

report on zoonotic agents in belgium in 2007

trends and sources

2007

working group on foodborne infections and intoxications

.be



trends and sources  
report on zoonotic agents  
in belgium in 2007

## Executive summary

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

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 food borne 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. Campylobacteriosis remained for the third consecutive year 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 zoonosis. Salmonella was for the first time only the second most important cause of foodborne outbreaks after norovirus outbreaks. The major sources of Salmonella in foodborne outbreaks are table eggs, poultry meat and pig meat. Salmonella reduction remains an important task. 2007 was the first year of implementation of the new Salmonella control programmes in breeding flocks of Gallus gallus by the Member States to meet the Salmonella reduction target to 1% set down by the Community legislation before the end of 2009.

## Table of contents

<u>Executive summary</u>	3	Leptospirosis in animals	46
<u>Preface</u>	7	Leptospirosis in humans	46
<u>Introduction</u>	8	Conclusions	49
<u>Belgian reference laboratories for zoonotic agents</u>	11	<u>Listeriosis</u>	51
<u>Acronyms, abbreviations and special terms</u>	14	Listeriosis	51
<b>General information</b>	<b>16</b>	Listeria monocytogenes in food	52
Susceptible human population	17	Listeria monocytogenes in humans	54
Susceptible animal populations	18	<u>MRSA</u>	57
Animals slaughtered 2003 – 2007	21	MRSA	57
<b>Bacterial diseases</b>	<b>24</b>	MRSA in pigs	58
<u>Brucellosis</u>	25	MRSA in humans	60
Zoonotic brucellosis	25	Conclusions.	61
Brucellosis in cattle	26	<u>Q-fever</u>	63
Brucellosis in sheep and goats	27	Coxiella burnetii	63
Brucellosis in pigs	27	Q-fever in animals	64
Brucellosis in wildlife	28	Q-fever in humans	64
Brucellosis in humans	28	<u>Salmonellosis</u>	67
<u>Campylobacteriosis</u>	29	Salmonella	67
Campylobacteriosis	29	Salmonella in animal feed	68
Campylobacter in food	30	Salmonella in poultry	69
Antimicrobial resistance	32	Salmonella in pigs	74
Campylobacter in humans	35	Salmonella in cattle	76
<u>Escherichia coli (VTEC) infections</u>	41	Salmonella in food (meat and meat products)	77
Verotoxin producing Escherichia coli	41	Salmonella in humans	80
Verotoxin producing Escherichia coli in cattle	42	Antimicrobial resistance	83
Escherichia coli O157 in food	43	<u>Tuberculosis</u>	91
Verotoxinogenic Escherichia coli in humans	44	Zoonotic tuberculosis	91
<u>Leptospirosis</u>	45	Mycobacterium bovis in cattle	92
Leptospirosis	45	Mycobacterium bovis in wildlife	93
		Mycobacterium bovis in humans	94
		Human tuberculosis	94

<u>Yersiniosis</u>	95	<u>Echinococcosis</u>	127
Yersinia enterocolitica	95	Echinococcus in food animals	128
Yersinia enterocolitica in food	96	Echinococcus in wildlife (foxes)	129
Yersiniosis in humans	96	Echinococcus in humans	129
<b>Viral diseases</b>	<b>100</b>	<u>Sarcosporidiosis</u>	<b>131</b>
<u>Avian influenza</u>	101	Sarcosporidiosis	131
Avian influenza	101	Sarcosporidiosis in animals	131
Monitoring in birds	102	<u>Toxoplasmosis</u>	<b>133</b>
Influenza in humans: monitoring	104	Toxoplasma	133
<u>Hantaviruses</u>	107	Toxoplasmosis in animals	134
Hanta disease	107	Toxoplasmosis in humans	134
Hantaviruses in animals	108	<u>Trichinellosis</u>	<b>137</b>
Hantaviruses in humans	108	Trichinella	137
<u>Rabies</u>	111	Trichinella in food animals	138
Rabies	111	Trichinella in wildlife	138
Rabies in animals	112	<b>Prion diseases</b>	<b>140</b>
. West Nile virus	115	<u>TSE</u>	<b>141</b>
West Nile virus	115	Transmissible spongiform encephalopathies	141
West Nile virus in animals	116	TSE in animals	142
West Nile virus in humans	116	Humans	145
<b>Parasitic diseases</b>	<b>118</b>	<b>Foodborne outbreaks</b>	<b>148</b>
<u>Cryptosporidiose</u>	119	<u>Foodborne outbreaks</u>	<b>149</b>
Cryptosporidiosis	119	Foodborne outbreaks in humans	150
Cryptosporidiosis in animals	120	Major etiological agents	150
Cryptosporidiosis in humans	120	Foodborne outbreaks in 2007	153
<u>Cysticercosis</u>	125	Working group on foodborne outbreaks	157
Cysticercosis	125		
Cysticercosis in cattle	126		
<u>Echinococcosis</u>	127		



trends and sources

# 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 FASFC, together with the federal scientific institutions CODA-CERVA and WIV-ISP agreed to yearly publish a booklet which contains this same information combined with data of previous years to indicate some trends of diseases or sources of infection. 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 sixth 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 the available data for 2007 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 first 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 campylobacteriosis) 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 (FAVV-AFSCA-FASFC);
- The Scientific Institute of Public Health (WIV-ISP-IPH);
- The Veterinary and Agrochemical Research Centre (CODA-CERVA-VAR).

This report was coordinated by L. Vanholme (FAVV-AFSCA), H. Imberechts (CODA-CERVA), K. Dierick and G. Ducoffre (WIV-ISP). Obviously, the professional help of many other experts was needed to assemble all available analytical data and information. The authors wish to thank all those who collaborated actively in any way to this Trends and Sources report, and especially (in alphabetical order):

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## Belgian reference laboratories for zoonotic agents

Zoonotic agent or domain	Contact	Address	E-mail address / Web site
Avian Influenza	T. van den Berg	CODA-CERVA Groeselenberg 99 1180 Brussels	thvan@var.fgov.be <a href="http://www.var.fgov.be/">http://www.var.fgov.be/</a>
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BSE / TSE	S. Roels	CODA-CERVA Groeselenberg 99 1180 Brussels	stroe@var.fgov.be <a href="http://www.var.fgov.be/">http://www.var.fgov.be/</a>
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Zoonotic agent or domain	Contact	Address	E-mail address / Web site
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Listeria monocytogenes	M. Yde	WIV-ISP, Bacteriology Section J. Wytsmanstraat, 14 1050 Brussels	Marc.Yde@iph.fgov.be <a href="http://www.iph.fgov.be/">http://www.iph.fgov.be/</a>
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Zoonotic agent or domain	Contact	Address	E-mail address / Web site
Q-Fever ( <i>Coxiella burnetii</i> )	M. Van Esbroeck	ITG-IMT, Klinische Biologie Kronenburgstraat, 43/3 2000 Antwerpen	mvesbroeck@itg.be <a href="http://www.itg.be/itg/GeneralSite/General-page.asp">http://www.itg.be/itg/GeneralSite/General-page.asp</a>
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Salmonella, public health	S. Bertrand	WIV-ISP, Bacteriology Section Rue J. Wytsman, 14 1050 Brussels	sophie.bertrand@iph.fgov.be <a href="http://www.iph.fgov.be/bacterio/">http://www.iph.fgov.be/bacterio/</a>
Salmonella, animal health	H. Imberechts	CODA-CERVA Groeselenberg, 99 1180 Brussels	heimb@var.fgov.be <a href="http://www.var.fgov.be/">http://www.var.fgov.be/</a>
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# Acronyms, abbreviations and special terms

BAPCOG	Belgian Antibiotic Policy Coordination Committee
CODA – CERVA - VAR	Veterinary and Agrochemical Research Centre <i>Centrum voor Onderzoek in Diergeneeskunde en Agrochemie</i> <i>Centre d'Etude et de Recherches Vétérinaires et Agrochimiques</i>
CRL	Community Reference Laboratory
DGZ Vlaanderen	Dierengezondheidszorg Vlaanderen, Flanders Regional Animal Health Association
ELISA	Enzyme Linked Immuno Sorbent Assay
FAVV – AFSCA - FASFC	Federal Agency for the Safety of the Food Chain <i>FAVV Federaal Agentschap voor de Veiligheid van de Voedselketen</i> <i>AFSCA Agence Fédérale pour la Sécurité de la Chaîne alimentaire</i>
FBO	Foodborne outbreak
FPS	Federal Public Service (Ministry) – Public Health, Food Chain Security and Environment
ITG – IMT - ITM	Institute of Tropical Medicine <i>Instituut voor Tropische Geneeskunde</i> <i>Institut de Médecine Tropicale</i>
NRL	National Reference Laboratory
Sanitel	National Bovine Database
WIV - ISP - IPH	Scientific Institute of Public Health <i>Wetenschappelijk Instituut Volksgezondheid</i> <i>Institut Scientifique de Santé Publique</i>





general  
information

# General information

## Susceptible human population

The Belgian human population remains fairly constant over the years.

The evolution of the total human population in Belgium categorised per age, sex and region from 2002 to 2007 is shown in table 1.

**Table 1.** Evolution in the total human population 2002-2007

(Source: National Institute for Statistics <http://statbel.fgov.be/>)

	2002	2003	2004	2005	2006	2007
<b>Total</b>	<b>10 309 725</b>	<b>10 355 844</b>	<b>10 396 421</b>	<b>10 445 852</b>	<b>10 511 382</b>	<b>10 584 534</b>
0-19	2 408 943	2 407 368	2 408 456	2 414 041	2 428 706	2 441 129
20-64	6 154 390	6 186 086	6 207 845	6 232 311	6 273 659	6 333 343
65+	1 746 392	1 762 390	1 780 120	1 799 500	1 809 017	1 810 062
<b>Male</b>	<b>5 042 288</b>	<b>5 066 885</b>	<b>5 087 176</b>	<b>5 111 325</b>	<b>5 143 821</b>	<b>5 181 408</b>
0-19	1 231 221	1 230 382	1 230 570	1 233 688	1 241 251	1 246 988
20-64	3 094 653	3 110 779	3 120 599	3 131 390	3 150 333	3 180 037
65+	716 414	725 724	736 007	746 247	752 237	754 383
<b>Female</b>	<b>5 267 437</b>	<b>5 288 959</b>	<b>5 309 245</b>	<b>5 334 527</b>	<b>5 367 561</b>	<b>5 403 126</b>
0-19	1 177 722	1 176 986	1 177 886	1 180 353	1 187 455	1 194 141
20-64	3 059 737	3 075 307	3 087 246	3 100 921	3 123 326	3 153 306
65+	1 029 978	1 036 666	1 044 113	1 053 253	1 056 780	1 055 679
<b>Brussels</b>	<b>978 384</b>	<b>992 041</b>	<b>999 899</b>	<b>1 006 749</b>	<b>1 018 804</b>	<b>1 031 215</b>
<b>Flanders</b>	<b>5 972 781</b>	<b>5 995 553</b>	<b>6 016 024</b>	<b>6 043 161</b>	<b>6 078 600</b>	<b>6 117 440</b>
<b>Wallonia</b>	<b>3 358 560</b>	<b>3 368 250</b>	<b>3 380 498</b>	<b>3 413 978</b>	<b>3 413 978</b>	<b>3 435 879</b>
<b>Foreigners</b>	<b>846 734</b>	<b>850 077</b>	<b>860 287</b>	<b>870 862</b>	<b>900 473</b>	<b>932 161</b>

The total number of immigrants in Belgium is important (932.161 in 2007). 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.

Figure 1 indicates a slight increase in all categories of human population during last years.

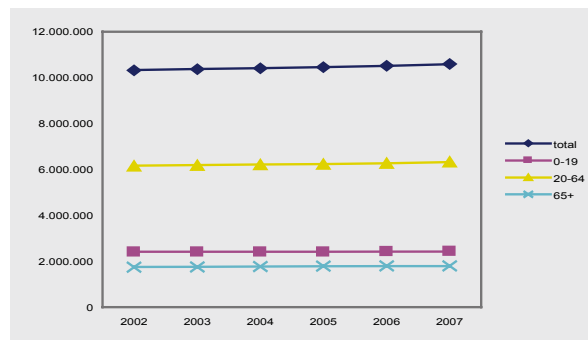


Figure 1. Evolution of human population 2002 – 2007

## Susceptible animal populations

### Ruminants and pigs

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

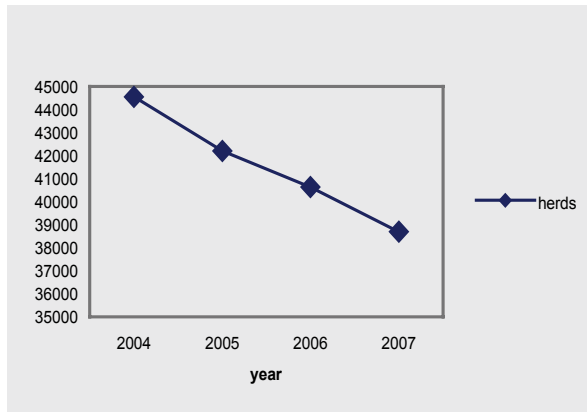
Table 2. Total number of herds and animals in the period 2004 – 2007

	2004		2005		2006		2007	
	herds	animals	herds	animals	herds	animals	herds	animals
<b>Cattle</b>	44.555	2.781.676	42.204	2.492.757	40.640	2.697.824	38.690	2.699.258
<b>Pigs</b>	10.614		10.792		10.631		9.950	
Breeding sows <sup>1</sup>		664.316		657.998		653.358		632.360
Fattening pigs <sup>2</sup>		4.998.124		4.989.016		4.850.501		5.007.614
<b>Sheep</b>	31.405	214.612	32.323	219.274	30.924	220.600	31.523	220.611
<b>Goats</b>	13.736	37.666	14.247	43.727	13.025	46.950	13.381	46.950
<b>Deer</b>	2.965	13.427	3.093	14.655	2.021	12.805	2.907	12.648

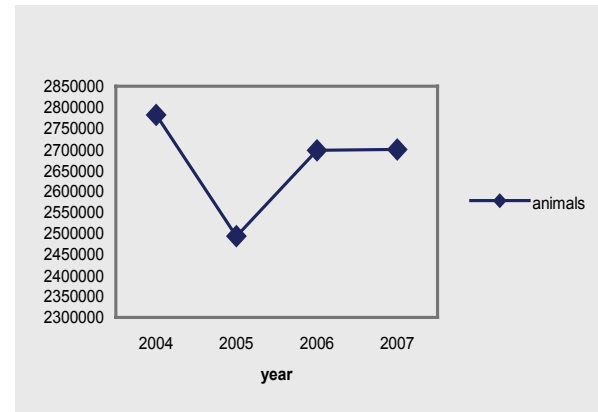
1 Total number of available places for sows and gilts in all herds

2 Total number of available places for fattening pigs in all herds

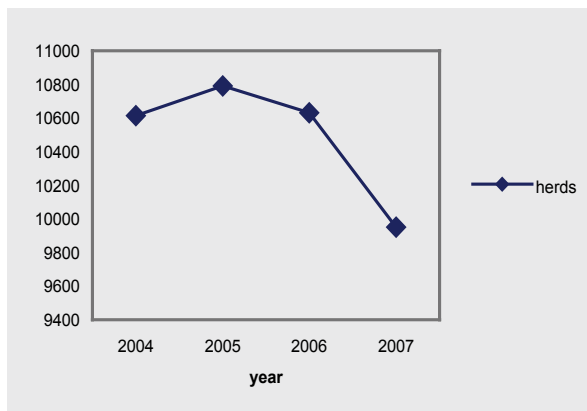
Figures 2 to 5 represent the evolution of total number of respectively bovine and porcine herds and animals over last years.



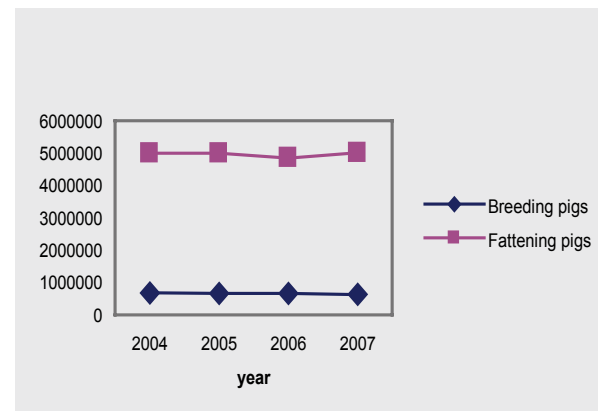
**Figure 2.** Evolution of the total number of cattle herds, period 2004 – 2007



**Figure 3.** Evolution of the total number of bovines, period 2004 – 2007



**Figure 4.** Evolution of the total number of pig herds, period 2004 – 2007



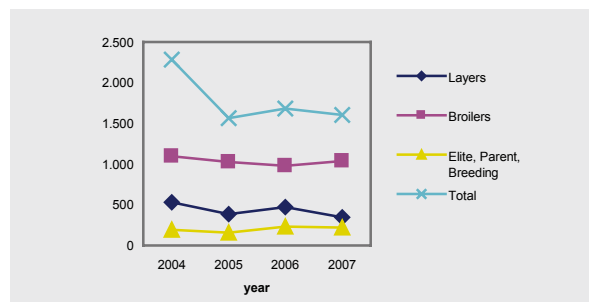
**Figure 5.** Evolution of the total number of porcines, period 2004 – 2007

## Poultry

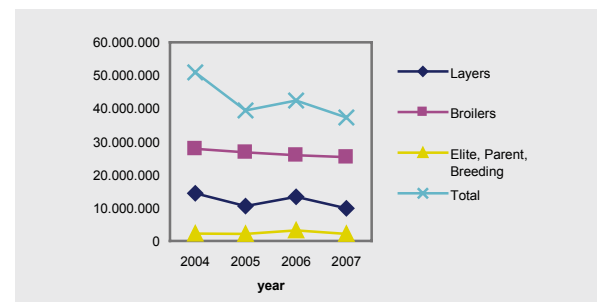
**Table 3.** Total number of herds and animals of poultry in the period 2004 – 2007

	2004		2005		2006		2007	
	herds	animals	herds	animals	herds	animals	herds	animals
<b>Gallus gallus</b>								
Layers	529	14.364.922	386	10.562.160	472	13.377.548	347	9.878.202
Broilers	1.097	27.873.988	1.024	26.754.817	978	25.894.597	1.036	25.311.775
Elite, Parent, Breeding	193	2.255.085	156	2.144.874	232	3.170.815	221	2.089.933
Total	2.284	50.947.719	1.566	39.461.851	1.682	42.442.960	1.604	37.279.910
Ducks	31	33.949	17	45.140	27	77.140	17	37.880
Geese	8	4.843	5	3.800	6	4.900	3	1.800
Turkeys	63	498.146	37	246.076	41	248.006	46	267.855
Guinea fowl	27	87.440	16	71.400	12	39.200	12	39.200
Partridges	2	123.300	4	129.000	4	136.000	5	136.000
Pheasants	14	206.649	16	226.049	25	268.000	23	268.000
Pigeons	4	1.520	2	1.300	2	2.500	3	2.500
Quails	7	56.020	1	1.700	13		13	

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 2004 – 2007



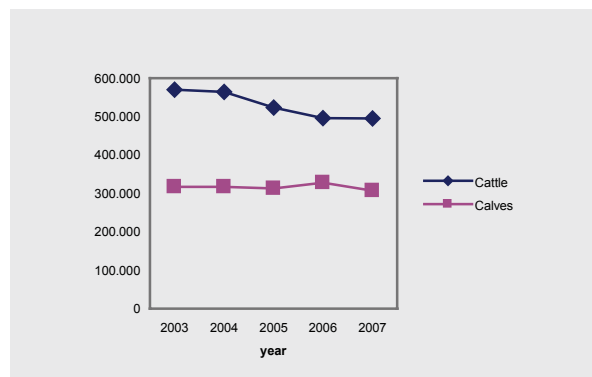
**Figure 7.** Gallus gallus, evolution total number of poultry, period 2004 – 2007

## Animals slaughtered 2003 – 2007

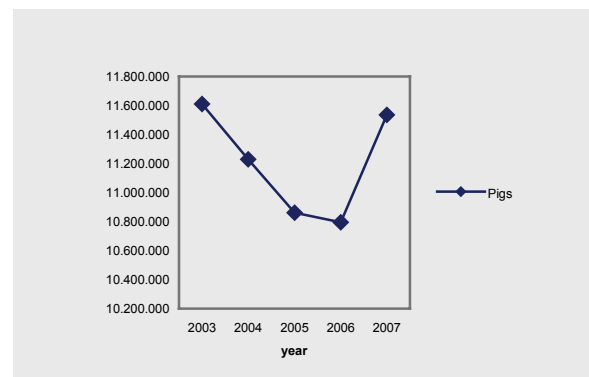
**Table 4.** Number of slaughtered animals in the period 2003 – 2007

	2003	2004	2005	2006	2007
Cattle	570.000	564.266	523.795	496.181	495.492
Calves	317.000	317.269	313.115	327.467	306.961
Pigs	11.609.933	11.229.149	10.861.234	10.794.757	11.536.172
Solipeds	12.304	11.655	11.542	10.728	10.064
Sheep & Goats	85.626	90.933	115.356	151.803	137.492
Broilers & Layers	242.038.535	272.641.500	267.578.340	279.986.675	274.505.734

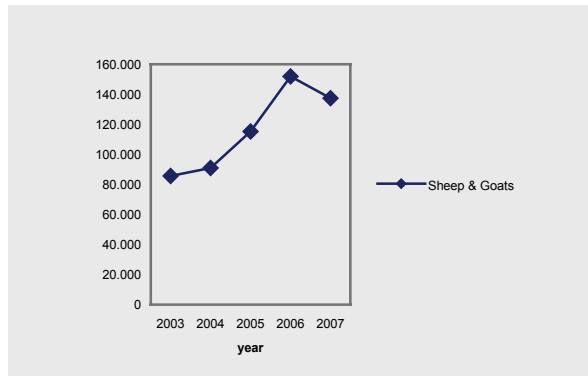
Figures 8 to 11 represent the evolution of the total number of slaughtered bovines, porcines, solipeds, sheep & goats and broilers & layers over the last years.



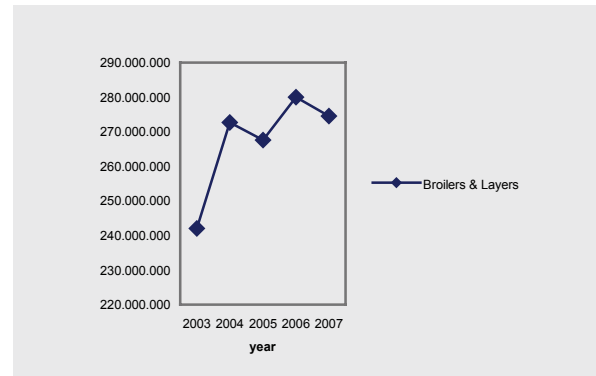
**Figure 8.** Evolution of slaughtered bovines 2003 – 2007



**Figure 9.** Evolution of slaughtered pigs 2003 – 2007



**Figure 10.** Evolution of slaughtered sheep & goats 2003 – 2007



**Figure 11.** Evolution of slaughtered broilers and layers 2003 – 2007





bacterial  
diseases

# Brucellosis

David Fretin, Annick Linden, 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 humans brucellosis is characterised by flu-like symptoms such as fever, headache, back pains and physical weakness. Nocturnal sweats are frequently observed. Infections of the central nervous systems or lining of the heart may occur.

- In the non-"officially brucellosis free" Mediterranean countries, the consumption of raw milk or raw cheese from sheep and goats is thought to be the major source of contamination (*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 wildlife

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.

### Surveillance programme and methods used

Since the official brucellosis free status, the eradication programme has been changed in a surveillance programme.

Dairy cattle are checked at least 4 times a year via tank milk. Tank milk is examined by means of the milk ring test. If tank milk is positive, all animals of the herd older than 2 years are tested by individual serological samples. 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 the individual serological tests and the bulk milk tests

	Individual serological tests	Bulk milk tests
2004	488.548	102.267 pools
2005	579.390	80.025 pools
2006	500.766	73.482 pools
2007	563.948	70.067pools

## Epidemiological investigations and results of 2007 surveillance

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 at the end of the year was 1.13% in 1988 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.

In the surveillance programme, animals are slaughtered for additional testing in case of serological and/or epidemiological suspicion. In 2007, the FASFC didn't have to instruct any test slaughter of animals, since no repeated positive serological tests were found.

## 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 of 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 (5% of the total population). Positive samples were subsequently tested with Rose Bengal test and Complement Fixation test. A sample is classified as positive for brucellosis only if it is positive in all three tests.

Since 2001, yearly serum samples from about 5% of the sheep and goats populations were tested at the National Reference Laboratory. In addition, serum samples from sheep for export were analysed. In 2007, 7.243 samples were tested. Serological positive reacting animals after serial and repeated testing were finally negative. The NRL has confirmed infections of *Yersinia enterocolitica* 0:9 in sheep. Those infections are associated with false positive serology in the tests ELISA, Rose Bengal and possibly CFT of brucellosis. The phenomenon of FPSR (false positive serological reactors) as known for bovines is also observed in sheep.

## 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, complement fixation test (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* O9 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, all samples were negative

## Brucellosis in wildlife

### Regional control programme

Since 2002, an annual surveillance programme is organised by the Network of Wildlife Disease Surveillance (Faculty of Veterinary Medicine, Liège) in collaboration with the NRL (CODA - CERVA, Uccle) with the aim to analyse brucellosis in wild boars (*Sus scrofa*) and lagomorphs in the South of Belgium. Blood samples and organs of hunted or found dead animals are analysed in order to follow seroprevalence and identify isolates of *Brucella* in these species. In 2007, 259 hunted wild boars were sampled and the apparent seroprevalence (ELISA) was 65.64% (IC<sub>95</sub> = 59.86 – 71.42). *Brucella suis* biovar 2 was isolated from spleen and tonsil of wild boars. In 2007, 11.31% of seropositive wild boars were positive for culture on spleen and/or tonsil. In hares, no *Brucella* was isolated from 154 spleen analysed between 2003 and 2006.

### Recommendation

Further attention should be given to brucellosis in wild species, as the potential for contact with *B. suis* can be high, particularly for people handling and/or slaughtering game animals. The species to be considered should include at least wild boar, deer and other wild ruminants as well as hares.

## 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.

# Campylobacteriosis

Katelijne Dierick, Geneviève Ducoffre, Olivier Vandenberg, Luc Vanholme, Karen Vereecken

## Campylobacteriosis

Campylobacteriosis continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in Belgium as in the previous three years. 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 reservoirs are the alimentary tracts of wild and domesticated birds and mammals. They 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* are 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 2007, 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 for *Campylobacter*.

Specially trained staff of the FASFC performed the sampling. Different contamination levels (25g, 0.01g and 600 cm<sup>2</sup>) 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 detect a minimal contamination rate of 1% with 95% confidence.

The results of the 2007 monitoring of the FASFC are shown in table 6.

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

Sample	Quantity of sample analysed	Percentage of positive samples
<b>Broiler</b>		
Carcasses at slaughter (n=236)	25g (caeca)	51.3%
Carcasses at slaughter (n=235)	0.01g	22.5%
Carcasses at retail (n=144)	0.01g	19.4%
Meat cuts (skinned or with skin) at processing plant (n=257)	0.01g	9.3%
<b>Layer</b>		
Carcasses at slaughter (n=74)	25g (caeca)	98.6%
Carcasses at slaughter (n=149)	0.01g	34.7%
Carcasses at retail (n=113)	0.01g	18.6%
<b>Poultry</b>		
Meat cuts (with skin) at retail (n=131)	0.01g	9.9%
Meat cuts (without skin) at retail (n=140)	0.01g	3.6%
Minced meat at retail (n=159)	Enumeration (M=100 cfu/g)	0.0%
Meat preparation at processing plant (n=275)	0.01g	2.9%
Meat preparation at retail (n=420)	Enumeration (M=100 cfu/g)	0.2%
<b>Pork</b>		
Carcasses at slaughter (n=213)	600 cm <sup>2</sup>	12.2%
Minced meat (intended to be eaten raw) at retail (all species) (n=128)	Enumeration (M=10 cfu/g)	0.0%
Minced meat (intended to be eaten cooked) at retail (all species) (n=127)	Enumeration (M=100 cfu/g)	0.0%
Raw milk cheese at retail (n=46)	25g	0.0%
Raw milk cheese at farm (n=23)	25g	0.0%
Live bivalve molluscs at retail (n=60)	25g	0.0%

The contamination rate of pig carcasses raised until 2006; in 2007, the contamination decreased a bit. From 2008, the *Campylobacter* spp. contamination will be enumerated.

