related species.

The number of non-pathogenic *Yersinia* strains did not vary markedly during the last years, in contrast to the obvious decrease of the pathogenic strains.

trends and sources

2008-2009



viral diseases

Avian influenza

Bénédicte Lambrecht, Annick Linden, Thierry van den Berg, Didier Vangeluwe, Françoise Wuillaume

General

A total of more or less 100,000 wild birds, from 27 Member States of the European Union was tested during the 2008 and 2009 active avian influenza (AI) surveillance. In contrast to 2007, where cases in wild birds were reported from four Member States (Germany, France, the Czech Republic and Hungary), in 2008 and 2009 H5N1 HPAI were reported from only two Member States, i.e. United Kingdom and Germany. In 2008, the incident was found following the testing of Mute Swans and a Canada Goose that were found dead or euthanized on welfare grounds. The H5N1 HPAI viruses were identified in a total of 11 birds over approximately six weeks in January and February 2008. In January 2009, an outbreak was demonstrated in one of 39 wild ducks shot during a hunt near the town of Starnberg in the German state of Bavaria. Finally in 2009 the virus was also detected in a wild duck on Lake Sempach, near Lucerne in Switzerland, during a regular detection programme. The common pochard that tested positive showed no clinical signs of the disease at the time of sampling. The other 200 birds caught in the same time and place were tested negative. The role of wild birds in the spread of virus has been subject to extensive international debate and uncertainty, but the likelihood is that both wild birds and activities associated with domestic poultry are responsible for the H5N1 spread.

General

Monitoring in birds

Influenza in humans: monitoring

An evolution of the H5N1 epidemiology in the poultry industry in Europe: was observed during 2006, 33 domestic poultry outbreaks were reported in five affected Member States and were preceded by the positive identification of virus in wild birds in the vicinity of the index case, giving a strong epidemiological link to the source of infection. During 2007, a decrease of number of reported outbreaks in domestic poultry have been observed (14) with no obvious epidemiological link to wild bird infection in the majority of cases. Since the end of 2007, however the virus continued to be detected in wild bird populations, one isolated case was reported in clinically healthy ducks in a mixed poultry holding in Saxony, Germany (2008). Surveillance of apparently healthy wild birds has not provided early warning of likely infection for the poultry industry, whereas searches for and reports of dead birds ("passive" surveillance) have provided evidence of environmental presence of the virus, but not necessarily its source.

Monitoring in birds

In Belgium, like in other EU Member-States, a large survey has been implemented since autumn 2005 including passive (dead birds) and active wild birds surveillance (swabs), exclusion diagnosis in the professional sector (following an abnormal mortality rate or treatment set-up) and active serological surveillance in poultry (H5 and H7 specific HI tests). This important monitoring is organised by the FASFC in close coordination with VAR. The active wild bird surveillance is a close cooperation between the Royal Belgian Institute of Natural Science, the Veterinary Faculty of Liege and VAR. All tests were performed at VAR.

Passive monitoring of dead wild birds

A Belgian expert group had determined the criteria for passive monitoring and further analysis of dead birds. These criteria are related to the number of dead birds, the finding place and the conditions in which the dead birds are found, in order to avoid an overload of samples to be sent to the laboratory. Species included each single dead swan, 5 waterfowls, 20 gulls or starlings. According to the findings during the spring and summer in Germany, grebes (single) were added to the initial list. During 2008-2009, a total of 62 suspicions complying with these criteria were analysed by Real Time RT-PCR and/or viral isolation, all with negative results.

Active monitoring of wild birds

Cloacal swabs (Figure 37; c) were taken systematically but oral swabs (Figure 37; b) were also taken on waterfowl during the high risk season (autumn migration period), as it is now well accepted that H5N1 is mostly excreted by the respiratory tract. As in previous years, the bird species taken into consideration were mallard, common teal, common shelduck, northern pintail, Canada goose, Egyptian Goose, coot, golden plover, lapwing, black headed gull, herring gull, terns and raptors. Sampling was organised in the whole territory of Belgium with a logically greater pressure on areas where waterfowl density is the highest. Three groups were targeted: birds wintering in or migrating through Belgium and potentially originating from regions where H5N1 occurs, birds-eating raptors susceptible to be good indicators of virus contamination and feral waterfowl representing a very important part of the biomass of Anatidae in Belgium, particularly during breeding season.

The active monitoring of wild birds was increased in 2008-2009 (Figure 37).

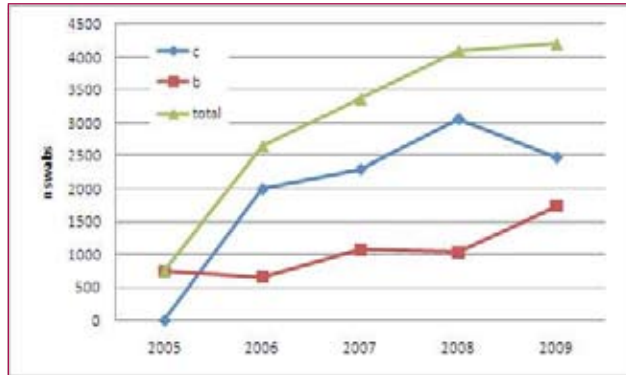


Figure 37. Number of cloacal (c) and buccal/oral (b) swabs analysed in 2005-2009

During these two last years, a total of 8305 swabs were taken during ringing activities of target wild birds and 1200 samples were taken from hunted waterfowls. No HP H5N1 was detected during this active surveillance program but an increased number of low pathogenic avian influenza have been isolated in 2008 and in 2009, corresponding to a viro-prevalence of about 0.5% (Figure 38). In Northern Europe, the H6 and H4 were the most frequently detected subtypes, followed by H7, H3, H11, H1, H2, and H5 subtypes, mainly Anseriformes. In Belgium a relatively large number of similar viruses were obtained from wild birds, principally from ducks (*Anas platyrhynchos*, *Tadorna tadorna* and swan). It is worth to note that the H13 and H16 subtypes reported essentially from gulls and other marine birds have also been detected in 2008 during this active surveillance.

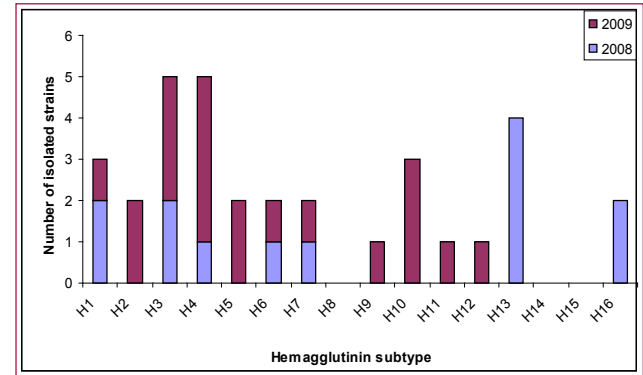


Figure 38. Number of isolated strains per H-subtype for 2008-2009

Surveillance of professional poultry flocks (syndromic surveillance)

In case of any abnormal symptoms in a domesticated poultry flock (chickens, ducks, geese and turkeys), the owner has to inform his veterinarian who is obliged to examine clinical symptoms and to evaluate the possible suspicion. In case of suspicion, samples are taken for further analysis. In 2008 and 2009, 77 and 106 possible cases were recorded and examined, respectively. The peak of sampling observed in 2007 (110 cases) was maintained during these two last years. But contrary to surveillance 2007 when LP AI H6N8 was demonstrated in a turkey, no LP AI have been detected during these last two years.

Serological screening of housed poultry flocks

Serological screening for antibodies against H5 and H7 viral antigens in poultry holdings was performed during the years 2008 and 2009. In total, sera from 11.630 birds were analysed by HI test, following standard procedures. In the case that flocks were found positive for H5 or H7, cloacal swabs were taken to identify possible active shedders. At the end of December 2008, two geographically distant holdings were found positive for H5. Subsequently, cloacal swabs were analysed by RRT-PCR and egg inoculation. A low pathogenic avian influenza virus of subtype H5N2 was isolated in the first holding and a H5 virus identified by real time PCR in the second holding. There were neither clinical symptoms nor mortality. The first farm housed both ornamental birds and poultry and the second one was a free range breeding geese farm. Control measures were taken as foreseen in Council Directive 2005/94/EC with notification in ADNS and to OIE. Stamping out of ducks, geese, chickens, pheasants and turkeys was done in the first farm whereas the other birds were isolated and tested again after 1 month. The quarantined birds were negative in this analysis. In the second farm, all the geese were culled and as precaution measures, this holding will be repopulated only after at least 6 months. These two farms are good examples of « at risk » farms as they were mixed population flocks and/or free range animals

Influenza in humans: monitoring

In 2008, the number of notifications for suspected cases of human A/H5N1 infections dropped significantly. The IPH duty service was only called twice during the year for a total three persons who developed a flu syndrome at their arrival in Belgium. One tested negative and the two other ones, a couple coming from Cambodia, were diagnosed with Influenza B.

In 2009, the A/H1N1v pandemic drawn the media and public attention, putting to the side A/H5N1 avian flu. In Belgium, the first case has been diagnosed on 12 Mei 2009. During the summer period, the circulation of the new variant virus was low to moderate, and most cases were imported from foreign countries. The epidemic developed from beginning of October to end of November, with a peak at the end of October. Children below 15 years were the most attacked by the new variant, while persons aged 65 and more showed low incidence rates.



Hantaviruses

Alexandre Doby, Geneviève Ducoffre, Paul Heyman, Luc Vanholme

Hanta disease

Wild (or laboratory) rodents are the reservoir for hantaviruses worldwide; humans are accidental hosts. The infection is chronic and apparently asymptomatic in host animals. A hantavirus serotype is hosted by a specific rodent species. According to the infectious agent and its region, hanta-viral diseases present with different level of severity, from mild infections to severe hemorrhagic fever with renal syndrome (HFRS). HFRS shows as an acute onset of fever, lower back pain, hemorrhagic manifestations and renal involvement. Hantavirus pulmonary syndrome (HPS) was also described as an infection predominantly involving the respiratory system. Outbreaks of HFRS and HPS are generally observed during years with dense rodent populations resulting from favorable climatic and environmental conditions and when this population is heavily infected by the virus. Human activities, such as rodent trapping, farming, cleaning rodent-infested areas, camping and hunting, are also associated with the occurrence of hantavirus disease.

Hanta disease

Hantaviruses in animals

Hantaviruses in humans

Hantavirus is excreted through urine, faeces or saliva of rodents. The transmission of hantaviruses to humans mainly occurs via inhalation of infected excretions. Person-to-person transmission is rare. The virus can survive hours or days in the environment.

Strategies to prevent hanta-viral infections consist in controlling rodents in and around the houses, and cleaning houses with bleach. Preventive measures in endemic areas rely essentially on information campaigns and rodent control.

Hantaviruses in animals

Rodents and insectivores are currently the only known reservoirs of hantaviruses. Other small mammals (e.g. rabbits, foxes, dogs, cats, moose, wild boar, bats, etc) can be infected as well, but they figure as dead-end hosts i.e. they do not transmit the virus to other animals or humans. It is not known whether other mammal species than humans show clinical symptoms after infection, but recent reports demonstrated impaired breeding success and decreased survival success in the carrier rodents.

A sero-epidemiological study of the presence of hantaviruses in domestic dogs and cats was recently performed by VAR. Sera of 410 dogs and 124 cats were obtained from private laboratories and tested by IgG ELISA. The positive results were confirmed with IFA. Hantavirus antibodies were found in both species but with a higher seroprevalence in cats than in dogs (17% vs. 5%)(Figure 39). The high seroprevalence in cats could be linked to their ability to catch small rodents. In Wallonia, more positive cats are mainly found in forested areas. In Wallonia, dogs have a higher seroprevalence than in Flanders.

The prevalence in cats and dogs is positively influenced by the presence of forests, as observed in humans. Companion animals could be candidates as bio-indicators for this cyclic zoonosis.

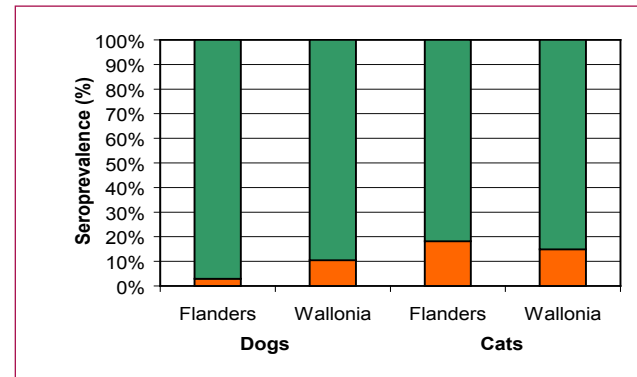


Figure 39. Seroprevalence in dogs and cats per geographical region

Other scientific research programs to monitor the seroprevalence of various animal species are still running.

Hantaviruses in humans

In 2008 and 2009, the Sentinel Laboratory Network and the NRL reported respectively 336 and 187 cases of hantavirus infection. This report indicates an increase of cases in 2008 as compared to 2007 (N=298) but not so high than in 2005 (N=372) (Figure 40).

Classically, hantavirus infections in Belgium display a seasonal peak in spring and summer and a periodic resurgence every 2 to 3 years. High seasonal peaks were reported in Belgium during the springs-summer of 1996, 1999, 2001, 2003, specially 2005 and 2008 (Figure 41).

