

trends and sources

# 2012-2013



report on zoonotic agents in belgium



- 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)

## Executive summary

Zoonoses are infectious diseases that are transmissible from animals to humans. Humans can be infected directly through animals or their environment, or indirectly through the ingestion of contaminated foodstuffs. It is important that persons in contact with animals are aware of possible transmission of a zoonotic infection 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. Programs and systems of animal disease monitoring, 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 should be available. Active collaboration between all actors of the food chain is necessary. All stakeholders, agriculture sectors and industries, scientists, experts of the national reference laboratories and from other laboratories, specialists of the competent authorities and technical committees have to bring together their expertise, experiences, methods and findings. Only a collaborative approach and effective partnership at all levels will successfully control zoonoses and improve food safety.

The most commonly reported zoonotic infections in humans are those caused by bacterial zoonotic agents that can be shed by asymptomatic farm animals. Since 2005, campylobacteriosis is the most frequently reported zoonotic disease in humans followed by salmonellosis .

The most important cause of food borne outbreaks (FBO's) in 2012 were Norovirus infections followed by Salmonella outbreaks and in 2013 bacterial toxins of *Bacillus cereus* and coagulase positive Staphylococci followed by Campylobacter infections. The major sources of Salmonella in food-borne outbreaks are table eggs, poultry meat and pig meat. Reducing Salmonella- and Campylobacter-infections remains an important challenge.

# Table of contents

Executive summary	3
Table of contents	4
Preface	8
Introduction	9
Belgian reference laboratories for zoonotic agents	11
Acronyms, abbreviations and special terms	14
General information	15
Susceptible human population	15
Susceptible animal population	16
Ruminants and pigs	16
Poultry	17
Animals slaughtered	18
<b>Part I Report on zoonotic agents and food-borne outbreaks 2012 - 2013</b>	<b>20</b>
Bacterial diseases	21
Brucellosis	21
Zoonotic brucellosis	21
Brucellosis in cattle	21
Brucellosis in sheep and goats	23
Brucellosis in pigs	24
Brucellosis in wildlife	24
Brucellosis in humans	24
Campylobacteriosis	25
Campylobacteriosis	25
Campylobacteriosis in food	25
Campylobacter in humans	27
Botulism	29
Botulism in animals	29
Clostridium botulinum in feed	31
Clostridium botulinum in food	31
Botulism in humans	31
Escherichia coli (VTEC) infections	32
Verotoxin and shiga-like toxin producing Escherichia coli	32
Verotoxin producing Escherichia coli in cattle	32
Verotoxin producing Escherichia coli O157 in food	33
Verotoxigenic Escherichia coli infections in humans	33

Leptospirosis	36
Leptospirosis	36
Leptospirosis in animals	36
Leptospirosis in humans	37
Listeriosis	38
Listeriosis	38
Listeria monocytogenes in food	38
Listeria monocytogenes in humans	41
Q-fever	43
Coxiella burnetii	43
Q-fever in animals	43
Q-fever in humans	45
Salmonellosis	46
Salmonellosis in feed	46
Salmonella in poultry	47
Salmonella in pigs	51
Salmonella in cattle	52
Salmonella in food	52
Salmonella in humans	56
Tuberculosis	59
Zoonotic tuberculosis ( <i>Mycobacterium bovis</i> )	59
Mycobacterium bovis in cattle	59
Mycobacterium bovis in wildlife	62
Mycobacterium bovis in humans	62
Human tuberculosis ( <i>Mycobacterium tuberculosis</i> )	63
Yersiniosis	64
Yersinia enterocolitica	64
Yersinia enterocolitica in food	64
Yersiniosis in humans	65
Viral diseases	67
Hantaviruses	67
Hanta disease	67
Hantaviruses in animals	67
Hantaviruses in humans	67
Hepatitis E Virus	70
Introduction	70
Hepatitis E Virus in animals	70
Hepatitis E Virus in humans	71
Rabies	72
Rabies	72
Rabies in animals	73
Tick-borne Encephalitis Virus	75
Introduction	75
Tick-borne Encephalitis Virus in animals and ticks in Belgium	75
Tick-borne Encephalitis Virus in humans in Belgium	76

West Nile virus	77
West Nile virus	77
West Nile virus in birds	77
West Nile virus in humans	79
<b>Parasitic diseases</b>	<b>80</b>
Cryptosporidiosis	80
Cryptosporidiosis	80
Cryptosporidiosis in animals	80
Cryptosporidiosis in humans	80
Cysticercosis	82
Cysticercosis in cattle	82
Echinococcosis	83
Echinococcosis	83
Echinococcus in animals	83
Echinococcus in wildlife	83
Echinococcus in humans	84
Toxoplasmosis	85
Toxoplasmosis in animals	85
Toxoplasmosis in humans	86
Trichinellosis	87
Trichinella	87
Trichinella in animals	88
Trichinella in wildlife	88
<b>Food-borne outbreaks</b>	<b>89</b>
Food-borne outbreaks in humans	89
Major etiological agents	89
Food-borne bacteria	89
Food-borne viruses	90
Marine biotoxins	91
Parasites	91
Food-borne outbreaks	91
Reported outbreaks	91
Causative agents	91
Source of the food-borne outbreaks	93
Setting of the food-borne outbreaks	93
Description of food-borne outbreaks of special interest	95
Trends in food-borne outbreaks 2006 - 2013	96
Working group on food-borne outbreaks	99

<b>Part II Report on antimicrobial resistance</b>	<b>100</b>
Antimicrobial resistance of <i>Campylobacter</i>	102
AMR of <i>Campylobacter</i> strains isolated from meat and meat products	102
Antimicrobial resistance in <i>Campylobacter</i> from poultry meat	103
Antimicrobial resistance in <i>Campylobacter</i> from pork	104
AMR of <i>Campylobacter</i> strains isolated from humans	104
Antimicrobial resistance of <i>Salmonella</i>	106
AMR of <i>Salmonella</i> strains isolated from food	106
AMR of <i>Salmonella</i> strains isolated from meat and meat products	106
AMR of <i>Salmonella</i> strains isolated from poultry meat	107
AMR of <i>Salmonella</i> strains isolated from pork	108
AMR of <i>Salmonella</i> strains of animals	109
Antimicrobial resistance of indicator commensal bacteria	114
Introduction	114
Sampling and laboratory testing	114
Statistical analysis	114
Prevalence of resistance in <i>E. coli</i> .	114
Prevalence of resistance in <i>Enterococci</i> spp.	115
Multiresistance in commensal flora	115
Conclusion	115
MRSA surveillance	118
MRSA	118
MRSA in cattle, surveillance 2012.	118
Methods	118
Prevalence	119
Antimicrobial resistance	119
Discussion	120
Conclusions	121
MRSA in pigs – surveillance 2013	122
Conclusions	124
MRSA surveillance in humans	125
Conclusions	126
Beta-lactam resistant phenotypes for <i>E. coli</i>	127
ESBL producing <i>E. coli</i> in animals	127
ESBL producing <i>E. coli</i> in food	127
Enumeration of <i>E. coli</i> and <i>E. coli</i> ESBL in poultry carcasses, 2012-2013	127
Detection of ESBL producing <i>E. coli</i> in beef, pork and veal, 2013	128
ESBL producing bacteria in humans	129

# Preface

All European member states have the obligation to annually 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<sup>1</sup>. 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 food-borne outbreaks.

Similarly, based on article 1 of Decision 2119/98/EC<sup>2</sup>, data on zoonotic infections in humans are officially reported each year to the European Centre for Disease Prevention and Control (ECDC).

Based on these two official reports, the Federal Agency for the Safety of the Food Chain (FASFC), together with the federal scientific institutions CODA-CERVA (Veterinary and Agrochemical Research Centre) and WIV-ISP (Scientific Institute of Public Health) agreed to publish a report which contains the same information combined with data of previous years to indicate some trends of diseases or sources of infection. This Belgian Trends and Sources report will focus especially on the data of 2012 and 2013 as this report is a bi-annual edition for the third time. The aim of this booklet is to inform professionals as well as persons who have a general interest in zoonotic diseases and in the safety of our food.

In this 2012 – 2013 edition botulism, Hepatitis E Virus infection

and Tick-borne Encephalitis Virus infection will be treated for the first time. For a second time, as antimicrobial resistance and the use of antimicrobials became an important topic, antimicrobial resistance will be published separately in this edition in a second part of this report. All AMR information on different zoonotic pathogens will be compiled instead of being treated per pathogen in different chapters as in some previous reports.

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

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1 Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/ http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L\_2003.325.01.0031.01.ENG

2 Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community, http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1407159991084&uri=CELEX:31998D2119

# Introduction

This report compiles especially the data of 2012 and 2013 and compares these to some data of previous years on zoonoses and zoonotic agents, and is derived from the official documents reported to EFSA and ECDC. For this reason, it is a unique document in which laboratory results from the primary production, from food, from feed and from public health 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 zoonotic diseases or food-borne agents that may be of interest to the reader, e.g. Norovirus and Hanta virus.

Together with the general descriptive information on the diseases or the infections and their evolution over time, some recommendations on prevention of infection are provided. This report 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 made 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 of the cold chain and simple hygiene measures in the kitchen may be very efficient in preventing food-borne contaminations and illness.

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

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

This report was coordinated by L. Vanholme (FASFC), H. Imberechts (CODA-CERVA), K. Dierick and T. Braeye (WIV-ISP) and reviewed by X. Van Huffel (FASFC), L. De Zutter (University of Ghent) and R. Mak (Agency for Care and Health, Flemish Ministry). 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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Campylobacter (non human)	N. Botteldoorn	WIV-ISP Foodborne Pathogens J. Wytsmanstraat, 14 1050 Brussels	Nadine.Botteldoorn@wiv-isp.be  <a href="http://www.wiv-isp.be/">http://www.wiv-isp.be/</a>
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Cryptosporidium	M. Van Esbroeck	ITG-IMT Klinische Biologie Kronenburgstraat, 43/3 2000 Antwerpen	mvesbroeck@itg.be  <a href="http://www.itg.be/itg">http://www.itg.be/itg</a>
Echinococcus multilocularis	C. Truyens	Fac. de Médecine U.L.B. Parasitologie Route de Lennik, 808 1070 Brussels	ycarlier@ulb.ac.be  <a href="http://www.ulb.ac.be/">http://www.ulb.ac.be/</a>
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Mycobacterium (animal health)	D. Fretin	CODA-CERVA Groeselenberg, 99 1180 Brussels	David.Fretin@coda-cerva.be  <a href="http://www.coda-cerva.be/">http://www.coda-cerva.be/</a>
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Q-Fever (Coxiella burnetii) (animal health)	D. Fretin	CODA-CERVA Groeselenberg, 99 1180 Brussels	david.fretin@coda-cerva.be  <a href="http://www.coda-cerva.be/">http://www.coda-cerva.be/</a>
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Salmonella (public health)	S. Bertrand	WIV-ISP Bacterial diseases Rue J. Wytsman, 14 1050 Brussels	sophie.bertrand@wiv-isp.be  <a href="http://www.wiv-isp.be/">http://www.wiv-isp.be/</a>
Salmonella (animal health)	V. Jasson	CODA-CERVA Groeselenberg, 99 1180 Brussels	Vicky.Jasson@codacerva.be  <a href="http://www.var.fgov.be/">http://www.var.fgov.be/</a>
Salmonella (food)	K. Dierick	WIV-ISP Food Pathogens J. Wytsmanstraat, 14 1050 Brussels	Katelijne.Dierick@wiv-isp.be  <a href="http://www.wiv-isp.be/">http://www.wiv-isp.be/</a>
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Staphylococcus aureus (food)	S. Denayere	WIV-ISP Foodborne Pathogens J. Wytsmanstraat, 14 1050 Brussels	Sarah.Denayere@wiv-isp.be  <a href="http://www.wiv-isp.be/">http://www.wiv-isp.be/</a>
Tick-born encephalitis virus	S. Van Gucht	WIV-ISP Viral diseases Engelandstraat, 642 1180 Brussels	steven.vangucht@wiv-isp.be  <a href="http://www.wiv-isp.be">http://www.wiv-isp.be</a>
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Yersinia enterocolitica	J. Verhaegen  M. Delmée	UZ Leuven Microbiology Herestraat, 49 3000 Leuven  UCL St-Luc Microbiology Unit Av. Hippocrate, 54.92 1200 Brussels	Jan.Verhaegen@uzleuven.be  <a href="http://www.uzleuven.be/">http://www.uzleuven.be/</a>  Michel.Delmee@uclouvain.be  <a href="http://www.saintluc.be">http://www.saintluc.be</a>
West Nile Virus	M. Van Esbroeck	ITG-IMT Klinische Biologie Kronenburgstraat, 43/3 2000 Antwerp	mvesbroeck@itg.be  <a href="http://www.itg.be/itg">http://www.itg.be/itg</a>
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# Acronyms, abbreviations and special terms