**Table 7.** Evolution of the pork *Campylobacter* prevalence 2004-2007

		Sampling level	2004	2005	2006	2007
Pork	Carcasses	600 cm <sup>2</sup>	4.9%	7.2%	13.4%	12.2%

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

### Surveillance programme and method used

In 2007, 205 *Campylobacter* strains isolated in the zoonoses monitoring programme and 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.

Twenty strains were isolated from pork meat or carcasses, 185 strains were isolated from poultry (broiler meat or carcasses, spent hens and turkey). *C. coli* was the most prevalent strain isolated from pork carcasses (75%), while for poultry meat *C. jejuni* was the most isolated *Campylobacter* strain (60%) and *C. coli* represented 19% of the isolates. *C. lari* was determined in 4% of the isolates and was exclusively isolated from spent hens.

Minimum Inhibitory Concentrations (MIC) were determined by using E<sup>o</sup>-test on blood agar plates. The antimicrobials tested and the breakpoints (following the CLSI standards) used are listed in the following table.

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

Antimicrobial	Breakpoints (µg / ml)
Ampicillin	8 – 32
Tetracycline	4 – 16
Nalidixic acid	16 – 32
Ciprofloxacin	1 – 4
Erythromycin	1 – 8
Gentamicin	4 – 16

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

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

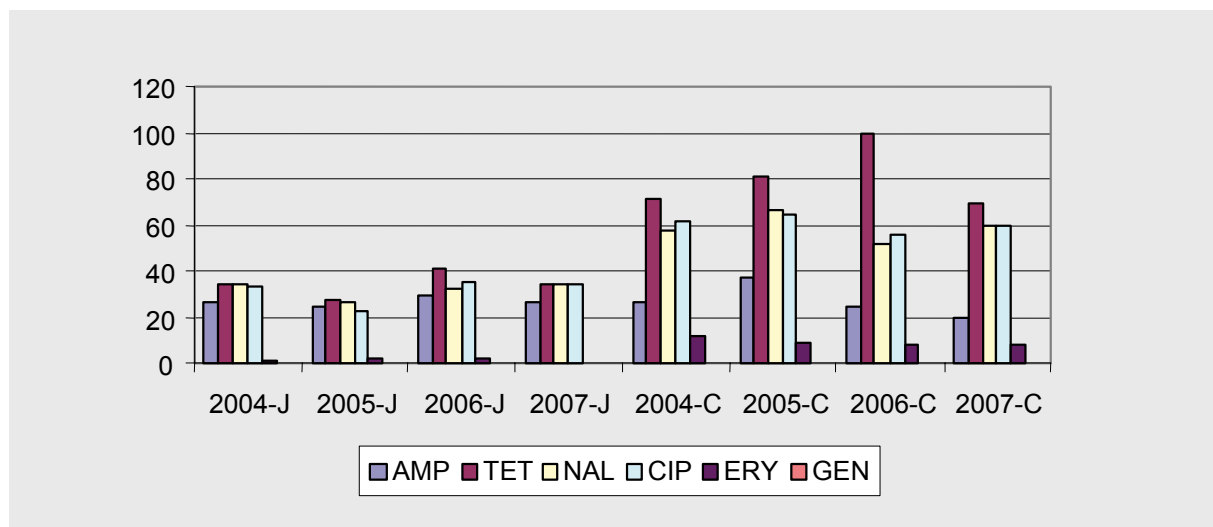
	Poultry meat		Pork
	<i>C. jejuni</i> (n=111)	<i>C. coli</i> (n=50)	<i>C. coli</i> (n=15)
Tetracycline	34%	70%	80%
Ciprofloxacin	34%	60%	20%
Nalidixic acid	34%	60%	20%
Gentamicin	0%	0%	0%
Erythromycin	0%	8%	7%
Ampicillin	27%	20%	7%

## Antimicrobial resistance in *Campylobacter* from poultry meat

185 *Campylobacter* strains were isolated in poultry meat and carcasses and tested for antimicrobial susceptibility (111 *Campylobacter jejuni* and 50 *Campylobacter coli* strains). In total 39% of the *C. jejuni* strains were sensitive for all tested antibiotics. Tetracycline, ciprofloxacin and nalidixic acid resistance were present in 34% and ampicillin resistance was noticed in 27% of the *C. jejuni* strains. No resistance was detected against erythromycin and gentamicin. Overall the antibiotic resistance within *C. coli* was higher than in *C. jejuni*, with a much higher percentage of resistance against ciprofloxacin (60%), nalidixic acid (60%) and tetracycline (70%). Resistance against erythromycin was found in 8% of

the *C. coli* strains. The ampicillin resistance was much higher in strains isolated from broiler meat and carcasses than in strains isolated from pork meat. Eleven *Campylobacter* strains were isolated from turkey, 55% of the strains were fully sensitive and no resistance against ampicillin and gentamicin was measured. Resistance was observed for erythromycin (18%) ciprofloxacin (36%), nalidixic acid (36%) and tetracycline (27%).

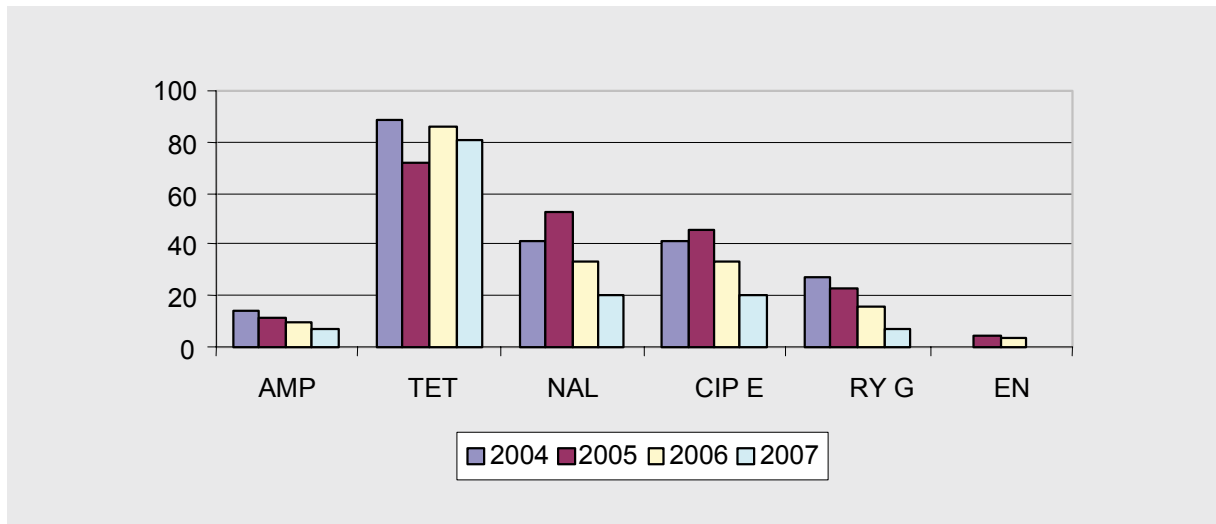
Compared to the results observed in previous years no remarkable differences were noticed in the resistance pattern of the *Campylobacter* strains isolated in poultry.



**Figure 12.** Evolution of the percentage resistant *Campylobacter jejuni* and *Campylobacter coli* strains in poultry

## Antimicrobial resistance in *Campylobacter* from pork

In the *C. coli* isolates (15) from pork, resistance was observed for all antibiotics except for gentamicin. Only 2 strains were sensitive to all tested antibiotics.

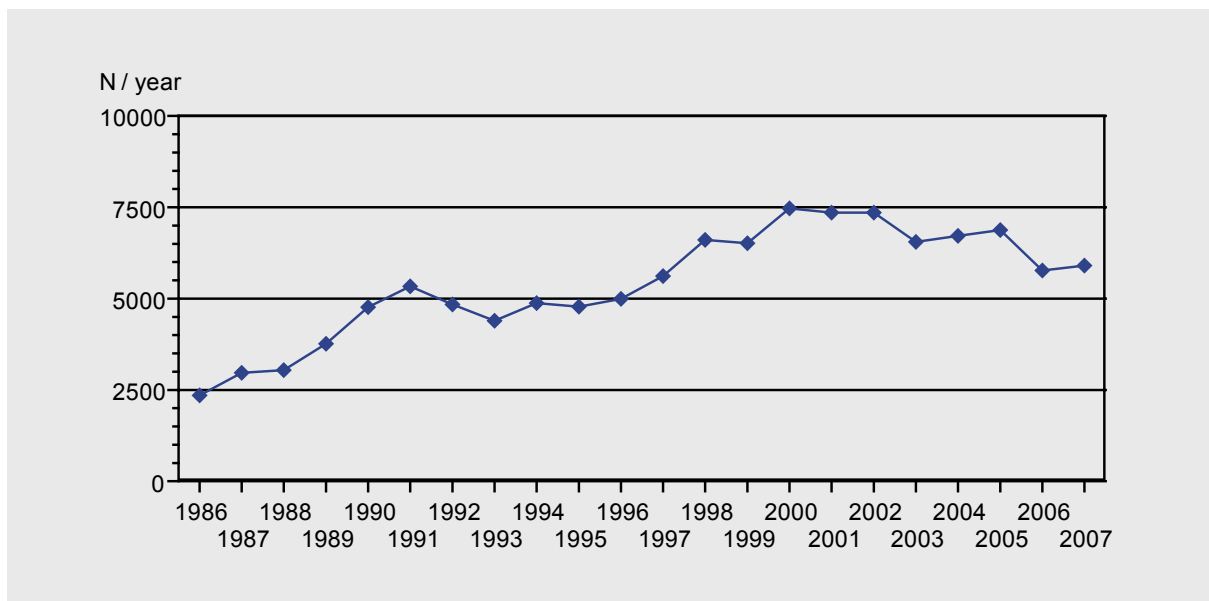


**Figure 13.** Evolution of the percentage resistant *Campylobacter coli* strains isolated in pork

The resistance against tetracycline (81%) was high followed by ciprofloxacin (20%) and nalidixic acid (20%). Compared to previous years a decrease in the resistance is noticed except for tetracycline where the resistance stays very high.

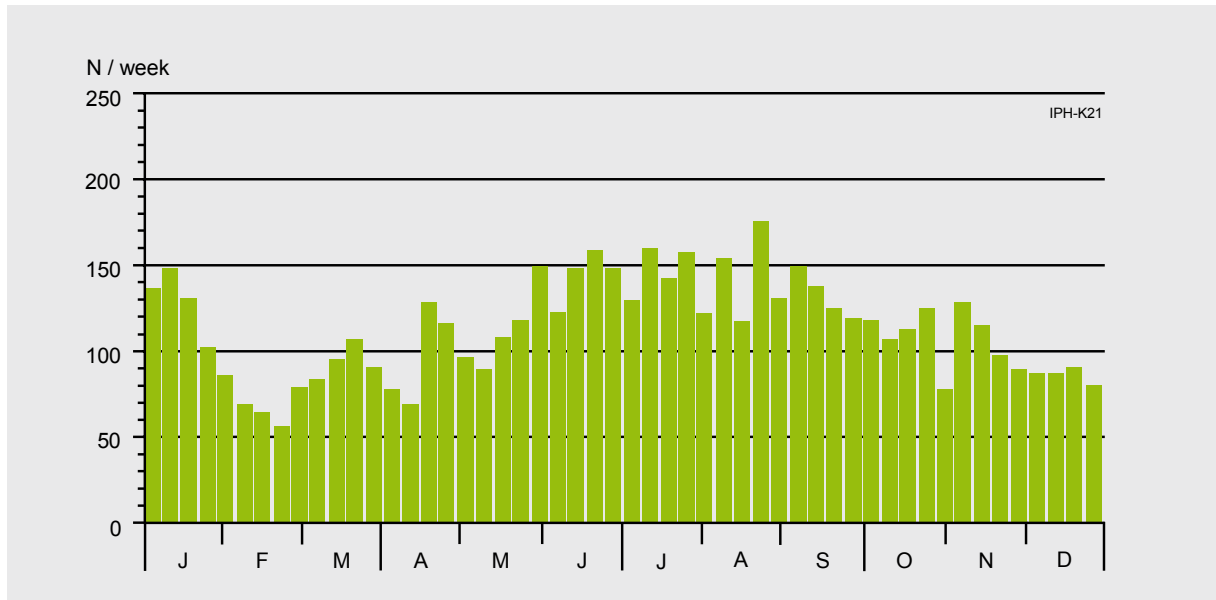
## Campylobacter in humans

In 2007, the Belgian Sentinel Laboratory Network consisted of 110 laboratories reporting Campylobacter. 5,906 strains of Campylobacter were isolated which represent at country level an isolation rate of 56 per 100.000 inhabitants (in 2006: N=5,771). The number of Campylobacter infections shows a significant decreasing trend since 2000 at national and regional level ( $p < 0.05$ ; Figure 14). Since 2005 Campylobacteriosis remains the most frequently reported zoonosis in humans.



**Figure 14.** Total number of Campylobacter infections in humans by year (1986-2007). (Source: Sentinel Laboratory Network)

Cases are reported during the entire year, with a peak in the summertime (Figure 15).



**Figure 15.** Weekly number of *Campylobacter* infections in humans, 2007. (Source: Sentinel Laboratory Network)

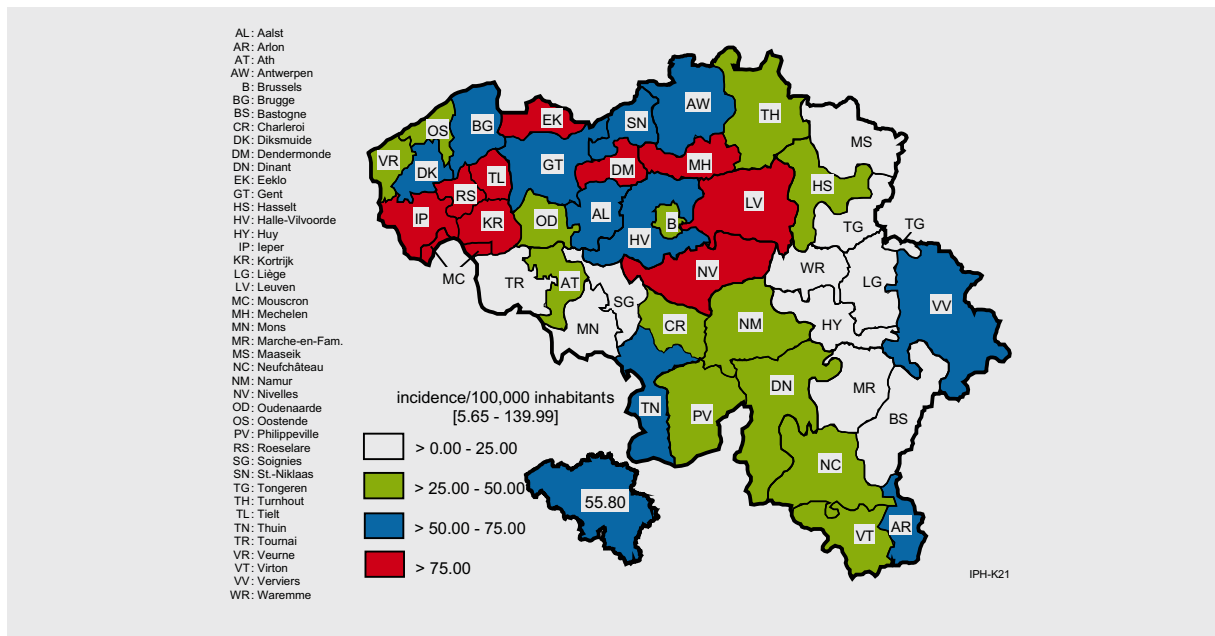
*Campylobacter* isolation rates are slightly higher in men (53%) than in women (47%); 21% of cases are diagnosed in children of 1-4 year old and 20% in adults of 25-44 year old. This distribution is observed since many years. There is no explanation for this observation (Table 10).

**Table 10.** Number of *Campylobacter* infections in humans by sex and by age groups, 2007. (Source: Sentinel Laboratory Network)

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	192	6,3	145	5,3	337	5,8
1 - 4	682	22,3	555	20,2	1237	21,3
5 -14	463	15,1	343	12,5	806	13,9
15 -24	308	10,1	369	13,4	677	11,7
25 -44	584	19,1	588	21,4	1172	20,2
45 -64	498	16,3	403	14,7	901	15,5
65	336	11,0	344	12,5	680	11,7
<b>Total</b>	<b>3063</b>	<b>100,0</b>	<b>2747</b>	<b>100,0</b>	<b>5810</b>	<b>100,0</b>

Since the beginning of the registration (1983), the incidence in Flanders is higher than in Wallonia. This was confirmed in 2007 with an estimated incidence of 66/100.000 inhabitants in Flanders, 41/100.000 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 2007: 140/100.000 inhabitants in Mouscron, 128/100.000 inhabitants in Eeklo, 122/100.000 inhabitants in Mechelen and 115/100.000 inhabitants in Leuven (Figure 16). It would be useful to make a study to explain the reason(s) of those high numbers of infections in certain districts of the country.

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**Figure 16.** Incidence of *Campylobacter* infections in humans by district (N/10<sup>5</sup> inhab., 2007). (Source: Sentinel Laboratory Network)

## Epidemiology Enteric Campylobacter

Data were obtained from the NRL for Human Enteric Campylobacter. Since clinical laboratories are not obliged to send human isolates to confirm the presence of Campylobacter, a correct epidemiology of Campylobacter in human populations cannot be estimated.

Therefore, data about human Campylobacter cases were obtained from the clinical laboratories of Brugmann, Queen Fabiola, Bordet and Saint-Pierre University Hospitals located in Brussels from January 2007 to December 2007.

During this period, a total of 5.136 stool specimens from 4.987 patients were routinely examined for Campylobacter spp. using one selective medium and a filtration method.

As a result, Campylobacter was isolated in 446 patients. Among these, 77.2% were *C. jejuni*, 11.4% were *C. coli* and 4.5% were *C. upsaliensis*. The reported prevalence of non-

*jejuni / coli* Campylobacter of 0.53% is higher than reported from other European countries and is probably due to the comprehensive isolation procedure used.

## Antimicrobial resistance of human isolates

The Campylobacter isolates from the above mentioned study (Epidemiology Enteric Campylobacter) were examined for their resistance against three antibiotics of therapeutic interest. The testing method was disk diffusion according to Kirby-Bauer, following SFM recommendations.

Resistance was mostly found against ampicillin (57.4%) and ciprofloxacin (49.8%). Most Campylobacter were susceptible to erythromycin (84.8%). However, an increasing resistance against erythromycin and ciprofloxacin was recorded. These results underscore the need to monitor antibiotic resistance in Campylobacter from patients (Table 11).

**Table 11.** Resistance of Campylobacter in Belgium fecal isolates, trend from 2001 till 2007.

(Source: NRL (Laboratory for Microbiology, Saint-Pierre University Hospital, Brussels)

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

## Invasive *Campylobacter* in Humans

In 2007, the NRL for *Campylobacter* confirmed 13 invasive *Campylobacter* isolates. Among these, *C. fetus* was recovered in 7 patients. The remainders were *C. jejuni* and *C. coli* in 4 and 2 cases respectively (Table 12).

**Table 12.** *Repartition by biotype of invasive Campylobacter in humans, 2007. (Source: NRL)*

N	<i>Campylobacter jejuni</i>				<i>C. coli</i>		<i>C. fetus</i>
	I	II	III	IV	I	II	subsp.fetus
13	3	1				2	7



# Escherichia coli (VTEC) infections

Hein Imberechts, Denis Pierard, Luc Vanholme, Karen Vereecken

## Verotoxin producing Escherichia coli

Verotoxigenic Escherichia coli (VTEC) is a group of E. coli that are 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 are 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. Some other pathogenic serotypes of E. coli, e.g. O26, O91, O103, O111 and O145 may also be involved.

Reported 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.

Verotoxin producing Escherichia coli

Verotoxin producing Escherichia coli in cattle

Escherichia coli O157 in food

Verotoxinogenic Escherichia coli in humans

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.

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 are 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. In previous years, epidemiological investigations and additional examinations at the farm of origin, excretion of VTEC strains by other bovines was never detected.

In Belgium, approximately 40 sporadic human cases are registered per year.

## Verotoxin producing Escherichia coli in cattle

### Surveillance programme, measures and methods used

The surveillance starts when an E. coli O157 (stx1, stx2, eaeA, enterohemolytic) is isolated from a carcass at the slaughterhouse. In such case, the farm of origin is traced back via Sanitel, the computerised registration and identification database for farm animals, managed by the FASFC. FASFC officials inform the owner that E. coli O157 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.

Carcasses contaminated with E. coli O157 should be destroyed or may be heat treated. In all other cases, no specific measures are taken.

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

## Epidemiological investigations and results of 2007 surveillance

In 2007, no cattle farms were sampled to identify the source of an *E. coli* O157 outbreak. In addition, not a single animal VTEC strain was sent to the NRL (animal health) for typing.

## 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 compulsory.

### Results of the 2007 monitoring

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

**Table 13.** Zoonosis monitoring programme - *E. coli* O157, 2007

	Sample	Prevalence
<b>Beef</b>	Carcasses (n=1 611)	0.3%
	Fresh meat at cutting plant (n=286)	0.0%
	Minced meat (steak tartare) at retail (n=152)	0.0%
	Meat preparations (steak tartare with herbs and sauce) at retail (n=150)	0.0%
<b>Milk</b>	Raw cow's milk, at farm (n=52)	1.9%
	Raw goat's, sheep's or horse's milk at farm (n=25)	0.0%
<b>Cheese</b>	From raw milk, at farm (n=24)	0.0%
	From raw milk, at processing (n=48)	0.0%
	From raw milk, at retail (n=83)	0.0%
	From raw sheep's milk, at retail (n=25)	0.0%
	From raw goat's milk, at retail (n=25)	0.0%
	From raw sheep's or goat's milk, at farm (n=17)	0.0%
<b>Butter</b>	At farm (n=111)	0.0%
	From raw milk, at retail (n=25)	0.0%
<b>Cream</b>	At farm (n=45)	0.0%

## 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 2007, the NRL confirmed 47 verotoxinogenic E coli isolated from 46 patients. Among these:

- 40 typical VTEC isolates, positive for two factors of additional virulence: the presence of the gene eae (intimin) gene and enterohemolysin (EHEC virulence plasmid) gene.
- 7 atypical VTEC isolates, negative for intimin and enterohemolysin.

The number of isolates analysed annually by the NRL has been rather constant, corresponding to a large rate of underdiagnosis (Table 14).

**Table 14.** E. coli: evolution in number of isolates in humans, 1998-2007.

Source: NRL

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Number of isolates	48	53	47	46	46	47	45	47	46	47
Number of typical isolates	38	46	33	36	37	40	36	36	36	40
Number of O157 isolates	25	33	26	29	26	21	29	27	21	25

In 2007, 10 strains (6 from serotype O157:H7, 2 O145, one O26, and one O111) were associated with haemolytic uremic syndrome (HUS). Five patients were less than 5 years old (of which one was infected with two strains, see below), two

were older children (8 and 12 years old) and two were adults (54 and 81 years old).

In addition, five children with HUS were diagnosed by serology alone: three with antibodies against O157 and two against O145.

According to the information available at the NRL, all but three of these cases were not related. In three related HUS cases, a double infection with VTEC O26 and O145 was confirmed in one case, while in the other two, evidence was found only for O145 infection. The infection source was found to be ice cream produced locally in a farm, where the same VTEC O26 and O145 strains were also found in animals and in the environment. Two more children suffered from HUS and seven of uncomplicated diarrhea, but no samples were obtained for laboratory investigation.

# 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 leptospirae 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

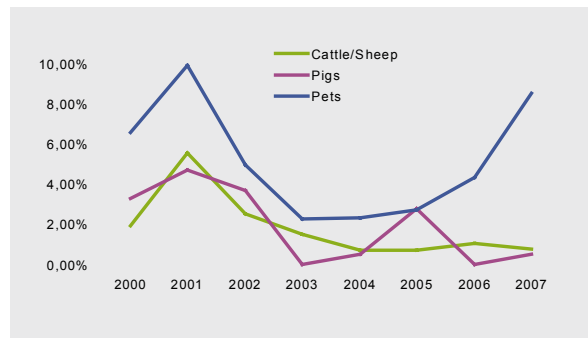
Leptospirosis in humans

## 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 it. Other serological tests used are rapid agglutination tests and ELISA's, 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

In Belgium, the number of human cases remains so far limited (see below). In animals, leptospirosis was considered widely present in cattle and pigs before 2001, resulting in abortions and reproductive disorders, leading to frequent infections in animal handlers (known as milker's fever). Since 2001, leptospirosis is seldom found in cattle and pigs. Surprisingly, a sudden increase in clinical cases was noted in Belgian dogs since 2006 (Figure 17) with a mortality ranging from 15 to 20%. Prophylaxis with the available inactivated leptospirosis vaccines containing serovar canicola and icterohemorrhagiae is ineffective, as other serovars are involved. Also in horses, the number of clinical cases has increased since 2006, with fatal cases in foals younger than 3 months. The same observations are made in Germany, France and Italy. This increase in mainly canine leptospiroses requires further attention.



**Figure 17.** Seropositive laboratory confirmed cases in animals at the NRL, animal health between 2000 and 2007. (Total number of samples received: mean of 1700 yearly (range 1390-2090))

## 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.

### **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 with internal organs. Generally the disease has a good prognosis.

### **Diagnostics**

Diagnosis is based on clinical symptoms, risk factors and laboratory analyses. Bacterial culture is difficult and takes a long time. Since it is useless for diagnostic purposes, serology is done. 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 reference laboratory. It is best to analyse two samples taken within one or two weeks of each other.

### **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 is expected about a month after treatment begin.

### **Prevention**

No vaccine to protect against leptospirosis is available in Belgium. Professionals carrying out activities associated 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 2007 surveillance

At the NRL, a total of 631 human sera have been examined for the presence of antibodies to *Leptospira* by the microscopic agglutination technique (MAT).

Eight confirmed or probable cases have been diagnosed in 2007. All patients were men. The age of the patients ranged from 25 to 58 years with a median age of 39.5 years. Five patients were exposed in Belgium, 3 patients contracted the disease during or after a stay in a (sub)tropical region (Togo, South East Asia, Cambodia). Five patients were exposed to water during recreational or professional activities, 3 patients were exposed in a garden or at a farm. The infections with exposure in Belgium were diagnosed between June and October.

### Study performed by the NRL, public health

A study was carried out by the Epidemiology Unit of the IPH using the sentinel laboratory network in collaboration with the NRL. The aim of the study was to find out if the sentinel laboratories were capable of diagnosing leptospirosis and if yes, the number of cases diagnosed between 2002 and 2007. In addition, the study aimed to find out if these laboratories sent their samples for confirmation and if yes, to which laboratory.

Ninety-four of 110 contacted sentinel labs responded to the survey. With the exception of seven laboratories, all sent their samples to a larger centre for leptospirosis diagnosis. Six of the seven laboratories that carried out their own analyses sent their positive samples to a reference laboratory for confirmation. The number of cases diagnosed by the reference laboratory between 2003 and 2007 varied between 3 and 14 and the number of cases that were contaminated in Belgium was seven in 2006 and five in 2007 (Table 15). Among the 22 cases diagnosed in 2006 and 2007, 15 (68%) were contaminated by water and the others by contact with domestic animals or by working in the garden or on a farm.

The 2005 European Centre for Disease Control and Prevention annual report mentioned that leptospirosis incidence in Belgium was estimated in 2005 at 0.1 per 105 inhabitants (N=12). This is comparable to the European incidence which varied between 0.1 and 0.22 per 105 inhabitants between 1995 and 2004.