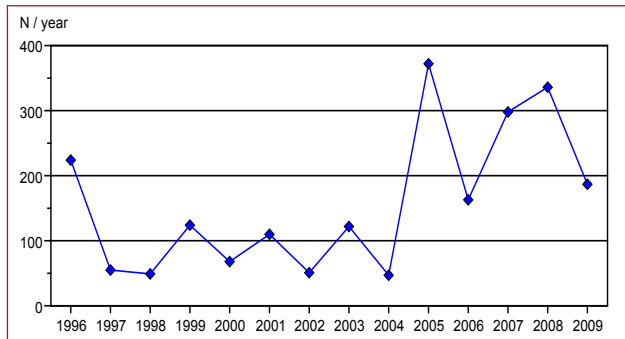


Figure 40. Total number of Hantavirus infections in humans by year (1996-2009). Sources: Sentinel Laboratory Network and NRL

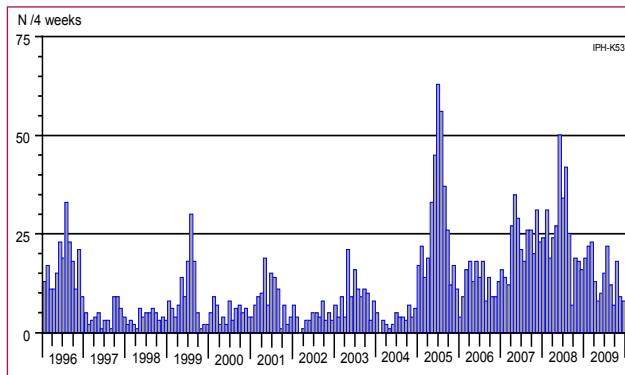


Figure 41. Distribution of Hantavirus infections in humans (N/4 weeks), 1996-2009 Sources: Sentinel Laboratory Network and NRL

Generally, the majority of cases are male adults over 19 years. In 2009, there were 54% of females and 46% of males (Table 32).

Table 32. Number of cases of Hantavirus infections in humans by sex and age groups, 2009. Sources: Sentinel Laboratory Network and NRL

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	0	0,0	2	2,0	2	1,1
1 - 4	1	1,2	1	1,0	2	1,1
5 -14	0	0,0	7	7,0	7	3,8
15 -24	7	8,3	12	12,0	19	10,3
25 -44	37	44,0	36	36,0	73	39,7
45 -64	25	29,8	27	27,0	52	28,8
≥ 65	14	16,7	15	15,0	29	15,8
Total	84	100,0	100	100,0	184	100,0

Among the cases reported in 2008, 73% (N=242) resided in Wallonia, 11% (N=38) in Flanders and 15% (N=51) in Brussels (5 unknown). The highest incidence rates are reported in Brussels and in the districts of Thuin (N=46), Liège (N=26) and Arlon (N=26). Two of these areas are known to be endemic for the disease, but cases in the district of Liège are only reported since 2003 on and those in Brussels since 2008 on (Figure 42 on the next page).

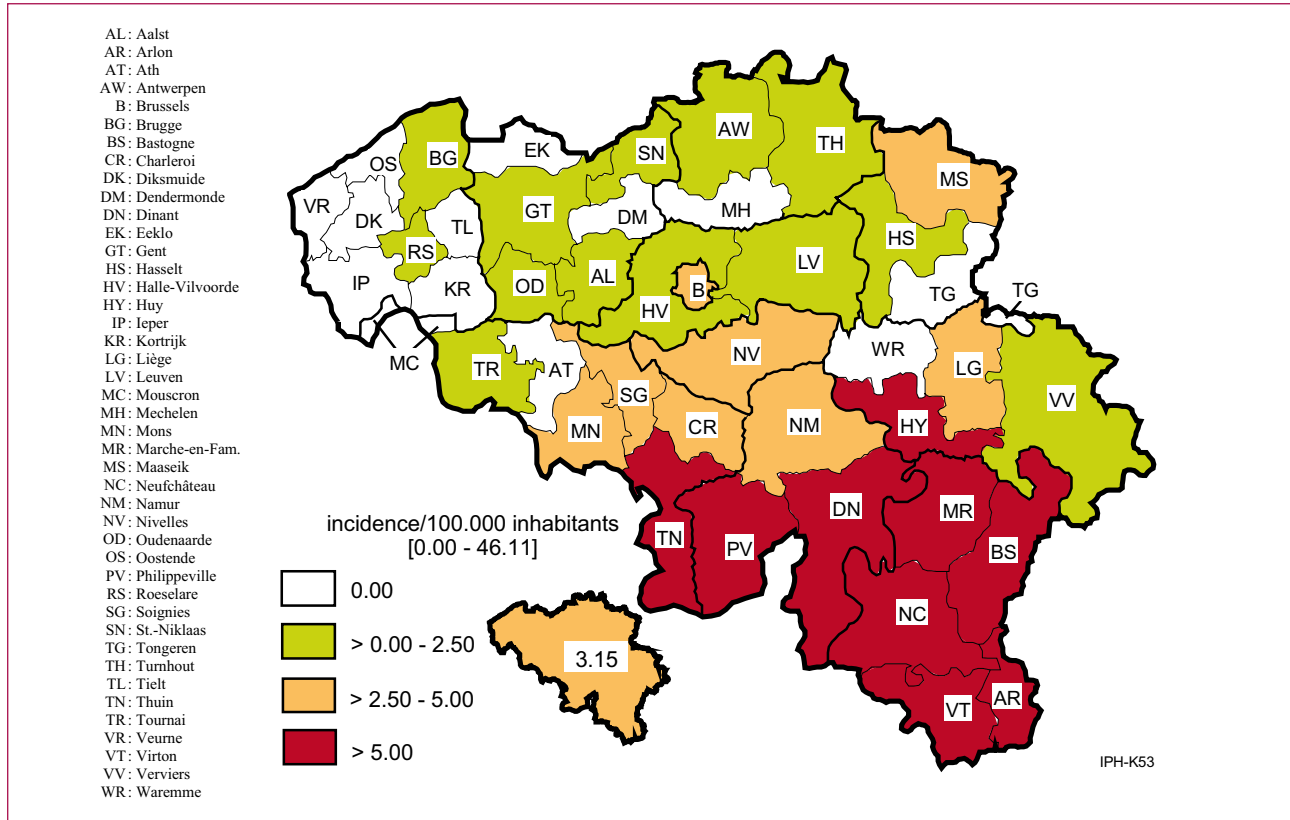


Figure 42. Incidence of Hantavirus infections in humans by district (N/105 inhab., 2008)

Sources: Sentinel Laboratory Network and NRL

Among the cases reported in 2009, 66% (N=123) resided in Wallonia, 11% (N=20) in Flanders and 18% (N=34) in Brussels (10 unknown). The highest incidence rates are reported in Brussels and in the districts of Liège (N=19) and Arlon (N=15) (Figure 43).

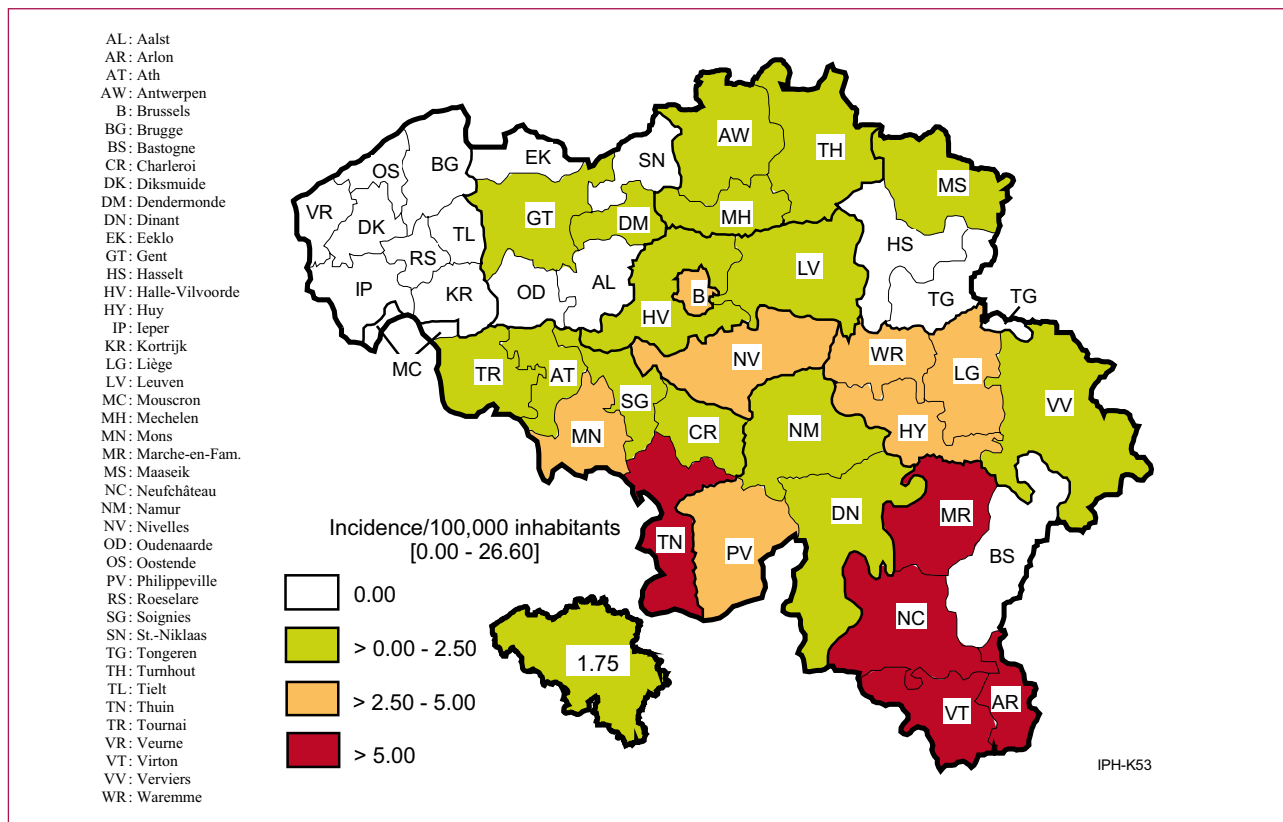


Figure 43. Incidence of Hantavirus infections in humans by district (N/105 inhab., 2009)

Sources: Sentinel Laboratory Network and NRL

Part of the increase observed since 2005 could be due to a greater awareness among health professionals and to a higher hantavirus testing. However, under-diagnosing of hantavirus infections remains a problem.



Rabies

Steven Van Gucht, Luc Vanholme

Rabies

Rabies is a zoonotic viral disease caused by a rhabdovirus of the genus Lyssavirus. The animal reservoir are carnivores (typically foxes) and bats. Other animals may be infected also, but do not play a role in the maintenance of the disease.

The genus Lyssavirus, within the Rhabdoviridae family, is subdivided into several genotypes based on RNA sequencing:

genotype 1 – ‘Classic’ rabies virus, worldwide spread

genotype 2 – Lagos bat virus, Africa

genotype 3 – Mokola virus, Africa

genotype 4 – Duvenhage virus, Africa

genotype 5 – European bat lyssavirus 1 (EBLV-1), Europe

genotype 6 – European bat lyssavirus 2 (EBLV-2), Europe

genotype 7 – Australian bat lyssavirus, Australia.

‘Classic’ rabies virus (RABV), genotype 1, causes an acute viral encephalomyelitis of warm blooded animals (e.g. foxes, dogs, cats, wildlife) and humans.

Rabies

Rabies in animals

Rabies is transmitted to other animals and humans through close contacts with saliva from infected animals, especially via bites or scratches, or less frequently via licks on injured skin or on 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. Once symptoms of the disease develop, rabies is fatal to both animals and humans. In humans, initial symptoms may include anxiety, headaches and fever. In a later phase, the effects of the encephalitis intensify. The inability to swallow liquids has given the disease the name of hydrophobia. Respiratory failure finally leads to death. Therefore it is important for any person who has been bitten by a 'suspected' animal (abnormal behaviour) to seek medical attention and start the necessary treatment consisting of wound treatment, passive immunization and vaccination. Some people may die despite post-exposure treatment using modern vaccines and/or rabies immunoglobulins. Pre-exposure vaccination should be offered to persons at risk, such as laboratory workers, veterinarians, animal handlers, international travellers. Currently available vaccines are safe and effective against both the classic rabies virus and the bat lyssaviruses.

Lyssaviruses and rabies in European bat species.

Over one thousand species of bats are known worldwide. Bats are listed as endangered and protected animals across Europe. Rabies that may be detected in bats in some European countries is caused by two independent *Lyssa* virus genotypes 5 and 6 (EBL-1 and EBL-2) that are related to the Classical rabies virus. Some but not all the bat species carry the viruses. Bat rabies is a public health concern: after infection e.g. due to a bat bite, the disease is fatal in humans. Post-exposure vaccination and treatment following a bat bite or after exposure to bats is highly recommended.

Education and recommendations should be given to travellers in order to reduce the risk of infection. Although dogs represent a more serious threat in many countries, the risk of rabies infection by bat bites should not be underestimated.

In July 2001, Belgium has obtained the official status of rabies-free country according to the OIE guidelines and the WHO recommendations. The last indigenously acquired case of rabies occurred in Belgium in a bovine in July 1999. Unfortunately, the official rabies-free status of Belgium was suspended at the end of October 2007 due to the detection of a rabid dog illegally imported from Morocco. The clinical diagnosis was confirmed by laboratory testing after euthanasia of the animal. Finally 32 persons and 18 pet owners with possible contact with the rabid animal were detected. Medical information and follow-up by experts of the IPH of all 'contact' persons was realised. Belgium regained its official free rabies status on 28 October 2008.

No indigenous cases of human rabies have been reported since 1923 although imported cases are diagnosed from time to time.

Rabies in animals

Surveillance programme and methods used

Food animals with nervous symptoms are suspect for rabies and therefore should be notified to the FASFC. Affected animals are killed and their brain is examined by immunofluorescence and virus cultivation in neuroblastoma cells at the NRL. The remaining nervous tissue of rabies-negative animals is afterwards transmitted to the NRL for TSE diagnosis.

Wildlife found dead or shot is transferred to the clinical veterinary laboratories for autopsy. In case of suspected behaviour or lesions, brain samples are examined at the NRL.

Vaccination policy

Since there were no more cases of rabies for the last years, vaccination of foxes by baits was stopped by the end of 2003. Vaccination started in 1989. Vaccine baits (Raboral, Rhône-Mérieux) were dispersed for the oral vaccination of foxes. Twice a year, in April and October, a zone of approximately 1.800 km² along the German border was covered by spreading 32 000 baits by means of a helicopter (17.78 baits per km²).

In the south of the country, below the rivers Sambre and Meuse, vaccination of dogs is compulsory.

Epidemiological investigations and results of 2008-2009 surveillance

Passive surveillance of rabies

In 2008 and 2009, a total of respectively 713 and 582 brain samples were examined for rabies virus at the NRL. The majority of samples originated from wildlife (n=367 and 324), especially foxes (n=245 and 183), cattle (n=214 and 181) and sheep and goats (n=108 and 116). Respectively 25 and 29 dead-found bats were also examined. The high number of cattle and small ruminants analysed is in consequence of the surveillance system for transmissible spongiform encephalopathy (TSE). In these species all suspected cases were first examined for rabies. Rabies must be considered in the differential diagnosis of TSE, although the course of the disease is usually shorter.