ADNS	Animal Disease Notification System <a href="http://ec.europa.eu/food/animal/diseases/adns/index_en.htm">http://ec.europa.eu/food/animal/diseases/adns/index_en.htm</a>
ARSIA	Association Régionale de Santé et d'Identification Animales, Walloon Regional Animal Health Association <a href="http://www.arsia.be">http://www.arsia.be</a>
BAPCOC	Belgian Antibiotic Policy Coordination Committee <a href="http://www.bapcoc.be">http://www.bapcoc.be</a>
CFT	Complement Fixation Test
CLSI	Clinical and Laboratory Standards Institute <a href="http://clsi.org">http://clsi.org</a>
CODA – CERVA	Veterinary and Agrochemical Research Centre Centrum voor Onderzoek in Diergeneeskunde en Agrochemie Centre d'Etude et de Recherches Vétérinaires et Agrochimiques <a href="http://www.coda-cerva.be">http://www.coda-cerva.be</a>
DGZ Vlaanderen	Dierengezondheidszorg Vlaanderen, Flanders Regional Animal Health Association <a href="http://www.dgz.be">http://www.dgz.be</a>
ECDC	European Centre for Disease Prevention and Control <a href="http://www.ecdc.europa.eu">http://www.ecdc.europa.eu</a>
EFSA	European Food Safety Authority <a href="http://www.efsa.europa.eu">http://www.efsa.europa.eu</a>
ELISA	Enzyme Linked Immuno Sorbent Assay
EURL	European Reference Laboratory
FASFC - FAVV – AFSCA	Federal Agency for the Safety of the Food Chain Federaal Agentschap voor de Veiligheid van de Voedselketen Agence Fédérale pour la Sécurité de la Chaîne alimentaire <a href="http://www.favv.be">http://www.favv.be</a>
FBO	Food-borne outbreak
FPS	Federal Public Service (Ministry) – Public Health, Food Chain Security and Environment <a href="http://www.health.belgium.be/eportal">http://www.health.belgium.be/eportal</a>
IFA	Indirect Fluorescent Antibody assay
IFN	Interferon
IPH - WIV - ISP	Scientific Institute of Public Health Wetenschappelijk Instituut Volksgezondheid Institut Scientifique de Santé Publique <a href="https://www.wiv-isp.be">https://www.wiv-isp.be</a>
ITM - ITG – IMT	Institute of Tropical Medicine Instituut voor Tropische Geneeskunde Institut de Médecine Tropicale <a href="http://www.itg.be">http://www.itg.be</a>
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats
NRCSS	National Reference Centre for Salmonella and Shigella, IPH
NRL	National Reference Laboratory
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
RBT	Rose Bengal Test
RFLP	Restriction Fragment Length Polymorphism
RT PCR	Reverse Transcriptase Polymerase Chain Reaction
Sanitel	Registration and Identification Database of farm animals
SAT	Slow Agglutination Test
SFM	French Society for Microbiology <a href="http://www.sfm-microbiologie.org">http://www.sfm-microbiologie.org</a>
WHO	World Health Organization <a href="http://www.who.int">http://www.who.int</a>

# General information

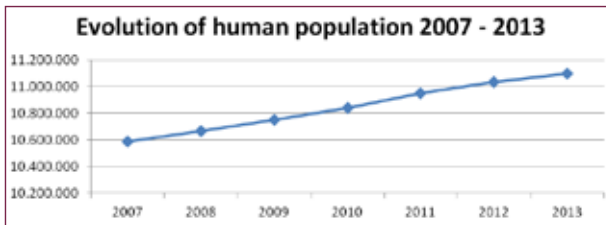
## Susceptible human population

The evolution of the total human population in Belgium from 2007 to 2013 is shown in table 1 and figure 1.

**Table 1.** Evolution in the human population 2007-2013 Source: National statistical authority, <http://statbel.fgov.be/>

	2007	2008	2009	2010	2011	2012	2013
<b>Total</b>	<b>10.584.534</b>	<b>10.666.866</b>	<b>10.753.080</b>	<b>10.839.905</b>	<b>10.951.266</b>	<b>11.035.948</b>	<b>11.099.554</b>
Male	5.181.408	5.224.309	5.268.651	5.312.221	5.370.234	5.413.801	5.447.488
Female	5.403.126	5.442.557	5.484.429	5.527.684	5.581.032	5.622.147	5.652.066
Brussels	1.031.215	1.048.491	1.068.532	1.089.538	1.119.088	1.138.854	1.154.635
Flanders	6.117.440	6.161.600	6.208.877	6.251.983	6.306.638	6.350.765	6.381.859
Wallonia	3.435.879	3.456.775	3.475.671	3.498.384	3.525.540	3.546.329	3.563.060
Foreigners	932.161	971.448	1.130.260	1.057.666	1.119.256	1.169.064	1.195.122

The total number of immigrants in Belgium is important. Immigration and tourism may play an important role in the introduction and the transmission of zoonotic or other infectious diseases.



**Figure 1.** Evolution of the total number of cattle herds, period 2007–2011

# Susceptible animal population

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

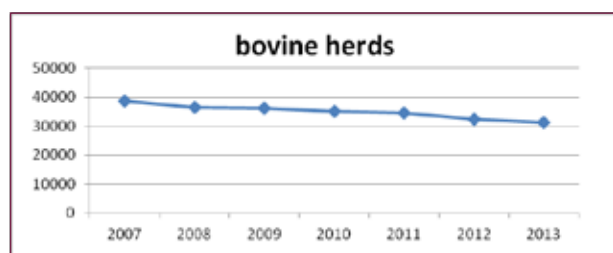
## Ruminants and pigs

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

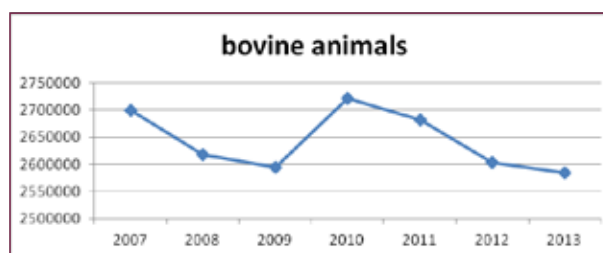
**Table 2.** Total number of herds and animals in the period 2007 – 2011

	2007		2008		2009		2010		2011		2012		2013	
	herds	animals	Herds	animals	herds	animals	herds	animals	herds	animals	herds	animals	herds	animals
Cattle	38.690	2.699.258	36.423	2.618.040	36.064	2.594.358	35.217	2.721.130	34.540	2.682.370	32.475	2.603.148	31.363	2.585.003
Pigs	9.950		9.419		9.243		9.063		9.075		8.690		8.332	
Breeding sows		632.360		615.298		598.857		589.049		583.919		566.600		542.187
Fattening pigs		5.007.614		5.123.189		5.113.202		5.286.829		5.375.356		5.362.090		5.324.618
Sheep	31.523	220.611	31.037	205.624	30.626	215.262	29.556	209.263	29.150	204.128	28.223	201.209	27.582	167.681
Goats	13.381	46.950	12.692	48.379	12.530	57.371	11.869	60.753	11.710	48.989	11.255	42.950	10.841	47.664
Deer	2.907	12.648	2.825	10.834	2.810	9.502	2.717	9.239	2.667	9.174	2.605	9.591	2.555	9.747

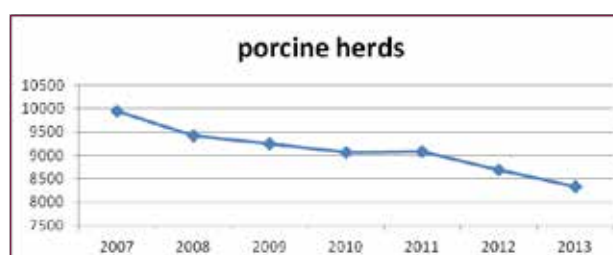
Next figures represent the evolution of total number of respectively bovine and porcine herds and animals over the last years.



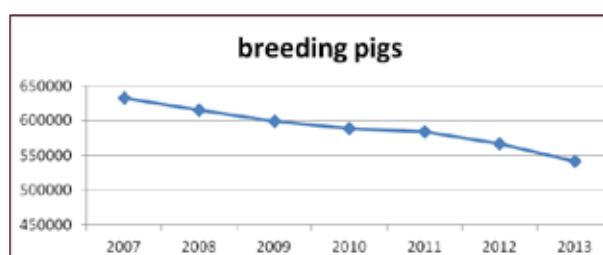
**Figure 2.** Evolution of the total number of cattle herds, period 2007–2013



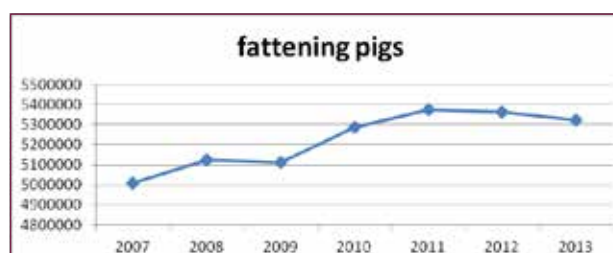
**Figure 3.** Evolution of the total number of bovines, period 2007–2013



**Figure 4.** Evolution of the total number of porcine herds, period 2007 – 2013



**Figure 5.** Evolution of the total number of breeding pigs, period 2007–2013



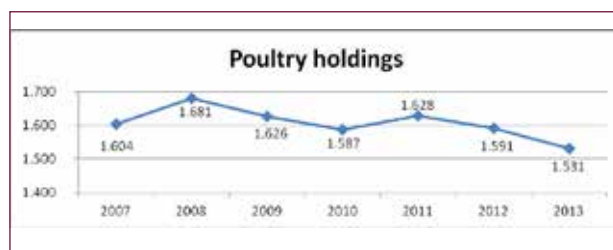
**Figure 6.** Evolution of the total number of fattening pigs, period 2007–2013

## Poultry

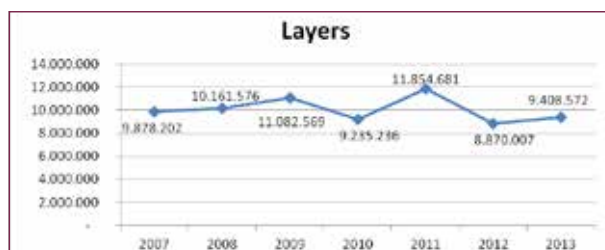
**Table 3.** Total number of herds and animals of *Gallus gallus* in the period 2007–2013

	2007	2008	2009	2010	2011	2012	2013
Holdings	1.604	1.681	1.626	1.587	1.628	1.591	1.531
Layers	9.878.202	10.161.576	11.082.569	9.235.236	11.854.681	8.870.007	9.408.572
Broilers	25.311.775	29.385.921	27.134.405	26.269.807	26.291.305	25.445.919	27.349.030
Breeders	2.089.933	1.865.111	2.031.572	1.979.233	1.844.109	1.472.600	2.564.649

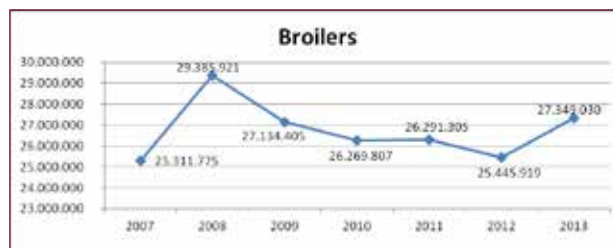
Next figures represent the evolution of total number of respectively poultry holdings, layers, broilers and breeders over the last years.