**Table 15.** Evolution of the number of cases of leptospirosis,

*ITM, 2003-2007*

Leptospirosis cases of humans	N (total)	N (contamination in Belgium)
2003	3	*
2004	4	*
2005	3	*
2006	14	7
2007	8	5

\*: data not available

## Conclusions

Leptospirosis is a disease that is probably under-diagnosed and under-reported in Belgium. Physicians need to suspect this disease when a patient presents with flu-like symptoms after a possible exposure to contaminated animal urine or contaminated freshwater.

Currently, this disease is not an important public health issue but it is crucial to continue to monitor the number of cases reported in Belgium as well as in the rest of Europe. This disease may become more important in the near future due to global warming, increasing of intercontinental travels and water sports activities.

A fact sheet is available on the IPH website ([www.iph.fgov.be/epidemie/lab0](http://www.iph.fgov.be/epidemie/lab0)).



# Listeriosis

Geneviève Ducoffre, Karen Vereecken, 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.

Listeriosis

*Listeria monocytogenes* in food

*Listeria monocytogenes* in humans

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 characterized by septicemia and meningoenzephalitis. In pregnant women the infection can spread to the foetus, which may either be born severely ill or die in the uterus and result in abortion. 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.

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 surroundings.

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 *L. 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 analysed for *Listeria monocytogenes* 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 2007 monitoring

The results of the national monitoring program of *Listeria monocytogenes* in other foods of animal origin are as follows:

- at retail: meat salad (n=48), crustacean salad (n=48), chicken salad (n=51), dried follow-on formula (n=47), nursing bottles (n=119), bakery products with cream (n=158), salty preparations based on raw eggs (n=55), desserts based on raw eggs (n=119 positive)
- at processing plant: sandwich spreads (meat, chicken, crustacean) (n=182), bakery products with cream (n=78).

All results were negative, except for desserts based on raw eggs at retail (8.4% positive), sandwich spreads at processing plants (10.4% positive) and bakery products with cream at processing plant (3.8% positive).

**Table 16.** Zoonosis monitoring programme - *Listeria monocytogenes* in food, 2007.

Sample		Quantity analysed	Percentage of positive samples
Beef	Minced meat at retail intended to be eaten raw (steak tartare) (n=159)	Enumeration (M=100 cfu/g)	0.0%
	Meat preparation at retail intended to be eaten raw (steak tartare with herbs and sauce) (n=157)	Enumeration (M=100 cfu/g)	3.2%
Pork	Cooked ham at processing plant (n=59)	25g	1.7%
	Cooked ham at retail (n=56)	Enumeration (M=100 cfu/g)	0.0%
	Raw ham at processing plant (n=78)	25g or enumeration (M=100 cfu/g) (*)	1.3%
	Raw ham at retail (n=33)	Enumeration (M=100 cfu/g)	0.0%
	Pâté at processing plant (n=56)	25g	0.0%
	Pâté at retail (n=58)	Enumeration (M=100 cfu/g)	0.0%
	White pudding at processing plant (n=49)	25g	8.2%
	White pudding at retail (n=72)	Enumeration (M=100 cfu/g)	0.0%
	Sausages at processing plant (n=95)	25g or enumeration (M=100 cfu/g) (*)	6.3%
	Sausages at retail (n=35)	Enumeration (M=100 cfu/g)	0.0%
Meat (unspecified)	Minced meat intended to be eaten raw, at retail (n=157)	Enumeration (M=100 cfu/g)	1.9%
Cheeses	Cheeses made from raw milk at retail (n=83)	Enumeration (M=100 cfu/g)	0.0%
	Cheeses made from pasteurised milk at retail (n=123)	Enumeration (M=100 cfu/g)	0.0%
	Cheese made from raw sheep's or goat's milk at farm (n=16)	Enumeration (M=100 cfu/g)	0.0%
	Cheese made from raw sheep's milk at retail (n=25)	Enumeration (M=100 cfu/g)	0.0%
	Cheese made from raw goat's milk at retail (n=25)	Enumeration (M=100 cfu/g)	0.0%
	Cheeses made from raw milk at farm (n=24)	Enumeration (M=100 cfu/g)	0.0%
	Cheeses made from pasteurised sheep's milk at retail (n=19)	Enumeration (M=100 cfu/g)	0.0%
	Cheeses made from pasteurised goat's milk at retail (n=20)	Enumeration (M=100 cfu/g)	0.0%
	Cheeses made from raw milk at processing plant (n=48)	25g	0.0%
	Cheeses made from pasteurised milk at processing plant (n=140)	25g	0.0%

Sample		Quantity analysed	Percentage of positive samples
Dairy products	Butter at farm (n=158)	Enumeration (M=100 cfu/g)	0.0%
	Butter made from raw milk at retail (n=39)	Enumeration (M=100 cfu/g)	0.0%
	Cream at farm (n=19)	Enumeration (M=100 cfu/g)	0.0%
	Ice cream at farm (n=43)	Enumeration (M=100 cfu/g)	0.0%
	Ice cream at retail (n=77)	Enumeration (M=100 cfu/g)	0.0%
	Milk desserts at processing plant (n=44)	25g	0.0%
	Milk desserts at retail (n=60)	Enumeration (M=100 cfu/g)	0.0%
	Yoghurt at retail (n=50)	Enumeration (M=100 cfu/g)	0.0%
	Yoghurt at farm (n=27)	Enumeration (M=100 cfu/g)	0.0%
	Milk powder at processing plant (n=20)	Enumeration (M=100 cfu/g)	0.0%
Fish	Smoked salmon at retail (n=150)	Enumeration (M=100 cfu/g)	1.3%
	Fresh fish at processing plant (n=49)	25g or enumeration (M=100 cfu/g) (*)	8.2%
	Fresh fish at retail (n=78)	Enumeration (M=100 cfu/g)	1.3%

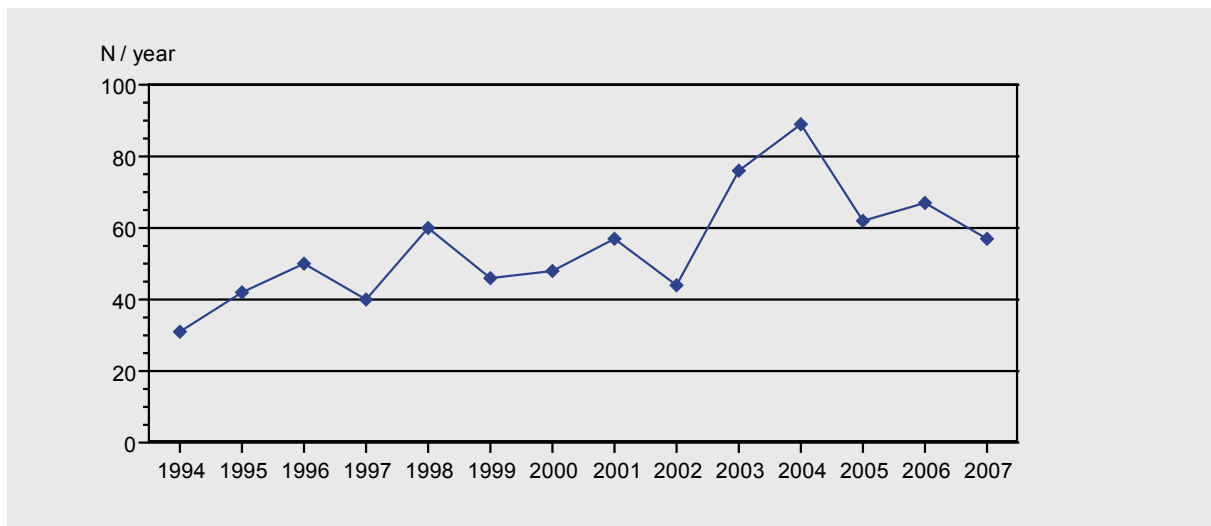
(\*) Depending on the values of the pH and the water activity (Cf Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs)

## Listeria monocytogenes in humans

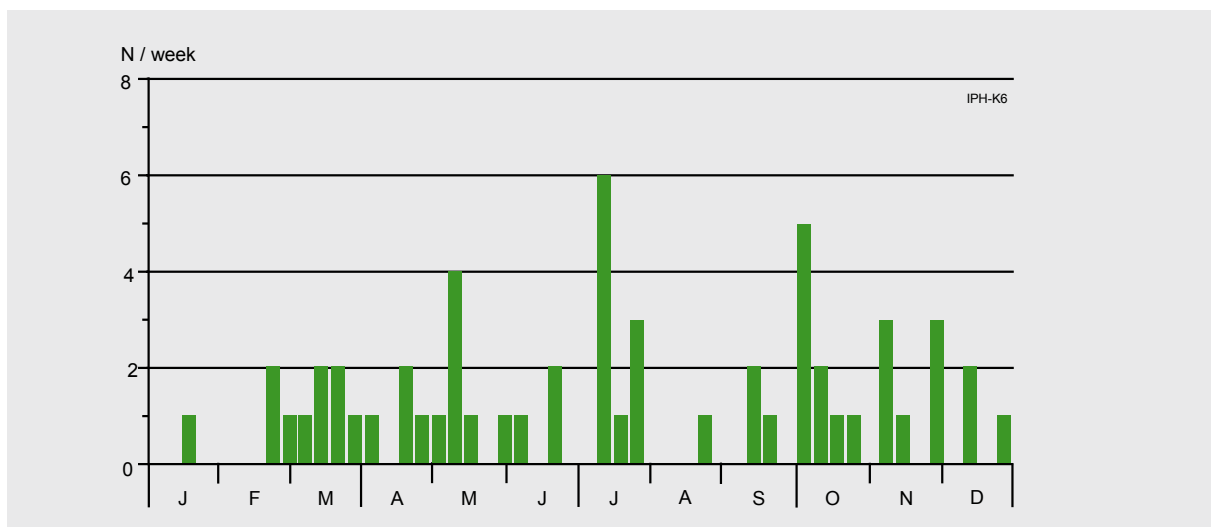
In 2007, the Sentinel Laboratory Network and the NRL reported 57 cases of listeriosis. This number is less than in 2003 (N=76) and 2004 (N=89), when particularly high numbers of listeriosis cases were recorded. For the period 1994-2007, the annual number of cases reported is depicted in Figure 18, corresponding to an annual mean number of 55 cases.

Cases are reported all over the year (Figure 19).

Listeria monocytogenes isolation rates are higher in men (54%) than in women (46%); 28% of cases are diagnosed in adults older than 64 year (Table 17 on page 56). Old 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 2007 is as follows: one case was reported in Brussels, 37 cases were reported in Flanders and 17 in Wallonia (2 from unknown geographic origin) (Figure 20 on page 56).



**Figure 18.** Number of *Listeria monocytogenes* infections in humans by year (1994-2007). (Sources: Sentinel Laboratory Network and NRL)

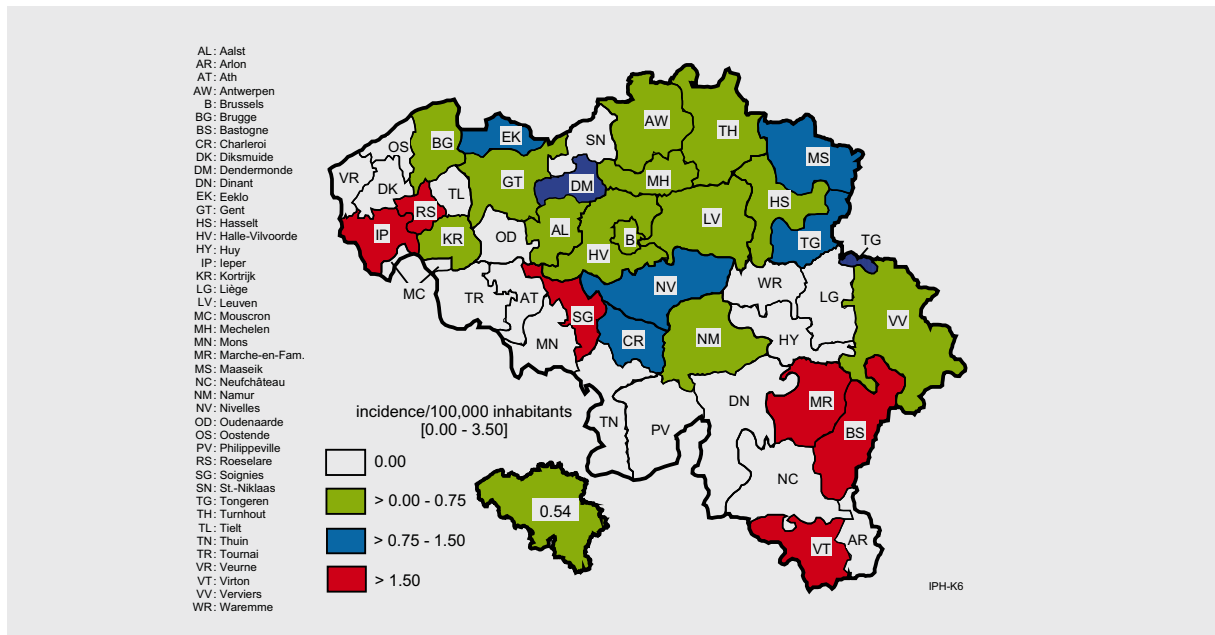


**Figure 19.** Weekly number of *Listeria monocytogenes* infections in humans, 2007. (Sources: Sentinel Laboratory Network and NRL)

**Table 17.** Number of *Listeria monocytogenes* infections in humans by sex and by age groups, 2007. Sources: Sentinel Laboratory Network and NRL

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	1	3,3	3	11,5	4	7,1
1 - 4	0	0,0	1	3,8	1	1,8
5 -14	0	0,0	0	0,0	0	0,0
15 -24	0	0,0	0	0,0	0	0,0
25 -44	2	6,7	6	23,1	8	14,3
45 -64	10	33,3	5	19,2	15	28,8
65	17	56,7	11	42,3	28	50,0
<b>Total</b>	<b>30</b>	<b>100,0</b>	<b>26</b>	<b>100,0</b>	<b>56</b>	<b>100,0</b>

In 2007 the NRL serotyped 52 clinical strains of *Listeria monocytogenes*. Contrary to 2006, no strain of *Listeria ivanovii* of human origin was received. The serovar 4b and 1/2a were the most prevalent (63% and 29% respectively). One clinical strain presented with an unusual serovar (4e). Six strains were related to pregnancy (3 strains isolated from the mother and 3 strains from the newborn). Six strains were isolated from patients with a meningo-encephalitis form of listeriosis; 38 strains were isolated from blood and 2 strains from pus. In 2007 the NRL still received strains from two outbreaks of listeriosis that peaked in 2006. Two new outbreaks of listeriosis (serovar 4b) emerged with 4 and 2 cases respectively. In any of these episodes no source of contamination could be determined



**Figure 20.** Incidence of *Listeria monocytogenes* infections in humans by district ( $N/10^5$  inhab., 2007. Sources: Sentinel Laboratory Network and NRL

# MRSA

Patrick Butaye, Marc Struelens, 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.

In 2007, a study was undertaken to estimate the prevalence of MRSA in pigs in Belgium.

## MRSA in pigs

### 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. Fifty farms were sampled and on each farm, faecal samples of 30 animals of different age groups were investigated.

The samples were analysed by DGZ Vlaanderen who placed them in enrichment broth medium made of BHI broth supplemented with NaCl 7.5%. After 24h incubation, broth was sub-cultured for another 16 h onto a selective chromogenic agar for MRSA (MRSA-ID, BioMérieux, France). Subsequently the plates were further analysed at CODA-CERVA. Suspected colonies were picked up and subcultured on a blood agar plate, demonstrating the typical characteristics of *S. aureus*. The strains were further identified by PCR, including

confirmation of the presence of the gene causing methicillin resistance. A subset of strains was typed by spa typing.

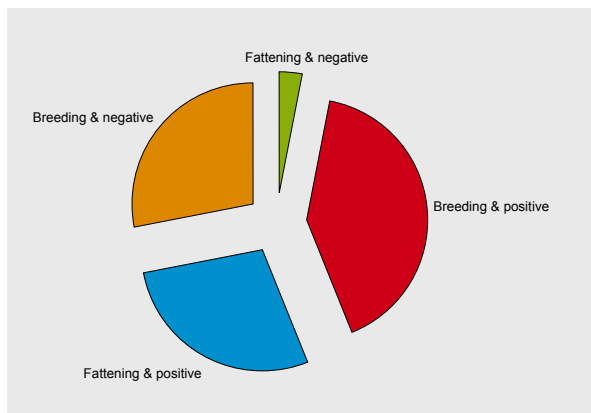
Of the 1500 samples analysed 663 were positive for MRSA, originating from 34 farms. The number of positive farms and the number of positive animals amongst the different age groups sampled are shown in Table 18. The data dealing with farm type and with age group are shown in Figure 21 and Figure 22.

Approximately 70% of the farms were positive for MRSA, with the majority of the fattening farms being positive.

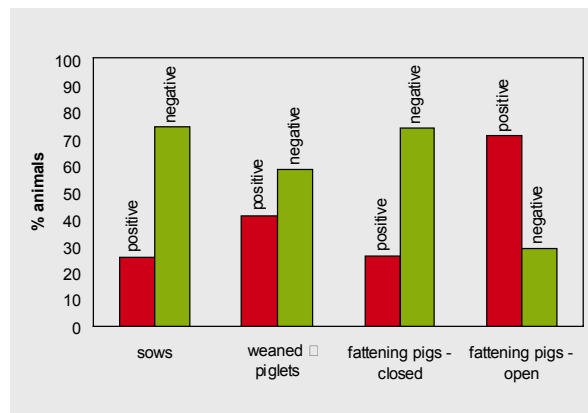
At the animal level, the prevalence in weaned piglets was significantly higher than in sows or in fattening pigs. When comparing differences in fattening pigs between farm types, significantly more fattening pigs were positive in the open farms.

In the open farms, clearly more farms are affected and more animals are positive. The regrouping of animals from different origins may be the cause of this, but also other factors should be taken into account since fattening pigs from closed farms are expected to be less positive compared to piglets. The reason for this discrepancy needs further investigation, looking at the factors causing colonisation differences.

The MRSA strains were as expected untypable by PFGE. All strains were of sequence type ST398. Spa-typing revealed in all but one farm t011. In one farm a combination of t011 and t034 was seen. Here 1 strain was t011 and four t034.



**Figure 21.** Distribution of tested farms according to farm type



**Figure 22.** Percentage animals positive and negative for the different age groups

**Table 18.** MRSA prevalence data in pigs

	MRSA	Non MRSA	Total	% MRSA
<b>Farms</b>	34	16	50	68
Closed farms	19	15	34	56
Open farms (fattening farms)	15	1	16	94
<b>Animals</b>				
Sows	88	252	340	26
Weaned piglets	141	199	340	41
Fattening pigs (closed farms)	87	245	332	26
Fattening pigs (open farms)	347	141	488	71

These results show that MRSA are highly prevalent in Belgian pig farms. There is clearly an age effect with piglets being more positive than sows or fattening pigs. There is also a difference in MRSA prevalence between closed and open farms, indicating that, differences in farm management are important for colonisation.

## 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 inhibition concentrations (MIC determination), phenotyping of glycopeptides susceptibility (population studies)
  - **Genotypic methods:** identification of different Staphylococci species by sequence analysis of gene *rpoB*, detection of gene *nuc* (to identify *S. aureus*), resistance gene *mecA* (coding for oxacillin resistance), resistance gene *mupA* (coding for mupirocin resistance) and of resistance genes against macrolides-lincosamides-streptogramins (MLS), tetracycline and aminoglycoside.
- Detection of genes coding for toxins: PCRs for exfoliative toxins A and B, Panton Valentine Leucocidin (PVL), Toxic Shock Syndrome Toxin-1 (TSST-1) and 18 enterotoxins among which sea, seb, sec, sed, see, seg, seh, sei, selj, selk, sell, selm, seln, selo, selp, selq, selr.
- Molecular typing, genomic micro restriction by Pulsed Field Gel Electrophoresis (PFGE), by *spa* sequence and typing of the Staphylococcal Chromosome Cassette *mec* (SCC*mec* typing), detection of the *agr* group, detection to 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 of patients with wound, skin or other clinical infections (arthritis, peritonitis, respiratory tract infections) or Staphylococcal strains originating from local epidemiological studies.

In the period 2006 – 2007 the NRL participated at a survey on the clonal diversity of *S. aureus* isolates (MRSA and MSSA) originating from invasive infections. This survey, as part of the EARSS programme (European Antimicrobial Resistance Surveillance System), was in collaboration with the Epidemiology Division of the IPH. The aim of this study was to identify the spread of predominant clones of *S. aureus* in Europe and to visualize this spread by mapping. Detailed information on the results of this EARSS programme is available on the website <http://www.earss.rivm.nl/>.

In 2007, the NRL participated at the first national prevalence study of the specific MRSA ST398 clonal lineage in swine and their caretakers. This study was executed in collaboration with IPH, VAR, DGZ Vlaanderen and the BAPCOC working group to determine the prevalence and the risk factors of MRSA type ST398 carriage by professionals and their environment and to compare human and animal isolates based on the polymorphism of the gene coding for protein A (*spa* typing). Some results of this study are mentioned in *MRSA in pigs*.

## Conclusions

The number of reported PVL-positive MRSA strains, 25 à 30 a year, remain stable since 2005. These strains belonged principally to clone MRSA ST80-SCCmec IV, a predominant strain in Europe. These PVL-positive MRSA-strains caused mainly infections of the skin and some non-lethal invasive infections (bacteraemia, osteomyelitis).

In 2007, the resistance percentage of *S. aureus* against oxacillin, isolated from patients with bacteraemia hospitalised in Belgium, stabilised at about 23% as in 2006 and this after a decrease of the important prevalence peak in 2004 (33%). This information corresponded to the results of a national surveillance programme realized by IPH. Since 2004 a decline of almost 50% of MRSA strains between all clinical isolates of *S. aureus* (from 34 hospitals in 2007) was found.

Within the framework of the European survey on clonal diversity of the *S. aureus* strains responsible for invasive infections, the combined genotyping method spa typing/SCCmec indicated the presence of 4 important genotypes within the MRSA-strains, which represented more than 90% of all strains. These genotypes are: spa CC008-ST8-SCCmec IV (pulsotype A20), spa CC740-ST45-SCCmec IV (pulsotype B2), spa CC002-ST5-SCCmec IV (pulsotype C) and spa CC002-ST5-SCCmec II (pulsotype G). MSSA-strains displayed a more extensive clonal diversity.

Concerning MRSA-strains of health care centers, genotyping of these strains coming from local epidemiological surveys and associated to MRSA-strains of epidemic/clustered cases in hospitals, mainly belonged to three epidemic nosocomial clones A20, B2 and G10.

Since 2003, MRSA clone ST398 strains of animal origin are regularly isolated in human patients. This MRSA-strain spread from animals to humans. This spread occurred particularly in the Flemish Region where an intensive farming of pigs takes place. The swine population appeared to be highly colonized by the porcine-related clone ST398 (see *MRSA in pigs*). Not only spread from animals to humans but also between humans may occur. This ST398 clone was also isolated from a number of other animal species such as bovine, equine, poultry and pets.



## Q-fever

David Fretin, Marjan Van Esbroeck, Luc Vanholme

### 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 spread the disease 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 secreta 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* does not usually 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 diagnosis 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 2007, the NRL analyzed 220 sera of bovine animals, 1 serum of a sheep and 1 serum of a dog. In total 73 bovine sera were positive. Also the serum of the dog tested positive. The clinical suspicion of the sheep could not be confirmed by analysis.

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

- Public education and information on sources of infection
- 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 and laboratories used in housing potentially infected animals
- Put aborted animals in quarantine
- Analysis of placenta and aborted foetuses in case of any abortion
- Appropriate disposal of placenta, birth products, foetal membranes and aborted foetuses
- Use only pasteurized milk and milk products
- Infected holding facilities should be located away from populated areas. Measures should be implemented to prevent airflow to other occupied areas.

## 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 milk or dairy products leads to infection and seroconversion but rarely to clinical symptoms.

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. The spontaneous evolution is usually a complete recovery but in immunocompromised hosts a chronic infection can develop with endocarditis as the major clinical form.

Consumption of pasteurized milk or raw milk only from Q-fever free herds as well as proper hygiene when in contact with infected animals are the best preventive measures.

In the ITM (NRL), a total of 1656 human sera have been examined for the presence of phase I and II IgM and IgG antibodies to *Coxiella burnetii* by IFAT (Focus Technologies).

Fourteen probable cases have been detected in 2007. Additionally 51 possible cases have been found merely on the basis of one serological result (due to the lack of follow-up samples) and without clinical information.

The age of the 14 patients with a probable infection ranged from 20 to 68 years with a median age of 44.7 years. Ten patients (71%) were male. Four patients stayed abroad before the start of their illness (Egypt (2), Democratic Republic of Congo, Senegal). The travel history of the remaining 10 patients is not known.



# Salmonellosis

Sophie Bertrand, Patrick Butaye, Katerijne Dierick, Hein Imberechts, Luc Vanholme, Karen Vereecken, 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 characterized 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 are 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

Each year, an official monitoring for the detection of Salmonella in compound feedingstuffs and in raw materials is organised by the Federal Agency for the Security of the Food Chain. Microbiological testing on 25g samples is done in the FASFC laboratories. In case of isolation of Salmonella in official samples no certification is provided.

Out of 68 samples of feed materials of animal origin (e.g. dairy products, meat and bone meal, poultry offal meal, feather meal, blood meal, animal fat and egg powder), none was found positive. Sixty nine fish meal and fish oil samples were analysed in 2007, all results were negative.

One out of 104 samples from feed materials of oil seed origin was found contaminated (with *S. typhimurium*).

In addition, 405 compound feedingstuffs were tested. Only two compound feed for poultry were found contaminated with Salmonella (*S. Agona* was identified).

# Salmonella in poultry

## Salmonella in breeders and hatcheries

### Surveillance programme in breeding flock

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

All breeder flocks are routinely examined for Salmonella at delivery as day-old birds (imported and domestic flocks). At the farm, pieces (5 by 5 cm) of the inner linings of the delivery boxes of the day-old chickens 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. Breeding chicks during the rearing period are sampled at the age of 16 weeks by technicians of DGZ and ARSIA. For this purpose, a pooled faecal sample of 60 x 1g or, alternatively, 2 pairs of overshoes is taken. Technicians of DGZ and ARSIA also officially sample all breeding hens in production; i.e. a pooled faeces sample of 60 x 1g, or 2 pairs of overshoes every six weeks. In addition, every two weeks each flock is sampled on mandatory basis with 2 pairs of overshoes by the owner.

All samples are immediately analysed in the laboratories of DGZ or ARSIA according to ISO 6579:2002 FDAM.

The official programme also controls the hygiene level of hatcheries 4 times a year. This is done during visits of the technician at non-hatching days and comprise 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 cm<sup>2</sup> in order to facilitate comparisons. In addition, a specific Salmonella control is done 4 times a year, on pooled samples from dead-in-shell chicks and on fluff and meconium. These samples are sent to the laboratory by the owner.

In 1999 the royal and ministerial decrees concerning the sanitary qualification (Gezondheidskwalificatie - Qualification sanitaire, Royal Decree of 10 August 1998, Ministerial Decree of 19 August 1998) came into force. They prescribe minimal requirements for infrastructure and general hygienic measures including specific sampling for Salmonella detection on farms with more than 5 000 birds. Thus, all poultry flocks before arrival at the slaughterhouse (i.e. breeders, layers and broilers) undergo a bacteriological examination.

### 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 one-day-old chickens, at 16 weeks (rearing) or at the occasion of one of the official samplings during production. If at least one sample in a flock is positive, the whole flock is considered as positive.

Confirmatory samples (5 faeces samples and 2 dust samples) during rearing or production may be requested by the farmer, and are taken by the competent authority. The results of these analysis are binding.

The isolation of zoonotic Salmonella is notifiable since January 2004 and should be reported to the FASFC.

Several measures are taken on the positive breeder flock: the hatching eggs are no longer incubated, but are removed and destroyed, and not yet incubated hatching eggs may be pasteurised. In addition, positive flocks are logistically slaughtered within the month. After removal the houses are thoroughly cleaned and disinfected and a Salmonella control is performed using 2 samples of 25 swabs.