None of the samples was found positive.

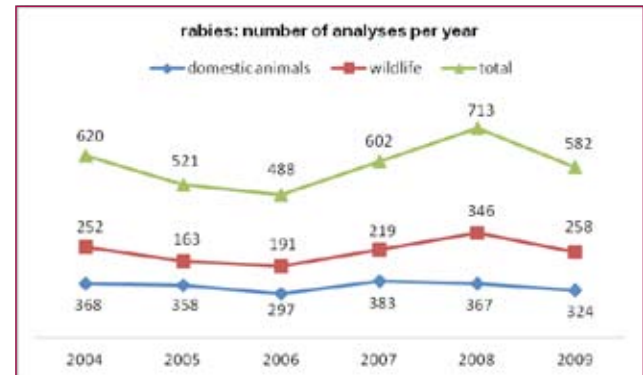


Figure 44. Number of rabies analyses 2004-2009

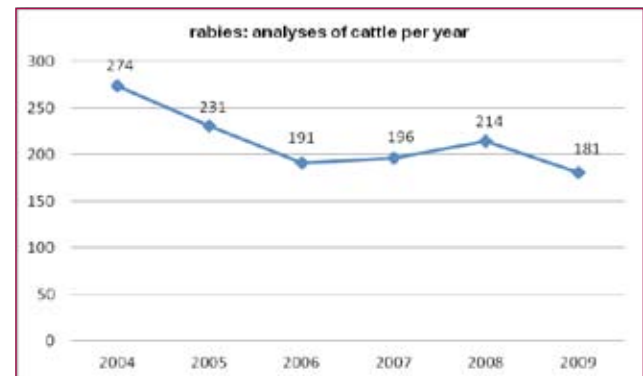


Figure 45. Number of rabies analyses of cattle 2004-2009

Surveillance of wildlife

Wildlife found dead or shot for signs of illness and/or aggression are autopsied by the network of wildlife surveillance. In addition, brain samples are transmitted to the NRL. In 2008 and 2009, the network has transmitted 367 and 324 samples of wild animals (foxes, wild cervids, badgers, mink and raccoon) to the NRL. All cases were negative (immunofluorescence and virus cultivation).

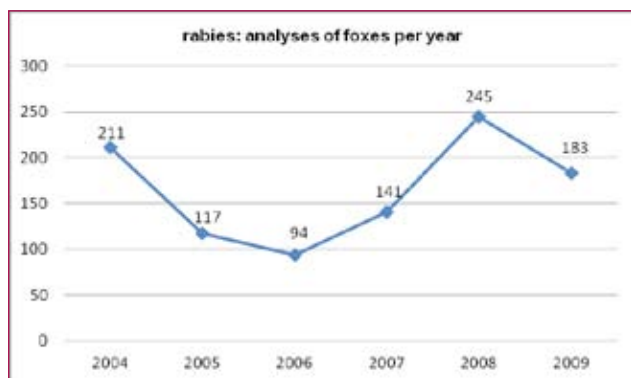


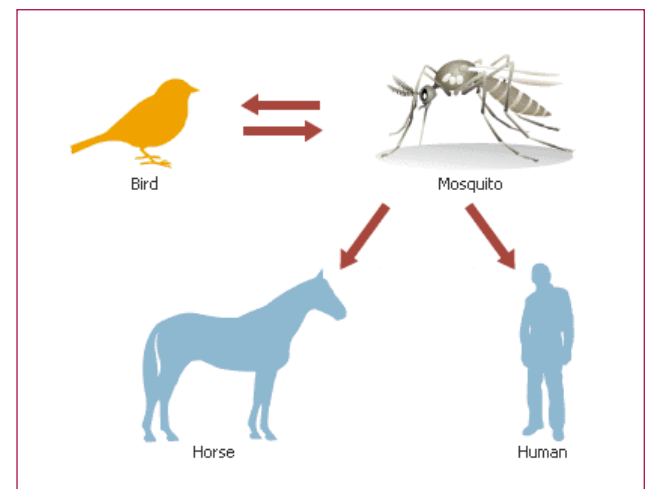
Figure 46. Number of rabies analyses of foxes 2004-2009

West Nile virus

Bénédicte Lambrecht, Olivier Poncin, Thierry van den Berg, Didier Vangeluwe, Marjan Van Esbroeck

West Nile virus

The West Nile virus (WNV) is a zoonotic mosquito-transmitted arbovirus of the genus *Flavivirus* in the family *Flaviviridae* with a wide geographical range. Transmission occurs mainly through the bite of ornithophilic mosquitoes of the *Culex* genus but the virus has been occasionally isolated from other arthropods, such as ticks (Figure 47).



West Nile virus

West Nile virus in animals

West Nile virus in humans

Figure 47. Transmission scheme of WNV

Migratory birds are involved in the transmission cycle of this virus as reservoir and amplifying hosts of the virus. Humans and horses are considered to be accidental dead-end hosts. Migratory birds wintering in or passing through WNV endemic areas could allow the dissemination of the virus from Africa to the temperate zones of Europe and Asia during spring migrations. Infected mammals like humans and horses are incidental hosts, unable to transmit the virus, the viremia being weak and of short duration. These hosts do not contribute to the transmission cycle. In humans, the majority of WNV infections cause a non-symptomatic or a mild flu-like illness. However some infections can cause encephalitis which may lead to death, particularly in elder patients. The incubation period is 3-6 days. In human infections, transmission by direct contact does not occur. However, virus transmission by transfusion of blood and blood products as well as by organ transplantation and breastfeeding has been observed. Most equine infections are subclinical or unapparent, as approximately 10% of the infected horses develop clinical neuro-invasive disease. The fatality rate of clinically affected horses can reach 40%.

Until the end of the 1990s, WN disease was considered as a minor risk for humans and horses because it only appeared sporadically. Since a large outbreak in Romania in 1996 and the emergence of WNV in America in 1999, WN fever has become a major public health and veterinary concern in Europe. Based on this recent spread of the disease, it can not ruled out that the disease will show up in our country, knowing that the vector mosquitoes and the ecological conditions are present.

West Nile virus in animals

Early warning system

The feasibility of an epidemiological surveillance program in wild birds and horses has been analysed in Belgium in 2008-2009. This programme was based on passive surveillance of abnormal bird mortalities (corvids and raptors) and equine encephalitis, as well as on active serological surveillance among wild birds (corvids) and poultry sentinels (free range chicken, ducks and goose).

The surveillance, in collaboration with the Royal Belgian Institute of Natural Sciences, also targeted the circulation of WNV in migratory birds, by virological investigation of *Silvidae*, captured during spring when they come back from sub-Saharan Africa. In this approach, there was need to improve serological (ELISA test) and virological tools (seroneutralisation and RT-PCR) to detect WN infection and its related immune response as well as to set up capture and detection systems for migratory passerines and corvids.

The collection of wild bird carcasses was done in collaboration with 2 non-profit organizations running a bird hospital. Specific baited trapping cages were built to catch *Corvidae* in rural area as well as in the centre of Brussels and sera were collected. For the active monitoring, trapping of *Sylviidae* (Figure 48) in spring migration from the Mediterranean basin or sub-Saharan Africa was performed (April and May 2008-2009) and oral swabs (b) were collected.

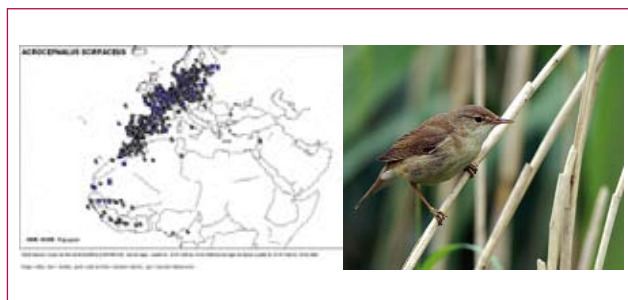


Figure 48. Trapping of Sylviidae in spring migration

The serological tests include competition ELISA (ID Screen West Nile Competition, ID VET) and seroneutralisation assay as confirmation test for positive ELISA reactions. RNA was extracted from the swabs and RT-PCR targeting the NS2A region was performed.

For passive surveillance, 32 and 28 corvid and raptor brains respectively were also tested negative by RT-PCR. For active surveillance, a total of 144 buccal swabs were obtained from 11 Sylviidae species and tested by RT-PCR with negative results (table 33).

Sera (s) and buccal swabs (b) from different corvid species tested negative in serology and virology, respectively (Table 34).

In conclusion, the results indicate that there is no evidence of WNV circulation in Belgium to date. The sampling of wild birds will be extended. This first monitoring will start in 2010 by the FASFC in close coordination with VAR. As is the case for the Avian Influenza monitoring, the active and passive wild bird surveillance will be a close cooperation between the Royal Belgian Institute of Natural Science and VAR.

Table 33. Active surveillance of WNV by buccal (b) swabs of Sylviidae species in 2008-2009

Year	2008	2009	Total
Species	b	b	
Acrocephalus arundinaceus		1	1
Acrocephalus palustris		2	2
Acrocephalus scirpaceus	21	54	75
Acrocephalus shoenobaenus		1	1
Muscicapa striata		2	2
Phylloscopus collybita	1		1
Phylloscopus trochilus	1	1	2
Sylvia atricapilla	44		44
Sylvia borin	1	10	11
Sylvia communis	1	2	3
Sylvia curruca	1	1	2
Total	70	74	144

Table 34. Active surveillance of WNV by sera (s) and buccal (b) swabs of Corvid species in 2008-2009

Year	2008			2009		
	b	s	Total	b	s	Total
Espèce Type						
Corvus corone	10	95	105	217	290	507
Corvus monedula	4	50	54	32	1	33
Pica pica					2	2
Total	14	145	159	249	293	542

West Nile virus in humans

In the ITM (NRL), a total of 135 and 128 human sera have been examined for the presence of antibodies to West Nile virus by ELISA in 2008 and 2009 respectively.

Most of the positive serological results can be attributed to cross-reactions with dengue antibodies. In 2008 one possible WNV case has been detected

trends and sources

2008-2009



parasitic diseases

Cryptosporidiosis

Leen Claes, Geneviève Ducoffre, Marjan Van Esbroeck, Luc Vanholme

Cryptosporidiosis

Cryptosporidiosis is a parasitic disease caused by the protozoan *Cryptosporidium* spp. Many species of *Cryptosporidium* can infect humans and a wide range of animals. *Cryptosporidium parvum* and *C. hominis* are the most prevalent species causing disease in humans. Infections by *C. felis*, *C. meleagridis*, *C. canis* and *C. muris* have also been reported. This parasite can affect the intestines of all mammals. It is spread by the fecal-oral route. It is probably one of the most common waterborne diseases caused by recreational and drinking water worldwide.

People get infected after ingestion of the parasite. It is typically an acute, self-limiting short-term infection of the intestines in persons with intact immune systems. Symptoms appear from two to ten days after infection and last up to two weeks. The most common symptom is watery diarrhea. Other symptoms are stomach pains or cramps, nausea, vomiting, dehydration, weight loss and a mild fever. Treatment by oral or intravenous fluid therapy is primarily supportive to rehydrate infected persons. Some individuals are asymptomatic after infection but are nevertheless active shedders of sporulated oocysts of the parasite. Even after symptoms have subsided, a person may still be infective for some weeks.

Cryptosporidiosis

Cryptosporidiosis in animals

Cryptosporidiosis in humans

In immunocompromised persons, such as AIDS patients, infection frequently causes a particularly severe and permanent form of watery diarrhea coupled with a greatly decreased ability to absorb key nutrients through the intestinal tract. This results in a progressively severe dehydration, electrolyte imbalance, malnutrition, wasting and in some cases will lead up to death within 3 to 6 months.

The most important zoonotic reservoirs are cattle, sheep and goats. Also contaminated pets or exotic animals (e.g. snakes, tortoises) can spread the disease.

Prevention can be done through good personal hygiene, avoiding unsafe water sources, washing hands carefully after going to the toilet or contacting stool, and before eating or preparing food. It is recommended to wash and peel all raw fruits and vegetables before eating. If possible, contact should be avoided with infected humans or infected animals. Water from lakes, rivers, springs, ponds or streams should not be drunk. If safety of the drinking water is questionable, water should be boiled. Suspect water supplies can also be carefully filtered before drinking.

Cryptosporidiosis in animals

No official monitoring program of cryptosporidiosis in animals was organised. Laboratory diagnosis confirmed some clinical suspicions of cryptosporidiosis.

Cryptosporidiosis in humans

Infections with *Cryptosporidium parvum/hominis* occur worldwide. Most reports describe endemic outbreaks caused by contaminated water and food. The infectious oocysts may survive in the environment for months.

In immunocompetent persons, *Cryptosporidium* infection is frequently asymptomatic. The most severe courses of diarrhea are observed in immunosuppressed patients.

In 2008 and 2009, the Sentinel Laboratory Network reported 396 and 473 cases of cryptosporidiosis, corresponding to a national incidence estimated at 4.4 per 100,000 inhabitants in 2009 (Figure 49). The incidence was very high in 1998 and 1999 (8.2 per 100,000 inhabitants).

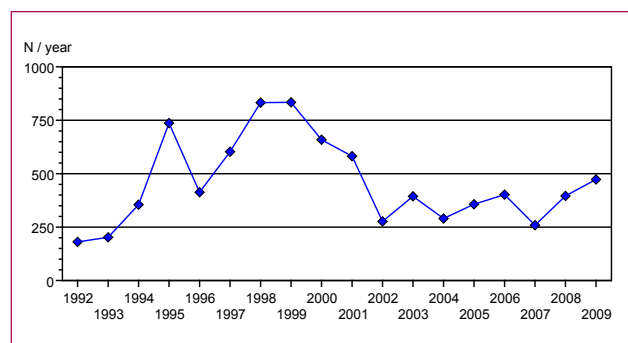


Figure 49. Total number of *Cryptosporidium* infections in humans by year, 1992-2009. Source: Sentinel Laboratory Network

Cases were observed all over the year (Figure 50).