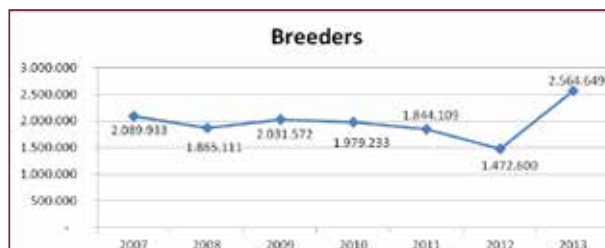
**Figure 7.** Evolution of the total number of poultry holdings, period 2007–2013



**Figure 9.** Evolution of the total number of layers, period 2007–2013



**Figure 8.** Evolution of the total number of broilers, period 2007 – 2013



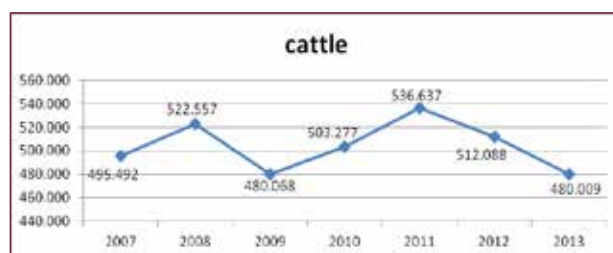
**Figure 10.** Evolution of the total number of breeders, period 2007–2013

# Animals slaughtered

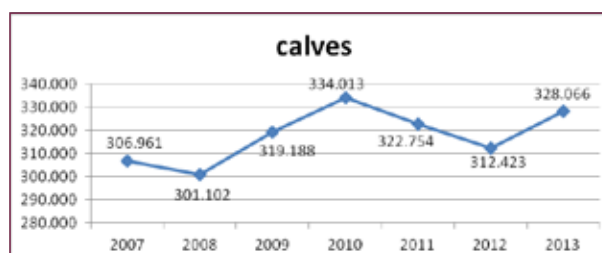
**Table 4.** Total number of slaughtered animals in the period 2007–2013.

	2007	2008	2009	2010	2011	2012	2013
Cattle	495.492	522.557	480.068	503.227	536.637	512.503	480.009
Calves	306.961	301.102	319.188	334.013	322.754	312.430	328.066
Pigs	11.536.172	11.588.072	11.677.883	11.924.052	11.801.106	11.724.493	11.945.169
Solipeds	10.064	9.173	8.910	8.970	9.669	9.201	8.800
Sheep		133.192	135.071	143.196	127.250	116.120	121.265
Goats		6363	6143	7.962	6.701	7.588	6.783
Sheep & Goats	137.492	139.555	141.214	151.158	133.951	123.708	128.048
Poultry	274.505.734	274.427.724	290.556.915	312.465.992	304.719.679	313.096.763	294.853.925

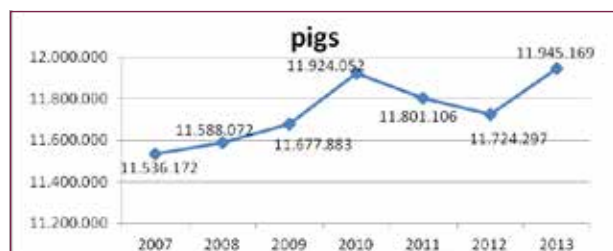
Figures 11 to 16 represent the evolution of the total number of slaughtered bovines, porcines, solipeds, sheep & goats and broilers & layers over the last years.



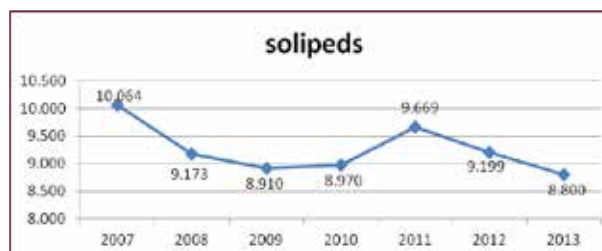
**Figure 11.** Evolution of total number of slaughtered cattle, period 2007–2013



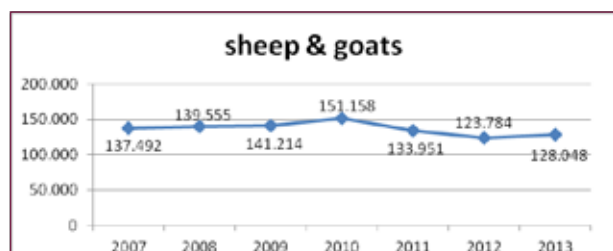
**Figure 12.** Evolution of total number of slaughtered calves, period 2007–2013



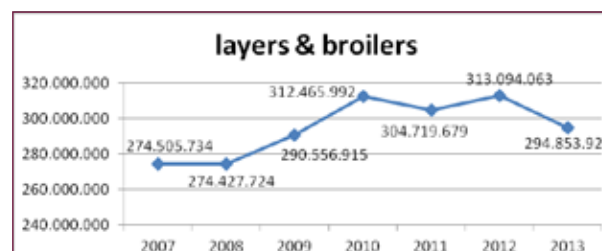
**Figure 13.** Evolution of total number of slaughtered pigs, period 2007–2013



**Figure 14.** Evolution of total number of slaughtered solipeds, period 2007–2013



**Figure 15.** Evolution of total number of slaughtered sheep & goats, period 2007–2013



**Figure 16.** Evolution of slaughtered broilers and layers 2007–2013



# Part I

## Report on zoonotic agents and food-borne outbreaks 2012 - 2013



trends and sources 2012 - 2013

# Bacterial diseases

## Brucellosis

David Fretin, Annick Linden, Marcella Mori, Luc Vanholme

### Zoonotic brucellosis

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

In people brucellosis is characterised by flu-like symptoms such as fever, headache, back pain and physical weakness. Nocturnal sweating is frequently observed. Infections of the central nervous system or lining of the heart may occur.

- In the “non-officially brucellosis free” Mediterranean countries, the consumption of raw milk or raw cheese from sheep and goats is considered as 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*.

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

#### **New sanitary policy since 2009.**

In 2009, a study was realized to evaluate the national surveillance program of bovine brucellosis. If a Member State has maintained the officially free status of brucellosis for at least 5 consecutive years, the existing surveillance program can be re-evaluated and some modifications on the sampling design are allowed on condition of further proof of freedom of disease and early detection of re-emerge or re-infection of the disease (Council Directive 64/432/EEC).

The study showed that in order to prove freedom of disease consistently at a 99% confidence level an important decrease in total number of serologically tested animals could be realised. A new surveillance program has been applied from winterscreening 2009–2010 on.

#### **Surveillance programme and methods used**

From 2010 on, dairy cattle herds are no longer checked via tank milk controls by the surveillance programme. Also beef cattle older than 2 years are no longer serologically monitored once every three years from herds selected on their geographical location. Furthermore, all female animals older than 1 year and breeding bulls are no longer systematically tested at purchase by serology. Testing at purchase can be realised on a voluntary base.

Due to the brucellosis outbreak end 2010 (one primary outbreak due to *Brucella abortus* biovar 3, no secondary outbreaks) and the outbreaks in spring 2012 (one primary outbreak and 4 secondary breakdowns due to *Brucella abortus* biovar 3 and 1 breakdown due to *Brucella suis* biovar 2), the surveillance programme had to be adapted. The number of individual serological tests increased importantly in 2012 because of follow-up blood testing of all contact herds of the outbreak herds. It was also decided to restart surveillance of brucellosis on all dairy herds, three times a year in 2012 and twice a year in 2013 by an ELISA of tankmilk. Also passive surveillance by analysis of abortions was further stimulated to early detect probable new infections.

The surveillance programme in 2012 was based on:

- an ‘at random’ selection of a number of herds for examination by serology for brucellosis and other diseases of some specific age categories and number of animals by disease;
- 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.

This risk category was further especially aimed at in 2012 and 2013. The FASFC financed the collection of aborted fetuses or premature stillbirths and the analyses of these matrices (abortion protocol);

- analysis at purchase of animals originating from or born in a non-brucellosis-free Member State or region or imported from a third country;
- repeated monitoring during 3 consecutive years of animals purchased from a non-brucellosis-free Member State or region or imported from a third country;
- at random monitoring of animals originating from a free Member State or purchased by national trade;
- due to the brucellosis outbreaks during the first months of 2012, from June to December 2012 a serological examination became again mandatory at purchase of animals older than 18 months of age and a mandatory analysis before participation to any animal fair, show or market;
- at random selection of a number of herds where no abortion notification was realised over the last 3 years and where female animals are present. This risk category was also especially aimed at in this reporting period to stimulate the notification and the analysis of any abortion by the farmer.

In 2013 the surveillance remained in line with the program of 2012 except that the serological examination at purchase was not mandatory anymore except for animals imported from non free MS or third countries.

Blood sera are analysed for brucellosis by micro-agglutination and an indirect ELISA as screening test. In case of a positive result, a competition ELISA is performed as confirmatory test at the NRL. Bacteriological examination is done in case of serological and/or epidemiological suspicion. An animal is legally suspected of brucellosis in case of a positive confirmatory ELISA realised by the NRL. If, according to the epidemiology an animal or herd is found to be at risk, a bacteriological investigation always takes place. Hence, a brucellosis infected 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.

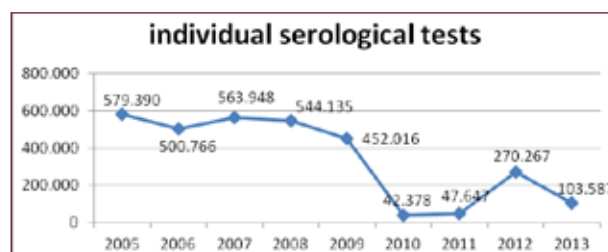
Table 5 indicates the evolution of the total number of individual serological tests related to the monitoring programme of beef cattle and the mandatory examination at purchase until 2010 and the monitoring of dairy cattle by bulk milk testing by a Milk Ring test until 2010 and by an ELISA from 2011 on. In line with a new sanitary policy, in 2010 no further testing of bulk milk samples took place. Due to an outbreak of brucellosis end 2010, to quickly evaluate the sanitary situation of brucellosis in

dairy cattle, a one-shot monitoring of all dairy herds (9460) was performed by an ELISA of tank milk in 2011. No additional brucellosis cases were detected. This monitoring by bulk milk of dairy herds was continued in 2012 and 2013. A few dairy herds with positive tankmilk had to be screened by individual serology of the animals but all results were finally negative except for one herd where an animal was found positive by culture.