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 2007 surveillance

In 2007, 206 rearing flocks and 496 production flocks were tested. 2 rearing flocks were positive for Salmonella (1 for *S. Enteritidis*, 1 for *S. O3,10*) and 19 flocks in production were positive for Salmonella of which 1 for *S. Enteritidis*, 3 for *S. Typhimurium*, 2 for *S. Hadar*, 4 for *S. Worthington* and 1 flock for each of the following serotype: *S. Braenderup*, *S. Coeln*, *S. Havana*, *S. Mbandaka*, *S. Meleagridis*, *S. Montevideo*, *S. Rissen*, *S. Senftenberg* and *S. O4:t*.

## Salmonella in layers and broilers

### Surveillance programme in commercial poultry flocks

The national control programme for Salmonella in layers and broilers is performed according to the sanitary qualification act, which is applicable to farms with more than 5 000 birds. Sampling is done by the farmer and consists of an exit sample for Salmonella, within 3 weeks of slaughter. The owner can sample in 3 ways: (1) pooled faeces (60 x 1g) taken with swabs, (2) a pooled faeces (60 x 1g) taken by hand, or (3) two pairs of overshoes. All samples have to be examined by an accredited laboratory within 48h.

In addition, layer and broiler flocks may be sampled as day-old chicks at the farm (entry control). In this purpose, the owner samples pieces of inner linings of the delivery boxes in the same way as is done for breeder flocks. After transport of reared layers to the production unit, a 60 x 1g faecal sample may be taken from the delivery boxes. Every flock is sampled taking into account the different origins of rearing.

As of July 2007, all layer flocks on farms with more than 199 laying hens are sampled every 15 week during production with two pairs of overshoes (pooled) and at the end of rearing.

#### Case definition, notification, sanitary measures and vaccination

A poultry layer flock is declared positive if *S. Enteritidis* is isolated at one day of age, during rearing or during production. In addition, the flock is positive if *Salmonella* belonging to any serotype is isolated within 3 weeks before slaughter. As for broilers, a flock is declared positive if in one of the samples *Salmonella* is isolated. *Salmonella* is notifiable to the FASFC since January 2004.

In case of positive findings in layers, the poultry house must be cleaned and disinfected after removal of the positive flock and eggs are placed on the market as B-eggs for heat treatment. If *Salmonella* was detected in a broiler flock at 3 weeks before slaughter, the birds were slaughtered at the end of the day (logistic/sanitary slaughter).

Vaccination against *S. Enteritidis* is mandatory for layers. Both attenuated and inactivated vaccines are available.

#### Epidemiological investigations and results of 2007 surveillance

In laying hen flocks within 3 weeks before slaughter, 17 out of 340 samples were positive for *Salmonella*, corresponding to 17 out of 307 flocks (5,54%) and 16 out of 175 farms. Serotype data were not available; see results from NRL. As of July 2007, 109 flocks were sampled at 16 weeks, 4 were positive for *Salmonella* spp, of which 2 for *S. Enteritidis*, 1 for *S. Mbandaka*,

1 not typable and 1 for *Salmonella* vaccine strain. During production, 378 flocks were sampled, 21 were positive for *Salmonella* spp (*S. Enteritidis* 11, *S. Braenderup* 2, *S. Livingstone* 1, *S. Mbandaka* 1, *S. Senftenberg* 1, *S. Virchow* 1, *S. Infantis* 1, *S. Typhimurium* 2, *S. O6,7* 1, not typable 2).

As for broilers, three weeks before slaughter 275 (3,12%) of 8809 flocks were found to be contaminated with *Salmonella*, corresponding to 123 out of 978 farms. Serotype data were not available; see results from NRL.

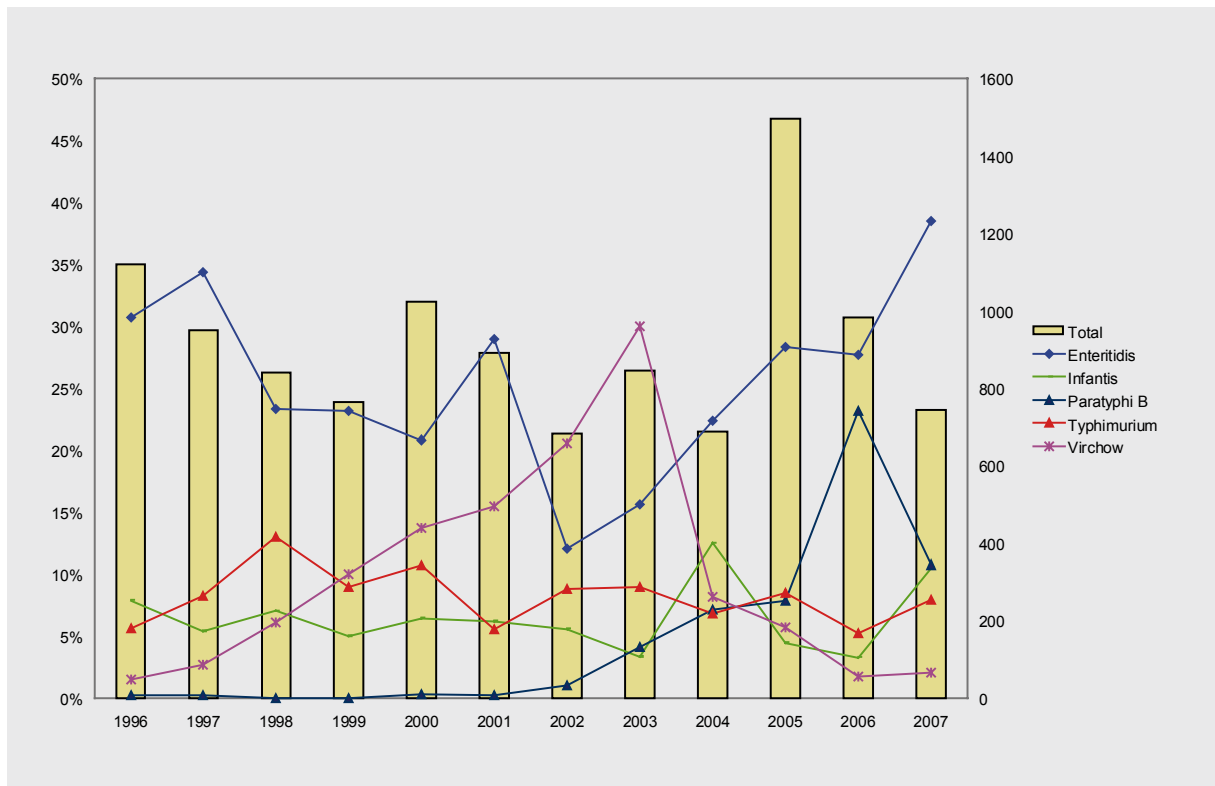
Laboratory findings of the NRL show that in total 745 *Salmonella* strains from poultry origin were analysed in 2007, which is 24% less than in 2006, when the European coordinated monitoring programme in broilers was organised.

The proportion of serotype *Enteritidis* (38,5%), *Infantis* (10,5%) and to a lesser extent *Typhimurium* isolates (7,9%) increased as compared to 2006 (27,7%, 3,3% and 5,2%, respectively); that of *S. Paratyphi B* decreased from 23,2% in 2006 to 11,3% in 2007.

The origin of 336 *Salmonella* poultry isolates was known in more detail. Only 15 strains were from breeders, and serotype *Enteritidis* was once identified. Three isolates belonged to serotype *Typhimurium*. The majority of layer isolates (n=36) were *S. Enteritidis* (44,4%), but also serotypes *Braenderup* (11,1%) and *Typhimurium* (8,3%) were found. In addition, 3 *S. Gallinarum* strains were typed. The majority of broiler isolates were *S. Enteritidis* (64,2%), and also serotype *Infantis* (9,8%) was frequently identified. Other frequent serotypes were *Paratyphi B* (all var. Java) (7,7%) and *Typhimurium* (6,7%).

Yearly, the number of poultry isolates sent to the NRL was approximately 700 to 1 100, except for 2005 when the European coordinated monitoring among layers caused a significant rise of isolates (almost 1 500 in total). Among poultry, *S. Enteritidis* is still the most prevalent serotype in Belgium, and its proportion is unmistakably raising, reaching its highest

value in 2007. The proportion of *Typhimurium* strains fluctuates between 5.2% and 13.0%. The raise of *S. Virchow* strains in 2000-2003 has come to an end, whereas *S. Paratyphi B* and eventually *S. Infantis* may become more important, especially in broilers (Figure 23).



**Figure 23.** Evolution of the percentages of the principal *Salmonella* serotypes isolated from poultry between 1996 and 2007 - 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 (see before). Sanitary Qualification A is mandatory for all commercial breeding flocks. Flocks are at least sampled as day-old chickens, 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 be sampled also.

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 analyzed separately according to ISO 6579:2002.

At 26 weeks, 60 blood samples were taken of each breeder flock. If one or more blood sample are positive, faecal samples are taken to confirm the results. The owner 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.

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.

From October 2006 to September 2007, the European coordinated monitoring of turkey flocks was undertaken according to article 5 of Directive 2003/99/EC. Details of a report of this baseline study on the prevalence of Salmonella in turkey hen flocks can be found at the website of the European Food Safety Authority) (<http://www.efsa.europa.eu>).

### Case definition, sanitary measures and vaccination policy

A turkey flock is considered positive if zoonotic Salmonella serotypes were isolated. Measures are taken only at time of slaughter: if the flock is Salmonella positive, 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 2007

For meat turkey flocks following results were found which were not a part of the baseline study: 51 flocks were sampled at the end of production of which 4 were positive for Salmonella spp.

12 flocks of guinea fowl were tested. One was positive for Salmonella spp.

Data from the NRL Salmonella indicate a high number of turkey strains (n=74) due to the European coordinated monitoring. Of these, 41.9% were *S. Paratyphi B* strains, of which only 1 isolate was tartrate positive (var. Java) and 30 tartrate negative. Another 41.9% belonged to serotype Kottbus, a serotype only identified among birds and poultry.

#### Salmonella in ducks and partridges

The surveillance programme for breeder animals of ducks, and for meat producing ducks is similar to that of turkeys (sanitary qualification A for breeders and B for meat production).

One meat production flock of ducks was tested, and found negative.

## Salmonella in pigs

### Serology

#### Surveillance programme in fattening pigs

Similar to former years, in 2007 the blood samples from fattening and growing pigs that were taken in the framework of the monitoring of Aujeszky's disease were also analysed for Salmonella. Blood samples from pigs were taken every 4 months. Depending on the number of pigs in the farm, 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 the same commercially available ELISA kit, following the manufacturer's instructions.

The aim of the current mandatory surveillance programme is to identify maximum 10% of pig farms with the highest Salmonella prevalence and the identification Salmonella-specific risk factors in these herds. Indeed, it is likely that those herds, when participating in the supportive control programme, will benefit the best results in terms of decreasing the risk for Salmonella infections. A herd is considered as a 'Salmonella risk herd' if 3 consecutive mean S/P ratio's are above 0,6. The S/P ratio gives a value to the presence of antibodies against Salmonella spp.

Pigs were not vaccinated in 2007, since no vaccine was authorised in Belgium.

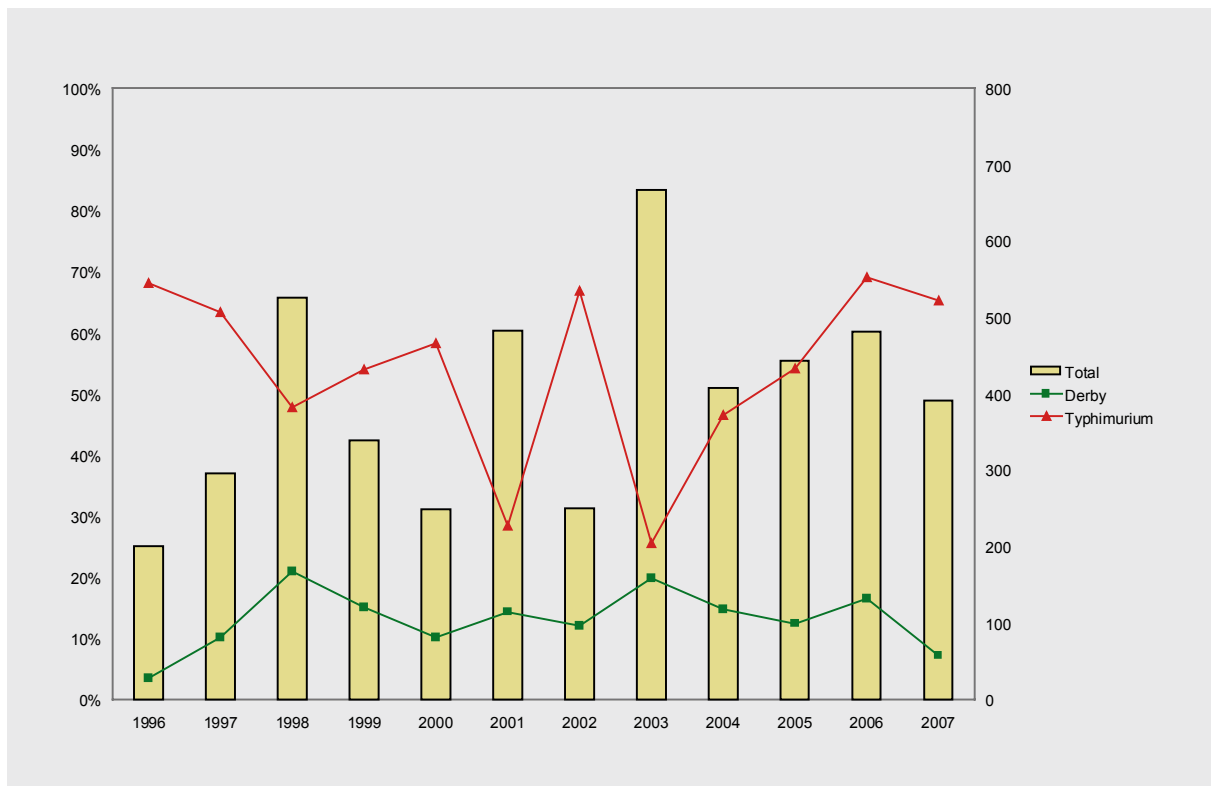
#### Results in 2007

A total of 200.697 serological analyses were performed. Of these, 33.516 samples (16,7%) had a S/P ratio above 0,6. 31% of the 6978 farms monitored had at least once a mean S/P ratio above 0,6. .

### Bacteriology

There was no surveillance system for Salmonella in pigs based on bacteriology. However, several samples were taken for research activities.

Laboratory findings from the NRL showed that *S. Typhimurium* continues to be the most prevalent serotype among pig isolates, representing more than 60% of the total number of pig Salmonella. Serotype Derby is the second most important serotype, but represents less than 10% of the strains (Figure 24). As compared to 2006, about 20% less pig strains were analysed in 2007 (n=391; n=481 in 2006).



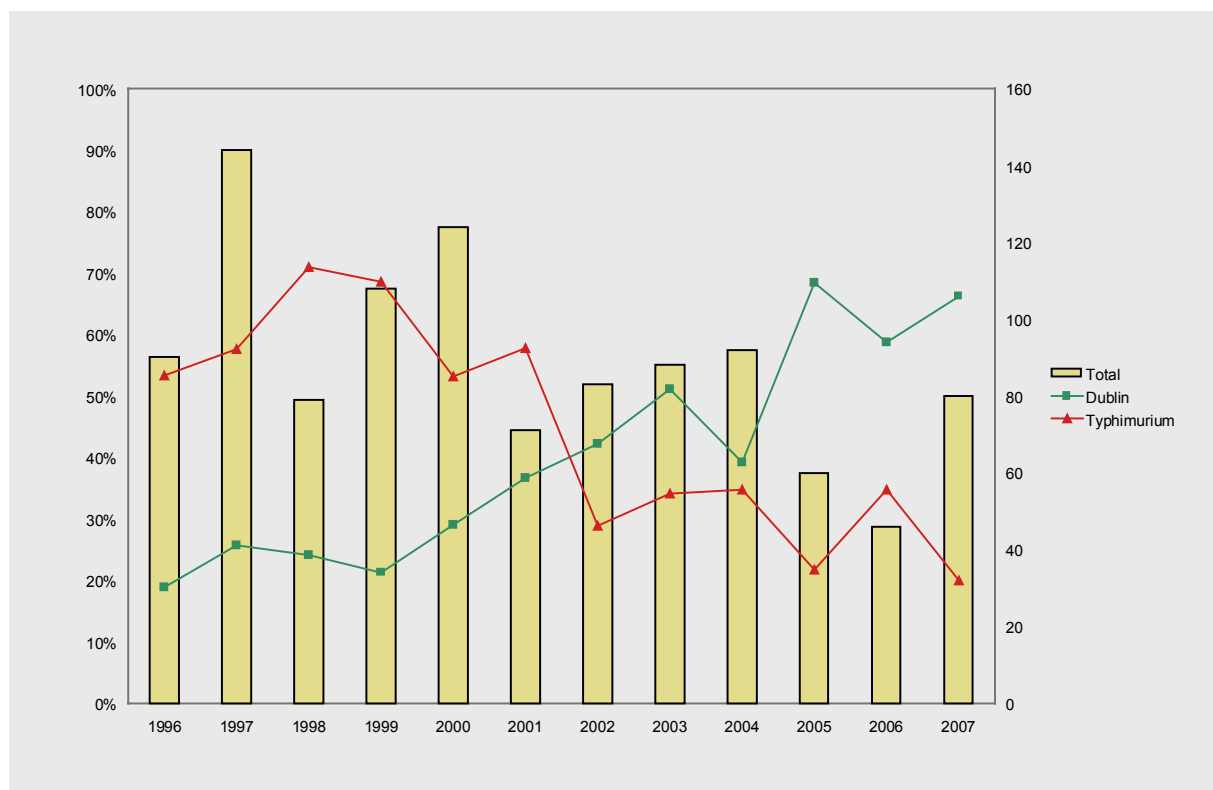
**Figure 24.** Evolution of the percentages of the principal *Salmonella* serotypes isolated from pigs between 1996 and 2007 - The bars represent the total number of pig isolates per year, and refer to the right axis; the lines represent the percentage of each serotype per year and refer to the left axis

## Salmonella in cattle

There was no official monitoring programme for Salmonella in cattle in 2007. Salmonella isolates were sent on a voluntary basis to the NRL for serotyping.

In Belgium no Salmonella vaccine is authorised in cattle.

According to the NRL, S. Dublin continues to be the principal serotype isolated in cattle since 2002, and reaching a proportion of more than 60%. S. Typhimurium (about 30%) is the second most important (Figure 25). In 2007, the number of Salmonella isolates from cattle (n=80) has almost doubled as compared to 2006 (n=46).



**Figure 25.** Evolution of the percentages of the principal Salmonella serotypes isolated from cattle between 1996 and 2007 - The bars represent the total number of cattle isolates per year, and refer to the right axis; the lines represent the percentage of each serotype per year and refer to the left axis

## Salmonella in food (meat and meat products)

### Monitoring programme

In 2007, Salmonella food 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, carcasses of spent hens 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 spent hens at slaughter consisted of neck skin. Different contamination levels were analysed: 25g, 600 cm<sup>2</sup>, 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.

### Epidemiological investigations and results of 2007 surveillance

**Table 19.** The results of the monitoring 2007 – Salmonella in meat and meat products

Species	Quantity of sample analysed	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
<b>Beef</b>				
Minced meat (steak tartare) at retail (n=128)	25g	1.6%		
Meat preparation (steak tartare with herbs and sauce) at retail (n=132)	25g	2.3%		
<b>Pork</b>				
Carcasses at slaughter (n=293)	600cm <sup>2</sup>	16.0%	Typhimurium	Derby, Anatum, Rissen, London
Trimmings at processing plant (n=411)	25g	4.1%	Typhimurium	Derby, Livingstone, London
Raw ham at retail (n=31)	25g	0.0%		
Raw ham at processing plant (n=65)	25g	1.5%		
<b>Broilers</b>				
Carcasses at slaughter (n=58)	1g	10.3%	Livingstone	Indiana, Mbandaka
Carcasses at slaughter (n=59)	25g (caeca)	13.6%	Enteritidis	Typhimurium O5+, Paratyphi var. Java
Carcasses at retail (n=145)	25g	6.9%		

Species	Quantity of sample analysed	Prevalence	Predominant serotype	Other serotypes (in decreasing order)
Meat cuts (skinned or with skin) at processing plant (n=170)	1g	6.5%	Typhimurium	Virchow, Indiana, Hadar, Montevideo, Paratyphi B
Spent hens				
Carcasses at slaughter (n=176)	0.1g	45.4%	Enteritidis	Typhimurium, Infantis, Agona
Carcasses at slaughter (n=142)	25g (caeca)	46.5%	Enteritidis	Typhimurium O5+, Paratyphi var. Java
Carcasses at retail (n=113)	25g	10.6%		
Poultry				
Meat cuts (with skin) at retail (n= 131)	25g	9.2%		
Meat cuts (without skin) at retail (n= 140)	25g	4.3%		
Minced meat at retail (n=70)	10g	13.0%		
Meat preparation at retail (n=27)	10g	11.0%		
Meat preparation at processing plant (n=53)	10g	17.0%		
Meat products intended to be eaten cooked at processing plant (n=32)	10g	0.0%		
Meat products intended to be eaten cooked at retail (n=86)	10g	5.8%		
Minced meat intended to be eaten raw (all species) at retail (n=129)	25g	6.2%		
Minced meat intended to be eaten cooked at retail (all species) (n=136)	10g	7.4%		
Meat preparation intended to be eaten raw (all species) at retail (n=47)	25g	2.1%		
Meat preparation intended to be eaten cooked (all species) at retail (n=40)	10g	2.5%		
Meat preparation intended to be eaten raw (all species) at processing plant (n=88)	25g	11.4%		
Meat preparation intended to be eaten cooked (all species) at processing plant (n=80)	10g	0.0%		
Mechanically separated meat (n=126)	10g	23.8%		

The contamination of pig carcasses and trimmings increased in 2007. The contamination of spent hen carcasses raised again in 2007. The contamination of broiler cutting meat decreased in 2007.

**Table 20.** Evolution of the food *Salmonella* prevalence, 2004-2007

	Samples	Sampling level	2004	2005	2006	2007
Pork	Carcasses	600cm <sup>2</sup>	12.3%	9.3%	7.1%	16%
	Trimmings	25g	10.4%	7.3%	2.4%	4.1%
Broilers	Carcasses	1g	7.9%	5.7%	1.4%	10.3%
	Fillets	25g	20.6%	14.2%	13.3%	7.4%
Spent hens	Carcasses	1g	19.6%	22.6%	35.6%	45.4%

## Salmonella in other food of animal origin

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

- at retail: dried follow-on formula (n=38), nursing bottles (n=119), cheese of pasteurised milk (n=122), cheese of pasteurised sheep's milk (n=19), cheese of pasteurised goat's milk (n=20), cheese of raw milk (n=81), cheese of raw sheep's milk (n=10), cheese of raw goat's milk (n=10), butter of raw milk (n=16), milk desserts (n=40), ice cream (n=58)
- at processing plant: cheese of raw milk (n=78), cheese of pasteurised milk (n=118), milk desserts (n=35), milk powder (n=20)
- at farm: cheese of raw milk (n=76), goat's or sheep's cheese of raw milk (n=17), butter (n=71), ice cream (n=29), raw

goat's, sheep's or horse's milk (n=34), raw cow's milk (n=59), cream (n=19)

All results were negative.

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

- at retail: fresh fish (n=62, 1.6%), cooked molluscs (n=60), cooked crustaceans (n=29), meat salad (n=44), chicken salad (n=47), crustacean salad (n=49), bakery products with cream (n=82), live bivalve molluscs (n=60), raw shrimps (n=31), chocolate (n=30), confectionary with chocolate (n=30), table eggs (n=117), liquid egg products (n=76), powdered egg products (n=24), salty preparation based on raw eggs (n=58), dessert based on raw eggs (n=115)
- at processing plant: gelatine (n=10), cooked crustaceans and molluscs (n=67), raw shrimps (n=32), sandwich spreads (crustacean, meat, chicken) (n=159), fresh fish (n=30), chocolate (n=15) and confectionery (n=24), bakery products with cream (n=44), egg products (n=82)

All results were negative, except for fresh fish at retail (1.6% positive), crustacean salad at retail (2% positive), live bivalve molluscs at retail (1.7%), liquid egg products at retail (1.3% positive), salty preparation bases on raw eggs at retail (1.7% 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 Virchow. Two additional serotypes (Brandenburg and Derby) were also randomly sampled, all isolates of *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 Enter-net international surveillance network) and helped detecting outbreaks and targeting preventive strategies.

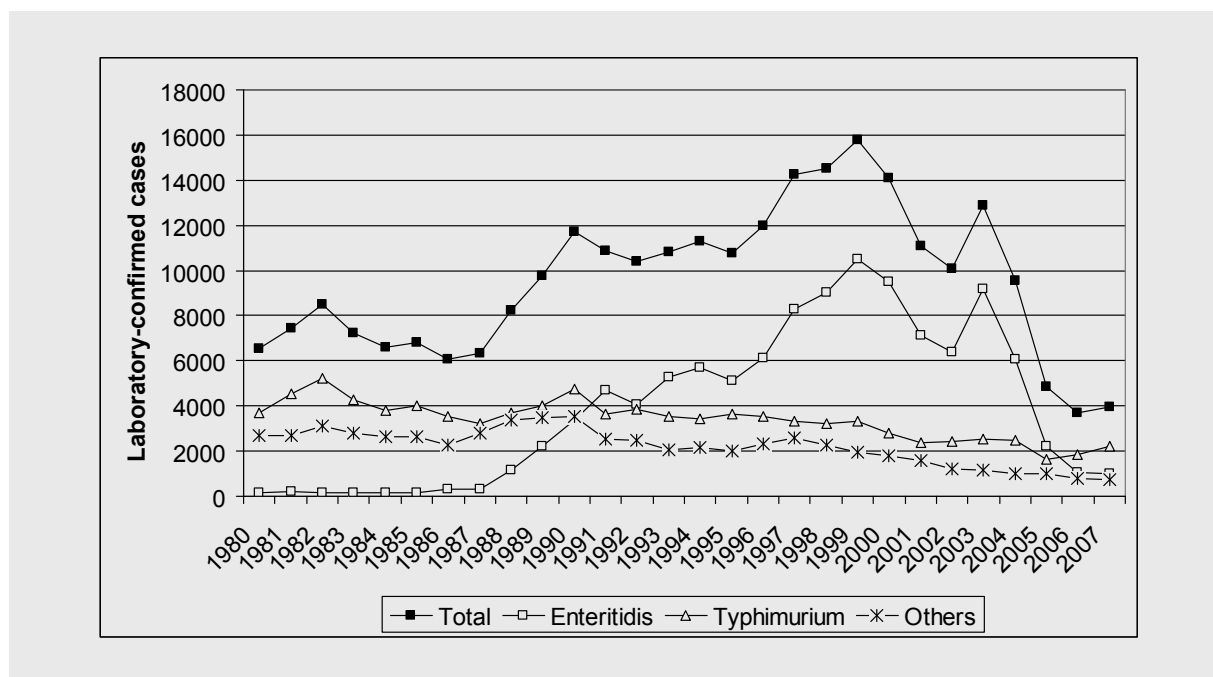
### Epidemiological investigations and results of 2007 surveillance

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 26, Table 21). In that year, exceptionally high numbers of *S. Enteritidis*. Between 2000 and 2007, the total number of laboratory-confirmed cases varied between 14,088 and 3,975 (Table 21). 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 Salmonella Enteritidis infections compared with the annual number of cases in the period 2000-2004 was recorded. This decrease persisted in 2007 where the total number of cases caused by Salmonella spp. and by *S. Enteritidis* decreased to 3693 and 987 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 21). 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 37 and 38 cases in 2006 and 2007, respectively. 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 vs about 29 from 2003 to 2007) cases was noted over the last years. Similarly, the number of *S. Derby* cases is shrinking since the beginning of 2000 but remained stable over the period 2004-2007.