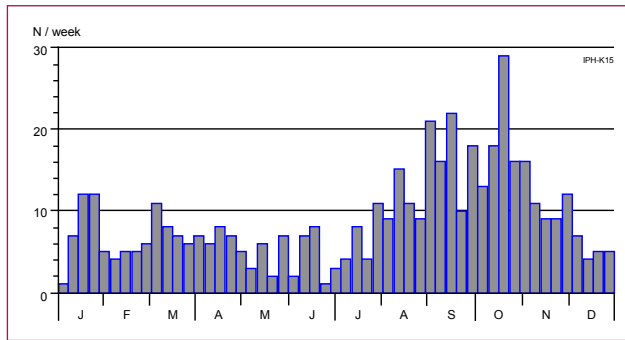


Figure 50. Weekly number of *Cryptosporidium* infections in humans, 2009
Source: Sentinel Laboratory Network

More than one quarter of cases is reported in 1 to 4 year old children and one quarter in 25-44 year old adults (Table 35).

Table 35. Number of *Cryptosporidium* infections in humans by sex and by age groups, 2009. Source: Sentinel Laboratory Network

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	13	5,4	6	2,8	19	4,2
1 - 4	94	39,2	61	28,2	155	34,0
5 -14	47	19,6	35	16,2	82	18,0
15 -24	15	6,3	23	10,6	38	8,3
25 -44	53	22,1	67	31,0	120	26,3
45 -64	14	5,8	19	8,8	33	7,2
≥ 65	4	1,7	5	2,3	9	2,0
Total	240	100,0	216	100,0	456	100,0

In 2009, the incidence was 6.4 per 100,000 inhabitants in Flanders, 1.6 per 100,000 inhabitants in Wallonia and 1.1 per 100,000 inhabitants in Brussels-Capital Region (Figure 51 on page 124).

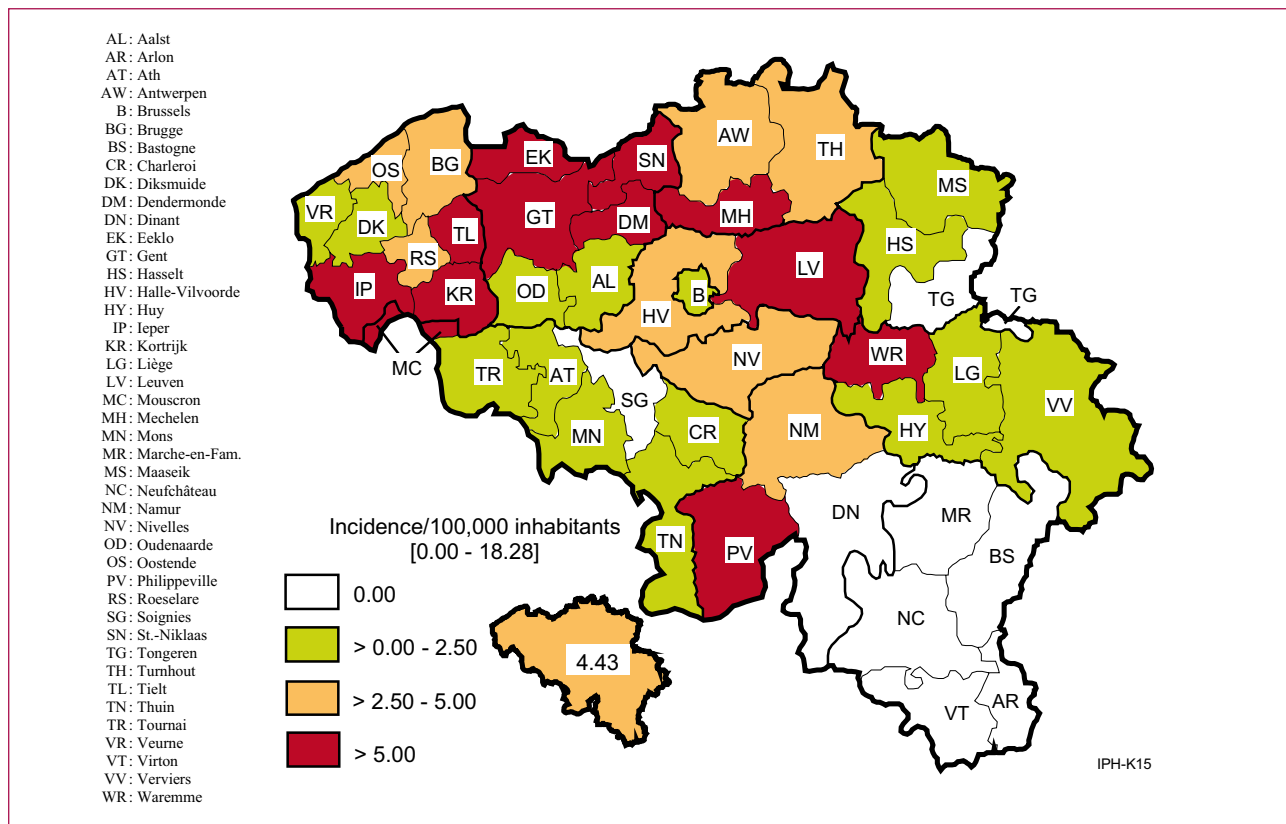


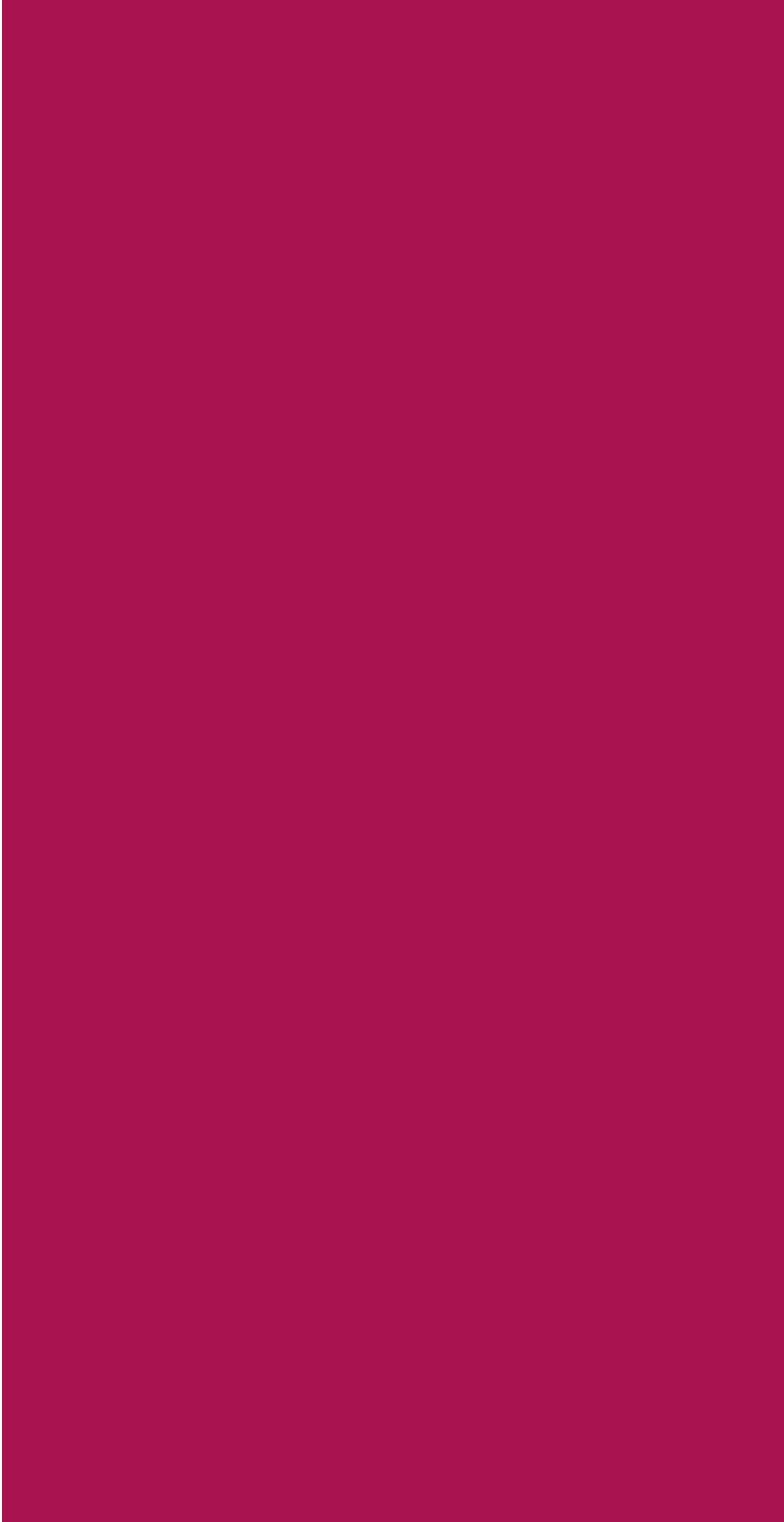
Figure 51. Incidence of *Cryptosporidium* infections in humans by district (N/105 inhab, 2009)

Source: Sentinel Laboratory Network

In the ITM (NRL), respectively 2048 and 2017 human faecal samples have been examined for *Cryptosporidium* after concentration and staining according to Heine in 2008 and 2009.

In 2008, *Cryptosporidium* was detected in 20 samples. Ten samples were from patients consulting the outpatient clinic of the ITM and 10 samples have been sent to the NRL by external laboratories. Twelve patients (60%) were male. The age of the patients ranged from 0 to 62 years, with a median age at 27.8 years. No travel history is known from the patients whose sample was sent to the reference laboratory by external laboratories. Half of the patients who consulted at the outpatient clinic of the ITM, travelled abroad before the start of their illness.

In 2009, *Cryptosporidium* was detected in 15 samples. Seven samples were from patients consulting the outpatient clinic of the ITM and 8 samples have been sent to the NRL by external laboratories. Eight patients (53%) were male. The age of the patients ranged from 0 to 49 years, with a median age at 20.4 years. No travel history is known from the patients whose sample was sent to the NRL by external laboratories. Three of the 7 patients who consulted at the outpatient clinic of the ITM, travelled abroad before the start of their illness.



Cysticercosis

Leen Claes, Luc Vanholme

Cysticercosis

Cysticercus bovis in muscular tissue of cattle is the larval stage of the tapeworm, *Taenia saginata*, a parasitic cestode of the human gut (taeniasis). The risk factor for bovine cysticercosis infection in cattle is the ingestion of feed contaminated with *T. saginata* eggs shed in human faeces. Cattle can become infected when grazing contaminated pastures. Free access of cattle to surface water, the flooding of pastures and the proximity of wastewater effluent have been identified as risk factors for bovine cysticercosis.

Humans contaminate themselves by the ingestion of raw or undercooked beef containing the larval form (cysticerci). Usually the pathogenicity for humans is low. The tapeworm eggs contaminate the environment directly or through surface waters. Human carriers should be treated promptly. Strict rules for the hygienic disposal or sanitation of human faeces with a method that inactivates *T. saginata* eggs should be developed. The spreading of human excrement on land should not be allowed.

Cysticercosis

Cysticercosis in cattle

Macroscopic examination is routinely done in adult cattle as well as in calves and sheep in the slaughterhouse. Serological examination is possible and confirmation of the lesions by PCR can be done. The introduction of serological techniques for the detection of cysticerci antigens in the serum of cattle should be developed. This would allow the detection of more cases than visual inspection of carcasses at the slaughterhouse, which has a low sensitivity.

Although *Cysticercus ovis* in sheep is not transmissible to humans, its presence causes total rejection of the carcass. No sheep were found to be infected over the last years.

The Belgian pig population is free of *Cysticercus cellulosae*. *Taenia solium* is not autochthonous in Belgium.

Cysticercosis in cattle

Post-mortem, macroscopic examination of carcasses is routinely done in the slaughterhouse. In 2008 and 2009, respectively 522.557 and 480.068 adult cattle and 301.102 and 319.188 veal calves were examined.

Figures 52 and 53 from the FASFC show that in 2008 and 2009, 18 and 9 carcasses of adult cattle were rejected for generalised cysticercosis. In addition, the meat of 2.356 and 1.811 adult cattle was treated by a 10 days freezing before human consumption.

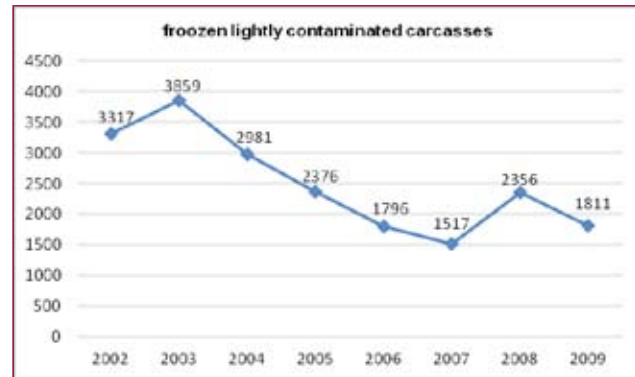


Figure 52. Cysticercosis: detection of lightly contaminated bovine carcasses at slaughterhouse

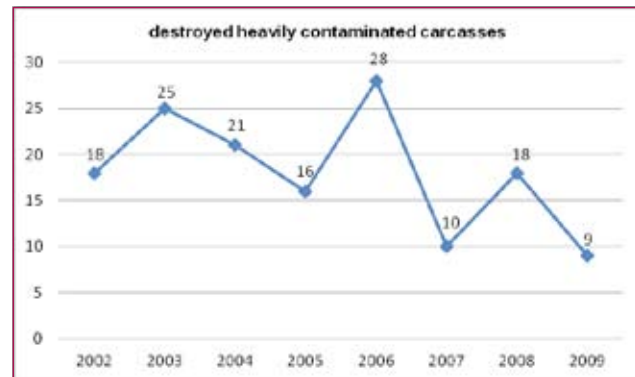


Figure 53. Cysticercosis: detection of heavily contaminated bovine carcasses at slaughterhouse



Echinococcosis

Yves Carlier, Leen Claes, Steven Van Gucht, Luc Vanholme

Echinococcosis

Human echinococcosis, known as hydatid disease, is caused by the larval stages of the small tapeworms of the species *Echinococcus granulosus* or *Echinococcus multilocularis* of the genus *Echinococcus*.