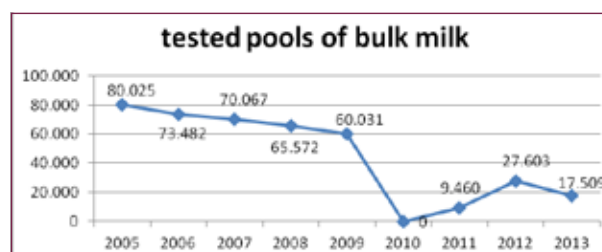
**Table 5.** Evolution of individual serological tests and testing of pools of bulk milk samples

	individual serological tests	pools of bulk milk tests
2005	579.390	80.025
2006	500.766	73.482
2007	563.948	70.067
2008	544.135	65.572
2009	452.016	60.031
2010	42.378	0
2011	47.647	9.460
2012	270.267	27.603
2013	103.587	17.509

The number of individual serological tests was very high in 2012 due to the urgently re-instated mandatory examination at purchase of all animals older than 18 months of age from June to December 2012 in consequence of the brucellosis incident in spring 2012.



**Figure 17.** Evolution of individual serological tests for bovine brucellosis, 2005–2013



**Figure 18.** Evolution of pools of bulk milk tests for bovine brucellosis, 2005–2013

Figures 17 and 18 represent the evolution of individual serological tests and testing of pools of bulk milk samples.

Regarding the passive surveillance by examination of abortions, next figure 19 represents the total number of notified abortions in the period 2005 – 2013.

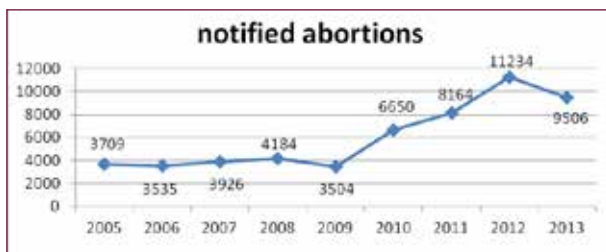


Figure 19. Number of notified abortions, 2005–2013.

### Additional information

End 2010 a brucellosis breakdown herd was detected after analyzing an abortion. The infected herd was totally depopulated. Intensive epidemiological investigations and serological follow-up of contact herds in 2010 and 2011 could not give any indication on the origin of the infection neither could any additional infected animal or herd be detected.

In March 2012, again a breakdown of brucellosis was detected after analysis of an abortion. No epidemiological link could be found with the breakdown herd of 2010. Tracing-back and -on and an epidemiological inquiry lead to the detection of 4 other secondary breakdowns linked to this primary case. All these 5 brucellosis breakdown herds were infected with an identical *Brucella abortus* biovar 3.

Another infected herd of brucellosis was detected by surveillance of all dairy herds by an ELISA of tankmilk. In consequence of a positive tankmilk with serological follow-up an infection with *Brucella suis* biovar 2 was detected in one animal of a dairy herd. All 64 contact herds were blood sampled but no other herd was detected with a *Brucella* infection. This breakdown could be considered as an isolated case. This biotype is endemic in Belgian wildboar population. Also bovines are susceptible to this biovar.

There was a total stamping-out realised on all the 6 infected herds with brucellosis independently of the serotype. Due to this brucellosis incident in 2012, a lot of microbiological testing by culture was realised on slaughtered animals. Of 1475 cultures, 29 isolates were identified as *Brucella abortus* biovar 3 and 1 isolate was identified as *Brucella suis* biovar 2. The rest of the cultures were negative for *Brucella* spp. This rather limited number of positive cultures of suspected test-slaughtered animals could indicate an early detection of infection on the infected herds.

In January 2013 a breakdown herd was detected as contact herd of the primary breakdown herd of 2012. Both herds had the same veterinarian. The breakdown herd of 2013 was already examined twice by serology in 2012 with negative results. In 2013 a third follow-up screening by serology indicated some positive results. This positive serology could be confirmed by culture after test-slaughter of the reactors. Finally 6 bovines were infected. There was a stamping-out of all the animals of this infected herd. 67 contact herds of this breakdown herd of 2013 had to be screened by serology. No other infected animal or herd has been detected.

### Brucellosis in sheep and goats

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

### Surveillance programme

Serum samples taken in the framework of national monitoring for a Visna-Maedi/CAE voluntary eradication programme and in the framework of export were examined for *Brucella melitensis* specific antibodies by means of an ELISA. Positive samples were subsequently tested with Rose Bengal test (RBT) and Complement Fixation test (CFT). A sample is classified as positive for brucellosis only if it is positive in all three tests.

Since 2001, serum samples from sheep and goats brucellosis were tested at the NRL.

In 2012, 6,329 samples were tested. Serological positive reacting animals after serial and repeated testing were finally negative.

In 2013, 7,151 samples were tested. Serological positive reacting animals in the ELISA were finally negative after RBT and CFT.

Next figure 20 represents the total number of tests realised by the NRL for *Brucella melitensis*.

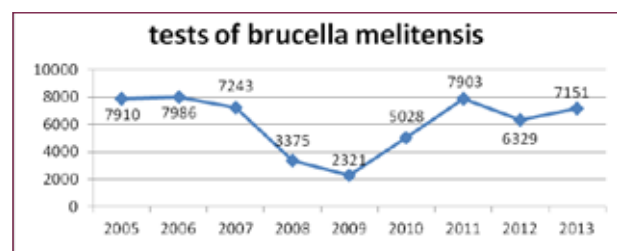


Figure 20. Number of tests of *Brucella melitensis*, 2005 – 2013

## Brucellosis in pigs

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 and CFT. Bacteriological examination for *Brucella* and *Yersinia* is done in case of positive serology.

Sometimes, false positive serological reactions are reported. These are due to a *Yersinia enterocolitica* O:9 infection and may be 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 2012 and 2013, *Brucella* was not detected in domestic pigs. All pigs positive by serological testing were finally negative in bacteriology at the NRL for *Brucella* (see table 6).

**Table 6.** Number of serological and bacteriological tests of *Brucella* in pigs, 2012 – 2013

Domestic pigs	Serology		Bacteriology	
	2012	2013	2012	2013
	Pos / Total number	Pos / Total number	Pos / Total number	Pos / Total number
	10/243	25/323	0/7	0/25

## Brucellosis in wildlife

In consequence of the brucellosis incident in 2012 in a cattle herd, the surveillance Network of Wildlife focused on analyses of deer and wildboar from the surrounding areas of some of the outbreak herds. Deer (9, spleen) and wild boars (32, tonsils) were analysed by culture. All results were negative for *Brucella abortus* but 7 wild boars were positive for *Brucella suis* by this targeted wildlife surveillance. In 2013, at the NRL in total 22 out of the 46 analysed wild boars were positive by serology and 1 out of the 109 analysed wild boars were positive by culture for *B. suis* biovar 2, which is endemic in the Belgian wild boar population.

All results of wildlife species tested by serology and bacteriology for brucellosis at the NRL in 2012 and 2013 are mentioned in the next table 7.

**Table 7.** Number of serological and bacteriological tests of *Brucella* in wildlife, 2012 – 2013

Wildlife species	Serology		Bacteriology	
	2012	2013	2012	2013
	Pos / Total number	Pos / Total number	Pos / Total number	Pos / Total number
Wild boar	3/22	22/46	7/39	1/109
Deer	0/24	2/293	0/24	2/293
Alpaca	0/140	1/141		
Llama	0/6	0/22		
Exotic mammals	0/54	0/88		

## 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 2012 three brucellosis cases were reported in humans, one following isolation of the strain (*Brucella melitensis* biovar 3), and two by serological case definition in association with epidemiological data derived from a cluster case of bovine brucellosis (suspicion of *Brucella abortus*). The first case was clearly associated with travelling in Turkey where brucellosis is endemic. Since *B. melitensis* is not detected in the Belgian small ruminant population, it is highly likely that the *B. melitensis*-patients were infected abroad.

No cases were detected in 2013.

# Campylobacteriosis

Toon Braeye, Vera Cantaert, Katelijne Dierick, Delphine Martiny, Olivier Vandenberg, Luc Vanholme, Julie Wits

## Campylobacteriosis

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

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

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

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

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

## Campylobacteriosis in food

In 2012 and 2013, 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 cheeses and live bivalve molluscs were also analysed.

Specially trained staff of the FASFC performed the sampling. Different sample sizes (25g, 1g, 0.01g and 600 cm<sup>2</sup>) were analysed and in some cases an enumeration was performed. For carcasses at slaughter and cutting meat at processing plants, independent samples were taken per matrix in order to estimate prevalence of the contamination at 95% confidence level.

### Results of the monitoring 2008 – 2013

The results of the monitoring of the FASFC are shown in table 8 on the next page.

The *Campylobacter* spp. contamination rate of pork carcasses observed at the end of the slaughtering process is not representative for the final contamination rate of meat ready to be marketed. Carcasses are submitted to an intensive cold treatment directly after slaughter and, due to a certain sensitivity, *Campylobacter* germs do not survive easily that industrial refrigeration process. In 2013 there was a significant increase in contamination rate of pork carcasses at slaughter compared to the 3 former years.

From 2008 on, the *Campylobacter* spp. contamination of poultry carcasses and meat cuttings has been enumerated. Risk analysis indicated that especially highly contaminated carcasses of broilers represent a significant risk for human health. The indicative value for carcass contamination of broilers and meat cuttings with skin was determined between 100 to 1.000 cfu/g.

**Table 8.** Zoonosis monitoring program – *Campylobacter* in food, 2009 - 2013

Sample	Sample size	Percentage of positive samples 2009	Percentage of positive samples 2010	Percentage of positive samples 2011	Percentage of positive samples 2012	Percentage of positive samples 2013
<b>Broiler</b>						
Carcasses at slaughter	25g (caeca)	31% (n=337)	-	-	-	-
Carcasses at slaughter	1g	32% (n=261)	37.8% (n=388)	38.8% (n=335)	9.8% (n=449)	21.8% (n=206)
Carcasses at retail	0.01g	25.4% (n=118)	19.1% (n=115)	16.5% (n=91)	16.5%* (n=91)	17.4%* (n=92)
Meat cuts (skinned or with skin) at processing plant	1g	8.6% (n=513)	8.9% (n=358)	14% (n=711)	2.2% (n=714)	7.7% (n=782)
<b>Layer</b>						
Carcasses at slaughter	25g (caeca)	-	-	-	-	-
Carcasses at slaughter	1g	37% (n=317)	35% (n=300)	48.9% (n=321)	4.3% (n=440)	8.7% (n=445)
Carcasses at retail	0.01g	15% (n=60)	-	28.6% (n=119)	18.6%* (n=113)	28.6%* (n=118)
<b>Poultry</b>						
Meat cuts (with skin) at retail	0.01g	21.7% (n=92)	5.9% (n=119)	16.0% (n=119)	11.3%* (n=115)	11.8%* (n=59)
Meat cuts (without skin) at retail	0.01g	0.0% (n=91)	1.1% (n=93)	1.1% (n=90)	3.2%* (n=91)	4.3%* (n=46)
Minced meat at retail	Enumeration (M=100 cfu/g)	0.0% (n=71)	0.0% (n=47)	-	-	-
Meat preparation at processing plant	0.01g	0.0% (n=112)	0.0% (n=45)	0.0% (n=59)	0.0%* (n=59)	0.0%* (n=25)
Meat preparation at retail	Enumeration (M=100 cfu/g)	0.0% (n=100)	0.0% (n=46)	0.0% (n=59)	0.0%* (n=59)	0.0%* (n=59)
<b>Pork</b>						
Carcasses at slaughter	600 cm <sup>2</sup>	14% (n=656)	10% (n=847)	10% (n=667)	10.1% (n=611)	16.6% (n=506)
Meat cuts at processing plant	25g	-	0.7% (n=268)	-	-	-
<b>Others</b>						
Minced meat (intended to be eaten raw) at retail (all species)	Enumeration (M=10 cfu/g)	0.0% (n=184)	0.0% (n=55)	0.0% (n=44)	0.0%* (n=46)	0.0%* (n=45)
Minced meat (intended to be eaten cooked) at retail (all species)	Enumeration (M=100 cfu/g)	0.0% (n=183)	0.0% (n=55)	0.0% (n=43)	0.0%* (n=45)	0.0%* (n=46)
Raw milk cheese at retail	25g	0.0% (n=49)	4.1% (n=49)	0.0% (n=22)	0.0%* (n=22)	0.0%* (n=11)
Raw milk cheese at farm	25g	0.0% (n=40)	-	0.0% (n=24)	0.0%* (n=11)	0.0%* (n=20)
Live bivalve molluscs at retail	25g	0.0% (n=94)	0.0% (n=88)	0.0% (n=87)	0.0%* (n=92)	0.0%* (n=91)

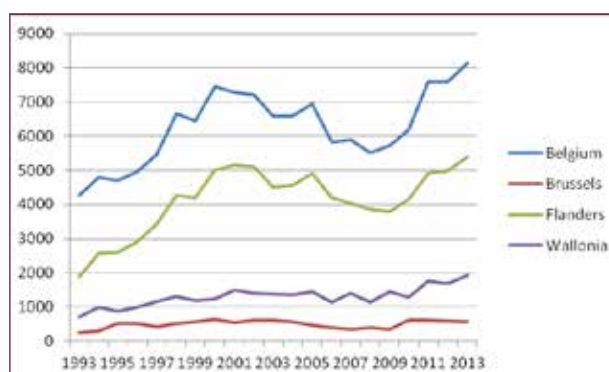
\* Enumeration (cfu/g), sample results > limit of quantification are considered as positive.