**Table 21.** Trends for the most prevalent *Salmonella* serotypes from 1986 to 2007

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
<b>Total</b>	6092	6360	8247	9752	11695	10891	10391	10840	11294	10754	12008	14239	14514	15774	14088	11065	10075	12792	9543	4916	3693	3975
<b>Enteritidis</b>	298	320	1163	2236	3382	4721	4084	5260	5700	5138	6145	8284	9003	10492	9503	7112	6398	9118	6075	2226	1052	987
<b>Typhimurium</b>	3512	3233	3699	4018	4756	3652	3835	3528	3418	3623	3522	3347	3221	3348	2799	2370	2438	2486	2459	1659	1826	2233
<b>Others</b>	2282	2807	3385	3498	3557	2518	2472	2052	2176	1993	2341	2608	2290	1934	1786	1583	1239	1188	549	691	815	596
<b>Derby</b>	131	169	168	177	161	134	139	103	113	107	118	157	162	138	169	158	92	100	64	67	52	64
<b>Brandenburg</b>	167	151	159	255	302	176	161	147	204	241	214	296	274	279	322	200	148	66	63	76	47	29
<b>Virchow</b>	152	170	235	293	302	224	295	273	308	245	178	114	115	86	147	143	132	152	91	65	46	28
<b>Infantis</b>	168	173	168	275	249	224	225	160	150	174	267	263	180	169	120	126	74	52	107	58	37	38

**Figure 26.** 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

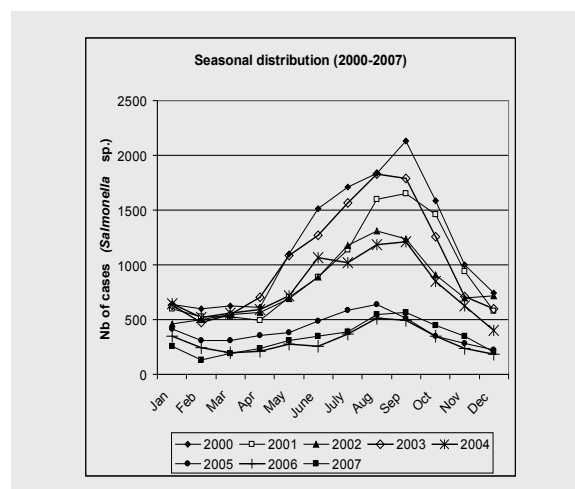
## Age and seasonal distribution

Most cases of salmonellosis were reported in children less than 5 years old (48.4% of cases), with no significant gender difference

**Table 22.** Human cases of *Salmonella*: Age and gender distribution 2007. 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	330	162	163	1.0	58	28	27	1.0	168	75	91	0.8
1 to 4 y	1594	788	780	1.0	313	163	147	1.1	1114	541	551	1.0
5 to 14 y	685	367	314	1.2	215	128	86	1.5	409	212	195	1.0
15 to 24 y	176	80	87	0.9	38	21	17	1.2	89	42	43	1.0
25 to 44 y	306	146	155	0.9	109	45	62	0.7	79	42	35	1.2
45 to 64 y	291	155	132	1.2	101	55	43	1.3	96	58	37	1.6
≥ 65 y	374	155	208	0.7	99	35	59	0.6	159	77	78	1.0
unknown	291	84	94	0.9	54	20	29	0.7	119	47	45	1.0
<b>Total</b>	<b>3975</b>	<b>1937</b>	<b>1933</b>	<b>1.0</b>	<b>987</b>	<b>495</b>	<b>470</b>	<b>1.0</b>	<b>2233</b>	<b>1094</b>	<b>1075</b>	<b>1.0</b>

Regarding the seasonal distribution (Figure 27), about 200 to 400 cases were monthly reported between January and July 2007. From August until September, the monthly number of isolates increased, to reach about 500 isolates. From October to December, the monthly number of isolates gradually decreased.



**Figure 27.** Seasonal distribution 2002 – 2007

## Antimicrobial resistance

### Antimicrobial resistance in isolates from living animals

#### Methods used

Antibiotic susceptibility data of Salmonella strains from livestock came from the NRL. Tests were performed by the disk diffusion test, using Neo-Sensitabs (Rosco). Tests and interpretation were done according to the manufacturers guidelines using an inoculum and breakpoints as described by CLSI (Kirby-Bauer). Internal control was performed with quality control strain E. coli ATCC25922. Results were accepted when results with the QC strain were within the limits as proposed by Rosco.

**Table 23.** 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
Sulfonamides	240µg	20 - 22
Trimethoprim - Sulfonamides	5.2µg + 240µg	27 - 31
Nalidixic acidid	130µg	21 - 24
Enrofloxacin	10µg	20 - 22
Chloramphenicol	60µg	21 - 24
Florfenicol	30µg	15 - 18

#### Epidemiological investigations and results of 2007 surveillance

The susceptibility of 891 Salmonella isolates was tested in 2007. Within the same LIMS dossier only one isolate belonging to the same serotype was selected for susceptibility testing, and therefore strains were likely to be independent from each other. The antimicrobials used are mentioned in Table 23.

A total of 550 Salmonella isolates (61.7%) were fully susceptible to all antimicrobial drugs tested. Most resistance was found against Ap (27.6%), Su (26.8%), Tc (24.8%), St (24.5%), but also against TSu (16.6%) and Nal (11.6%). Seventy-two strains were found resistant against Cm (8.1%); about 57% of these isolates were also resistant against Ff. Moreover, twenty-nine isolates were found Cef resistant (3.3%). Most of the cephalosporin resistant strains originated from poultry (n=26) (14 S. Infantis, 6 S. Paratyphi B var. Java) and two from pigs. In addition, five Enr resistant strains (0.6%) (two bovine S. Typhimurium, two S. Paratyphi B from poultry and one S. Dublin) were detected. Finally, four strains were resistant to neomycin and one to gentamicin.

Most (88.6%) S. Agona isolates (n=35) were fully susceptible for all antimicrobials tested. Three strains were multiresistant and had the profile Ap St Tc Su Cm Ff.

Most of S. Derby strains (n=21) were sensitive (71.4%), although some resistance against Tc (23.8%), Su (19.0%), St (14.3%) and Ap (14.3%) was noticed.

As for S. Dublin isolates (n=31; most from cattle), 41.9% were found completely susceptible. Resistance against Su (48.43%), Cm and St (both 45.2%) and Nal (32.3%) was noticed.

Most *S. Enteritidis* isolates (n=105) were susceptible (95.2%). Resistance was only found against Ap (3.8%; 4 isolates) and against Nal (1.0%).

All *S. Hadar* (n=19) strains were found resistant against Nal (100%). In addition, Tc (94.7%) and St (63.2%) were frequently found, and to a lesser extent against Ap (26.3%). Twelve isolates (63.2%; 12 isolates) were resistant to Nal St and Tc.

Less than 10% of the *S. Indiana* strains (n=31) were fully susceptible. Twenty-six (83.9%) of these strains had the profile Ap St Tc Su TSu.

About three quarter (76.3%) of the *S. Infantis* strains (n=59) were susceptible. All resistant strains (n=14) originated from poultry and were Ap and Cef resistant. Few strains showed co-resistance to St, TSu, Su and Tc.

As for *S. Paratyphi B* (n=33) almost only tartrate positive (i.e. var. Java; n=30) strains were tested. Only 10% of variety Java were sensitive. Resistance was mainly observed against Su (76.7%), TSu (73.3%), St (70.0%) and Nal (66.7%). Also resistance against Ap was frequently observed (56.7%), and six strains (all from poultry) were Cef resistant. All three *S. Paratyphi B*, tartrate negative isolates (2 from poultry, one from turkeys) had the profile Ap Su TSu Nal.

Forty-four percent of *S. Typhimurium* isolates (n=248) were found susceptible; classic variant (O5+) strains were found slightly more often susceptible (45.3%) than Copenhagen variant (O5-) isolates (41.6%). Pentaresistance Ap St Tc Su Cm

was encountered in 13.8% and 20.2% of O5+ and O5- isolates, respectively. Cef resistance was found in one poultry O5+ and one pig O5- isolate.

Only 18.2% of the *S. Virchow* isolates (n=11) were susceptible to the antimicrobials tested. Most resistance was found against Nal (72.7%) and Ap (54.5%).

Some strains belonging to other serotypes were also tested, but to a lesser extent. Most of these isolates were fully sensitive for all the antimicrobials tested.

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

During 2007, all 1009 strains of *Salmonella enterica* isolated during the zoonosis monitoring program from different matrices like poultry (broiler meat and carcasses from spent hens) pork, beef and other foods (shrimps, milk, RTE food, e.g.) were sent to the IPH for serotyping and determination of antimicrobial resistance. Minimum Inhibitory Concentrations (MIC) were determined by the use of E-test (n=638) for most isolates. In view of the harmonisation of the testing at European level sensititre (n= 371) was used for the second part of the strains as recommended by the CRL antibiotic resistance. The antimicrobials tested were ampicillin, ceftriaxon, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline and trimethoprim and gentamicin. Interpretation of the results was according to CLSI. Quality control was performed by using an *Escherichia coli* ATCC 25922 strain. Breakpoints used are listed in the following table.

**Table 24.** *Salmonella* from food: list of antimicrobials tested with their breakpoints

Antimicrobial	Breakpoints (µg / ml)
Ampicillin	16
Ceftriaxon	2
Streptomycin	32
Kanamycin	64
Tetracycline	16
Sulfamethoxazol	256
Trimethoprim	16
Nalidixic acid	32
Ciprofloxacin	4
Chloramphenicol	32
Gentamicin	4

The level of resistance of *Salmonella* isolates from broilers and pork is influenced by the serotype distribution in the corresponding matrix. The presence of highly resistant serotypes as Hadar, Virchow, Paratyphi B and Typhimurium contributed mainly to the high resistance levels in some matrices. The results for poultry (broiler and carcasses from spent hens), pork and beef are summarized in the next table. Other matrices where *Salmonella* was isolated were frog's legs, shrimps, ready to eat dishes, mussels, milk powder, eggs and animal feed. In total about 60 different serotypes were identified, with *S. Enteritidis* being the most prevalent serotype mainly isolated from carcasses of spent hens. For broiler meat and poultry meat a large variation in serotypes were identified with Typhimurium, Paratyphi B and Virchow as most prevalent. Typhimurium and Derby are the most prevalent *Salmonella* serotypes isolated from pork samples.

**Table 25.** Antimicrobial susceptibility testing of *Salmonella* spp. isolated from different food matrices: percentage of resistant strains

Antimicrobial tested	Poultry meat Broiler (n=341)	Poultry meat Spent hens (n=118)	Pork (n=343)	Beef (n=22)
Ampicillin	52	19	42	14
Ceftriaxon	16	3	3	0
Streptomycin	39	12	36	18
Kanamycin	3	0	1	0
Tetracycline	26	10	45	23
Sulfamethoxazol	42	14	45	23
Trimethoprim	45	10	22	14
Nalidixic Acid	33	8	3	0
Ciprofloxacin	0.3	0	0	0
Chloramphenicol	5	8	14	4

#### Antimicrobial resistance in strains isolated from poultry meat

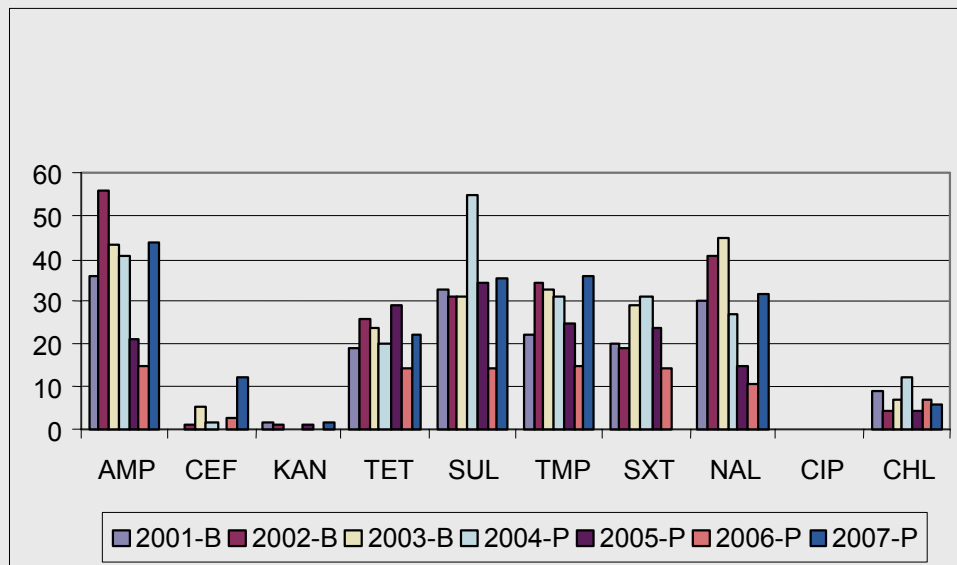
In 2007, 459 *Salmonella enterica* isolates from poultry meat were tested for their antimicrobial susceptibility. Of all tested strains 35% were sensitive for all tested antibiotics. Most resistance was found to ampicillin (44%), streptomycin (36%), trimethoprim (36%), sulfamethoxazole (35%), nalidixic acid (32%) and tetracycline (22%). Chloramphenicol resistance was observed in 6% of the *Salmonella* strains isolated from poultry meat. The resistance against the 3rd generation cephalosporin ceftriaxon was very high with 57 (12%)

resistant *Salmonella* strains. Especially the serotype Paratyphi B expresses resistance against ceftriaxon. For ciprofloxacin only one strain was resistant and kanamycin resistance was observed in 2% of the strains. In comparison with 2006 the percentage of resistance increased considerably for almost all the antibiotics tested except for chloramphenicol where a slight decrease in the resistance was noticed.

For 2007, 80 *S. Enteritidis* isolates from poultry meat were tested for their susceptibility to all antimicrobials. The resist-

ance in this serotype is very low as was found in previous years. Only two strains showed resistance, one against ampicillin and the other strain against streptomycin and nalidixic acid.

From the 52 *S. Typhimurium* isolates from poultry meat 31% were sensitive to the tested antibiotics. A high degree of multi-resistance was observed with AmpStrSulTetTrmp (14%) and AmpStrSulTet (11%) as most prevalent antibiotic resistance profiles.

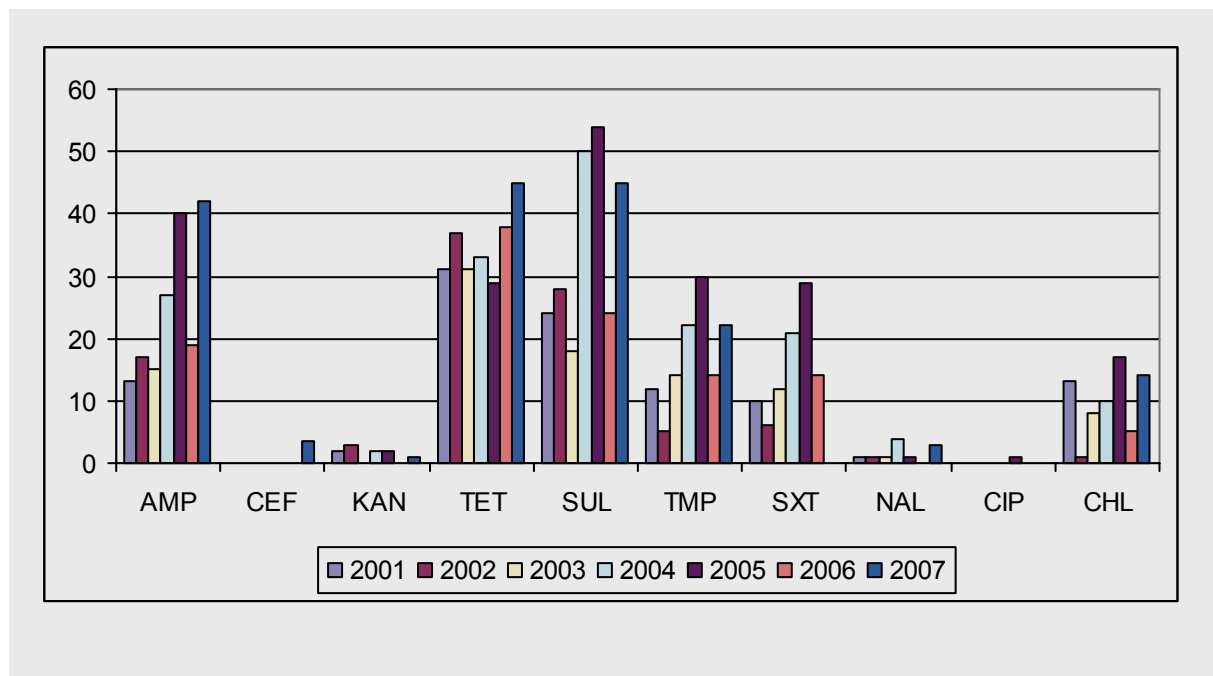


**Figure 28.** Percentage resistant *Salmonella* strains in broiler meat (2001-2003) and poultry meat (2004-2007)

### Antimicrobial resistance in strains isolated from pork

In total 343 *Salmonella* strains from pork were tested for their susceptibility. *S. Typhimurium* (178) and *S. Derby* (69) were the two most frequently isolated serotypes from pork. In total 57% of the strains were sensitive to all tested antibiotics. A high degree of resistance was determined for tetracycline 45%, sulfamethoxazole 45%, ampicillin 42% and streptomycin 36%. No resistance was noticed to ciprofloxacin. Chloramphenicol resistance was observed in 14% of the strains.

Low percentages of resistance were observed for kanamycin 2%, ceftriaxon 3.5% and nalidixic acid (3%). Multiresistance was observed in 22% of the strains (> 4 antimicrobials). Compared to 2006 a general increase in antimicrobial resistance was observed. 2007 was also the first year that resistance against the third generation cephalosporins was observed in the serotypes Paratyphi B (n=8), Typhimurium (n=2), Gold-coast and Derby.



**Figure 29.** Percentage resistant *Salmonella* strains in pork (2001-2007)

*S. Typhimurium*: very high resistance percentages were observed for ampicillin (63%) sulphonamides (63%), streptomycin (55%) and tetracycline (57%). Four strains resistant to kanamycin were detected but these strains were multi resistant with the following resistance profile was observed AmpChlKanStrSulTetTrmp.

*S. Derby*: In general a lower percentage of antibiotic resistance is observed in *S. Derby* strains compared to *S. Typhimurium* strains isolated in pork. Resistance against tetracycline (26%) sulphonamide (20%), streptomycin (19%) and ampicillin (9%) were the most common observed. Only two strains were multi resistant with the profile AmpStrSulTetTrmp and AmpCefStrSulTet.

In 2007 also a European survey about the prevalence of *Salmonella* in slaughter pigs was performed to harmonise the sampling and analysis methods in the different member states. In total 137 *Salmonella* strains isolated from ileo-caecal lymph nodes (n= 72) and carcasses (n= 65) were analysed for the antibiotic resistance. *S. Typhimurium*, with 61% of the strains was the most prevalent serotype. Within the serotype *Typhimurium*, 40% belonged to the variant Copenhagen. In total 41% of the strains were sensitive to all tested antibiotics. Resistance against ciprofloxacin and gentamicin was not observed. High resistance percentages were observed for tetracycline (49%) sulphonamide (50%), ampicillin (45%) and streptomycin (39%). Resistance against cefotaxime, a 3rd generation cephalosporin, was observed in two strains: one *S. Anatum* strain with following resistance profile AmpCef, and one *S. Typhimurium* strain that was multi resistant to the following antibiotics AmpCefChlStrSulTetFlor.

## Antimicrobial resistance of human isolates

### Methods used

A total of 1039 human *Salmonella* isolates randomly selected from the six most important serotypes in 2007 (*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. Thirteen antibiotics of therapeutic or epidemiological interest were tested in disk diffusion according to Kirby-Bauer, following CLSI procedures (Table 26).

**Table 26.** 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 2007 surveillance

Resistance was mostly found to tetracycline (25.8%), sulfonamides (25.7%), ampicillin (26.5%), streptomycin (24.9%), and to a lesser extent to trimethoprim (7.9%).

The vast majority (87.2%) of human *S. Enteritidis* isolates (n=476) was fully sensitive to all antimicrobials tested.

*S. Typhimurium* (n=308) showed a high level of resistance; especially resistances to ampicillin (68.8%), sulfonamides (66.2%), tetracycline (63.3%) and streptomycin (61.7%) are striking. More than the half of the isolates (58.7%) was found resistant to four or more antimicrobial agents. In addition, almost 18.1% of the isolates showed multi-resistance to at least ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. About 76.7% of these multi-resistant isolates (ACSSuT) were of phage type DT104.

Except two strains, all *S. Hadar* isolates (n=33) were resistant to at least one antibiotic. Resistance to tetracycline, nalidixic acid, ampicillin and streptomycin reached values from 54.5% up to 87.9%. Simultaneous resistance to these four antibiotics was observed in 45.4% of these isolates. The isolates from this serotype remained fully sensitive to cefotaxime, ciprofloxacin, chloramphenicol and gentamicin.

In *S. Virchow* (n=29), multi-resistance was less common as compared to 2003 (34.5% of the strains in 2007 instead of 60% of the 2003 isolates). The highest incidence of resistance was observed for nalidixic acid (75.9%). Resistances to ampicillin, tetracycline, sulfonamides, trimethoprim and trimethoprim+sulfonamides were common (approximately

30%). One strain of *S. Virchow* showed resistance to cefotaxime.

In contrast, the vast majority of *S. Brandenburg* (n=29) and *S. Derby* (n=65) isolates remained sensitive to the vast majority of tested antibiotics: 72.4% and 83% sensitive or resistant to one antibiotic, respectively.

*S. Infantis* (N= 38) displayed in general a low level of multi-resistance.

The vast majority of *S. Paratyphi B* (n=26) and *S. Newport* (n=21) isolates remained sensitive to the vast majority of tested antibiotics: respectively 64% and 71.4% were fully sensitive to all antimicrobials tested. However, two isolates of *S. Newport* displayed resistance to at least 8 antibiotics but remained sensitive to cefotaxime and ciprofloxacin.

No tendency could be highlighted 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 2007 were comparable to those from 2002-2006.

### Phagotyping of human isolates

A total of 476 human *S. Enteritidis* isolates were phage typed. Of these, 17.2% were PT 21 and 14.7% were PT 4. In addition, 308 *S. Typhimurium* isolates were phage typed and most prevalent types were DT104 (23.6%), DT120 (16.2%), DT12 (13.6%), DT193 (7.4%), and U302 (7.4%).

**Table 27.** Antimicrobial resistance in human *Salmonella* of serotypes Enteritidis, Typhimurium, Brandenburg, Derby, Hadar, Virchow, Infantis, Typhi, Newport, Paratyphi B and A isolated in 2007.

Abbreviations antimicrobial; AMP, ampicillin; AMC, amoxicillin + clavulanic acid; CTX, cefotaxime; TET, tetracycline; CIP, ciprofloxacin; TMP, trimethoprim; SPE, Spectinomycin; AZY: Azythromycin; NAL, nalidixic acid; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SUL, sulfonamides.(ND: not determined).

	Total	N	Amp	Amx	Ctx	Tet	Cip	Tmp	Azy	Spe	Nal	Chl	Gen	Kan	Stp	Sul	Stx
Enteritidis	987	476	5,0	0,0	0,8	3,2	0,0	1,9	ND	2,3	6,9	1,1	0,0	0,2	2,9	3,2	1,9
Typhimurium	2233	308	68,8	2,9	0,0	63,3	1,0	13,6	ND	42,5	4,5	32,8	0,0	2,3	61,7	66,2	12,7
Derby	65	65	6,2	0,0	0,0	15,4	0,0	12,3	ND	4,6	3,1	0,0	0,0	1,5	9,2	16,9	12,3
Hadar	41	33	54,5	0,0	0,0	72,7	0,0	3,0	ND	0,0	87,9	0,0	0,0	0,0	78,8	3,0	3,0
Infantis	38	38	13,2	0,0	2,6	13,2	0,0	10,5	ND	13,2	13,2	0,0	0,0	5,3	13,2	23,7	10,5
Virchow	29	29	31,0	0,0	3,4	27,6	0,0	27,6	ND	17,2	75,9	6,9	6,9	3,4	13,8	34,5	27,6
Brandenburg	29	29	3,4	0,0	0,0	20,7	0,0	13,8	ND	3,4	6,9	0,0	0,0	0,0	3,4	24,1	13,8
Newport	21	21	4,8	4,8	0,0	14,3	0,0	9,5	ND	14,3	28,6	4,8	14,3	4,8	14,3	14,3	9,5
Paratyphi B	26	25	12	0	0	12	0	20	0	ND	16	0	0	0	24	12	20
Typhi	11	9	11,1	0,0	0,0	11,1	0,0	0,0	0,0	ND	33,3	11,1	0,0	0,0	11,1	11,1	0,0
Dublin	9	9	0	0	0	0	0	0	ND	55,6	22,2	55,6	0	0	55,6	55,6	11,1
Paratyphi A	6	6	0	0	0	0	0	0	0	ND	66,7	0	0	0	0	0	0

# Tuberculosis

Maryse Fauville-Dufaux, Marc Govaerts, Luc Vanholme, Maryse Wanlin

## 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 among elderly persons as a result of the reactivation of dormant *M. bovis* within old lesions and among migrants from high-prevalence countries. Agricultural workers may acquire infection of *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.

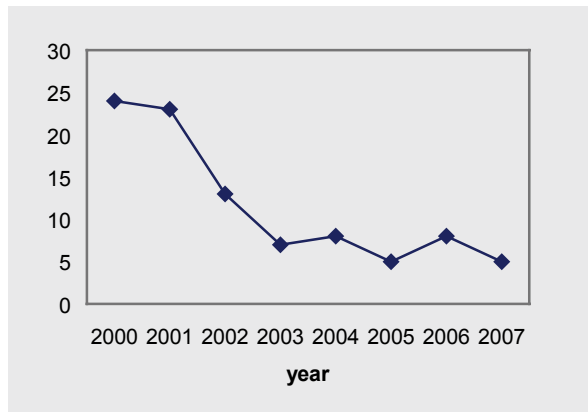
In Belgium, vaccination against tuberculosis is prohibited.

### Epidemiological investigations and results of 2007 surveillance

At the slaughterhouse, 444 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 detected in 89 animals all belonging to the outbreak herds.

**Table 28.** Evolution of bovine tuberculosis outbreaks in cattle herds in Belgium

2000	2001	2002	2003	2004	2005	2006	2007
24	23	13	7	8	5	8	5



**Figure 30.** Evolution of bovine tuberculosis outbreaks 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 Pasteur Institute Department (IPH). As a consequence, a comprehensive database of the vast majority of *M. bovis* types isolated in Belgium since 1995 is maintained.

Out of the 89 *M. bovis* isolates originating from the outbreak herds in 2007, 41 were typed by spoligotyping, 37 of which

were additionally typed by IS6110 RFLP. They belonged to 3 combined molecular types based on three distinct RFLP profiles and spoligotypes SB0162 and SB0121. One outbreak herd displayed a totally new genotype, novel to the historical 1995-2006 collection. The second genotype shared by two outbreak herds had been previously isolated (102 times) in 1995-96 and 1999-2004 from 39 breakdown herds in various parts of the country. The last genotypic profile was shared by the remaining two, geographically clustered outbreak herds, and similarly known (23 times) from 4 identically localized outbreaks in 1999-2000 and 2003.

## Mycobacterium bovis in wildlife

A number of wild life animal species (deer, wild boars, badgers) might contribute to the spread and/or maintenance of *M. bovis* infection in cattle.

In the South of Belgium, the control of wild animal diseases is carried out by "the Network of Wildlife Disease Surveillance" of the Faculty of Veterinary Medicine (University of Liège).

In 2007, targeted organs of 385 hunted wild cervids (*Cervus elaphus* and *Capreolus capreolus*), 134 found dead/shot for sanitary reason cervids, 184 hunted wild boars (*Sus scrofa*), 17 found dead/shot for sanitary reason wild boars and 4 found dead badgers (*Meles meles*) were checked for suggestive lesions of tuberculosis. Samples from 14 wild boars showing suspect lesions were sent to the NRL for analysis but none proved culture positive for *Mycobacterium bovis*.

One tigon (*Panthera tigris* x *Panthera leo*), one leopard (*Panthera pardus*) and one African Grey Parrot (*Psittacus erithacus*) suspect of tuberculosis were received in 2007 from wildlife centers and proved negative for *M. bovis* in culture.