Echinococcus granulosus, the agent of cystic echinococcosis, produces unilocular human hydatidosis. *E. granulosus* is a small tapeworm (6 mm) that lives in the small intestine of dogs, foxes and other canids which are the definitive hosts. The adult tapeworm releases eggs that are passed in the faeces. Sheep, goats, pigs, cattle, horses and wild boar serve as intermediate hosts in which ingested eggs hatch and release the larval stage (oncosphere) of the parasite. Humans also can acquire infection by accidental ingestion of typical taeniid eggs, which are excreted in the faeces of infected dogs and foxes. When eggs are ingested by the intermediate hosts or by humans, the larval stages (oncospheres) liberated from the eggs migrate via the bloodstream to the liver, lungs and other tissues to develop hydatid cysts. These cysts may develop unnoticed over many years, and ultimately rupture. Clinical symptoms and signs of the disease depend on the location of the cysts and are often similar to those induced by slow growing tumours. Within these cysts brood capsules and protoscoleces develop. Each protoscolex is a potentially infective organism for canids. The definite hosts become infected by ingestion of these cyst-containing organs (of the infected intermediate hosts).

Echinococcosis

Echinococcus in food animals

Echinococcus in wildlife (foxes)

Echinococcus in humans

Indigenous unilocular hydatidosis in man has been sporadically reported in Belgium. Recommendations for basic risk-mitigation actions are destruction of contaminated viscera found at the slaughterhouse in order to avoid the infection of dogs.

Echinococcus multilocularis has a similar life cycle as *E. granulosus*. Foxes and to a lesser extent dogs are the definitive hosts of this parasite. Small rodents and voles are the intermediate hosts. Foxes are infected by ingestion of contaminated rodents. *E. multilocularis* is the agent of highly pathogenic alveolar (multilocular) echinococcosis in humans. Humans can be infected by ingestion of eggs. Alveolar echinococcosis is of particular public health relevance as it results in a chronic cancer-like liver disease. Ingestion of the eggs by humans can result in the development of invasive cysts in the liver. Most untreated cases in humans are fatal. In the intermediate hosts, the larval form of the parasite remains in the invasive proliferative stage in the liver, thus invading the surrounding tissues. With regards to domestic animals, cats have been ruled out as hosts of *E. multilocularis*, since the parasite does not fully develop in their intestine.

Possible risk factors include contact with dogs hunting for game, and ingestion of contaminated water or contaminated unwashed fresh products (in particular raspberries and strawberries) and vegetables. Chewing grass is another practice to be associated with alveolar echinococcosis. Contamination of the hands during gardening, through contact with contaminated soil, may also carry some risk.

Recommendations to improve the protection of public health are the use of good general hygiene practices such as washing fruit and vegetables before consumption, cooking berries or mush-

rooms (washing alone is not sufficient, neither does freezing at -18°C !), hand-washing after gardening and before the consumption of meals. Also hand-washing after contact with dogs, especially if they have direct contact with wildlife or if they live in areas where wildlife, in particular, foxes, rodents or voles, is abundant. Planned treatment of dogs with taenicides and subsequent hygienic disposal of their faeces in endemic areas is recommended.

Echinococcus in food animals

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

Echinococcus in wildlife (foxes)

E. multilocularis is known to be endemic in a wide area of central Europe. This area extends as far as the south of Belgium (Wallonia), the southeast of the Netherlands, the east of France and Switzerland.

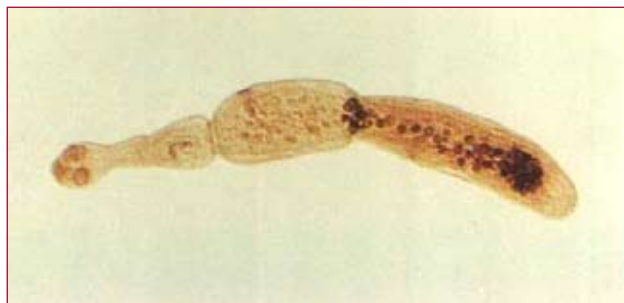


Figure 54. Fox tapeworm (Source: IPH)



Figure 55. Intestinal scraping technique (Source: IPH)

In Wallonia, the cestode seems to maintain itself stably in the fox population. Between 2003 and 2004, 25% of 990 examined foxes tested positive (Hanosset et al., 2008). The highest prevalences (41 to 62%) are found in the Ardennes and Fagne-Famenne, areas which are rather densely forested and located at relatively high altitudes between 400 to 700 meters above sea level. The musk rat seems to be an important intermediate host.

In Flanders and Brussels, the parasite remains rare and there is no proof of a recent increase in prevalence. In 2007-2008, 187 foxes from the regions of Brussels (n = 56) and Flanders (n = 131) were examined by the IPH with the intestinal scraping technique. In Flanders, most foxes were healthy specimens that were shot during the hunting season. Storage of cadavers and sampling of the intestines was done by the Research Institute for Nature and Forest (INBO). In Brussels, most examined foxes were traffic victims that were deposited by police officers. Sampling was intensified in the south of Flanders and the southwest of Brussels.

Indeed, in the southwest of Brussels 3 positive foxes were found during a former study in 1996-1999 (Vervaeke et al., 2003). None of the examined foxes from 2007-2008 carried *E. multilocularis* cestodes.

The possible reasons for the difference in prevalence between the north and south are not known, but may include differences in altitude, climate, fox diet or low abundance of suitable intermediate hosts.

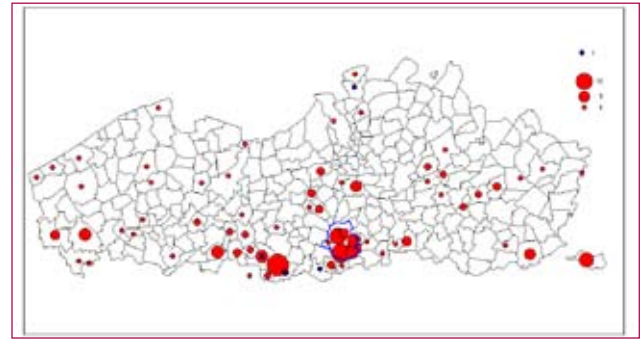


Figure 56. Distribution of the foxes examined for *E. multilocularis* in Flanders and Brussels between 2007 and 2008.

Each circle represents one to thirteen foxes. The red circles represent foxes examined between 2007 and 2008. All foxes were negative. The blue dots indicate the location of the four foxes which were found positive in a prevalence study in the same study area between 1996 and 1999.

Echinococcus in humans

Till the end of 2003, 8 autochthonous cases of alveolar echinococcosis have been diagnosed. It concerned patients between 36 and 90 years old, 4 women and 4 men. Two cases presented a rapid evolution due to immunosuppression. Two other cases were lethal due to surgical complications.

In 2004, a serological study among 115 forest guards working in Belgium did not identify any suspected case of echinococcosis in this specific risk group.

Since 2004, the NRL performs on average 250 serological analyses/year for antibodies against *Echinococcus multilocularis*. Interestingly, a slight tendency to an increased demand is observed. In screening tests using a crude antigenic extract, 3 to 7% of the samples are positive, i.e. 7 to 12 patients/year. More specific tests (in collaboration with the Institute for Parasitology of the Faculty of Medicine, Berne, Switzerland) show that most of these positive cases correspond in fact to cross reactions between *Echinococcus multilocularis* and *Echinococcus granulosus*, i.e. most of these patients suffer from hydatidosis.

In 2006 and 2007, 2 new autochthonous cases of alveolar echinococcosis were confirmed in patients of 64 and 82 years old.

In 2008 and 2009, no new autochthonous cases of alveolar echinococcosis were confirmed by the NRL.

Sarcosporidiosis

Leen Claes, Luc Vanholme

Sarcosporidiosis

The following species are of zoonotic importance: *Sarcocystis bovi-hominis* (man final host, bovine intermediate host), *Sarcocystis sui-hominis* (man final host, pig intermediate host). Man is infected with *Sarcocystis* spp. by ingesting undercooked infected meat. *Sarcocystis* spp. infections are mostly asymptomatic but may cause mild aspecific gastrointestinal symptoms like nausea and diarrhoea.

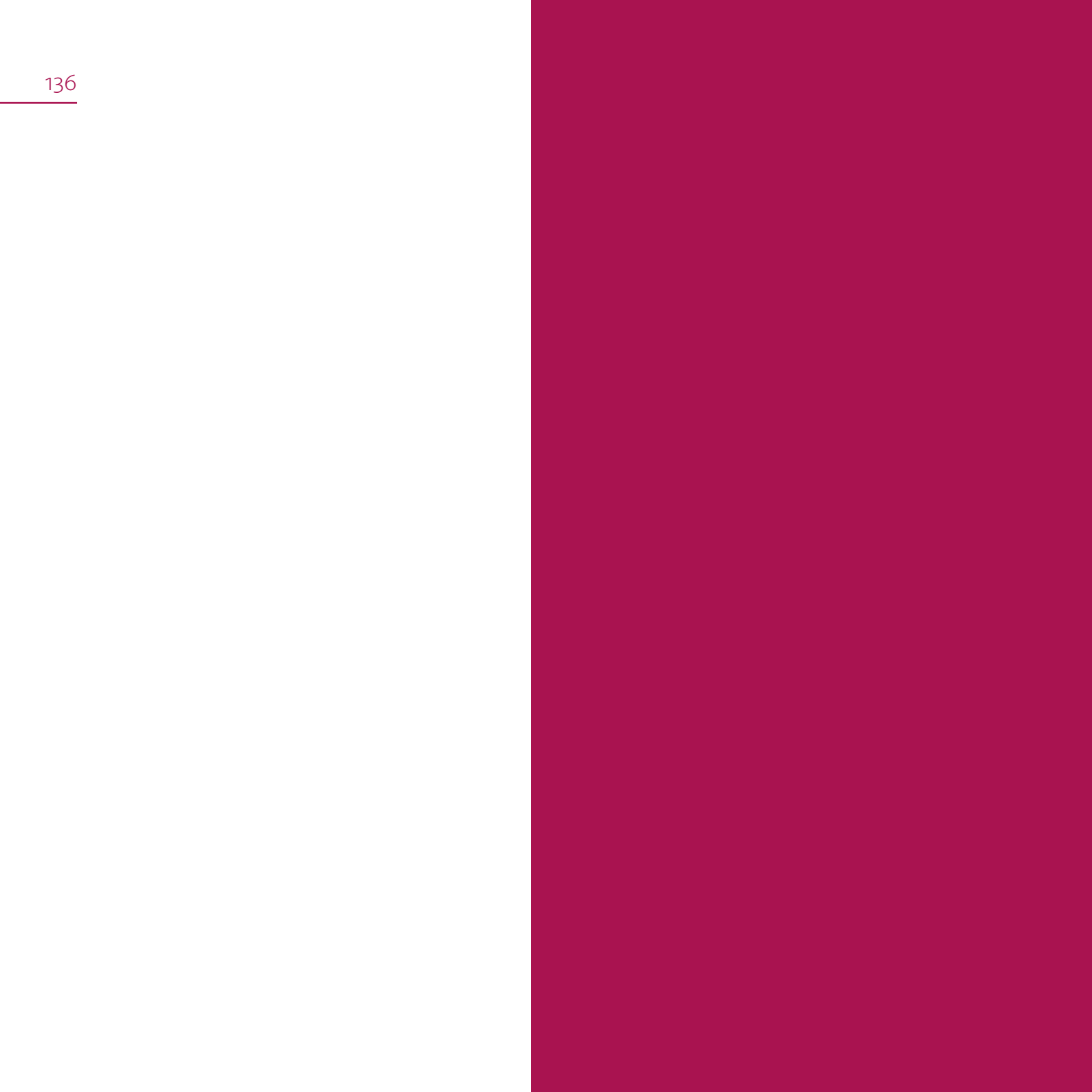
Sarcosporidiosis in animals

Surveillance programme in food animals

Carcasses are partially or entirely condemned when myositis eosinophilica (green colouring spots of the carcass) is observed at post-mortem examination in the slaughterhouse. Myositis eosinophilica may be linked with sarcosporidiosis, although the association is not unequivocally proven.

Sarcosporidiosis

Sarcosporidiosis in animals



Toxoplasmosis

Leen Claes, Stephan Decraeye

Toxoplasma gondii

Toxoplasma gondii is an obligate intracellular organism that can be found worldwide. The final hosts are the felidea (more commonly cats); humans and almost all warm-blooded animals are intermediate hosts. The sexual cycle takes place exclusively in the intestines of felidea. As a result, millions of oocysts are shed into the environment with the cat's faeces within the first two weeks after infection. These oocysts sporulate and are very resistant to environmental damage and can persist for several years. Oral ingestion of oocysts by a seronegative host leads to toxoplasmosis. The infection has an acute and a chronic phase. The latter characterised by the persistent presence of tissue cysts in the host (in muscle, brain, heart ...). Carnivorous ingestion of infected tissues by a seronegative host (final or intermediate) will lead to development of the disease.

Toxoplasma gondii

Toxoplasmosis in animals

Toxoplasmosis in humans

Toxoplasmosis in animals

The majority warm blooded animals are indiscernible carriers of tissue cysts. The prevalence depends on the feeding of the animal: herbivores are more exposed to oocysts and carnivores more exposed to tissue cysts.

There is a need for efficient serological and molecular biological methods for both indirect and direct detection of *T. gondii* in animals and food. Veterinary samples can be screened serologically to see if the animals have been infected. The presence of tissue cysts can be detected by PCR, but bio-assay is still needed to demonstrate the presence of viable and thus infectious cysts in meat products. Screenings are not routinely done; there is no data on the status of toxoplasmosis in the livestock in Belgium. The European Food Safety Authority has recommended the initiation of monitoring programmes in the pre-harvest sector on sheep, goats, pigs and game.

The consequences of veterinary toxoplasmosis depend on the animal species, its feeding habit and the *T. gondii* strain. In sheep and goats, primary infection during gestation leads to abortion or stillbirth, with important economic losses. In general, more than 70% of sheep and goats are seropositive. The outcome of toxoplasmosis in cattle is less clear: although 30-50% is seropositive, there is less association with transmission to humans. The prevalence in pigs (and other animals) depends on the production system, with a higher infection rate in animal friendly farms ("free run"). In general animals with outdoor access have a higher prevalence of toxoplasmosis.