## Campylobacter in humans

In 2012, the Sentinel Laboratory Network consisted of 100 active laboratories (56% of all laboratories of microbiology) and 91 of them reported Campylobacter. 7,601 strains were isolated, which represent at country level an isolation rate of 68.87 per 100,000 inhabitants.

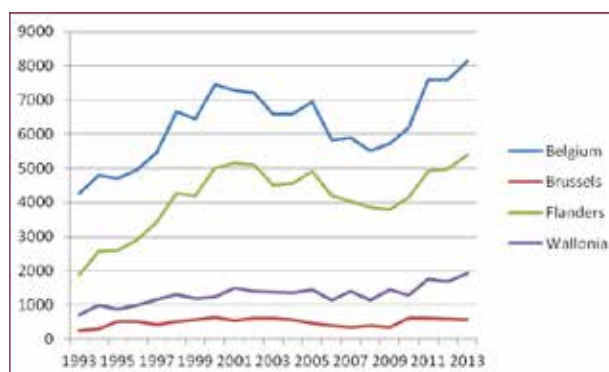
In 2013, the Sentinel Laboratory Network consisted of 99 laboratories (55% of all laboratories of microbiology) and 85 of them reported Campylobacter. 8,147 strains were isolated, which represent at country level an isolation rate of 73.40 per 100,000 inhabitants.

The number of Campylobacter infections shows a significant increase trend since 2008 ( $p < 0.05$ ; figure 21). Since 2005, Campylobacteriosis remains the most frequently reported zoonosis in humans.



**Figure 21.** Number of Campylobacter infections in humans by year (1993-2013). Source: Sentinel Laboratory Network

Cases are reported during the entire year, with a peak in the summertime (Figure 22)



**Figure 22.** Monthly number of Campylobacter infections in humans, 2012-2013. Source: Sentinel Laboratory Network

In 2012, Campylobacter isolation rates are higher in men (50%) than in women (46%); 21% of cases are diagnosed in children of 1-4 years old and 13% in adults aged over 65 years. (Table 9)

**Table 9.** Number of Campylobacter infections in humans by sex and by age groups, 2012. Source: Sentinel Laboratory Network

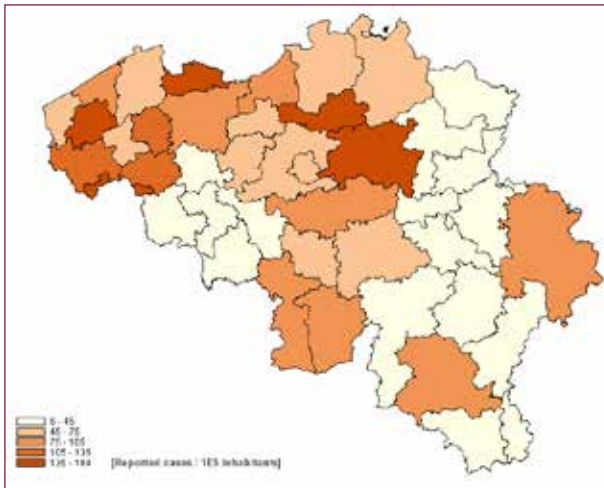
AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	745	20.5	970	24.8	1726	23.00
05-09	267	7.35	301	7.7	571	8.00
10-14	163	4.49	247	6.32	410	5.00
15-19	220	6.05	256	6.55	480	6.00
20-24	341	9.38	250	6.39	595	8.00
25-29	248	6.82	201	5.14	452	6.00
30-34	185	5.09	158	4.04	344	5.00
35-39	159	4.38	114	2.91	275	4.00
40-44	151	4.16	143	3.66	296	4.00
45-49	156	4.29	203	5.19	361	5.00
50-54	125	3.44	164	4.19	289	4.00
55-59	131	3.6	152	3.89	285	4.00
60-64	138	3.8	134	3.43	273	4.00
65-++	483	13.29	470	12.02	963	13.00

In 2013, Campylobacter isolation rates are higher in men (51%) than in women (48%); 20% of cases are diagnosed in children of 1-4 year old and 14% in adults more than 65 years old. (Table 10). This distribution was also observed in previous years.

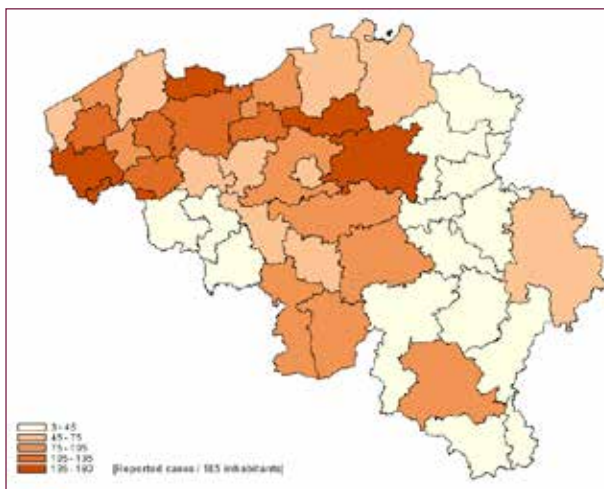
**Table 10.** Number of Campylobacter infections in humans by sex and by age groups, 2013. Source: Sentinel Laboratory Network

AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	709	18.24	925	22.4	1660	20.00
05-09	226	5.82	324	7.85	561	7.00
10-14	150	3.86	235	5.69	396	5.00
15-19	254	6.54	243	5.89	501	6.00
20-24	395	10.16	267	6.47	678	8.00
25-29	306	7.87	226	5.47	539	7.00
30-34	228	5.87	181	4.38	415	5.00
35-39	169	4.35	170	4.12	344	4.00
40-44	161	4.14	161	3.9	329	4.00
45-49	191	4.92	178	4.31	371	5.00
50-54	173	4.45	164	3.97	341	4.00
55-59	160	4.12	191	4.63	353	4.00
60-64	137	3.53	178	4.31	334	4.00
65-++	533	13.72	568	13.76	1114	14.00

Since the beginning of the registration (1983), the incidence in Flanders is higher than in Wallonia. This was confirmed in 2012 and 2013 with an estimated incidence of 79.2/100,000 and 85.7/100,000 inhabitants in Flanders, 53.2/100,000 and 54.6/100,000 inhabitants in Brussels-Capital Region and 48.2/100,000 and 51/100,000 inhabitants in Wallonia, respectively. The difference observed in estimated incidence over districts is partly due to an uneven distribution of sentinel laboratories (Figures 23 and 24).



**Figure 23.** Incidence of *Campylobacter* infections in humans by district ( $N/10^5$  inhab., 2012). Source: Sentinel Laboratory Network



**Figure 24.** Incidence of *Campylobacter* infections in humans by district ( $N/10^5$  inhab., 2013). Source: Sentinel Laboratory Network

### Invasive *Campylobacter* in humans, data from the national reference center for 2013

Data were obtained from the National Reference Center (NRC) for human Enteric *Campylobacter*. Since there is no obligation for clinical laboratories to send human isolates to the NRC to confirm the presence of *Campylobacter*, a correct epidemiological situation of *Campylobacter* in human populations cannot be made.

#### Results

Enteric *Campylobacter* was isolated in 60 patients. Among the *Campylobacter* and related organisms identified by the NRC, 47% of them were *C. jejuni*, 35% were *C. coli* and 10% were *C. fetus*. Other species were also found in small numbers.

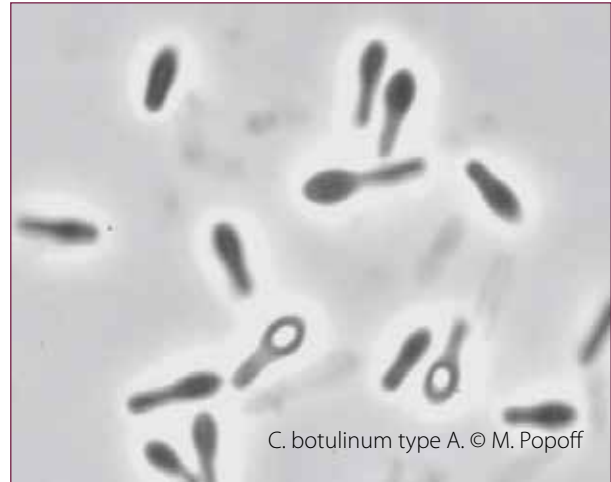
# Botulism

Laurence Delbrassinne, Sarah Denayer, Katelijne Dierick, Youssef Fikri

Botulism is a severe neuromuscular disease caused by several heat-labile botulinum neurotoxins (BoNTs) produced by various strains of *C. botulinum* and rare strains of *C. butyricum* and *C. baratii*. The bacterium is a spore-forming obligate anaerobe and is present in the environment generally under the form of spores.

The three main forms of botulism are: (1) foodborne botulism caused by the ingestion of BoNTs produced during the anaerobic growth of bacteria in stored food, (2) infant botulism and adult intestinal botulism resulting from the colonisation of the intestine by *C. botulinum* and 'in situ' production of toxins, and (3) wound botulism, the rarest type, elicited by the toxins produced by the bacteria growing in a contaminated wound. Botulism affects humans and animals with a difference in susceptibility towards the various neurotoxins. Toxin types A, B, E and F cause disease in humans whereas toxins of types C and D are the most common causes of botulism in animals (mammals, birds). Type B and type E have also been observed in animals. Four genotypically and phenotypically distinct groups of BoNT-producing *C. botulinum* have been defined and are designated as group I to IV. *C. botulinum* group I (proteolytic *C. botulinum*) and *C. botulinum* group II (non proteolytic *C. botulinum*) mainly cause human botulism. Group III (types C and D) causes animal botulism while group IV, also referred to *C. argentinensis*, has not generally been associated with illness.

The method of reference to detect BoNTs is the mouse bioassay. If symptoms of botulism are detected in mice, a seroneutralisation test is performed by adding antitoxins (e.g. antitoxins of type A, B, C, D, E, and F) to the samples and/or cultures in order to specifically neutralize the toxin and thereby to identify the toxin type. The laboratory of zoonotic Clostridia (WIV-ISP) has a long experience with the detection and the identification of botulism in human, animal, food, feed and environmental samples and is National Reference Laboratory (NRL) for botulism in Belgium. In 2011 with the creation of the National Reference Centres (NRC) for clinical microbiology, the laboratory was officially designated as NRC for human *C. perfringens* and *C. botulinum*.