## Mycobacterium bovis in humans

*Mycobacterium bovis* is capable of infecting humans. In humans, infection with *M. bovis* causes a disease similar to infections with *M. tuberculosis*, which is the primary agent of tuberculosis in humans.

The main transmission routes of *M. bovis* to humans are contaminated food (especially raw milk and raw milk products) or through direct contact with animals.

In 2007, 3 human cases of bovine tuberculosis were reported to the Belgian Register and identified by molecular techniques in the NRL. No link between these patients and bovine tuberculosis in a Belgian herd could be detected.

One patient had a pulmonary disease and the two other ones (born in Morocco) had an extra-pulmonary form of the disease. Among them, one patient already detected in 2005 (abdominal tuberculosis), was infected by a multidrug resistant isolate. The MIRU-VNTR profile and spoligotype of this isolate were identical to the genetic profiles observed in 2005 and 2006, but the strain acquired resistance to isoniazid and to rifampicin in 2007.

As previously emphasized, the number of *M. bovis* detected in humans could be underestimated as some clinical laboratories identify mycobacterial cultures with molecular tests specific for the entire complex *M. tuberculosis*, unable to distinguish *M. bovis* from *M. tuberculosis*.

## Human tuberculosis (*Mycobacterium tuberculosis*)

The incidence of human tuberculosis shows little variation over the last years. In 2001, 2002, 2003, 2004, 2005, 2006 and 2007 respectively 1321, 1309, 1128, 1226, 1144, 1127 and 1030 new notified cases of active human tuberculosis were detected. Over the 60% were male patients. In 2007, 48% 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

Michel Delmée, Geneviève Ducoffre, Luc Vanholme, Karen Vereecken

## Yersinia enterocolitica

The bacterial 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). *Y. pseudotuberculosis* and specific types of *Y. enterocolitica* cause foodborne enteric infections in humans. 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 considered as 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 serotypes. Clinical disease in animals is uncommon.

Infection is most often acquired by eating contaminated food, particularly raw or undercooked pork. The ability of the organism to grow at + 4°C makes refrigerated food with a relatively long shelf life a probable source of infection. 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 are non-pathogenic types. It is, therefore, most important that investigations discriminate between which strains are pathogenic for humans. Biotyping of the isolates is essential to determine whether or not isolates are pathogenic to humans, and this method 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 Competent Authority organised a monitoring of meat since 1997, which showed a very low prevalence of *Yersinia enterocolitica* in pork, beef and poultry. In 2007, the monitoring programme concentrated on one matrix, i.e. minced meat containing pork meat.

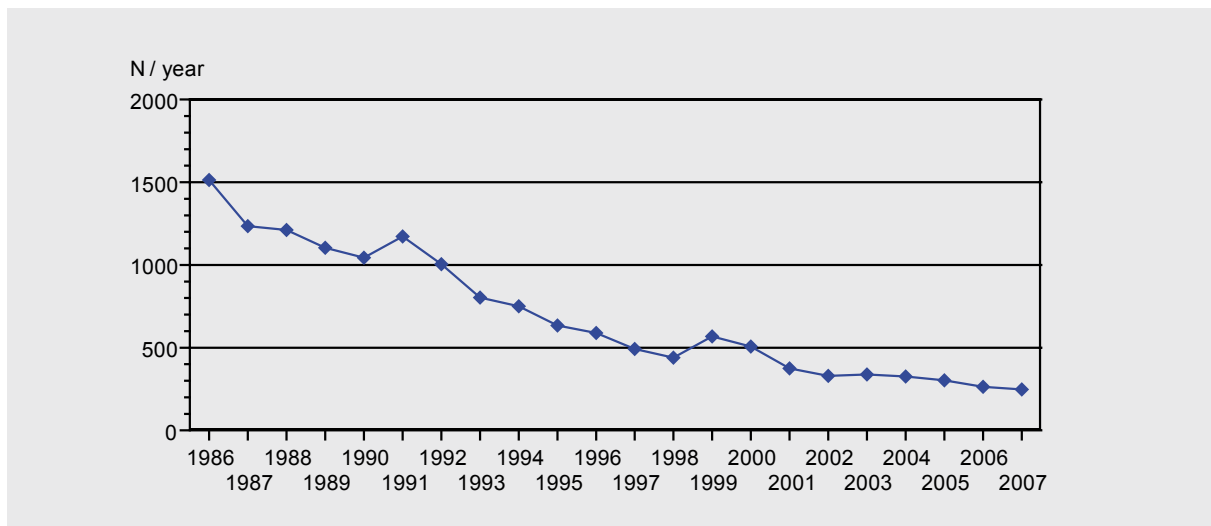
**Table 29.** Monitoring *Yersinia enterocolitica* in pork meat.

Sample	Quantity analysed	Percentage of positive samples
Minced meat (containing pig meat) intended to be eaten raw at retail (n=129)	1g	0.0%
Minced meat (containing pig meat) intended to be eaten cooked at retail (n=131)	1g	0.0%

## Yersiniosis in humans

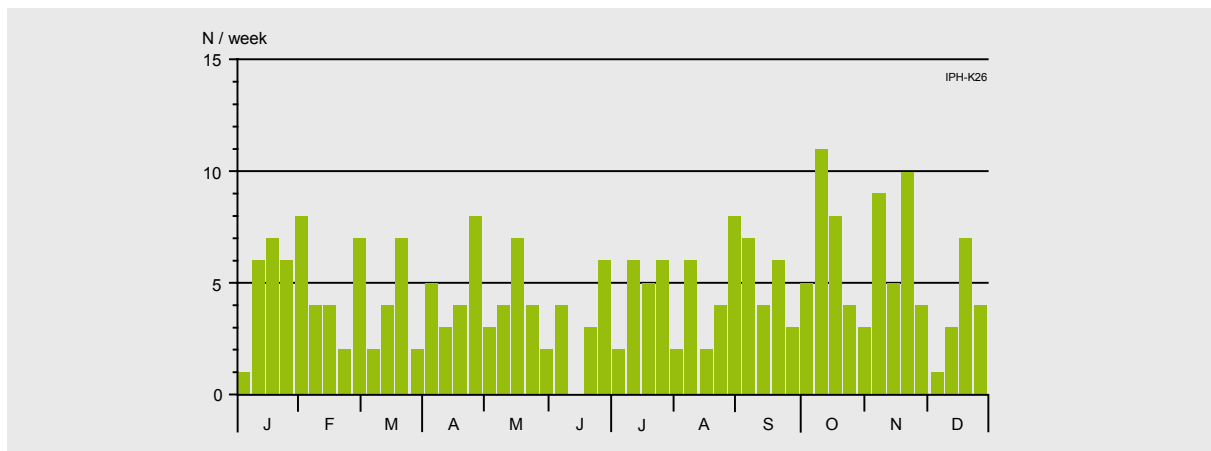
In 2007, the Sentinel Laboratory Network registered 248 cases, corresponding to a national incidence estimated at 2.5 per 100.000 inhabitants.

Since 1986, when 1.514 cases were reported by this network, the number of human infections in Belgium significantly decreased (Figure 31).



**Figure 31.** Total number of *Yersinia enterocolitica* infections in humans by year (1986-2007). Source: Sentinel Laboratory Network

Cases were observed all over the year (Figure 32).



**Figure 32.** Weekly number of *Yersinia enterocolitica* infections in humans, 2007. (Source: Sentinel Laboratory Network)

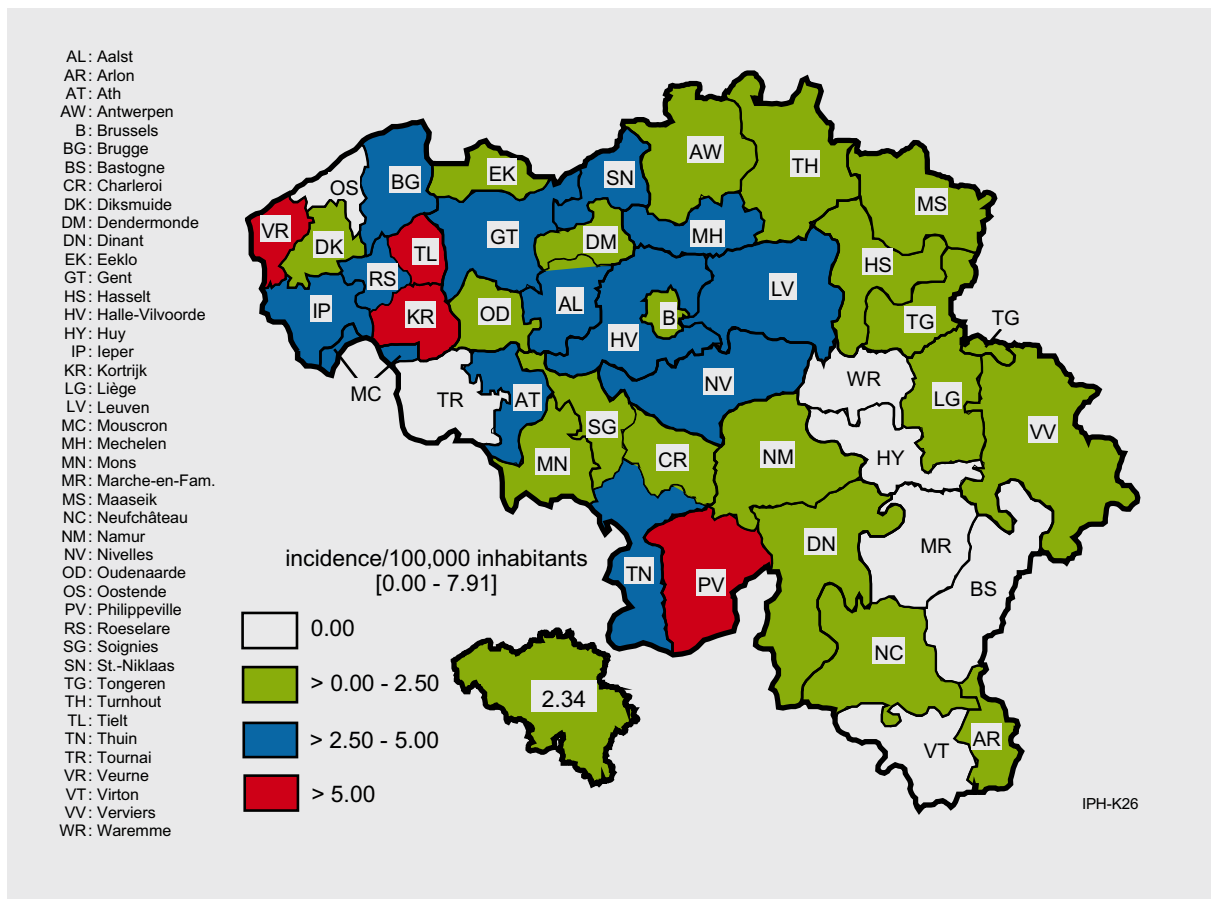
Forty percent of cases were 0 to 4 year old children (Table 30).

**Table 30.** Number of *Yersinia enterocolitica* infections in humans by sex and by age groups, 2007. (Source: Sentinel Laboratory Network)

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	1	0,8	5	4,1	6	2,5
1 - 4	57	47,9	36	29,3	93	38,4
5 -14	30	25,2	27	21,0	57	23,6
15 -24	9	7,6	13	10,6	22	9,1
25 -44	8	6,7	16	13,0	24	9,9
45 -64	12	10,1	15	12,2	27	11,2
65	2	1,7	11	8,9	13	5,4
<b>Total</b>	<b>119</b>	<b>100,0</b>	<b>123</b>	<b>100,0</b>	<b>242</b>	<b>100,0</b>

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 33).

Bio-serotyping was performed by the National Reference Laboratories. In 2007, 72.6% of the 376 isolates tested belonged to the pathogenic bio-serotypes (including 5 *Yersinia pseudotuberculosis*) with serotype O:3 / biotype 4 accounting for 68.62 % of the total. The remaining 103 strains (27.4%) belonged to non-pathogenic bio-serotypes and their number did not vary markedly during the last years, in contrast to the obvious decrease of the pathogenic strains.



**Figure 33.** Incidence of *Yersinia enterocolitica* infections in humans by district ( $N/10^5$  inhab., 2007).

(Source: Sentinel Laboratory Network)



viral  
diseases

# Avian influenza

Sophie Quoilin, Thierry van den Berg

## Avian influenza

A total of 307 wild birds have been tested positive in the EU during 2007. The vast majority was found in Germany (298), with small numbers in France (7) the Czech Republic (1) and Hungary (1). Almost all these cases were reported in a seven-week period between mid-June and beginning of August. This is in contrast to 2006, when 14 Member States reported a total of 748 positive cases of A/H5N1 in wild birds between February and August, indicating a broader distribution of the virus in the EU, mostly in a wave that rose and fell in the first half of the year. Grebes grabbed the attention in 2007 as compared to swans in 2006. The apparent lower level of virus circulation in wild birds during 2007 has not been reflected in the reported number of domestic poultry outbreaks in the EU. Indeed, 14 outbreaks in domestic poultry have been reported in six Member States: Hungary, Germany, the Czech Republic, Poland, Romania and UK. Although the 3 first countries have also reported cases in wild birds, the timing and location of cases in domestic poultry offer no obvious epidemiological link to wild bird infection in the majority of cases. This is in contrast to 2006, when most of the 33 domestic poultry outbreaks reported in the five affected Member States (Hungary (29), Sweden (1), German (1), Denmark (1) and France (1)) 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.

General

Monitoring in birds

Influenza in humans: monitoring

It seems, therefore, that unlike 2006, where proactive surveillance in wild birds established the presence of virus in several locations prior to the virus being identified in poultry, epidemiological data from 2007 did not detect infection in local wild birds before infection in domestic flocks. This is open to various interpretations, one being that EU wild bird surveillance, although extensive, has not been sufficient to trace infection in wild birds. However, in those areas where domestic flocks have been affected, even increased retroactive wild bird surveillance around cases in domestic poultry has, in most cases, failed to identify H5N1 infection in wild bird populations. This points to the possibility of other routes of entry into domestic poultry, such as through trade in poultry products, or via fomites. The latter has been suggested in the linked outbreaks in Hungary and the United Kingdom (UK) in February 2007 when an outbreak of HPAIV H5N1 occurred in a single turkey farm in Suffolk and trade in poultry meat was implicated as the most likely source of infection. Indeed, the source of virus was not clearly evident but epidemiological investigation, including sequence analysis, indicated that the introduction likely originated from importation of fresh turkey meat from a subclinically infected goose flock in Hungary.

In October 2007, a supermarket bargain action for frozen ducks in Brandenburg cities was at the origin of a silent spread of H5N1 virus in Germany. This insidious incursion of HPAIV H5N1 into the food chain was possible by the absence of clinical signs in infected ducks, making syndromic surveillance useless. These findings call for a regular virological monitoring of duck flocks as serology is often less indicative in this species. As some countries in eastern Europe also have

dense populations of domestic ducks and geese, which are in contact with migrating birds, they could lurk in these domestic fowl, causing periodic outbreaks of bird flu across Europe. This year after year changing epidemiological situation warns for a constant and sustained surveillance of avian influenza.

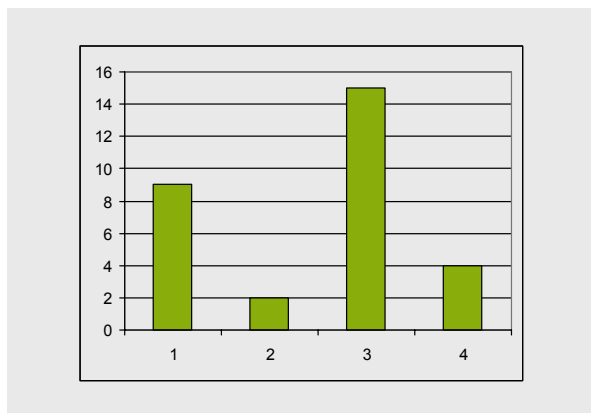
## Monitoring in birds

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 (upon 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 Liège and VAR. All tests are performed at VAR.

### Passive monitoring of dead wild birds

An 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 2007, a total of 30 suspicions complying with these criteria (and corresponding to more than 100 wild birds) were analysed by Real Time RT-PCR and/or viral isolation, all with negative results.



**Figure 34.** Number of suspicion dossier / trimester of 2007

### Active monitoring of wild birds

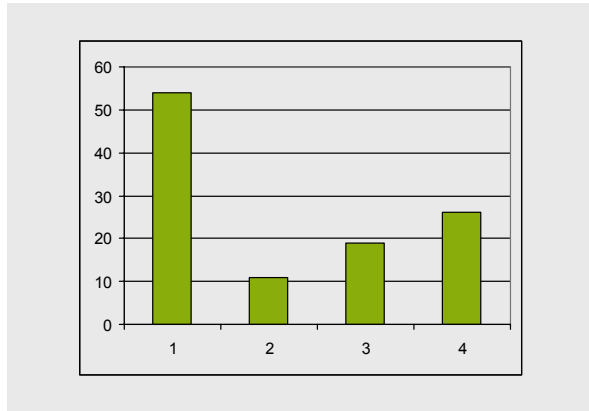
The active monitoring of wild birds was increased to 4.113 samples in 2007. Cloacal swabs were taken systematically but oral swabs were also taken on waterfowl during the high risk season (autumn migration period), as it is now well accepted that H5N1 is mostly excreted by the respiratory track. As in 2006, 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.

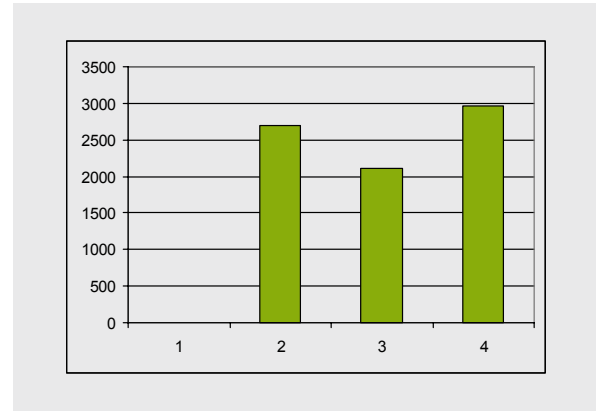
In total, 977 samples were taken from hunted waterfowls and 3136 swabs were taken during ringing activities of other wild birds. No HP H5N1 was detected during this active surveillance program but only low pathogenic avian influenza with an overall rate of about 2%. These included LPAI H1N1, H4N6 and H12N2 from mallards, H11N9 from northern pintails, H13N2 from black-headed gulls and H4N6 from a common shelduck. Interestingly, several APMVs were also isolated at this occasion from a goshawk (PPMV-1) or mallards.

### Surveillance of professional poultry flocks (syndromic surveillance)

In case of any abnormal symptoms in a domesticated poultry flock, the owner has to inform his veterinarian who is obliged to examine clinical symptoms and evaluate a possible suspicion. In case of suspicion, samples are taken for further analysis. In 2007, 110 possible cases were recorded and examined (Figure 35). All results were negative for H5 and H7 but a LPAI H6N8 was isolated from a turkey. The peak of sampling was consistent with the outbreak in UK in early 2007.



**Figure 35.** Number of exclusion diagnosis dossiers / trimester of 2007.



**Figure 36.** Serological screening of poultry / trimester of 2007

### Serological screening of housed poultry flocks

Serological screening for antibodies against H5 and H7 viral antigens of the avian influenza virus in poultry holdings was performed during the 3 last trimesters of 2007 like in 2006. In total, sera from 8500 birds were analysed by HI test, following standard procedures. One chicken, one duck and one goose flocks were found positive for H5 and 2 chicken flocks for H7.

Cloacal swabs were taken from these flocks to identify possible active shedders but were all negative.

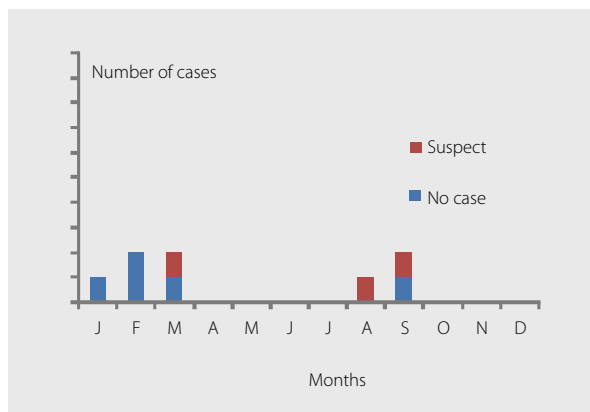
### Influenza in humans: monitoring

The surveillance of suspected cases of a virus infection by Influenza A/H5N1 is based on a standard operational procedure as made available for all clinicians.

([http://www.influenza.be/nl/document/Procedure\\_H5N1\\_voor\\_artsen\\_NL.pdf](http://www.influenza.be/nl/document/Procedure_H5N1_voor_artsen_NL.pdf))

([http://www.influenza.be/fr/document/Version%2029%20mars%202006%20Proc%C3%A9dure\\_suspicion\\_d\\_influenza\\_pour\\_Medecin.pdf](http://www.influenza.be/fr/document/Version%2029%20mars%202006%20Proc%C3%A9dure_suspicion_d_influenza_pour_Medecin.pdf))

In 2007, the epidemiologist on guard duty received 7 calls for suspicion of Influenza A/H5N1 infection. Among these calls, one involved two patients, this makes 8 cases for 7 calls of which 5 were classified 'No case' and 3 as 'Suspects' (Figure 37).



**Figure 37.** Number of cases for suspicion of infection with Influenza A/H5N1, represented per month and classified by case definition, January – December, 2007. (Source: Report of Influenza A/H5N1 surveillance 2007, IPH (Epidemiology Unit))

Each time the epidemiologist on duty receives a call about suspicion of Influenza A/H5N1 infection, the symptoms and risk factors are discussed with the medical doctor and the health inspector of the Community in order to determine the corresponding case definition level. When a case is classified as ‘no case’, tests for Influenza A/H5N1 are usually not performed. In 2007 we did one test for a ‘no case’.

Following the procedure, tests for suspected cases and one ‘no case’ were not performed in emergency except for one case due to the severity of the medical history of the patient. This patient was diagnosed as an Influenza A/H3N2 infection (Table 31).

**Table 31.** Results of lab-tests carried out on 4 samples of suspect cases for virus infection by Influenza A/H5N1, represented per case definition, January – December 2007. (Source: Surveillance of flu in Belgium, 2006–2007, IPH (Virology Unit))

Lab results		Case definition				Total
		No case	Suspect	Possible	Probable	
A	H3N2	0	1	0	0	1
Negative		1	2	0	0	3
Total		1	3	0	0	4

Among risk factors, three patients had a travel history (Table 32).

**Table 32.** Distribution of the suspected cases of virus infection by Influenza A/H5N1 per destination, January – December 2007.

Source: Report of Influenza A/H5N1 surveillance, 2007, IPH (Epidemiology Unit)

Country	Frequency	Lab results
China	1	Neg
Thailand	1	A/H3N2
Kuwait	1	Neg
No travel history	1	Neg
Total	4	

The patient who did not travel started pulmonary symptoms a few weeks after her chickens suddenly died.

The surveillance system for the suspected cases of virus infection by Influenza A/H5N1 was effective. A sharp decrease is observed in comparison to the 29 calls reported in 2006.

In Belgium, no human case of virus infection by Influenza A/H5N1 has been identified in 2007.



# Hantaviruses

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

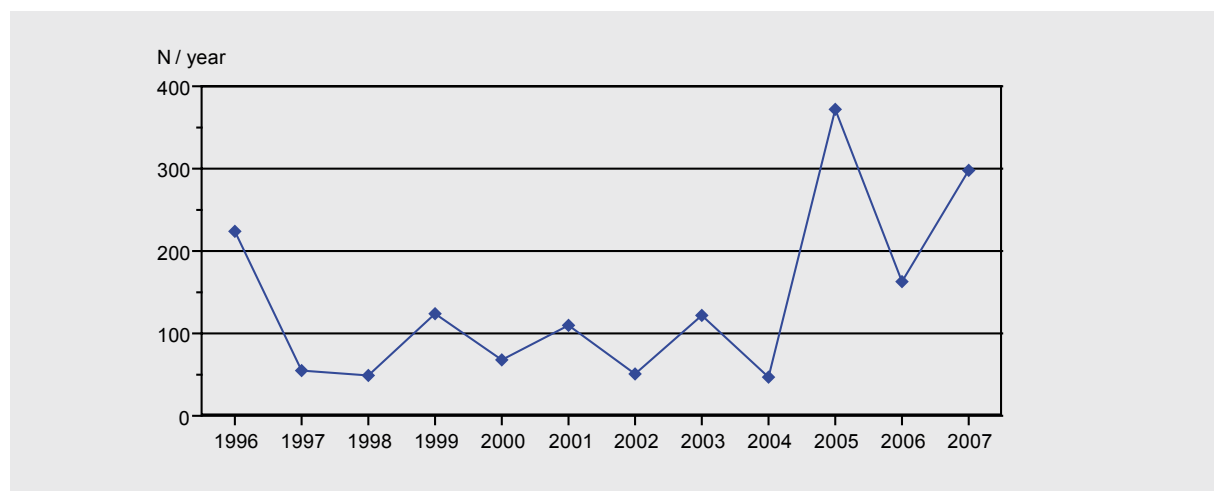
Rodents are the only known reservoirs of hantaviruses. Other small mammals (e.g. rabbits) can be infected as well, but are

less likely to transmit the virus to other animals or humans. While antibodies to hantaviruses are found in numerous other species of rodents and their predators, no evidence supports the transmission of infection to other animals or to humans from the “dead-end” hosts.

A scientific research program to screen the seroprevalence of some small animal species is still running.

## Hantaviruses in humans

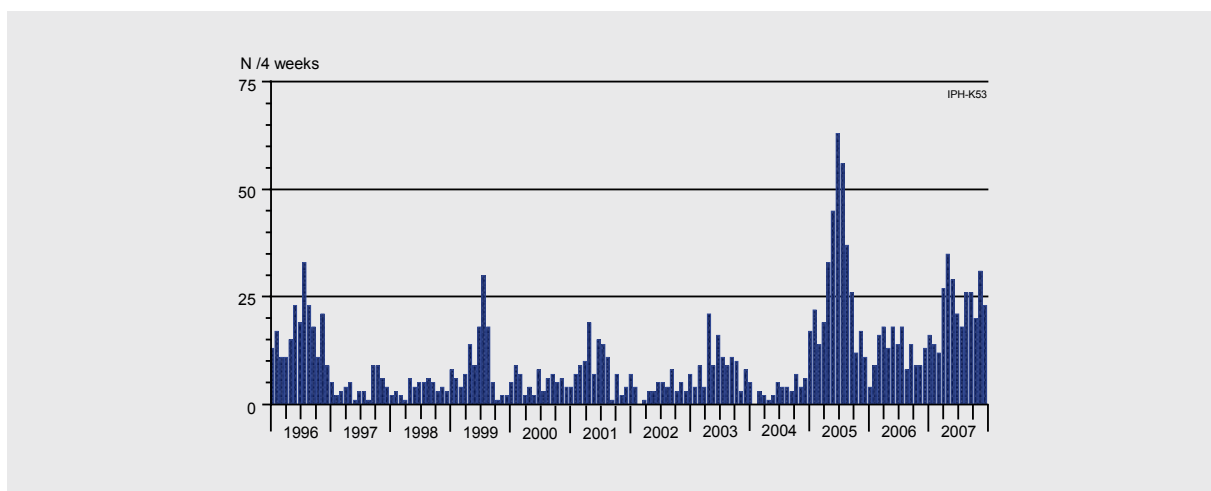
In 2007, the Sentinel Laboratory Network and the NRL reported 298 cases of hantavirus. This report indicates an increase of cases as compared to 2006 (N=163; Figure 38).



**Figure 38.** Total number of Hantavirus infections in humans by year (1996-2007). (Sources: Sentinel Laboratory Network and NRL)

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-summertime of 1996, 1999, 2001, 2003 and specially 2005 (Figure 39).

In 2007, the majority of cases are adults over 19 years (90%) and 73% are males (Table 33).