The presence of cats and rodents on the farm is a risk factor. Limitation of these factors are important as preventive measures. The seroprevalence in cats varies between 25 to 70%. A serological survey on house cats demonstrated an overall chance of 6% for a seronegative cat to become infected within the next life-year and actively shed oocysts (NRL for Toxoplasmosis at IPH).

Table 36. Animal sera tested for diagnosis of toxoplasmosis during 2008-2009 at the NRL for Toxoplasmosis at IPH

Species	IgM positive	IgG positive
Sheep	14/14	14/14
Goat	6/31	13/31
Cats	39/266	114/266
Dogs	124/318	153/318

Toxoplasmosis in humans

Humans are mostly infected with *T. gondii* by oral route: ingestion of oocysts excreted by cats (e.g. in cat litter trays or not properly washed raw vegetables) or by ingestion of tissue cysts present in inadequately cooked or raw meat.

The prevalence of toxoplasmosis increases with age as the risk that an individual is exposed to the parasite increases in time. About 50% of the Belgian population is seropositive. More than 80% of infections with *T. gondii* are asymptomatic. However, mild (flu-like) to moderate clinical symptoms are possible (lymphadenopathy, chronic fatigue, fever, retinochoroiditis). The majority of adult persons have acquired immunity to re-infection, but remain carriers for life.

Immunocompromised patients (HIV, organ transplantation) are much more susceptible to the disease, with possible severe medical complications like pulmonary toxoplasmosis and encephalitis. In 37% of the cases the consequences are fatal. When *T. gondii* seropositive patients have to take immunosuppressive drugs (for example transplant patients), the parasite present in tissue cysts reactivates. The disease can also be transmitted via infected donor tissues to seronegative patients.

When a primo-infection with *T. gondii* occurs during pregnancy, the parasite can cross the placental barrier and cause foetal infection. The pathological consequences for the foetus depend on the time of infection during pregnancy and the virulence of the strain. The earlier the infection (first trimester), the higher the risk of severe complications (ocular disorders, severe mental retardation, hydrocephalus, intra-uterine death). Infections at the end of the pregnancy are often without direct consequences. However, the child can be carrier of latent tissue cysts which can reactivate later in life and cause symptomatic toxoplasmosis (e.g. ocular disease). The costs associated with a congenital *T. gondii* infection have been estimated and in some cases price can be very high. The disease burden of toxoplasmosis is comparable to that of other foodborne diseases such as salmonellosis or campylobacteriosis.

There is a whole battery of tests available to diagnose toxoplasmosis. As the disease is generally asymptomatic, diagnosis relies mostly on serological tests (mostly ELISA). In case of immunocompromised patients or congenital toxoplasmosis, more direct tests like PCR and bio-assay are needed to evaluate the severity of the illness.

During 2008-2009 the NRL for Toxoplasmosis at IPH tested 277 serum samples in the context of possible congenital toxoplasmosis. Two hundred thirteen sera tested positive in Sabin-Feldman test and 36 tested positive for IgM. Compared to the high number of positive serum samples, only 24 out of 842 tissue and body fluids samples (placenta, amniotic fluid, blood, cerebrospinal fluid...) tested positive in bio-assay.

Only a very limited number of drugs can be used to control the infection: macrolides (mainly spiramycine) and inhibitors of folate metabolism (pyrimethamine and sulfamides). In addition, these are only active on the free form of the parasite, not on the cysts formed in the tissues. The treatment takes a long time, does not prevent the formation of new tissue cysts and is not without adverse effects. However, the effectiveness of antibiotic treatment in the case of congenital toxoplasmosis has been questioned. For this reason preventive measures are very important for high-risk patients. Efforts are made to avoid a primary *T. gondii* infection during pregnancy. The mode of acquiring toxoplasmosis from meat, cat faeces and contaminated soil is so circumscribed that simple but effective measures should be recommended during pregnancy: regular hand-washing, especially after contact with cats, meat, soil and water. Freezing meat (at -20°C for minimum 48 hours) before consumption or adequate heating of meat (internal temperature of 60°C) during preparation are other effective measures. Cleaning the cat litter should be avoided.



Trichinellosis

Leen Claes, Luc Vanholme

Trichinella

Trichinellosis is a zoonotic disease caused by parasitic nematodes of the genus *Trichinella*. In the EU, infections are mainly caused by the species *T. spiralis*, *T. nativa*, *T. britovi* and in a few cases by *T. pseudospiralis*. It is transferred to humans by the consumption of contaminated raw or undercooked meat or meat products from an infested animal contaminated with infectious larvae.

The clinical signs of acute trichinellosis in humans are characterised in a first phase by nausea, vomiting, diarrhoea, fatigue, fever and abdominal cramps. In a second phase, symptoms may include pains, headaches, fevers, eye swelling or facial oedema, aching joints, chills, cough, itchy skin, diarrhoea or constipation. In more severe cases, difficulties with coordination of movements as well as heart and breathing problems may occur.

Trichinella

Trichinella in food animals

Trichinella in wildlife

The parasite has a wide range of host species, mostly mammals. *Trichinella* undergo all stages of the life cycle, from larva to adult, in the body of a single host.

Trichinella is an intestinal parasite whose larvae can be present in the muscles of different animal species. Particularly, the following animals represent a risk for humans:

- game, in particular wild boar and carnivorous hosts such as the bear and fox;
- backyard pigs and pigs with extensive outdoor access including pigs from organic farms;
- horses.

Therefore, pork, wild boar and horse meat should always be examined before marketing. Carcasses found positive for the presence of *Trichinella* are declared unfit for consumption. Commission Regulation (EC) No 2075/2005 imposes systematic *Trichinella* examination of all pig carcasses intended for export and all horses, wild boar and other susceptible wildlife animals.

Trichinella has not been detected in carcasses of pigs and horses destined for human consumption in Belgium for many years. Improvements in the monitoring and the reporting of *Trichinella* in wildlife should be considered.

It is recommended to travellers not to import raw meat of susceptible animals, e.g. sausages or bear meat. Also the consumption abroad of meat of unknown quality should be avoided.

Trichinella in food animals

Surveillance programme and methods used

Pig carcasses intended for intra community trade or export, except when frozen, all locally slaughtered horses and wild boars placed on the market were checked for *Trichinella*.

The analysis is done by artificial digestion: the magnetic stirrer method of pooled 100 gram sample as described in Commission Regulation (EC) No 2075/2005, 1 gram per fattening pig, 2 grams per breeding sow or boar and 5 grams per horse or wild boar. Serology may be done in live pigs and for epidemiological studies on wildlife.

Notification to the FASFC is compulsory.

Results of the 2008-2009 surveillance

In 2008 and 2009, a total of respectively 11.547.720 and 11.677.883 pigs, 9.173 and 8.711 solipeds (mainly horses) and 15.177 and 10.744 wild boars were examined. All samples were negative.

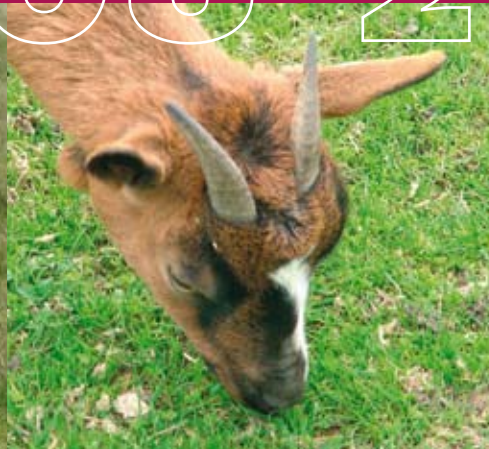
Trichinella in wildlife

In 2008 and 2009, respectively 61 and 142 foxes were analysed for *Trichinella*, all tested negative.

An important measure to avoid spreading of trichinellosis among wildlife is not to leave offal of animal carcasses in the field after skinning of hunted animals.

trends and sources

2008-2009



prion diseases

TSE

Patrick Cras, Sophie Quoilin, Stefan Roels

Transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies (TSEs) known as 'prion' diseases, are caused by an infectious agent whose molecular properties have not been fully determined. The animal TSEs include the archetype – scrapie in domestic sheep and goats – and animal diseases much more recently recognized, including transmissible mink encephalopathy (TME) and feline spongiform encephalopathy (FSE); chronic wasting disease (CWD) of deer and elk; and bovine spongiform encephalopathy (BSE).

Public health protection is covered by the testing of all animals in the slaughterhouse of 30 months of age and more in 2008 and 48 months of age and more in 2009, suspected animals (in farm and slaughterhouse) and fallen stock of 24 months and more in 2008 and 48 months and more in 2009. Additionally, all organs and tissues that can be infectious are routinely removed at the slaughterhouse, destroyed and therefore excluded from the (human and animal) food chain. This last measure is better known as the removal of specific risk material (SRM).

TSE
TSE in animals
Humans

TSE in animals

BSE became a notifiable disease in Belgium in 1990. In 1997, a Royal Decree described the regulations for the epidemiological surveillance for ruminant TSE in Belgium, including the herd slaughter and compensation policy. In the beginning of 2001, this 'passive' surveillance was supplemented with an 'active' surveillance (based on EU Regulation (EC) No 999/2001) controlling slaughtered animals and the fallen stock.

For the moment the NRL uses 5 tests for diagnosis, i.e. the 'rapid' ELISA test, histopathology, immunohistochemistry, electronmicroscopic detection of scrapie associated fibrils (SAFs) and western blotting. In Belgium, all private and regional animal health laboratories (primary 'active' screening) and the NRL are accredited (ISO 17025:2005) and the whole epidemiological surveillance is coordinated by the FASFC.

Table 37. Number of animals controlled in Belgium between 2001 and 2009

Year	Animal	At the slaughterhouse (healthy)	Suspected Animals: Herd screening / farm, slaughter, autopsies (with symptoms)	Fallen stock
2001	Cattle	360.948	3.522 / 379	13.060
	Small ruminants	0	11 / 45	0
2002	Cattle	410.379	3.277 / 377	36.386
	Small ruminants	2.195	428 / 85	780
2003	Cattle	357.389	1.126 / 250	33.691
	Small ruminants	2.447	205 / 52	499
2004	Cattle	358.120	172 / 254	35.322
	Small ruminants	39	333 / 170	1.650
2005	Cattle	325.302	15 / 234	41.729
	Small ruminants	703	8 / 86	1.588
2006	Cattle	320.541	8 / 185	44.066
	Small ruminants	8.076	81 / 246	3.064
2007	Cattle	313.570	0 / 240	46.099
	Small ruminants	7.291	111 / 155	2.584
2008	Cattle	317.781	0 / 311	49.188
	Small ruminants	0	0 / 117	3.512
2009	Cattle	199.621	0 / 217	26.202
	Small ruminants	0	0 / 121	1.633
Total	Cattle	2.972.660	8.120 / 2.475	325.743
	Small ruminants	20.751	1.177 / 993	15.310

In the next table 38 the total number of positive TSE cases is indicated and subdivided by category and place of detection.

Table 38. Positive TSE cases in cattle and sheep in Belgium until 2009

Year	Cattle	Sheep
1992	0	5 (First case) / 5C
1993	0	0
1994	0	0
1995	0	0
1996	0	0
1997	1 (First case) / C	2 / C
1998	6 / C	11 / 3C – 8Sc
1999	3 / C	12 / 2C – 10Sc
2000	9 / C	0
2001	46 / 28S – 10C – 7F – 1Sc	0
2002	38 / 17S – 5C – 16F	25 (1 atypical case) / 1S – 2C – 2F – 20Sc
2003	15 / 10S – 5F	2 / F
2004	11 / 6S – 3C – 2F	13 (1 atypical case) / 1S – 3F – 9Sc
2005	2 / 1S – 1C	2 (2 atypical cases) / F
2006	2 / S	3 (3 atypical cases) / 2F – 1S
2007	0	3 (2 atypical cases) / S
2008	0	0
2009	0	0
Total	133 cases (64 slaughterhouse / 38 clinical cases / 30 fallen stock / 1 second case in a farm)	78 cases (25 primary cases - 9 atypical cases) / (6 slaughterhouse / 14 clinical cases / 11 fallen stock / 47 second cases in a farm)

S = slaughterhouse control / C = suspected clinical / F = fallen stock / Sc = additional case in a herd

Laboratory and epidemiologic studies provided strong circumstantial evidence for a causal link between vCJD and the epizootic bovine spongiform encephalopathy (BSE) in cattle with the most likely route of primary human infection being through dietary exposure to highly infected bovine tissues.

Atypical BSE prion strains

While in scrapie strain variations were found, BSE showed homogeneity of the phenotype. Indeed, the incubation time, the lesion localisation ("lesion profile") and the biochemical signature of the prions were identical for all cases. This uniform aspect of the BSE prions during the epidemic was probably due to the contamination of the food chain by a single strain in United Kingdom.

However, due to the extensive active surveillance and new research techniques, rare variants of BSE have been recently reported. The analysis of their features showed that these emerging types represent different strains of BSE prions. Two atypical forms of BSE prions have been described. Western blot studies showed that, in comparison to the classic BSE prion (C-type), they are characterised by a higher or lower molecular weight of the unglycosylated PrPres. Therefore, they were named H-type and L-type BSE (L-type is also called bovine amyloid spongiform encephalopathy (BASE)). They also show a lower proportion of diglycosylated PrPres than C-type.

Such atypical types are very rare; only 42 cases were described worldwide. They were detected in old bovines (at least eight years in age) and reported on different continents. Indeed, atypical prions might correspond to natural "sporadic" forms of BSE, not originating from the contamination of the food chain. It is essential for the surveillance programmes to determine the prevalence of these atypical types on a global level.

In order to determine the prevalence of the two types of atypical BSE in Belgium, a retrospective analyses was carried out on samples from the Belgian collection of BSE-diagnosed animals originating from cattle of at least 7-years-old, using the latter criteria and techniques. Dobly et al. 2010 showed that till now, no atypical cases were present in the Belgian BSE collection.

The emergence of atypical types of BSE is partially due to a better knowledge of prion strains and more efficient diagnostic techniques. However the area in the brain where the PrPres is deposited differs drastically between the types and the atypical types are more susceptible to a proteinase treatment. It is therefore essential to ascertain that the routine sampling techniques and analyses are adapted to these new types. As these new strains seem more virulent than classic types, at least in mice models, they represent one of the next challenges in the field of prions.