## Botulism in animals

Bovine and equine botulism are associated with toxins of type B, C and D. The route of contamination in animals is the ingestion of preformed toxins in feed, water or carrions. Clinical symptoms induced by botulinum toxins vary according to the implicated toxin and the incubation period depends on the amount of ingested toxin. The incubation time is longer for ruminants and ranges from 3 to 17 days. Botulism of type C or D presents a quite variable clinical picture in cattle: paresis or paralysis of the muscles or sudden death. Botulism of type B causes mainly digestive disorders such as diarrhea, obstruction, belching and/or excessive salivation.

Although vaccination of cattle is possible since 2009, it is not legally obliged. There is no therapeutic treatment once the symptoms of botulism have appeared and it is therefore necessary to develop other preventive measures in order to avoid further cases.

Bird and poultry botulism is associated with toxins of types B, C and E. The type C is the most frequent and the most toxic for this group of animals. Flaccid paralysis is the main symptom of botulism in birds and leads to the inability to walk, fly and erect the neck.

Animal botulism is a notifiable disease under the zoonosis law (Royal Decrees of 14/11/2003 and 22/5/2014). Since 2014 (R.D 2014) botulism in cattle has been added to the list of notifiable diseases in Belgium.

In Belgium, a large number of animal botulism cases were confirmed between 2005 and 2013 (see Table 11). The BoNT type D was more frequently associated with bovine botulism while BoNT type C was more frequently found in birds and poultry. Sporadic cases of botulism type B were also confirmed in cattle and horses.

In the case of suspicion of animal botulism, feed and environmental samples (e.g. ensiled grass, flour, corn, water or manure) are collected in order to detect the source of the contamination. Those samples are analyzed in parallel with samples coming from the autopsy of dead animals.

It is important to underline that the various positive samples presented in the Table 11 were often associated with the same animal as, for instance, the stomach, the kidney, and the liver were extracted from one dead animal and were all found positive in the analysis (i.e. three positive samples concerning only one case of animal botulism).

**Table 11.** Reported animal botulism in Belgium (2005-2013)

Type of botulism	Samples	2005	2006	2007	2008	2009	2010	2011	2012	2013	
Bovine botulism	Number of samples tested <sup>a</sup>	32	62	136	177	201	61	88	64	64	
	Positive samples <sup>a,b</sup>	4	10	16	17	58	20	20	24	25	
	BoNT Type <sup>c</sup>	B				1	1	2			
		C		4	2	1	2				2
		D	4	6	12	15	54	18	20	23	23
C or D <sup>d</sup>				2		1			1		
Bird and poultry botulism	Number of samples tested <sup>a</sup>	36	25	15	18	14	19	3	5	7	
	Positive samples <sup>a,b</sup>	10	13	6	3	7	8	2	1	7	
	BoNT Type <sup>c</sup>	C	10	13	6	3	7	8	2	1	7
Equine botulism	Number of samples tested <sup>a</sup>	8	9	8	4	0	3	2	3	1	
	Positive samples <sup>a,b</sup>	0	2	0	1	0	1	0	0	0	
	BoNT Type <sup>c</sup>	B		2		1		1			

<sup>a</sup> Different samples can originate from a single animal

<sup>b</sup> Sample containing either a botulinum toxin or the germ BoNT-producing *Clostridium botulinum*

<sup>c</sup> BoNT type determined by the mouse bioassay

<sup>d</sup> C or D because a neutralisation was effective using both antitoxins C and D

In 2012, a total of 85 veterinary samples have been analysed for the presence of *C. botulinum* and/or for the presence of the toxin. Those samples were sent on the basis of a strong clinical suspicion of animal botulism. After the autopsy of the dead animals, the stomach, the liver, the kidney and/or the stomach content were sent to the NRL for analysis together with suspicious environmental and/or feed samples.

Among these samples, there was a vast majority of bovine samples (64 samples) that were mainly organs from autopsy, faeces but also some suspected feed and environmental samples related to the outbreaks. Besides the bovine samples, there was also one duck sample and 4 poultry samples, three horse samples, two sheep samples and other environmental and feed samples related to the outbreaks. Twenty-four samples

were confirmed as bovine botulism and the two environmental/feed samples that were sent with the tissues of a suspected case helped to confirm the case: a manure sample and a feed sample were both found positive (one of type D and one undetermined type were indeed related to a bovine botulism type D). The duck sample (liver) was positive for botulism type C whereas no equine botulism was confirmed.

In 2013, a total of 75 veterinary samples have been screened. Among them, there were 64 bovine samples (23 positive of type D and 2 of type C), 7 samples from bird, poultry and duck suspected cases (7 positive; all of type C), one sample from a horse (negative) but also one sample from a deer (negative), one from a rabbit (negative), and one from a sheep (negative). Among the environmental samples that were sent with the

suspected cases, two manure samples were positive for type D and related to a bovine outbreak while a water sample (type C) was related to one of the bird botulism cases. Another positive sample of water (type D) was related to a bovine case.

Since 2012, a real-time PCR method that detects the BoNT A, B, C, D, E and F, but also the hybrids C-D/D-C BoNTs was implemented at the NRL. This molecular assay was performed on 58 samples from 2010-2013 that were found positive for BoNT D in the mouse bioassay, and for which a sufficient amount was still available. Preliminary data show that the hybrid toxin D-C is present in the majority of the samples (38 samples of type D-C) but this molecular identification of BoNT D and C is still ongoing.

### Clostridium botulinum in feed

The contamination of ruminants mostly occurs through the ingestion of contaminated feed or contaminated water. Water supplies, litter or silos can be contaminated by dead poultry and birds or by carcasses of small animals like rodents. Those are at the origin of types C and D contamination while the toxin type B can be found in animal feed containing spores or in grass silage.

Feed samples are regularly sent to the NRL for analysis of *C. botulinum*. These samples are not related to suspected cases but are part of routine analysis. In 2012 and 2013, a total of 68 and 36 feed samples have been analysed respectively, for the presence of *C. botulinum* and/or the botulinum toxin. All of them resulted negative.

### Clostridium botulinum in food

*C. botulinum* and its spores are very common in the environment and may therefore easily contaminate food. Foodborne botulism is caused by the ingestion of foods containing the potent neurotoxin. Typical foods associated with foodborne botulism are usually home-canned vegetables, foods preserved in oil, fermented fish products, but also cooked foods stored at room temperature (potatoes, onions) and foods packaged in modified-atmosphere containers with inappropriate refrigeration.

The foodborne botulism type B seems to predominate in Europe. For example, from 2007 to 2009, 33 cases of botulism type B occurred in France while only 4 cases of botulism type A were confirmed.

In Belgium, the Federal Agency for the Safety of the Food Chain (FASFC) performs monitoring programs of food products and sends to the NRL a large variety of food samples for analysis.

The analysed samples were mostly canned vegetables: fruit vegetables (peppers, tomatoes, aubergines, peppers, zucchinis, gherkins), leafy vegetables (lettuce), bulb vegetables (garlic), stem vegetables (artichokes, asparagus) and root vegetables (parsnips) but also edible flowers and mushrooms.

A total of 200 food samples have been analysed in 2012 for the presence of *C. botulinum* and none of them was found positive. In 2013, a total of 114 food samples have been screened and no positive result has been obtained.

### Botulism in humans

Human botulism is rare but can be life threatening due to respiratory paralysis. The clinical symptoms of botulism in humans are progressive weakening and paralysis of skeletal muscles. Infant botulism is caused by BoNT produced in vivo and the initial symptoms are constipation, poor feeding and weak crying. The main risk is associated with the consumption of honey by children less than one year old.

From 1988 to 2011, 12 cases of human botulism type B, among which one case of infant botulism, were confirmed in Belgium while only one case of botulism type A was detected. Two other cases of botulism for which no BoNT type could be determined also occurred in this period.

In 2012, 17 human samples (serum and/or faeces) related to 11 suspected human cases (clinical criteria) were sent to the NRC for *C. perfringens* and *C. botulinum* analysis. However, no case of human botulism was confirmed on the basis of laboratory criteria.

In 2013, 12 human samples (serum and/or faeces) related to 8 suspect human cases were analysed. Again, no case of human botulism was laboratory confirmed.

# Escherichia coli (VTEC) infections

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## Verotoxin and shiga-like toxin producing Escherichia coli

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

Human EHEC infections mostly take place in a sporadic form. Infections may occur after consumption of contaminated food or water, after contact with contaminated water, through contact with infected animals or less frequently by direct transmission from person to person.

The clinical symptoms range from mild to bloody diarrhoea through haemorrhagic colitis, which is often accompanied by abdominal cramps, usually without fever. Zoonotic 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 cattle seems to be the foremost important reservoir for VTEC. The organism is intermittently excreted in the faeces. Food of bovine origin is frequently reported as a source for human VTEC infections. Other important food sources include faecally contaminated vegetables and drinking water.

Prevention mainly relies on biosecurity 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. Therefore, epidemiological investigations and additional sampling at the farm of origin is only done following EHEC infections in humans.

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

## Verotoxin producing Escherichia coli in cattle

No herd surveillance programme was in place in 2012 or 2013. In case a cattle farm is suspected to be at the origin of an EHEC outbreak in humans, a representative number of animals of this farm is sampled (faecal, feed, environmental samples). FASFC officials inform the owner that EHEC circulate on the farm and encourage the implementation of hygienic measures, i.e. cleaning and disinfection of milk reservoirs and milking equipment, and cleaning of animals before transport to the slaughterhouse.

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

In 2010 and 2011, respectively 17 and 545 E.coli isolates from cattle were PCR typed for diagnostic reasons. None of these isolates were serotyped.

In 2012, a total of 278 E. coli isolated from cattle for diagnostic reasons were PCR typed at the NRL for the presence of virulence genes. Eight VTEC strains were identified: six Stx1 and Eae positive strains (all negative for genes stx2), one Stx1 (negative for genes stx2 and eae) and one Stx2 (negative for genes stx1 and eae) strains. In 2013, 115 bovine E. coli isolates were PCR typed. Only three of them were identified as VTEC, i.e. Stx2 positive (all these isolates were negative for genes stx1 and eae). No serotype data are available for these strains.

## Verotoxin producing *Escherichia coli* O157 in food

### Results of the monitoring between 2009 – 2013

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

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

**Table 12.** Zoonosis monitoring programme - *E. coli* (STEC, EHEC), 2009 - 2013

	Sample	Prevalence 2009	Prevalence 2010	Prevalence 2011	Prevalence 2012	Prevalence 2013
Beef	Carcasses	1.0% (n=995)	0.0% (n=357)	0.7% (n=427)	0.2% (n=453)	0.4% (n=449)
	Fresh meat at cutting plant	0.0% (n=291)	0.0% (n=271)	0.3% (n=294)	0.5% (n=374)	0.0% (n=292)
	Minced meat (steak tartare) at processing plant	0.0% (n=294)	0.0% (n=267)	0.7% (n=296)	0.0% (n=297)	0.4% (n=282)
	Minced meat (steak tartare) at retail	0.0% (n=147)	0.0% (n=294)	1.0% (n=295)	0.2% * (n=582)	0.3% * (n=292)
Beef - Porc	Meat preparations (steak tartare with herbs and sauce) at retail	1.0% (n=147)	0.0% (n=296)	0.3% (n=294)	-	-
Cheese	From raw cow's milk, at farm	0.0% (n=59)	0.0% (n=73)	0.0% (n=74)	0.0% * (n=42)	0.0% * (n=130)
	From raw cow's milk, at retail	0.0% (n=106)	0.0% (n=102)	0.0% (n=102)	0.0% * (n=99)	0.0% * (n=88)
	From raw cow's milk, at processing	0.0% (n=40)	0.0% (n=98)	0.0% (n=98)	2.0% * (n=50)	0.0% * (n=53)
	From raw sheep's milk, at farm	0.0% (n=15)	0.0% (n=21)	0.0% (n=19)	10.0% ** (n=10)	0.0% ** (n=11)
	From raw sheep's milk, at retail	0.0% (n=63)	0.0% (n=84)	0.0% (n=86)	0.0% ** (n=80)	1.6% ** (n=62)
	From raw sheep's milk, at processing				0.0% ** (n=8)	0.0% ** (n=13)
	From raw goat's milk, at farm	4.0% (n=25)	0.0% (n=25)	0.0% (n=25)	0.0% ** (n=8)	0.0% ** (n=39)
	From raw goat's milk, at retail	0.0% (n=64)	0.0% (n=44)	0.0% (n=45)	0.0% ** (n=45)	0.0% ** (n=54)
Butter	At farm	0.0% (n=132)	0.0% (n=114)	0.0% (n=116)	0.0% ** (n=89)	0.0% ** (n=111)
	From raw milk, at retail	0.0% (n=24)	0.0% (n=22)	-	-	-
Cream	At farm	0.0% (n=46)	0.0% (n=45)	0.0% (n=45)	0.0% ** (n=35)	0.0% ** (n=41)
Vegetables	At processing plant	-	0.0% (n=6)	0.0% (n=6%)	-	-
	At retail	-	0.2% (n=821)	0.0% (n=815)	0.0% * (n=674)	0.0% * (n=532)
Sprouted seeds, lettuce, spinach, fresh herbs	At retail				0.0%** (n=307)	0.0%** (n=533)

\* STEC    \*\* *E. coli* O157:H7

In 2012, only *E. Coli* O157:H7 was detected. In 2013, serotypes O157 and O103 were detected.