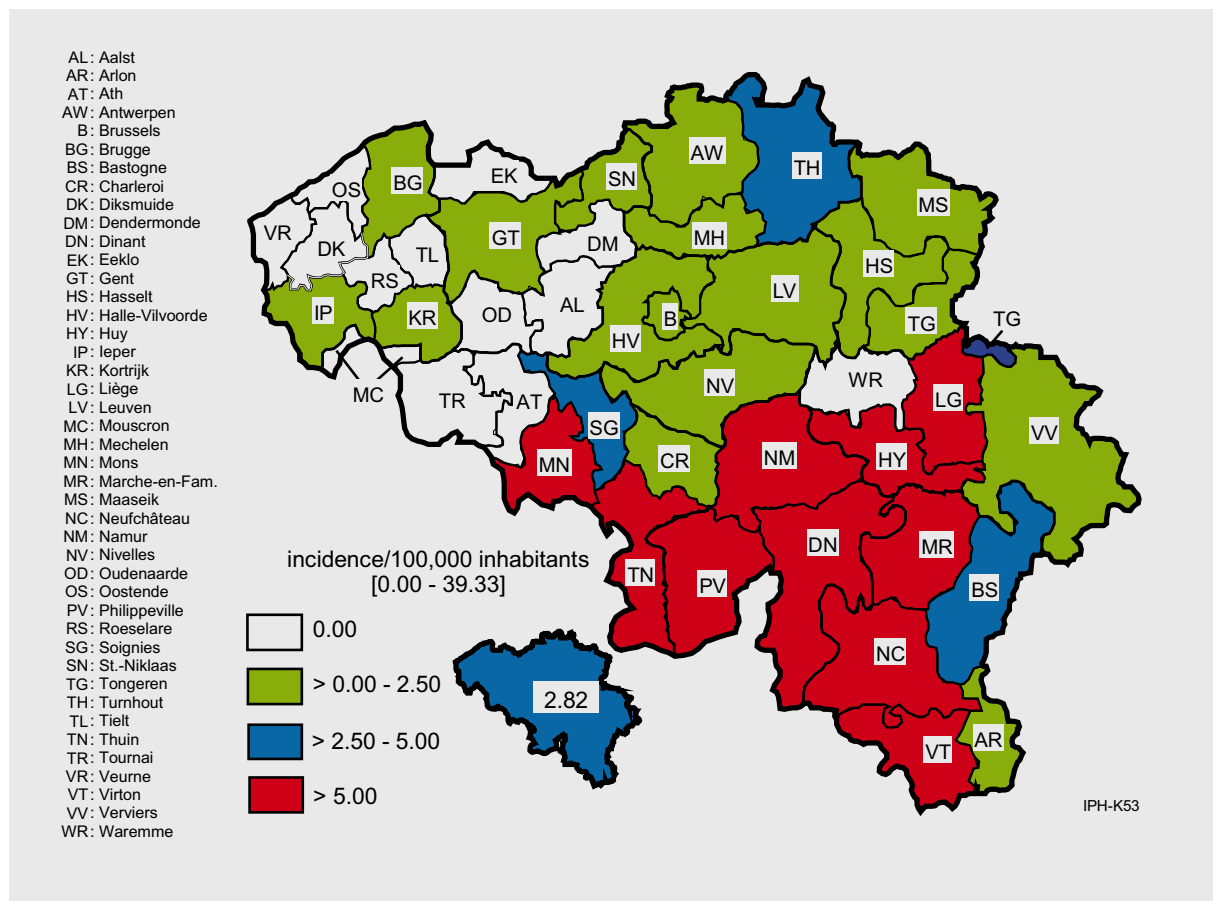
**Figure 39.** Distribution of Hantavirus infections in humans (N/4 weeks), 1996–2007. (Sources: Sentinel Laboratory Network and NRL)

**Table 33.** Number of cases of Hantavirus infections in humans by sex and age groups, 2007. (Sources: Sentinel Laboratory Network and NRL)

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	1	0,5	1	1,2	2	0,7
1 - 4	0	0,0	2	2,5	2	0,7
5 -14	9	4,1	4	4,9	13	4,4
15 -24	29	13,4	10	12,3	39	13,1
25 -44	87	40,1	31	38,3	118	39,6
45 -64	68	31,3	24	29,6	92	30,9
65	23	10,6	9	11,1	32	10,7
<b>Total</b>	<b>217</b>	<b>100,0</b>	<b>81</b>	<b>100,0</b>	<b>298</b>	<b>100,0</b>

Among the cases reported in 2007, 81% (N=234) resided in Wallonia, 16% (N=45) in Flanders and 3% (N=10) in Brussels (9 unknown). The highest incidence rates are reported in the districts of Thuin (N=58), Liège (N=40), Philippeville (N=24) and Mons (N=24). Most of these areas are known to be endemic for the disease, but cases in the district of Liège are only reported since 2003 on (Figure 40).

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.



**Figure 40.** Incidence of Hantavirus infections in humans by district (N/10<sup>5</sup> inhab., 2007). (Sources: Sentinel Laboratory Network and NRL)

# Rabies

Ingrid Leroux, 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.

Rabies

Rabies in animals

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

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.

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 immunofluo-

rescence 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 km<sup>2</sup> along the German border was covered by spreading 32 000 baits by means of a helicopter (17.78 baits per km<sup>2</sup>).

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

### Epidemiological investigations and results of 2007 surveillance

#### Passive surveillance of rabies

A total of 602 brain samples were examined for rabies virus at the NRL. The majority of samples originated from wildlife (n=219), especially foxes (n=141) and deer (n=41), cattle (n=196) and sheep and goats (n=160). Twenty-three dead-found bats were also examined. The high number of cattle and small ruminants is the 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.

In October 2007, a suspicion of rabies on clinical symptoms in a dog illegally imported from Morocco was confirmed. The clinical diagnosis was proved by laboratory testing after euthanasia of the animal. Finally 32 persons and 18 pet owners with possible contact with the rabid animal were traced. Medical information and follow-up by experts of the Institute of Public Health – Pasteur Department of all ‘contact’ persons was realised.

Due to the detection of this rabid dog, Belgium was no longer officially rabies-free since the end of October 2007. The officially free status was regained on 28 October 2008.

### 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 2007, the network has transmitted 219 samples of wild animals (foxes, wild cervids, badgers, mink and raccoon) to the NRL. All cases were negative (immunofluorescence and virus cultivation).

## Seroprevalence of bat lyssaviruses

In 2006, a preliminary study was undertaken to estimate the seroprevalence of EBL-1 and -2 in Belgian bats. Antibodies against EBL-1 were found in blood of 9 out of 58 bats captured in the South of Belgium. No antibodies against EBL-2 were found. Bats appeared in good health, indicating that EBL-1 circulates in Belgian bats without causing lethal disease.

# West Nile virus

Carine Letellier, Marjan Van Esbroeck, Luc Vanholme

## West Nile virus

The West Nile virus (WNV) is a zoonotic mosquito-transmitted arbovirus of the genus *Flavivirus* in the family *Flaviviridae*. WNV has a wide geographical range. It occurs throughout Africa, the Middle East, southern Europe, Russia, India and Indonesia and it was introduced into North America in 1999 in New York and has rapidly spread westwards across the continental United States and into Canada.

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.

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.

West Nile virus

West Nile virus in animals

West Nile virus in humans

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%.

Based on the recent spread of the disease in North America and Europe (cases in France, Hungary, Spain and Italy) 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. In this context, there is an urgent need for the improvement of diagnostic tools that are able to detect the 5 lineages of WNV.

## West Nile virus in animals

### Early warning system

Better analytical methods are essential to be used in an early warning system for the timely detection of WNV. A system based on 2 levels of surveillance (in collaboration with the Royal Belgian Institute of Natural Sciences, [http://www.naturalsciences.be/index\\_html](http://www.naturalsciences.be/index_html)) will be evaluated:

- a passive surveillance based on the investigation of cases of abnormal mortality of birds and/or of equine encephalitis cases, and
- an active serosurveillance based on the serological follow-up of poultry sentinels (domestic birds like ducks, goose) and wild birds (in particular Corvidae trapped in specific baited cages).

The surveillance also should target the circulation of WNV in migratory birds, by virological investigation of Silvidae, captured during spring when they come back from sub-Saharan Africa.

Horses should be monitored in case of WNV circulation in Belgium.

## West Nile virus in humans

In the ITM (NRL), a total of 115 human sera have been examined for the presence of antibodies to West Nile virus by ELISA in 2007.

No confirmed, probable or possible case have been detected.





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 low 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. Wash and peel all raw fruits and vegetables before eating is recommended. If possible, avoid contact with infected humans or infected animals. Do not drink water from lakes, rivers, springs, ponds or streams. If safety of the drinking water is questionable, water should be boiled. Simply bringing water to the boil will kill any cryptosporidium oocysts in it. Suspect water supplies can also be carefully filtered before drinking.

## Cryptosporidiosis in animals

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

## Cryptosporidiosis in humans

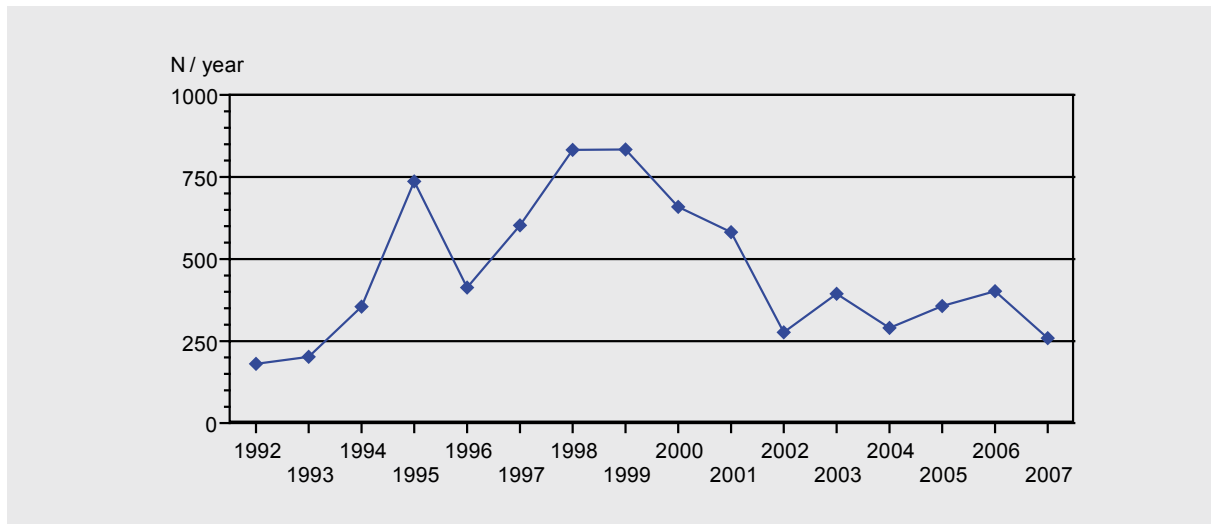
Infections with *Cryptosporidium parvum/hominis* occur worldwide. Most reports describe endemic outbreaks caused by contaminated water and food. The infectious oocysts may survive in the environment for months.

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

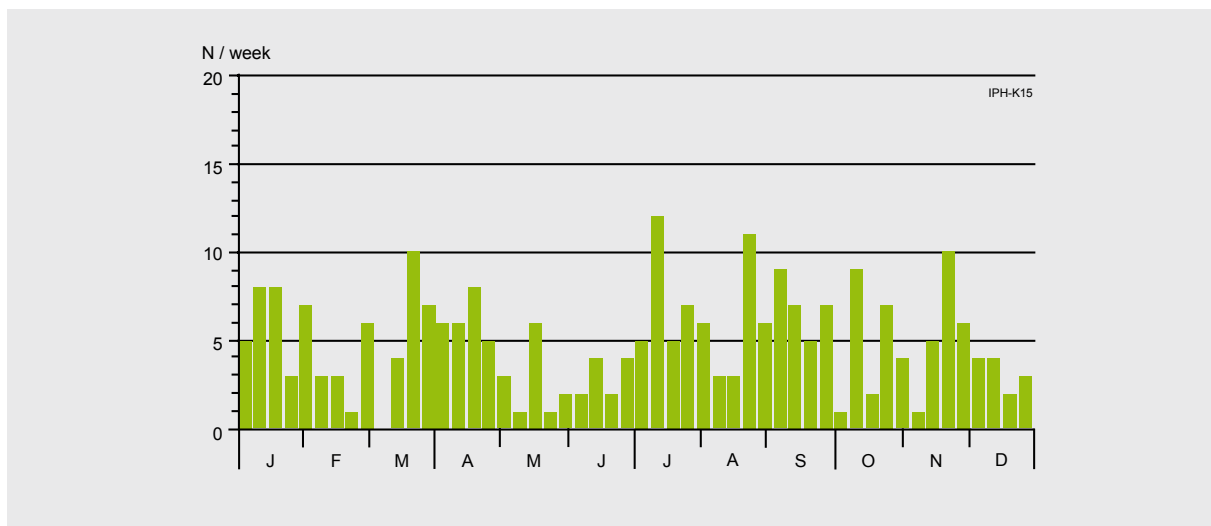
In 2007, the Sentinel Laboratory Network reported 259 cases of cryptosporidiosis, corresponding to a national incidence estimated at 2.5 per 100.000 inhabitants (Figure 41). The incidence was very high in 1998 and 1999 (8.2 per 100.000 inhabitants).

Cases were observed all over the year (Figure 42)

One quarter of cases is reported in 0 to 4 year old children and one quarter in 25-44 year old adults (Table 34).



**Figure 41.** Total number of *Cryptosporidium* infections in humans by year, 1992-2007. (Source: Sentinel Laboratory Network)



**Figure 42.** Weekly number of *Cryptosporidium* infections in humans, 2007. (Source: Sentinel Laboratory Network)

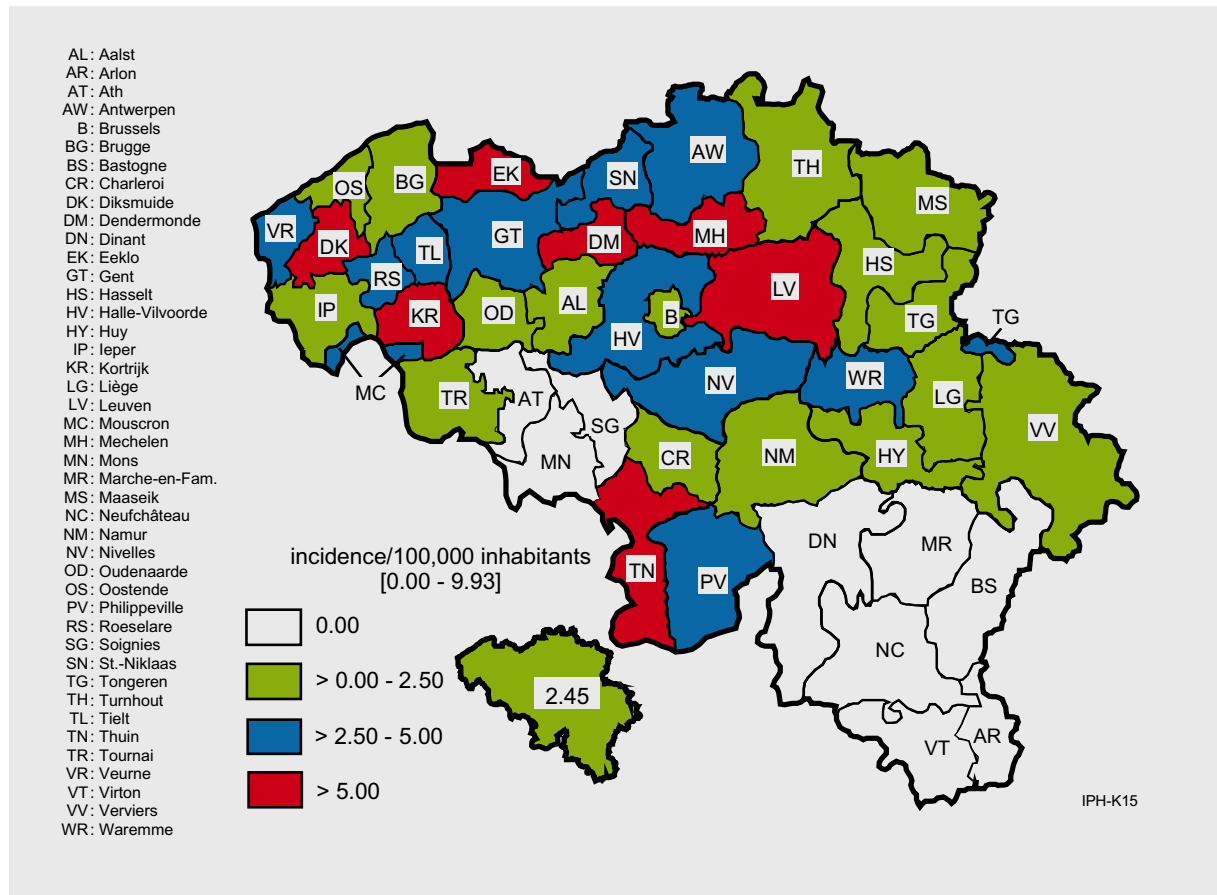
**Table 34.** Number of *Cryptosporidium* infections in humans by sex and by age groups, 2007. (Source: Sentinel Laboratory Network)

Age groups (year)	Males		Females		Total	
	N	%	N	%	N	%
< 1	5	3,9	5	3,9	10	3,9
1 - 4	34	26,8	26	20,3	60	22,5
5 -14	32	25,2	18	14,1	50	19,6
15 -24	12	9,4	21	16,4	33	12,9
25 -44	28	22,0	39	30,5	67	26,3
45 -64	11	8,7	10	7,8	21	8,2
65	5	3,9	9	7,0	14	5,5
<b>Total</b>	<b>127</b>	<b>100,0</b>	<b>128</b>	<b>100,0</b>	<b>255</b>	<b>100,0</b>

The incidence in Flanders is higher than in Wallonia. In 2007, the incidence was 3.2 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 43)

In the ITM (NRL), a total of 1851 human faecal samples have been examined for the presence of *Cryptosporidium* after concentration and staining according to Heine.

*Cryptosporidium* has been detected in 16 samples. Nine samples were from patients consulting the outpatient clinic of the ITM and 6 samples have been sent to the reference laboratory by external laboratories. Eleven patients (69%) were male. The age of the patients ranged from 0 to 66 years, with a median age at 27 years. No travel history is known from the patients whose sample was sent to the reference laboratory by external laboratories. Seven of the 9 patients who consulted at the outpatient clinic of the ITM, travelled to Africa before the start of their illness. Two patients did not travel abroad.



**Figure 43.** Incidence of *Cryptosporidium* infections in humans by district ( $N/10^5$  inhab., 2007).

(Source: Sentinel Laboratory Network)



# 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 in or around the farm. 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 cysticeri 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.

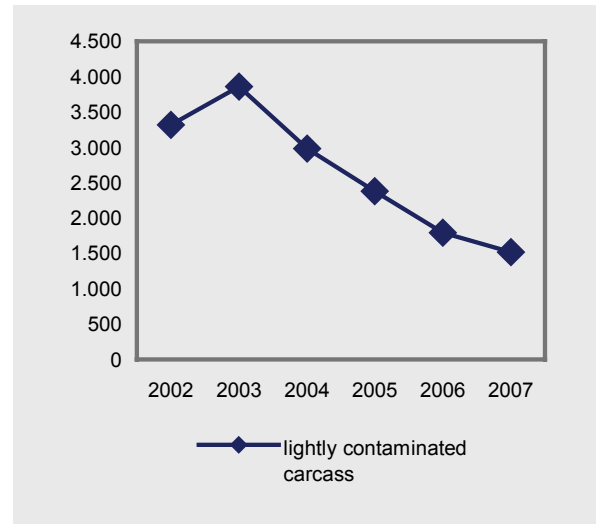
Although *Cysticercus ovis* in sheep is not transmissible to humans, its presence causes total rejection of the carcass. No sheep were found to be infected in 2006 and 2007.

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

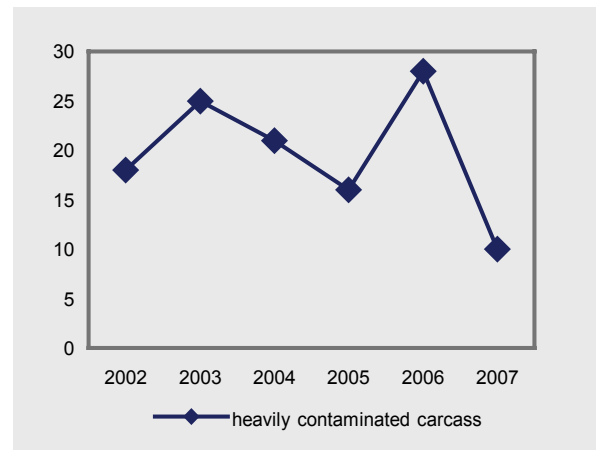
## Cysticercosis in cattle

Post-mortem, macroscopic examination of carcasses is routinely done in the slaughterhouse. In 2007, 495 492 adult cattle and 306 961 veal calves were examined.

Figures 44 and 45 from the FASFC show that in 2007, 10 carcasses of adult cattle were rejected for generalised cysticercosis. In addition, the meat of 1.517 adult cattle was treated by a 10 days freezing before human consumption.



**Figure 44.** *Cysticercosis: detection of lightly contaminated bovine carcasses at slaughterhouse*



**Figure 45.** *Cysticercosis: detection of heavily contaminated bovine carcasses at slaughterhouse*

# Echinococcosis

Leen Claes, Carine Truyens, 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 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.

Echinococcosis

Echinococcus in food animals

Echinococcus in wildlife (foxes)

Echinococcus in humans

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).

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 and small rodents and voles the intermediate hosts. *E. multilocularis* is the agent of highly pathogenic alveolar (multilocular) echinococcosis in humans. 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 mushrooms (washing alone is not sufficient, neither does freezing at  $-18^{\circ}\text{C}$ !), 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

### Surveillance programme and results

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)

In Belgium, the percentage of infested foxes varies according to the region, with an increasing rate from the North-West to the South-East (2% in the Flanders region, 33% in the Walloon region). The endemic region is situated under the river Meuse, on the heights of the Ardennes. As the population of foxes increased in the last few years, the opportunity for contact between humans and this wild carnivore, even in urban areas, has consequently increased. In 2007, no budget was available to perform analyses on Echinococcus in wildlife

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.

## 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/



# Sarcosporidiosis

Leen Claes, Luc Vanholme

## Sarcosporidiosis

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

## Sarcosporidiosis in animals

### Surveillance programme in food animals

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

Sarcosporidiosis

Sarcosporidiosis in animals



# Toxoplasmosis

Leen Claes, Stephan Decraeye

## Toxoplasma

*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.

Toxoplasmosis

Toxoplasmosis in animals

Toxoplasmosis in humans

## Toxoplasmosis in animals

The majority of grazing animals are indiscernible carriers of tissue cysts. There is a need for suitable microscopic, 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 or bio-assay. But this is not routinely done and there is no data on the status of toxoplasmosis in the livestock in Belgium. Recently, 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. In ovine and goats, primary infection during gestation leads to abortion or stillbirth, with important economic losses. In general, about 70% of sheep and goats are infected. 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 we can state that the more the animals have outdoor access, the higher the prevalence of toxoplasmosis. The presence of cats and rodents on the farm will also increase the infection rate. Limitation of these factors is important as preventive measures. The seroprevalence in cats is around 25 to 70%, depending on the cat's way of life. For dogs the prevalence is around 15-20%.

## Toxoplasmosis in humans

*Toxoplasma gondii* infects humans mostly by oral route: by ingestion of oocysts excreted by cats (e.g. in litter trays), by ingestion of cysts present in inadequately cooked meat or by eating not properly washed raw vegetables (e.g. salad) contaminated with cat faeces.

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, the majority of adult persons have acquired immunity to re-infection, but remain carriers. More than 80% of infections with *T. gondii* are asymptomatic. However mild (flu-like) to moderate clinical symptoms are possible (lymphadenopathy, chronic fatigue, retinochoroiditis).

In immunocompromised patients (HIV, organ transplantation) severe disease may occur, like pulmonary toxoplasmosis and encephalitis, with fatal consequences in 37% of the cases. In transplant patients (due to immunosuppressive drugs) the parasite reactivates or can be transmitted via infected donor tissues and results in high morbidity and mortality.

*T. gondii* is able to cross the placental barrier and cause foetal infection. An infection in seronegative, pregnant woman can develop into congenital toxoplasmosis, with pathological consequences for the foetus that depends on the time of the infection during pregnancy. In Belgium, congenital toxoplasmosis occurs in 9/10,000 pregnancies. The earlier the infection (first trimester), the higher the risk of severe complications (ocular disorders, hydrocephalus and severe

mental retardations, 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 that can re-activate later in life and cause symptomatic toxoplasmosis (e.g. ocular disease). Costs associated with congenital toxoplasma infection have been estimated at 1.26 million US\$ per case. 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. 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.

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 for primary prevention of toxoplasmosis 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  $-20^{\circ}\text{C}$  for 48 hours) before consumption or adequate heating of meat 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*. Human 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) N° 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) N° 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 2007 surveillance

A total of 11.512.404 pigs, 10.064 solipeds (mainly horses) and 13.731 wild boars were examined. All samples from pigs and solipeds were negative. 1 pooled sample, from 3 wild boar piglets, revealed a suspected *Trichinella* larva. This was morphologically confirmed by the NRL. Further species identification by the CRL failed.

## Trichinella in wildlife

In 2007, 35 badgers and 62 foxes were analyzed 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.





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, suspected animals (in farm and slaughterhouse) and fallen stock of 24 months and more. 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) N° 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, electron-microscopic detection of scrapie associated fibrils (SAFs) and western blotting.

In Belgium, all 19 private laboratories (primary 'active' screening) and the NRL are accredited (ISO 17025:2005) and the whole epidemiological surveillance is coordinated by the FASFC.

**Table 35.** Number of animals controlled in Belgium (2001 - 2007)

Year		Slaughterhouse	Suspected Animals:		Fallen stock
			Herd screening / farm, slaughter, autopsies		
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
Total	Cattle	2.455.258	8.120 / 1.947		250.353
	Small ruminants	20.751	1.177 / 755		10.165

**Table 36.** Positive TSE cases in cattle and sheep in Belgium (First case until 2007)

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
Total	133 (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 Sc)

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

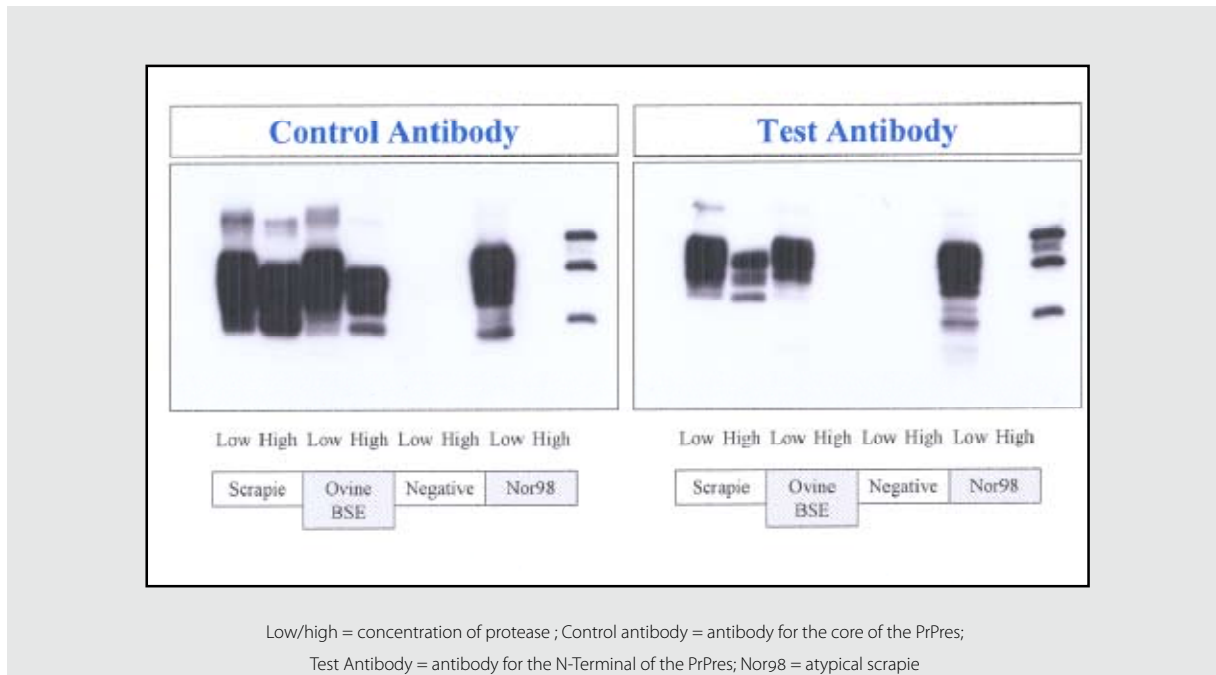
Laboratory and epidemiologic studies provided strong circumstantial evidence for a causal link between vCJD and the bovine spongiform encephalopathy (BSE) epizootic in cattle with the most likely route of primary human infection being through dietary exposure to highly infected bovine tissues.