Differential diagnosis with *Listeria monocytogenes*

In the field of differential diagnosis of BSE cases, a retrospective study was performed on the occurrence of *Listeria monocytogenes* infections in BSE suspected cattle (Roels et al. 2009). *L. monocytogenes* is an important foodborne pathogen both in humans and animals. In order to determine the presence and importance of this zoonotic bacterial agent in Belgian cattle, we have reviewed brain tissue specimens for the presence of histopathological lesions pathognomonic for *L. monocytogenes* meningoencephalitis. Additional *Listeria*-specific immunohistochemistry was performed in order to confirm the diagnosis of these cases.

Samples originated from 2 432 cattle clinically suspected of BSE and submitted to VAR during the period 1998-2006. While in recent years no listeriosis cases have been reported in cattle, this study indicated that meningoencephalitis due to listeriosis is still a non-negligible disease in the Belgian cattle population. The zoonotic character of *L. monocytogenes* justifies maintaining vigilance for this infection.

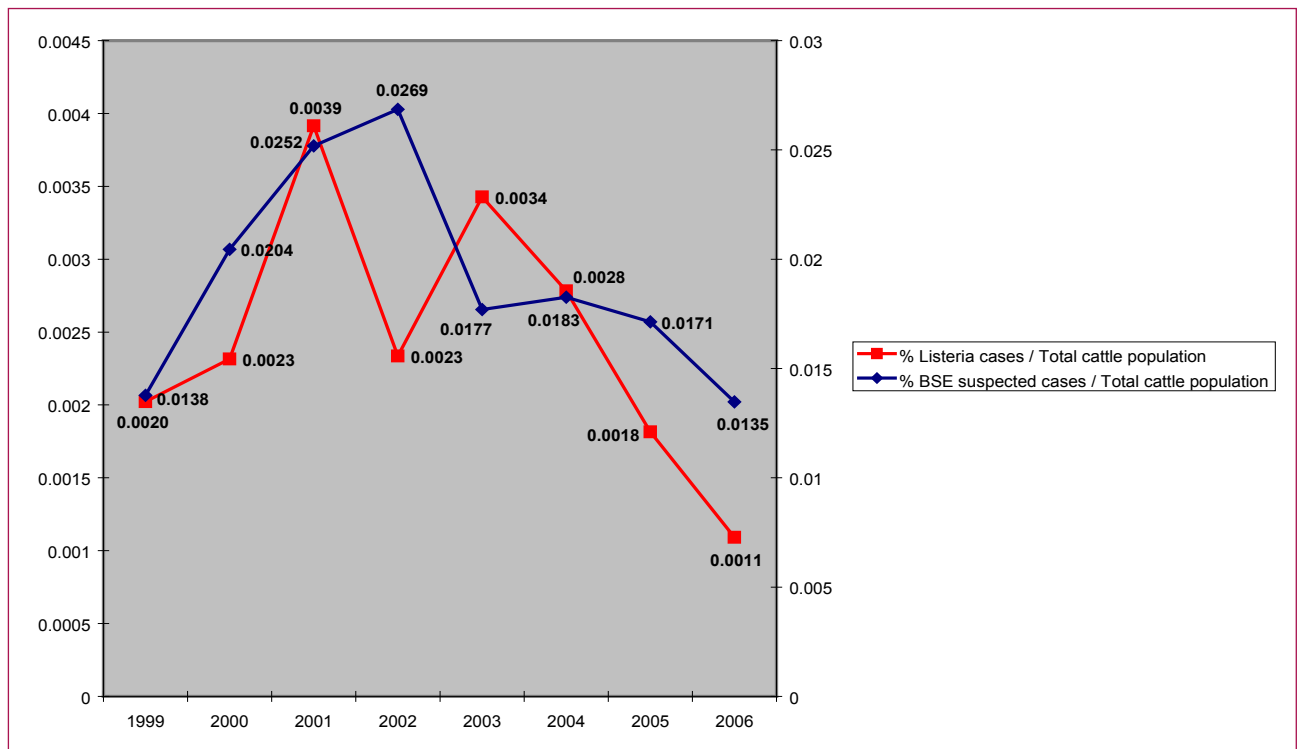


Figure 57. Yearly percentage of *Listeria* and BSE suspected cases as to the total cattle population of that year (1999-2006)

TSE in humans

Creutzfeldt-Jakob disease

The disease of Creutzfeldt-Jakob (CJD) is a transmissible spongiform encephalopathy (TSE), which is characterised by the fast deterioration of the cerebral functions. Prion diseases are rare when compared with other neurodegenerative diseases such as Alzheimer's disease. On the other hand, prion diseases are unique because they are transmissible, coming on the international public health stage when a new expression of CJD (variant CJD or vCJD) was diagnosed and linked to the consumption of beef contaminated by BSE (bovine spongiform encephalopathy) prion.

A Belgian CJD surveillance network was created in 1998 in order to follow the trends of the disease and the potential occurrence of variant CJD cases. The network is built on the collaboration of the seven academic reference centres of neurology/neuropathology and the IPH.

Academic reference centres offer a support for diagnosis of CJD by communicating to IPH data on patients with a probable or neuropathological confirmed diagnosis. Data are analysed and interpreted by IPH allowing an epidemiological follow up of the disease in the country. A procedure was set up in order to deal with possible public health consequences in case a variant CJD was diagnosed. Incidence data are shared with other European countries in a Collaborative Study Group (EuroCJD) and figures are communicated to ECDC.

Several forms of CJD are described according to their transmission way: sporadic, genetic and acquired including a.o. the iatrogenic and variant forms. The diagnosis of CJD is based on clinical presentation, evolution of the symptoms and laboratory tests but can only be confirmed by neuropathological investigation. Added to age of onset and disease duration, these elements allow making a differential diagnosis.

In Europe, the estimated incidence of sporadic CJD is around 1 to 2 cases per million-year. After 10 years surveillance, the observed incidence rate in Belgium is 1.39 cases by 1.000.000 inhabitants per year. The median number of cases per year in Belgium is 15 (range 3-22) with a total 174 reported cases on a 12 years period. About 97% of cases were sporadic, 2.5% genetic and only one is classified as iatrogenic. There was no preponderance in the sex ratio although year-to-year variations were observed. The median age at onset was 66 year (range 31-88). The mean disease duration was 6 month.

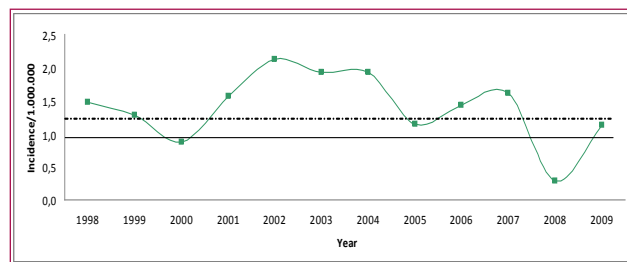


Figure 58. Incidence of CJD in Belgium, 1998-2009.
(Report of the surveillance network, IPH, 2009)

Since 1995, 215 patients died of variant CJD in 11 countries of which 7 are European. UK is most affected with 79% of the patients deceased of this disease. In Belgium no case of variant CJD has ever been diagnosed. Considering that the population has been exposed to BSE and that bordering countries are affected (France, the second most affected country and The Netherlands), the probability to diagnose a case in Belgium can not be excluded but the risk to face a major public health problem lessens. This assumption is illustrated by the European situation. After a peak in 2000, a continuous decline in the number of vCJD cases is observed in UK unlike other countries where the majority of the new cases are diagnosed since 2005 but in a limited extent (n=46).

Despite the declining incidence, vCJD remains a public health concern because prions could be present in the population thus highlighting today's risk of secondary transmission through medical acts (e.g. blood transfusion, surgery, ocular, ORL, dental care). Introduced as a foodborne disease 15 years ago, vCJD has the potential to become a more significant public health issue due to new transmission ways associated with medical care!

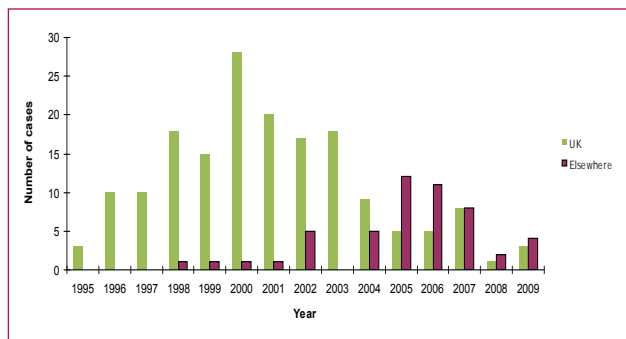


Figure 59. Number of vCJD in UK and outside UK, 1995 to 2009

Source: IPH

trends and sources

2008-2009



foodborne outbreaks

Foodborne outbreaks

Nadine Botteldoorn, Sarah Denayer, Katelijne Dierick, Jean-Yves Michelet, Maria Naranjo

In Belgium different authorities have competences related to FBO's.

The Federal Agency for Safety of the Food Chain (FASFC) deals with safety of foodstuffs, epidemiological investigation of FBOs and related animal health issues.

The Communities (Flemish, French and German speaking Community) which deal with person related matters as human health, can start an epidemiological investigation by its Public Health medical inspectors in case of a FBO.

At the IPH, the Scientific Service of Food Pathogens, which is NRL for FBO's, analyses the suspected food samples, coordinates the analyses of human samples, collects all data on FBOs and gives scientific support to the FASFC officers and the Public Health Inspectors.

Foodborne outbreaks

Major etiological agents

Foodborne outbreaks 2007

Working group on foodborne outbreaks

A national 'Platform for foodborne infections and intoxications and zoonoses transmitted by food', approved by the National Conference of Ministers of Public Health, was created to advance data exchange between different competent authorities on food safety, animal health and public health. Furthermore in 2007, for a better communication, a protected web-application was made available to exchange outbreak data and laboratory results in "real time" between the different authorities dealing with FBOs. In this web-application a common file is created for each individual outbreak, and the data and laboratory results are shared between food inspectors and human health inspectors.

Foodborne outbreaks in humans

A foodborne outbreak is defined as an incident, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of human cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source (Directive 2003/99/EC, Article 2(d)). Data are collected from FASFC, the communities (French, Flemish, Brussels and German), the sentinel laboratories network for human clinical microbiology, and the Federal Reference Centres for FBOs, Salmonella and Shigella, Listeria and *C. botulinum*. The reporting includes both general and household outbreaks.

The causative agents covered are Salmonella spp., Shigella spp., Campylobacter spp., Verotoxinogenic *E. coli*, Listeria monocytogenes, Clostridium botulinum, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Yersinia enterocolitica, Giardia, Norovirus, Hepatitis A virus, toxins of Staphylococcus aureus and Bacillus cereus, histamine and shellfish biotoxins.

Major etiological agents

Foodborne bacteria

Salmonella enterica

Although the number of human salmonellosis has drastically decreased in Belgium since 2005, it remains an important reported pathogen in FBOs. The onset time varies between 6 and 48 hours after ingestion of the contaminated food. Nausea, vomiting, abdominal cramps, diarrhoea, fever and headache are the symptoms in an acute outbreak and last for 1-2 days or longer. In case of an outbreak human samples (stool) and suspected food samples are tested for Salmonella. If Salmonella is detected, Pulse Field Gel Electrophoresis (PFGE) typing can confirm the clonal relationship between the human isolates and those isolated from food products. Raw or undercooked meat, poultry meat, eggs, shrimps, cream-filled desserts and chocolate are frequently associated with food borne Salmonella outbreaks. The food can be at the origin of contamination or can transmit the infection from a contaminated food handler.

Campylobacter jejuni and C. coli

Since 2005, Campylobacter is the most frequently reported illness of food borne origin in humans in Belgium. *Campylobacter jejuni* and *C. coli* infections cause diarrhoea, which may be watery or sticky and can contain blood. Other symptoms often observed are fever, severe abdominal pain, nausea, headache, malaise and muscle pain. Rarely, it can cause constipation leading to a misdiagnosis of appendicitis. The illness usually occurs 2-5 days after ingestion of the contaminated food or water and generally lasts

7-10 days, but relapses are not uncommon (about 25% of cases). Some rare but feared complications are acute colitis, reactive arthritis and Guillain-Barré syndrome.

Campylobacter frequently contaminates raw poultry meat and raw pork. Raw milk and cheeses made from raw milk are also sources of infections.

Yersinia enterocolitica

Yersiniosis is frequently characterised by symptoms as gastro-enteritis with diarrhoea and/or vomiting; however, fever and abdominal pain are typical symptoms. Yersinia infections can also cause pseudo-appendicitis and reactive arthritis. Illness onset is usually between 24 and 48 hours after ingestion of food or water, which are the usual vehicles of infection. Contaminated and undercooked pork is a common source of infection, but also ice-cream has been reported as the source of infection.

Clostridium perfringens

The common form of *Clostridium perfringens* poisoning is characterised by intense abdominal cramps and diarrhea which begin 8-22 hours after consumption of food contaminated with large numbers of vegetative *C. perfringens* cells capable of producing the food poisoning toxin. Toxin production in the human digestive tract is associated with sporulation. The illness is usually self-limited within 24 hours but less severe symptoms may persist in some individuals for 1 or 2 weeks. In most instances, the actual cause of poisoning by *C. perfringens* is temperature abuse of prepared foods. Indeed, small numbers of the spores are often present after cooking and multiply to food poisoning levels during cooling and storage of prepared foods at too elevated

temperatures under anaerobic conditions (e.g. fat layer on stock). Meat, meat products, and gravy are the foods most frequently implicated.

Staphylococcus aureus

Some Staphylococcus strains are capable of producing highly heat-stable enterotoxins in the food, which cause illness in humans. The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the ingested food, and the general health status of the victim. The most common symptoms are nausea, vomiting and abdominal cramping. Recovery generally takes two days. Foodstuffs at risk for staphylococcal food poisoning are those that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation. Contamination occurs by infected food handler or by the food itself (e.g. milk).