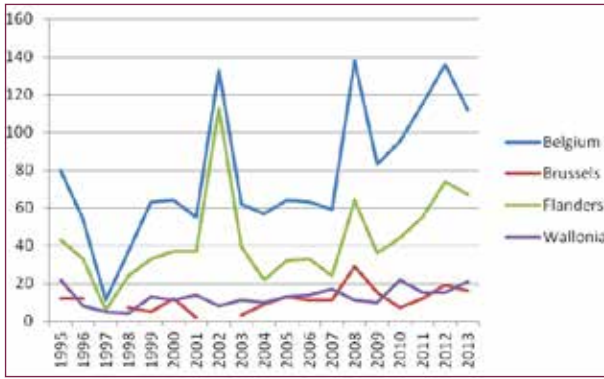
As a consequence of the German *E. Coli* O104:H4 outbreak in 2011, the control programme of the FASFC includes analyses on O104:H4 in different matrices such as sprouted seeds, lettuce, spinach, fresh herbs. Samples were mainly taken at retail level. In 2012 and in 2013 a total of respectively 307 and 533 samples were analyzed. In none of these samples *E. coli* O104:H4 was detected.

### Verotoxigenic *Escherichia coli* infections in humans

In 2012, the Sentinel Laboratory Network registered 136 cases, corresponding to a national incidence estimated at 1.2 per 100,000 inhabitants.

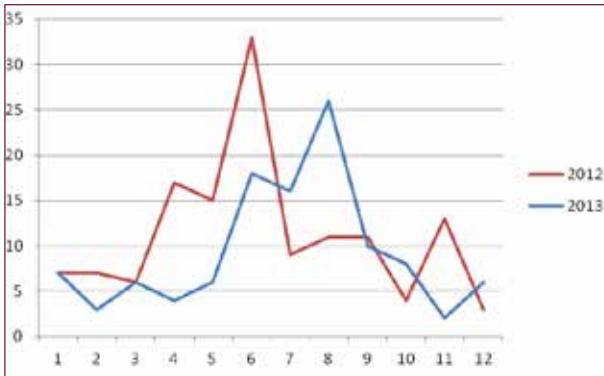
In 2013 the Sentinel Laboratory Network registered 112 cases, corresponding to a national incidence estimated at 1 per 100,000 inhabitants.

The number of cases since 1995 is shown in Figure 25. The peak observed in 2012 was related to a local outbreak in the East of Limburg.



**Figure 25.** Total number of *E. coli* infections (VTEC) in humans by year (1995-2013). Source: Sentinel Laboratory Network

Cases were observed all over the year (Figure 26). The peak observed in 2012 was related to a local outbreak in the East of Limburg.



**Figure 26.** Total number of *E. coli* infections (VTEC) in humans by year (1995-2013). Source: Sentinel Laboratory Network

Twenty-five percent for 2012 and 28% for 2013 of cases were children up to 4 years old. In 2012 27% of cases were male, 73% were female. In 2013 47% were male and 53% were female. The higher number of female cases as compared to male cases is also observed internationally and is not completely understood (Table 13 and 14)

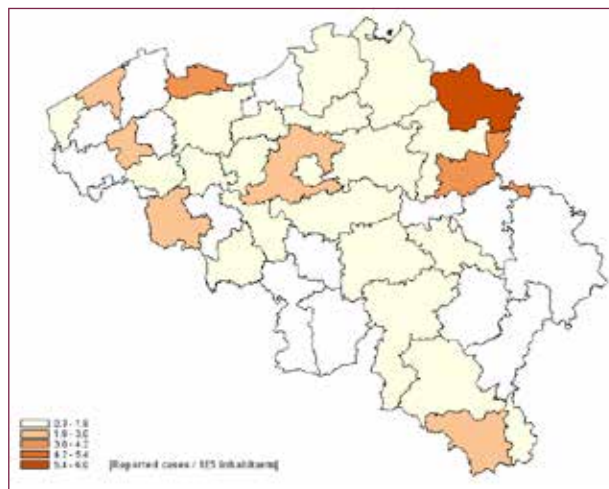
**Table 13.** Number of verotoxigenic *E. coli* infections (VTEC) in humans by sex and by age groups, 2012. Source: Sentinel Laboratory Network

AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	19	19	15	40	34	25
05-09	17	17	7	19	24	18
10-14	12	12	4	11	16	12
15-19	4	4	0	0	4	3
20-24	1	1	0	0	1	1
25-29	2	2	0	0	2	1
30-34	1	1	0	0	1	1
35-39	2	2	2	5	4	3
40-44	4	4	1	3	5	4
45-49	2	2	0	0	2	1
50-54	0	0	2	5	2	1
55-59	3	3	0	0	3	2
60-64	2	2	2	5	4	3
65-++	29	29	4	11	33	24

**Table 14.** Number of *E. coli* infections (VTEC) in humans by sex and by age groups, 2013. Source: Sentinel Laboratory Network

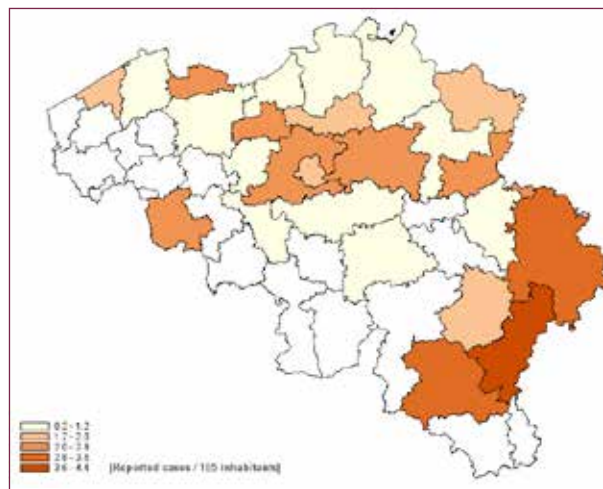
AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	14	23.73	28	52.83	42	38.00
05-09	11	18.64	11	20.75	22	20.00
10-14	5	8.47	1	1.89	6	5.00
15-19	4	6.78	1	1.89	5	4.00
20-24	3	5.08	2	3.77	5	4.00
25-29	4	6.78	0	0	4	4.00
30-34	0	0	0	0	0	0.00
35-39	1	1.69	0	0	1	1.00
40-44	0	0	0	0	0	0.00
45-49	2	3.39	1	1.89	3	3.00
50-54	1	1.69	0	0	1	1.00
55-59	0	0	0	0	0	0.00
60-64	2	3.39	0	0	2	2.00
65-++	10	16.95	6	11.32	16	14.00

As already reported in former years, the incidence in Flanders is higher than in Wallonia. Geographical distribution of cases



**Figure 27.** Incidence of *E. coli* infections (VTEC) in humans by district (N/105 inhab., 2012). Source: Sentinel Laboratory Network

is partly due to the location of the sentinel labs. The local outbreak of 2012 is visible in surveillance data (Figure 27 and 28).



**Figure 28.** Incidence of *E. coli* infections (VTEC) in humans by district (N/105 inhab., 2013). Source: Sentinel Laboratory Network

In 2012, the NRC confirmed 103 verotoxigenic *E. coli* isolates from the same number of patients. Among these:

- 82 typical VTEC isolates, positive for two additional virulence: the *eae* (intimin) gene and the enterohemolysin (EHEC virulence plasmid) gene.
- 21 atypical VTEC isolates, negative for intimin and/or enterohemolysin.

In addition, two patients had a positive serology for either serogroup O157 or O26.

The number of isolates confirmed annually by the NRC increased until 2009 and varied then between 82 and 111 isolates but still probably corresponds to a large rate of underdiagnosis (Table 15)

**Table 15.** *E. coli*: evolution in number of isolates in humans, 2000-2013. Source: NRC, UZBrussel

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of isolates	47	46	46	47	45	47	46	47	103	112	82	99	103	111
Number of typical isolates	33	36	37	40	36	36	36	40	73	86	68	81	82	93
Number of O157 isolates	26	29	26	21	29	27	21	25	55	61	50	64	64	60

In 2012, 14 strains (9 from serotype O157, one O26, O148, O145, O26, O55, O63) were associated with haemolytic uremic syndrome (HUS). Eleven patients were children (up till 12 years of age, 82%). The three other patients were adults aged between 46 and 87 years. Thirteen were female. In addition, two patients suffering from HUS had a positive serology for either serogroup O157 or O26.

In 2013, 14 strains (6 from serotype O157, four O145, one O182, O111, O104, O103) were associated with haemolytic uremic syndrome (HUS). Eight patients were children (57% up to the age of 12 years). With the exception of one 14 year old, the rest of the patients were older than 60 years. Six were female.

In addition, five patients suffering from HUS had a positive serology for either serogroup O157 (3 patients) or O26 (two patients).

# Leptospirosis

Toon Braeye, David Fretin, Hein Imberechts, Marcella Mori, 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 leptospire 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.

### Laboratory tests

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

## Leptospirosis in animals

In 2012, CODA-CERVA, the NRL Leptospirosis for animal health, has performed 1.457 serological analyses by means of MAT and 87 real time PCR (rtPCR) for the direct detection of the *Leptospira* pathogens. The majority of the samples originated from the following animal species: dogs (n=549), cattle (n=362, from which 291 from artificial insemination centres), pigs (n=279), horses (n=273) and muskrats (n=27). In dog samples, the micro-agglutination test demonstrated 109 positive sera. Antibodies against serogroups Australis, Icterohaemorrhagiae, Canicola, Grippytyphosa, Autumnalis, Pomona, Ballum, and Pyrogenes were found in respectively 60,0%, 32,9% (roughly the half due to vaccination), 17,1% (roughly the half due to vaccination), 15,7%, 10,0%, 7,1%, 4,3%, and 4,3% of the positive cases, and the rtPCR detected 1 positive in the 3 tested cases. In cattle and pigs, sera were found positive in 10 and 5 samples, respectively

and involved serogroups were Australis, Grippytyphosa, Sejroe and Tarassovi in cattle and serogroups Australis, and Bataviae in pigs. The one cattle abortion sample analysed by rtPCR was positive. In the 89 positive horse sera, antibodies against serovars Australis (33,0%), Grippytyphosa (18,7%), Ballum (12,1%), Pomona (9,9%) and Pyrogenes (7,7%) were detected. None of the few organs analysed by rtPCR post-mortem (n=6) was positive. The analysis of the muskrats was performed following environmental investigation related to a human cluster of cases occurring after an adventure camp in the South of Belgium. Positive sera were found in 5 out of the 9 animals and 3 out of 18 samples were detected positive by rtPCR.