Using the standard diagnostic techniques (see above) for diagnosis of TSE in small ruminants, it is not possible to differentiate between scrapie and BSE, i.e. TSE associated with the variant of Creutzfeldt-Jakob disease in man. Following the first confirmed BSE case in a French goat in 2002 (Vet Rec

2005; 156(16): 523-524), the need to implement a rapid test for discrimination between scrapie (no risk for public health) and BSE (threat for public health) in small ruminants became stringent. In the past, only strain typing in mice was available. This technique is very time consuming (up to 3 years before final evaluation) and needs specialist expertise. For these reasons, only a limited number of results are available and not all small ruminants positive for TSE could be tested. With the introduction in 2005 of 3 new (EC) validated tests based on western blotting, discriminative tests became available. According to EU regulation 999/2001, amendment 36/2005,

each Member State had to implement one of these 3 tests or to subcontract the testing to another Member State. In Belgium, the choice of the discriminatory test was based on the experience with an earlier, similar ELISA test. The principle of this new CEA (Bio-Rad) test is based on the difference in resistance against proteolysis of the N-terminal of the resistant PrP protein (PrPres) in scrapie (more resistant) and in BSE (less). In the presence of high concentrations of the enzyme (proteinase K), PrPres is destroyed in BSE and not in scrapie. Thus, revelation of the protease products by means of two antibodies (one against the N-terminal and one against the

core of the PrPres) differentiates between scrapie and BSE. Both antisera will detect scrapie PrP products in the presence of both the low and high concentration of the enzyme, whereas only the antiserum against the PrPres core will detect BSE PrPres in high concentration of protease. Both antisera will detect BSE PrPres in low concentration of enzyme. Additionally, this test can also identify atypical scrapie strains, based on the principle that in these strains the complete PrPres will be destroyed in combination with high enzyme concentrations, resulting in the total absence of a signal for both the N-Terminal and core. (Figure 46).



**Figure 46.** Final result of the (CEA) discriminatory test in small ruminants

The main objective to introduce these rapid discriminatory tests is to limit the cases submitted for mouse strain typing, as explained above.

Another development in 2007 in the field of TSE typing was the description and typing of atypical cases of BSE. In the past, BSE was considered an infection caused by one strain that was present all over Europe. This hypothesis changed when Italian researchers found cases of BASE or Bovine Amyloid Spongiform Encephalopathies based on western blotting that differentiate between classical and atypical BSE strains. In literature, two atypical BSE strains are described, i.e. H & L types, L type being the synonym of the former BASE cases. The H type could also be linked to the cases of TSE in mink (Transmissible Mink Encephalopathy).

## Humans

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

Subsequently a Belgian CJD surveillance network was created in 1998 in order to follow the disease trends 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 communicating data on patients with a probable or neuropathological confirmed diagnosis to IPH. Regarding the importance of having a confirmation of a clinical suspicion, a financial support facilitates an autopsy for suspected CJD patient. Data are analysed and interpreted by IPH allowing an epidemiological follow up of the disease in the country. A procedure was also set up in order to deal with possible public health consequences if a variant CJD was to be diagnosed. Incidence data are shared with other European countries in a Collaborative Study Group (EuroCJD) and figures communicated to the European Centre for Diseases Control.

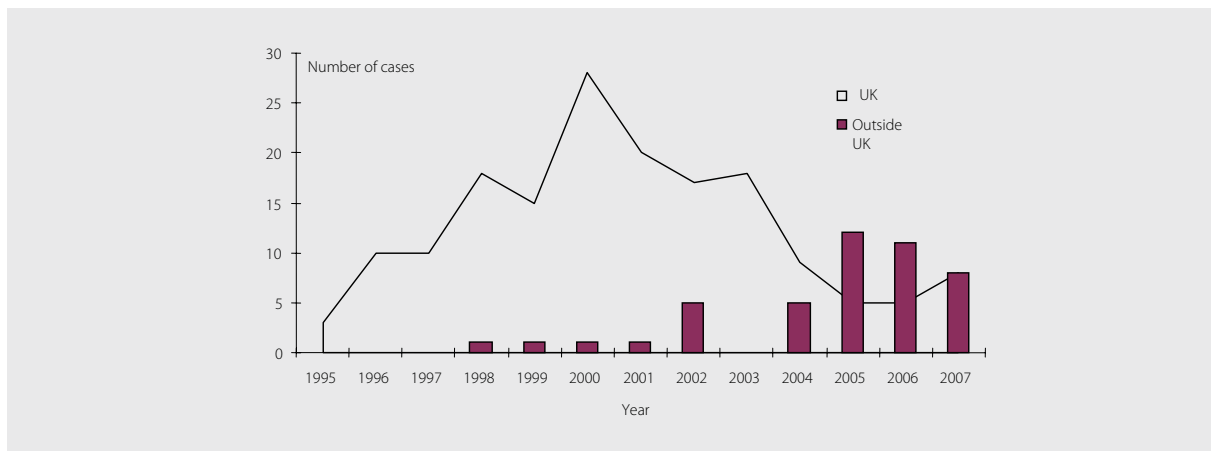
Several forms of CJD are described according to their transmission way: sporadic, genetic and acquired, this one including the iatrogenic form and variant. The diagnosis of CJD is based on clinical presentation, evolution of the symptoms and laboratory tests but can only be confirmed by neuropathological investigation. Added to the age of onset, these elements differ from a CJD form to another neuropathological disorder allowing 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 1.53 cases by 1.000.000 inhabitants per year. The median number of cases per year is 15.5 (range 9-22) with a total 159 reported cases on a 10 years period. Among them 97% are sporadic, 2.5% genetic and only one is classified as iatrogenic (0.5%). There is no preponder-

ance in the sex ratio although large year-to-year variations are observed. The median age at onset is 66 year (range 31-88). The mean disease duration is 6 month.

Since 1995, 203 patients died of variant CJD in 11 countries of which 7 are European countries. UK is the most affected country with 80% of the patients deceased of this disease. In Belgium no case of variant CJD has ever been diagnosed. Although the population has been exposed to BSE and considering that border countries are affected (France, the second most affected country and The Netherlands), the probability to diagnose a case in Belgium can not be excluded. It is also illustrated by the proportion of cases occurring outside UK since 2005. After a peak observed in 2002, a continuous decline in the number of vCJD cases is observed in UK unlike others countries where the majority of the new cases are now diagnosed.

Despite the efficacy of control measures on BSE epidemic, vCJD remains a major public health concern because prions could be present in the population highlighting today the risk of secondary transmission by medical acts (e.g.: blood transfusion, surgery digestive, 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 47.** Number of vCJD in UK and outside UK, 1995 to 2007 - Report of the surveillance network, IPH, 2007





foodborne  
outbreaks

# Foodborne outbreaks

Nadine Botteldoorn, Katelijne Dierick, Jean-Yves Michelet

In Belgium different authorities have competences related to FBOs.

The FASFC deals with safety of foodstuffs, epidemiological investigation of FBOs and related animal health issues.

The Communities (Flemisch, 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.

The IPH (NRL on FBOs) analyses all suspected food samples, collects all data on FBOs and gives scientific support to the FASFC officers and the Public Health Inspectors.

A national "Platform FBOs", 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 FBO. 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

Major etiological agents

Foodborne outbreaks 2007

Working group on foodborne outbreaks

## 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 Flemish Community, the French Community, the Brussels Common Community Committee, 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*, *Giardia*, Norovirus, toxins of *Staphylococcus aureus* and *Bacillus cereus*, histamine and shellfish biotoxins.

## Major etiological agents

### Foodborne bacteria

#### *Salmonella enterica*

Although the number of human salmonellosis is drastically decreased since 2005 in Belgium, 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, 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 foodborne *Salmonella* outbreaks. The food can be the origin of contamination or transmit the infection from a contaminated food handler.

#### *Campylobacter jejuni* and *C. coli*

Since 2005, *Campylobacter* is the most frequently reported illness of foodborn 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, abdominal pain, constipation, nausea, headache and muscle pain. 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). *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 characterized by symptoms as gastroenteritis with diarrhoea and/or vomiting; however, fever and abdominal pain are typical symptoms. *Yersinia* infections can also cause pseudo-appendicitis and arthritis. Illness onset is usually between 24 and 48 hours after ingestion of food or water, which are the usual vehicle 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 characterized 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. Small numbers of the organisms are often present after cooking and multiply to food poisoning levels during cooling and storage of prepared foods under favorable 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 a highly heat-stable enterotoxin in the food, that causes 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*

Although *Bacillus cereus* is a well-known cause of foodborne illness it is not commonly reported because the pathogen usually causes only mild symptoms. 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 characterized 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

Foodborne and waterborne viral infections are increasingly recognized as causes of illness in humans. This increase is partly explained by changes in food processing, consumption patterns, and globalisation of the food trade. Bivalve molluscan shellfish, especially oysters that are consumed raw, are notorious as a source of foodborne viral infections (filter-feeding shellfish can concentrate viruses up to 100-fold from large volumes faecally contaminated water). Several other foodstuffs however, have also been implicated as vehicles of transmission (fruits, berries, vegetables, salads, sandwiches). Raw and minimally processed fruits and vegetables are high risk food products.

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 become 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. It is often difficult to identify whether the food is contaminated at the source, as is common with oysters, or whether the food is contaminated by a sick food handler, or whether person to person transmission occurred.

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 and improved detection methods due to advances in molecular techniques, especially real-time RT-PCR which allow quantification, 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, self-limited gastro-enteritis but attack rates are high.

### 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 man, in extreme case resulting 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 produce in man, 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 too, 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. Often

illness 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 2007

### Prevention of foodborne outbreaks

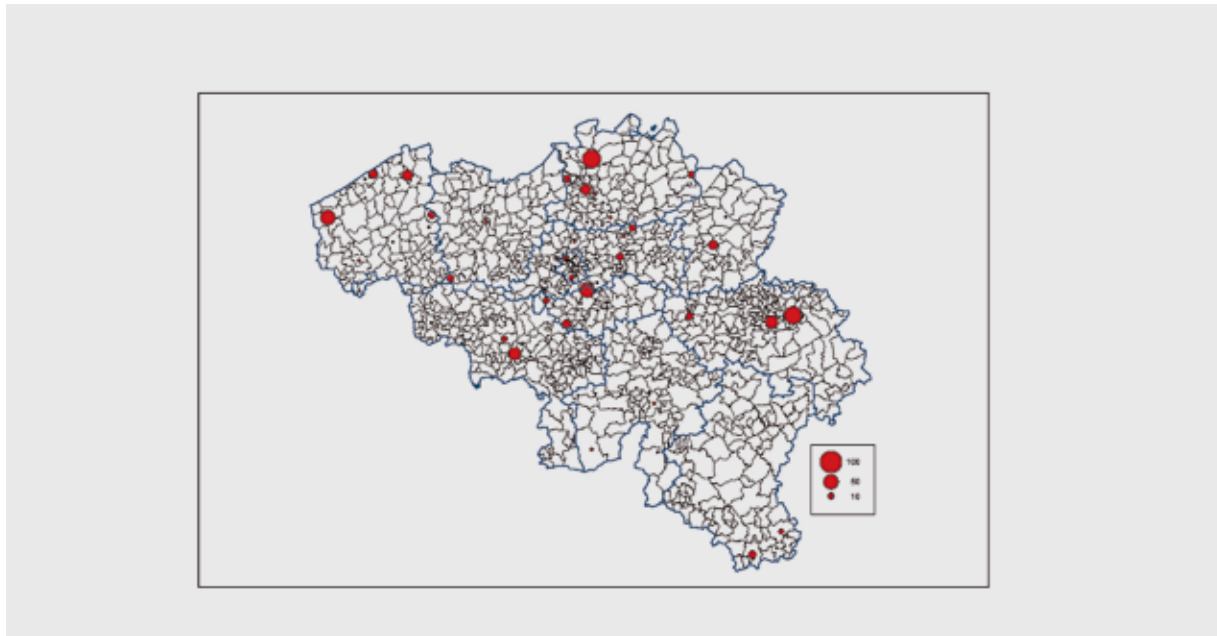
Since the most frequent causes of FBOs are disruption of cold chain, insufficient heating of the food, lack of personal hygiene, bad hygiene in the kitchen, long delay between preparation and consumption and raw materials of poor microbiological quality, outbreaks can be prevented by the application 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.

### Reported outbreaks in 2007

In 2007, a total of 75 outbreaks of foodborne infections and intoxications were recorded in Belgium. More than 846 people were ill and at least 67 persons were hospitalized. Until 2006, all listeriosis cases with a possible food link were included in the total number of outbreaks, even if only one human case was reported, together with perinatal cases affecting a mother and a newborn baby. However, according to the instructions of the European Food Safety Authority,

those were not considered as FBOs any more in 2007. This is partially the reason for the decrease in the number of FBOs in Belgium in 2007.

The geographic distribution of all foodborne outbreaks is shown in figure 48.



**Figure 48.** Geographical distribution of FBOs in Belgium – 2007

### Causative agents in 2007

For the first time Norovirus became the most frequently detected foodborne pathogen in FBOs: 10 outbreaks were reported. The virus was detected in the food and human samples ( $n=2$ ), in the food ( $n=3$ ) only or in human samples only ( $n=5$ ).

In 11% of the outbreaks Salmonella was the causative agent ( $n=8$ ), 99 persons were affected and 20 hospitalised. This confirms the decrease in importance of Salmonella as causative agent noticed in 2004 (53%), 2005 (20%) and 2006 (12%). *S. Enteritidis* was still the most dominant serotype and was detected in 87,5% of the Salmonella outbreaks. In 4 of the 7 Enteritidis outbreaks the link could be made with desserts

made with fresh eggs (Tiramisu (n=3) and chocolate mousse (n=1)). The person who prepared the chocolate mousse however, was carrier of the same strain, which makes it difficult to conclude whether the eggs or the food handler that contaminated the chocolate mousse, since the contamination was only found at the outside of the egg-shell. One Salmonella outbreak was linked with minced meat from a contaminated cutter and one was linked to travelling to Croatia. The only other serovar isolated in FBOs was *S. Typhimurium* var. Copenhagen and was linked with consumption of pitta meat.

Coagulase positive *Staphylococcus* spp caused 7% of the outbreaks in 2007 (n=5). Toxine A was produced by most of the strains.

Thermotolerant *Campylobacter* was responsible for 3% of the outbreaks.

*B. cereus* was the causative agent in seven outbreaks (9% of the outbreaks) and 57 persons became ill. Only in one outbreak the emetic type was detected, the six other ones were caused by enterotoxin producing strains.

Verotoxinogenic *E. coli* were detected in 2 outbreaks. O157 was the causative agent in the first one but the food source was unknown. Both *E. coli* O145 and O26 were detected in the second outbreak due to farm-made ice-cream birthday cakes, where 12 people were affected including 5 human cases of HUS.

In 53% of the 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 37.** FBOs in humans in Belgium in 2007

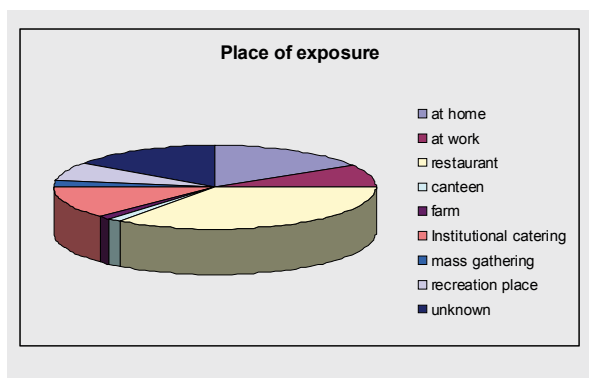
Causative agent	Outbreaks	Ill	Died	Hospitalised	Sources
<i>Bacillus cereus</i>	7	57	0	0	Mixed meals, meat
<i>Campylobacter</i>	2	10	0	1	Grilled meat
<i>Clostridium</i>	0	0	0	0	
Verotoxinogenic <i>E. coli</i>	2	15	0	6	Ice-cream, unknown
Norovirus	10	348	0	37	Sandwiches, mixed meals
<i>Listeria</i>	0	0	0	0	
Other agents	0	0	0	0	
Parasites	0	0	0	0	
<i>Salmonella</i>	8	98	0	14	Preparations with raw eggs, minced meat
<i>Staphylococcus</i>	5	69	0	5	Mixed meals, hamburgers
Histamine	0	0	0	0	
Unknown	40	220	0	4	
<i>Yersinia</i>	1	17	0	0	Unknown
Total	75	835	0	67	

## Source of the foodborne outbreaks

Most FBOs (56%) were due to the consumption of meals composed of different ingredients. Meat and meat based products were responsible for 19% of the outbreaks. Bakery products, including preparations with raw eggs such as tiramisu and chocolate mousse were responsible for 5% of the outbreaks. These preparations were the only egg related outbreaks in 2007, all with *S. Enteritidis*, and count for 4% of the total outbreaks. In 2006, 2005 and 2004 this was respectively 4%, 8% and 36%. This shows that the decrease in egg-related illness is maintained. Sandwiches were the vehicle in 4 out of 10 norovirus outbreaks.

## Setting of the foodborne outbreaks

In more than 90%, FBOs could be traced back to the place of exposure. Restaurants (38%) were the most frequently associated with FBOs, followed by shops (butchers, bakeries) representing 21% and institutional catering services (13%). Other locations of exposure were camping (3%) and one farm with an outbreak of *E.coli* O145 and O26 was detected.



**Figure 49.** Place of exposure in FBOs 2007

## Description of two outbreaks of special interest

A FBO caused by *Staphylococcus aureus* in frozen hamburgers in the summer of 2007 at a Scouts' camp in the south of Belgium

At least 15 children and adults became ill, showing severe nausea, vomiting and diarrhoea shortly after eating lunch. The FASFC sampled all leftovers of the suspected food (milk,

hamburgers, cheese, ketchup and pasta) as well as frozen hamburgers of the same production date, sampled at the supermarket. The samples were submitted to the NRL for FBOs for microbiological analysis and detection of toxins. Since the production site was in the Netherlands, an inspection was performed at the hamburger production plant by the Dutch Authorities. An extensive review of production and handling procedures and laboratory testing of different lots of snacks from the production plant was performed.

The hamburgers served at the camp were contaminated with high levels of *S. aureus*, and tested positive for *S. aureus* enterotoxin type A. The hamburgers sampled at the supermarket as well as the hamburgers sampled at the production plant contained varying levels of *S. aureus*, but no enterotoxins could be detected. The *S. aureus* isolates were subjected to molecular typing by PFGE and MLST. All food isolates and isolates from the production plant belonged to the same PFGE type indicating a common source of contamination. The inspection revealed that the cooling system used to rapidly cool the cooked hamburgers was contaminated with *S. aureus* and could not be properly cleaned.

An outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with the consumption of ice cream produced at a farm

In October 2007, an outbreak of verocytotoxin-producing *E. coli* (VTEC) O145 and *E. coli* O26 occurred among consumers of ice cream produced and sold in September 2007 at a farm in the province of Antwerp. The ice cream was consumed at two birthday parties and was at the farm. Five children,

between two and 11 years, developed haemolytic uraemic syndrome (HUS), and seven other co-exposed persons contracted severe diarrhoea. In three of the five HUS cases VTEC O145 infections were laboratory confirmed, one in association with VTEC O26. Identical isolates of *E. coli* O145 and O26 were detected with PCR and PFGE in faecal samples of patients and in ice cream leftovers from one of the birthday parties, in faecal samples taken from calves, and in samples of soiled straw from the farm at which the ice cream was produced. Ice cream was made from pasteurized milk and most likely contaminated by one of the food handlers.

De Schrijver K. et al, Euro Surveill. 2008 Feb 28; 13 (9)

## Working group on foodborne outbreaks

The working group was created in 1995 by the Institute of Public Health 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 matter and matters related to persons such as illness are the competence of the Flemish, French or German community, 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 foodborne outbreaks.

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 Trends and Sources Report to the European Food Safety Agency (EFSA).

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

## Tables

Table 1. Evolution in the total human population 2002-2007	17
Table 2. Total number of herds and animals in the period 2004 – 2007	18
Table 3. Total number of herds and animals of poultry in the period 2004 – 2007.	20
Table 4. Number of slaughtered animals in the period 2003 – 2007	21
Table 5. Evolution of the individual serological tests and the bulk milk tests	26
Table 6. Zoonosis monitoring programme – Campylobacter in food	31
Table 7. Evolution of the pork Campylobacter prevalence 2004-2007	31
Table 8. Campylobacter in meat and meat products: list of antimicrobials tested and breakpoints used	32
Table 9. Antimicrobial susceptibility testing of Campylobacter in food: Percentage of resistant strains	32
Table 10. Number of Campylobacter infections in humans by sex and by age groups, 2007.	37
Table 11. Resistance of Campylobacter in Belgium fecal isolates, trend from 2001 till 2007.	38
Table 12. Repartition by biotype of invasive Campylobacter in humans, 2007	39
Table 13. Zoonosis monitoring programme - E. coli O157, 2007	43
Table 14. E. coli : evolution in number of isolates in humans, 1998-2007	44
Table 15. Evolution of the number of cases of leptospirosis, ITM, 2003-2007	49
Table 16. Zoonosis monitoring programme - Listeria monocytogenes in food, 2007.	53
Table 17. Number of Listeria monocytogenes infections in humans by sex and by age groups, 2007	56
Table 18. MRSA prevalence data in pigs	59
Table 19. The results of the monitoring 2007 – Salmonella in meat and meat products	77

Table 20. Evolution of the food Salmonella prevalence, 2004-2007	79
Table 21. Trends for the most prevalent Salmonella serotypes from 1986 to 2007	81
Table 22. Human cases of Salmonella: Age and gender distribution 2007	82
Table 23. Animal Salmonella: list of antimicrobials tested	83
Table 24. Salmonella from food: list of antimicrobials tested with their breakpoints	85
Table 25. Antimicrobial susceptibility testing of Salmonella spp. isolated from different food matrices:	85
Table 26. List of antimicrobials used for susceptibility testing of Salmonella	88
Table 27. Antimicrobial resistance in human Salmonella isolated in 2007.	90
Table 28. Evolution of bovine tuberculosis outbreaks in cattle herds in Belgium	93
Table 29. Monitoring Yersinia enterocolitica in pork meat.	96
Table 30. Number of Yersinia enterocolitica infections in humans by sex and by age groups, 2007.	98
Table 31. Results of lab-tests carried out on 4 samples of suspect cases for virus infection by Influenza A/H5N1	105
Table 32. Distribution of the suspected cases of virus infection by Influenza A/H5N1 per destination, 2007	105
Table 33. Number of cases of Hantavirus infections in humans by sex and age groups, 2007	109
Table 34. Number of Cryptosporidium infections in humans by sex and by age groups, 2007	122
Table 35. Number of animals controlled in Belgium (2001 - 2007)	142
Table 36. Positive TSE cases in cattle and sheep in Belgium	143
Table 37. FBOs in humans in Belgium in 2007	155

## Figures

Figure 1. Evolution of human population 2002 – 2007	18
Figure 2. Evolution of the total number of cattle herds, period 2004 – 2007	19
Figure 4. Evolution of the total number of pig herds, period 2004 – 2007	19
Figure 3. Evolution of the total number of bovines, period 2004 – 2007	19
Figure 5. Evolution of the total number of porcines, period 2004 – 2007	19
Figure 6. Gallus gallus, evolution number of poultry herds, period 2004 – 2007	20
Figure 7. Gallus gallus, evolution total number of poultry, period 2004 – 2007	20
Figure 8. Evolution of slaughtered bovines 2003 – 2007	21
Figure 9. Evolution of slaughtered pigs 2003 – 2007	21
Figure 10. Evolution of slaughtered sheep & goats 2003 – 2007	22
Figure 11. Evolution of slaughtered broilers and layers 2003 – 2007	22
Figure 12. Evolution of the percentage resistant Campylobacter jejuni and Campylobacter coli strains in poultry.	33
Figure 13. Evolution of the percentage resistant Campylobacter coli strains isolated in pork	34
Figure 14. Total number of Campylobacter infections in humans by year (1986-2007)	35
Figure 15. Weekly number of Campylobacter infections in humans, 2007	36
Figure 16. Incidence of Campylobacter infections in humans by district (N/105 inhab., 2007)	37
Figure 17. Seropositive laboratory confirmed cases in animals at the NRL, animal health between 2000 and 2007	46
Figure 18. Number of Listeria monocytogenes infections in humans by year (1994-2007)	55
Figure 19. Weekly number of Listeria monocytogenes infections in humans, 2007	55

Figure 20. Incidence of <i>Listeria monocytogenes</i> infections in humans by district (N/105 inhab., 2007)	56
Figure 21. Distribution of tested farms according to farm type	59
Figure 22. Percentage animals positive and negative for the different age groups	59
Figure 23. Evolution of the percentages of the principal <i>Salmonella</i> serotypes isolated from poultry, 1996 – 2007	72
Figure 24. Evolution of the percentages of the principal <i>Salmonella</i> serotypes isolated from pigs, 1996 – 2007	75
Figure 25. Evolution of the percentages of the principal <i>Salmonella</i> serotypes isolated from cattle, 1996 – 2007	76
Figure 26. Trend of the human <i>Salmonella</i> isolates over the last twenty seven years in Belgium	81
Figure 27. Seasonal distribution 2002 – 2007	82
Figure 28. Percentage resistant <i>Salmonella</i> strains in broiler meat (2001-2003) and poultry meat (2004-2007).	86
Figure 29. Percentage resistant <i>Salmonella</i> strains in pork (2001-2007).	87
Figure 30. Evolution of bovine tuberculosis outbreaks in cattle herds in Belgium	93
Figure 31. Total number of <i>Yersinia enterocolitica</i> infections in humans by year (1986-2007)	97
Figure 32. Weekly number of <i>Yersinia enterocolitica</i> infections in humans, 2007	97
Figure 33. Incidence of <i>Yersinia enterocolitica</i> infections in humans by district (N/105 inhab., 2007)	99
Figure 34. Number of suspicion dossier / trimester of 2007	103
Figure 35. Number of exclusion diagnosis dossiers / trimester of 2007.	104
Figure 36. Serological screening of poultry / trimester of 2007	104
Figure 37. Number of cases for suspicion of infection with Influenza A/H5N1, January – December, 2007	105
Figure 38. Total number of Hantavirus infections in humans by year (1996-2007)	108

Figure 39. Distribution of Hantavirus infections in humans (N/4 weeks), 1996 –2007	109
Figure 40. Incidence of Hantavirus infections in humans by district (N/105 inhab., 2007)	110
Figure 41. Total number of Cryptosporidium infections in humans by year, 1992-2007	121
Figure 42. Weekly number of Cryptosporidium infections in humans, 2007	121
Figure 43. Incidence of Cryptosporidium infections in humans by district (N/105 inhab., 2007)	123
Figure 44. Cysticercosis, detection of lightly contaminated bovine carcasses at slaughterhouse	126
Figure 45. Cysticercosis: detection of heavily contaminated bovine carcasses at slaughterhouse	126
Figure 46. Final result of the (CEA) Discriminatory test in small ruminants	144
Figure 47. Number of vCJD in UK and outside UK, 1995 to 2007	146
Figure 48. Geographical distribution of FBOs in Belgium – 2007	154
Figure 49. Place of exposure in FBOs 2007	156

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Kruidtuinlaan 55 - 1000 Brussel

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Graphic design: FAVV Communication service

Print: Cartim, Gentbrugge

Printed on FSC compliant paper

D/2008/10.413/1







2007

- 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)