Bacillus cereus

Two types of food poisoning, an emetic and a diarrhoeal type, can be observed. For the emetic type, a heat-stable emetic toxin named cereulide, preformed in the food, is responsible for the symptoms similar to those of *Staphylococcus aureus* intoxication, and is characterised by a short incubation period. This type is probably the most dangerous since it has been associated with life-threatening acute conditions like acute liver failure. Heat-unstable enterotoxins, produced in the gut by vegetative cells cause the diarrhoeal type, with symptoms similar to those of the *C. perfringens* food poisoning, with a 6 to 24h incubation period. The emetic type is frequently associated with the consumption

of food rich in carbohydrates such as rice and pasta whereas the diarrhoeal type is often associated with cooked meat and meat products.

Foodborne viruses

Viruses cannot grow in or on food but may be present on fresh products by contact with polluted water in the growing area or during processing. Unhygienic handling during distribution or final preparation is also reported as a cause of contamination. People can be infected without showing symptoms. Person to person transmission is common and the high frequency of secondary cases following a FBO results in amplification of the problem.

Although numerous faecal-orally transmitted viruses exist, the risk of foodborne transmission is highest for Hepatitis A virus and Norovirus. European data show that oysters are frequently reported as a main source of contamination, but water, fruits and food handler contamination are also reported. Increased awareness towards viral infections is raised thanks to improved molecular detection methods. In particular real-time polymerase chain reaction (RT-PCR) allows quantification and has made diagnosis and outbreak management easier.

Noroviruses are among the most important causes of gastroenteritis in adults and often occur as outbreaks which may be foodborne. They are the most common cause of non-bacterial FBOs recognised in Europe and United States and have been diagnosed worldwide. Noroviruses can be transmitted from person-to-person, or indirectly via food or water contaminated with faeces or vomit. They are responsible of mild and usually self-

limiting gastroenteritis. Considering the high attack rates and the fact that Norovirus can cause disease in all age groups, the socio-economical burden of Norovirus infection is very important.

Marine biotoxins

Marine biotoxin poisoning in humans is caused by ingestion of shellfish containing algae toxins. Bivalve molluscs like mussels, oysters and scallops feed with phytoplankton. Some kinds of phytoplankton produce, under favorable climatic and hydrographic circumstances, natural toxins which are consequently absorbed by the bivalve molluscs. According to the effects that they cause, biotoxins are classified in different groups, the 3 main groups being the paralytic shellfish poisoning toxins (PSP), the diarrhoeic shellfish poisoning toxins (DSP) and the amnesic shellfish poisoning toxins (ASP).

The effects of these toxins are generally observed as acute intoxications: PSP are causing paralysis in human which in extreme cases results in death. These toxins are accumulated by shellfish grazing on algae producing these toxins. Symptoms of human PSP intoxication vary from a slight tingling or numbness to complete respiratory paralysis. In fatal cases, respiratory paralysis occurs within 2 to 12 hours of consumption of the PSP contaminated food. The responsible toxins are produced by worldwide present dinoflagellates.

DSP are characterised by the diarrhoea they cause in human, unpleasant but not lethal. Symptoms include diarrhoea, nausea, vomiting and abdominal pain starting 30 minutes to a few hours after ingestion. Complete recovery occurs within three days. Here also, worldwide present dinoflagellates are responsible for the production of the toxins. Europe and Japan seem to be the most affected areas.

ASP have been detected at the American and Canadian east-coast. The symptoms of the intoxication include abdominal cramps, vomiting, disorientation and memory loss (amnesia). A permanent loss of memory is possible. In extreme cases, for older people, a lethal result has been reported. These toxins are produced by a diatom and the main ASP toxin is domoic acid.

Parasites

Giardia lamblia

Giardia lamblia is a protozoan that may cause diarrhea within 1 week of ingestion of the cyst, which is the environmental survival form and infective stage of the organism. Illness often lasts for 1 to 2 weeks, but there are cases of chronic infections lasting months to years. Illness is most frequently associated with the consumption of contaminated water, contaminated vegetables that are eaten raw, or food contamination by infected or infested food handlers. Cool moist conditions favor the survival of the organism.

Foodborne outbreaks in 2008 and 2009

Reported outbreaks in 2008 and 2009

During 2008 and 2009, a total of 209 outbreaks of foodborne infections and intoxications were recorded in Belgium. More than 1753 people became ill and at least 55 were hospitalized. In 2009 one person died from Listeriosis.

The geographic distribution of all foodborne outbreaks is shown in the Figure 60 for 2008 and in Figure 61 for 2009.

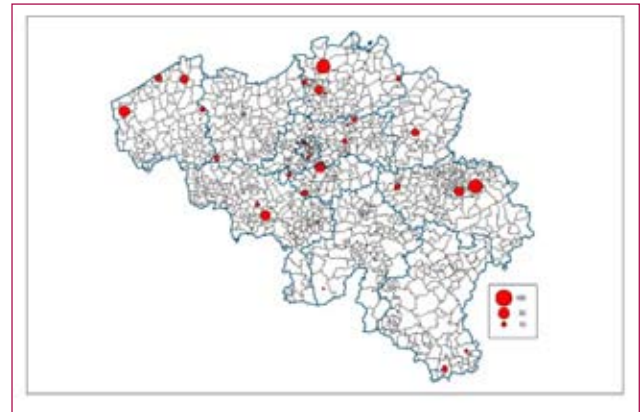


Figure 60. Geographical distribution of FBOs in Belgium – 2008

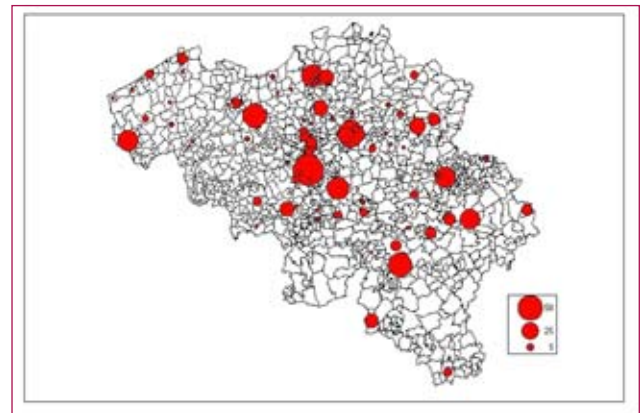


Figure 61. Geographical distribution of FBOs in Belgium – 2009

Causative agents in 2008 and 2009

Foodborne viruses stayed the most frequently detected agents in FBOs. In total 14 Norovirus outbreaks were reported in 2008 and 2009 and 3 outbreaks were caused by Hepatitis A. For Norovirus in 11 outbreaks the agent was confirmed in the human faecal samples and in 3 outbreaks the agent could also be detected in the food (meat, filling of the sandwiches or a witness meal).

Salmonella was the causative agent in 8 outbreaks. In 4 outbreaks *S. Enteritidis* was detected in food products based on raw eggs (tiramisu, chocolate mousse). *S. Ohio* was found in an outbreak caused by pork that was contaminated at the slaughterhouse. The slaughterhouse was closed during several weeks and disinfected. It took several weeks to get rid of the contamination. *S. Brandenburg* was found in an outbreak where the origin could not be detected.

Coagulase positive *Staphylococcus* spp. caused 4 outbreaks. Toxine A was produced by most of the strains. Spaghetti with sauce, pasta with ham, hamburger and beans were the identified vehicles.

Thermotolerant *Campylobacter* was responsible for 10 outbreaks. The agent was almost always detected in the human samples. In one outbreak it was very clear that undercooked stuffed quails were the cause of the outbreak.

Bacillus cereus was the causative agent in six outbreaks. Four of them were caused by *B. cereus* enterotoxin and 2 by the emetic toxin cereulide. Cold dishes, spices and sauces were the food vehicles.

Verotoxinogenic *E. coli* was detected in 3 outbreaks. One outbreak affected 6 patients of a psychiatric hospital. The cause was minced meat that had been consumed raw.

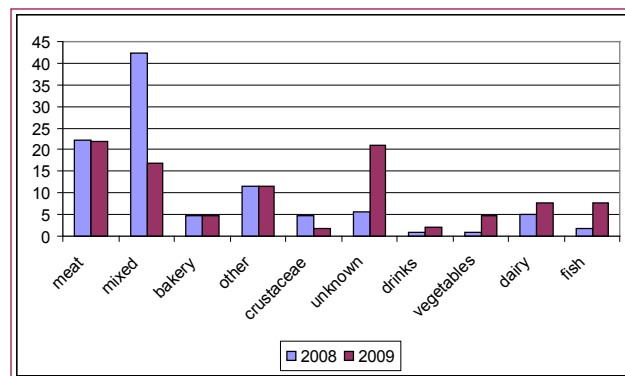
In many outbreaks no causative agent could be identified. An important reason for this was the absence of leftovers of the meal in many of those outbreaks.

Table 39. FBOs in humans in Belgium in 2008 and 2009

Causative agent	Outbreaks 2008				Outbreaks 2009			
	N	Human cases	Deaths	Hospitalisations	N	Human cases	Deaths	Hospitalisations
Bacillus cereus	2	10	0	3	4	53	0	1
Campylobacter	6	31	0	6	4	8	0	unknown
Clostridium perfringens	1				4	43	0	1
Verotoxinogenic E. coli	3	11	0	6	1	4	0	4
Hepatitis A Virus	1	49	0		2	10		1
Norovirus	7	439			7	91	0	0
Listeria	1	2	0	1	2	4	1	2
Other agents	4				3	0	0	0
Parasites	1	10	0	0	3	6	0	0
Salmonella	3	39	0	3	5	68	0	2
Staphylococcus	2	32	0	10	2	24	0	0
Histamine	0				1	11	0	2
Unknown	73				68	533	0	7
Total	104	841	0	35	105	912	0	20

Source of the foodborne outbreaks

Most FBOs were due to the consumption of meals composed of different ingredients (42% in 2008 and 17% in 2009). In 2008 and 2009 meat and meat based products were responsible for 22 % of the outbreaks. Bakery products, including preparations with raw eggs such as tiramisu and chocolate mousse, dairy products and crustaceae were each responsible for 5% of the outbreaks.

**Figure 62.** Food vehicles responsible for FBO in 2008 and 2009

Setting of the foodborne outbreaks

In 94% of FBOs, the place of exposure could be traced back. Restaurants (36%) were the most frequently associated with FBOs, 18% occurred at home and about 12% of the outbreaks occurred by an institutional catering. The place of exposure reported to the NRL FBO was almost identical during the two different years.

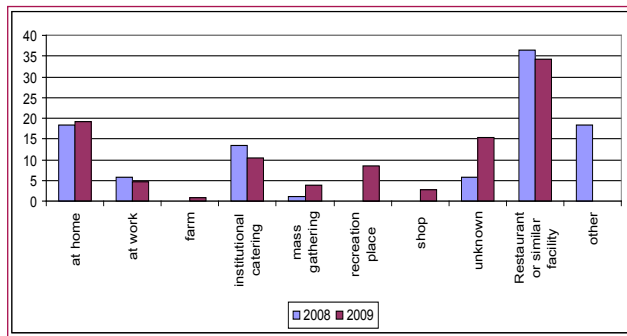


Figure 63. Place of exposure in FBOs 2008-2009

Description of outbreaks of special interest

In the summer of 2008, 4 patients of a psychiatric institution were hospitalized with the symptoms of bloody diarrhea. Two of them developed an hemolytic uremic syndrome (HUS) and, as specific biochemical characteristic, the strains were all urease +. PFGE revealed that consumption of raw minced meat was at the origin of the outbreak

In 2009, there has been a significant increase in registration of *Salmonella enterica* serovar Ohio infections in the Belgian population ($p < 0.01$). During the period from the 1st of August to the 31th of October 2009, 34 strains of *S. Ohio* isolated in clinical laboratories had been reported to the NRCSS. Most human strains caused self-limiting gastroenteritis, but two of the enquêted patients had to be hospitalized. One terminal cancer patient died due to the Salmonellosis and the other hospitalized case suffered from kidney insufficiency. With regard to the population, both sexes (18 males and 16 females) and all age groups (3 children aged < 5 years, 22 adults 15-64 years and 9 adults >65 years) were infected. A cluster of patients was identified around the city of Liège. At the same time, an increase of this serovar was also observed in the *Salmonella* isolates sampled during the monitoring program of the Food Agency for the Safety of the Food Chain. The samples containing *S. Ohio* were of pork origin suggesting that this species was responsible for the outbreak of the disease. PFGE typing confirmed the clonal relationship between the human isolates and those isolated from pork products. Further epidemiological investigations showed that one slaughterhouse was involved. In that slaughterhouse the carcasses were contaminated during the evisceration process by contaminated equipment and uncontrolled environmental conditions.

Prevention of foodborne outbreaks

Since the most frequent causes of FBOs are disruption of cold chain, insufficient heating of the food, lack of personal hygiene, bad hygiene in the kitchen, long delay between preparation and consumption and raw materials of poor microbiological quality, outbreaks can be prevented by the application of simple hygienic rules like adequate refrigeration of the food, hand washing before and during preparation, clean surfaces and materials in the kitchen, separation of raw and cooked food and sufficient heating during preparation.

Working group on foodborne outbreaks

The working group was created in 1995 by IPH and brings together, on a voluntary basis, the main actors in the field of foodborne infections and intoxications in Belgium.

Since its final reform in 1993, Belgium consists of Communities and Regions, each with their specific responsibilities and competences. Since food and food hygiene is a federal issue and matters related to human such as illness are the competence of the communities (Flemish, French or German), data on FBOs are dispersed. As a consequence, there was a need for a working group that assures the coordination, the streamlining of policy and the harmonization of the approach between the different partners implicated in outbreaks.

The group is composed of delegates representing

- the FPS - Public Health, Food Chain Safety and Environment,
- the FASFC,
- the Health Inspection Services of the Communities,
- the Brussels Community Coordination Commission,
- the Anti-poison Centre,
- the Food microbiology laboratory of the University of Ghent,
- the NRL for food microbiology at the University of Liège,
- the VAR.

The IPH houses the working group and is represented by the Epidemiology section, the Reference centres for Salmonella and Shigella, for Listeria and for FBOs.

The main goals of the working group are to exchange field information on detection, epidemiological investigation, controlling and reporting of outbreaks and eventually of sporadic cases of foodborne infections in the country. Significant effort has been put on the improvement of outbreak data collections and case-control studies. The working group also provides scientific support to the mandatory annual Belgian Zoonoses Report to EFSA.

Since 2004, the Belgian authorities recognized the working group as 'Platform for Foodborne infections and intoxications and food related zoonoses' reporting to the National Conference of Ministers of Public Health.

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