In 2013, a total of 1.347 sera and 50 samples were analysed with MAT and rtPCR, respectively. Animal species distribution was similar as in the former year; dog and horse samples were mostly taken for diagnostic reasons. Observed serogroups in the positive dog sera were reflecting the same trend as in 2012 and 2 out of 12 samples were positive with rtPCR. In horses, the MAT assay identified 123 (44,7%) positive sera, and an increase in the presence of antibodies against the serogroup Pyrogenes in positive samples (from 7,7% in 2012 to 18,5% in 2013) as compared to the other serogroups.

During this year, also a number of rodent samples (mouse, guinea pig, rats) were analysed subsequently to two human leptospirosis cases associated with infection contracted from these companion animals. Six out the 14 tested animals were positive with rtPCR and isolation of the *Leptospira* strains was achieved for 4 of the 6 positive pet animals.

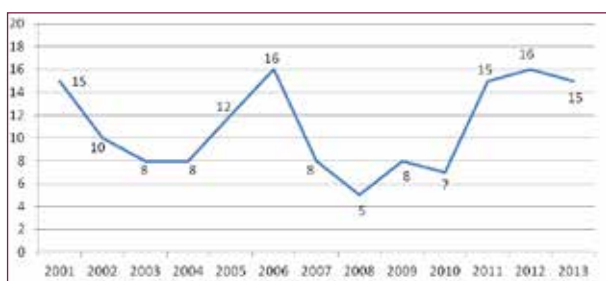
## Leptospirosis in humans

In 2012, 16 cases of leptospirosis were detected (14 confirmed cases and 2 possible cases). Twelve (75%) cases were male. The mean age was 37 years (range 12-74 years).

For 9 persons the contamination occurred in Belgium. Six patients suspected contact with or a bite from a rat. Three among them were kids camping in the Ardens. For 6 cases the contamination was suspected to have happened abroad (2 Thailand, Sri Lanka, Malaysia, Columbia, Philippines/Borneo). For the remaining cases, there is no information.

In 2013, 15 cases of leptospirosis were detected. Ten cases (66%) were male. The mean age of the cases was 35 years. For two cases the country of infection was unknown. For 7 cases Belgium was named as the likely country of infection. The remaining six cases named Thailand (3x), Greece, France and Ghana as likely country of infection.

Again contact with rodents was the most likely cause in case of domestic contamination. If the contamination occurred abroad, water sports were likely involved.



**Figure 29.** Evolution of possible and confirmed leptospirosis patients, period 2001-2013

# Listeriosis

Sophie Bertrand, Toon Braeye, Vera Cantaert, Katelijne Dierick, Wesley Mattheus

## Listeriosis

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

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

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

## *Listeria monocytogenes* in food

*Listeria monocytogenes* was analysed in beef, pork, dairy products, fish, ready-to-eat products and other products for infants. 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 monitoring 2009 – 2013.

**Table 16.** Zoonosis monitoring programme - *Listeria monocytogenes* in food, 2009 - 2013

Sample		Quantity analysed	Percentage of positive samples 2009	Percentage of positive samples 2010	Percentage of positive samples 2011	Percentage of positive samples 2012	Percentage of positive samples 2013
Beef	Minced meat at retail intended to be eaten raw (steak tartare)	Enumeration (M=100 cfu/g)	0.0% (n= 98)	-	-	0.2% (n=584)	0.3% (n=296)
	Minced meat at processing intended to be eaten raw (steak tartare)	25g or enumeration (M=100 cfu/g) (*)	-	-	-	-	4.5% (n=66)
	Meat preparation at retail intended to be eaten raw (steak tartare with herbs and sauce)	Enumeration (M=100 cfu/g)	0.0% (n=98)	-	-	-	-

Sample		Quantity analysed	Percentage of positive samples 2009	Percentage of positive samples 2010	Percentage of positive samples 2011	Percentage of positive samples 2012	Percentage of positive samples 2013
Pork	Cooked ham at processing plant	25g	2.7% (n=37)	1.0% (n=99)	1.7% (n=121)	-	-
	Cooked ham at retail	Enumeration (M=100 cfu/g)	0.0% (n=76)	0.0% (n=115)	0.0% (n=114)	-	-
	Raw ham at processing plant	25g or enumeration (M=100 cfu/g) (*)	3.1% (n=96)	3.2% (n=125)	3.2% (n=125)	0.86% (n=116)	5.4% (n=112)
	Raw ham at retail	Enumeration (M=100 cfu/g)	0.0% (n=34)	0.0% (n=112)	0.0% (n=113)	0.0% (n=114)	0.0% (n=113)
	Pâté at processing plant	25g	0.0% (n=38)	4.8% (n=42)	1.6% (n=128)	-	-
	Pâté at retail	Enumeration (M=100 cfu/g)	0.0% (n=84)	0.0% (n=113)	0.0% (n=114)	-	-
Meat (un-specified)	Minced meat intended to be eaten raw, at retail	Enumeration (M=100 cfu/g)	0.0% (n=200)	1,8% (n=55)	0.0% (n=72)	0.0% (n=72)	0.0% (n=72)
	Minced meat intended to be eaten raw, at processing plant	25g or enumeration (M=100 cfu/g) (*)	13.8% (n=72)	-	-	-	6.4% (n=62)
	Meat preparation at retail intended to be eaten raw (steak tartare with herbs and sauce)	Enumeration (M=100 cfu/g)		0.3% (n=295)	0.3% (n=294)	-	-
	Minced meat at retail intended to be eaten raw (steak tartare)	Enumeration (M=100 cfu/g)		0.3% (n=292)	0.0% (n=296)	-	-
	Sausages at processing plant	25g or enumeration (M=100 cfu/g) (*)	10.3% (n=185)	14,6% (n=130)	5,6% (n=124)	-	-
	Sausages at retail	Enumeration (M=100 cfu/g)	0.0% (n=69)	0.0% (n=106)	0.0% (n=113)	-	-
	Meat products- cooked – RTE at processing	25g or enumeration (M=100 cfu/g) (*)				1.0% (n=566)	1.7% (n=475)
	Meat products- cooked – RTE at retail	Enumeration (M=100 cfu/g)				0.2% (n=524)	0.0% (n=419)
Cheeses	Cheeses made from raw milk at retail	Enumeration (M=100 cfu/g)	0.0% (n=106)	0.0% (n=282)	0.0% (n=226)	0.0% (n=224)	0.0% (n=227)
	Cheeses made from pasteurised milk at retail	Enumeration (M=100 cfu/g)	0.0% (n=120)	0.0% (n=287)	0.0% (n=282)	0.0% (n=286)	0.4% (n=243)
	Cheeses made from raw milk at farm	Enumeration (M=100 cfu/g)	0.0% (n=58)	3,5% (n=85)	0,7% (n=137)	1,6% (n=62)	1,0% (n=192)
	Cheeses made from raw milk at processing plant	25g or enumeration (M=100 cfu/g) (*)	1,6% (n=63)	8,2% (n=49)	5,9% (n=101)	6,6% (n=105)	9,3% (n=54)
	Cheeses made from pasteurised milk at processing plant	25g or enumeration (M=100 cfu/g) (*)	0.0% (n=162)	0,7% (n=135)	0,0% (n=178)	0,0% (n=168)	0,0% (n=132)

Sample		Quantity analysed	Percentage of positive samples 2009	Percentage of positive samples 2010	Percentage of positive samples 2011	Percentage of positive samples 2012	Percentage of positive samples 2013
Dairy products	Butter at farm	Enumeration (M=100 cfu/g) (*)	0.0% (n=161)	2.4% (n=83)	0.8% (n=125)	3.3% (n=90)	3.2% (n=124)
	Butter made from raw milk at retail	Enumeration (M=100 cfu/g)	0.0% (n=25)	0.0% (n=23)	-	-	-
	Cream at farm	Enumeration (M=100 cfu/g) (*)	0.0% (n=20)	0.0% (n=40)	0.0% (n=49)	0.0% (n=35)	2.0% (n=51)
	Ice cream at farm	Enumeration (M=100 cfu/g)	-	0.0% (n=113)	0.0% (n=113)	0.0% (n=115)	0.0% (n=120)
	Ice cream at retail	Enumeration (M=100 cfu/g)	0.0% (n=80)	0.0% (n=114)	0.0% (n=114)	0.0% (n=114)	0.0% (n=118)
	Milk desserts at processing plant	25g	0.0% (n=94)	-	-	-	-
	Milk desserts at retail	Enumeration (M=100 cfu/g)	0.0% (n=160)	0.0% (n=224)	0.0% (n=228)	0.0% (n=113)	0.0% (n=110)
	Milk desserts at farm						0.0% (n=211)
	Yoghurt at processing plant	Enumeration (M=100 cfu/g)	-	0.0% (n=68)	0.0% (n=40)	0.0% (n=40)	0.0% (n=40)
	Yoghurt at retail	Enumeration (M=100 cfu/g)	0.0% (n=60)	0.0% (n=148)	0.0% (n=147)	0.0% (n=67)	0.0% (n=40)
	Yoghurt at farm	Enumeration (M=100 cfu/g)	0.0% (n=60)	0.0% (n=31)	0.0% (n=41)	0.0% (n=22)	0.0% (n=64)
	Milk powder at processing plant	Enumeration (M=100 cfu/g)	0.0% (n=56)	0.0% (n=54)	0.0% (n=45)	0.0% (n=45)	0.0% (n=45)
	White pudding at processing plant	25g	2.4% (n=41)	-	-	-	-
	White pudding at retail	Enumeration (M=100 cfu/g)	0.0% (n=76)	-	-	-	-
Fish	Smoked salmon at processing plant	25g	-	5.9% (n=17)	4.8% (n=21)	0.0% (n=23)	12.3% (n=106)
	Smoked salmon at retail	Enumeration (M=100 cfu/g)	1.0% (n=398)	0.0% (n=8)	0.0% (n=203)	0.0% (n=200)	0.0% (n=120)
	Fresh fish at processing plant	25g or enumeration (M=100 cfu/g) (*)	-	-	-	-	-
	Fresh fish at retail	Enumeration (M=100 cfu/g)	1.6% (n=62)	0.0% (n=293)	1.4% (n=293)	0.0% (n=293)	0.3% (n=293)
	Fishery products – cooked – RTE at retail	Enumeration (M=100 cfu/g)		0.0% (n=48)	0.0% (n=179)	0.0% (n=148)	0.0% (n=165)
	Fishery products – cooked – RTE at processing	25g or enumeration (M=100 cfu/g) (*)		3.4% (n=116)	5.8% (n=154)	0.6% (n=154)	2.5% (n=201)
Other products	Infant formula at retail					0.0% (n=289)	0.0% (n=279)
	Dietary foods for special medical purposes for infants at retail			0.0% (n=146)	0.0% (n=146)	0.0% (n=146)	0.0% (n=146)

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

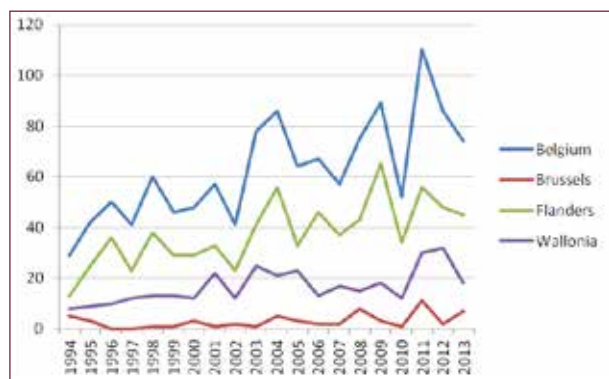
The national monitoring program for *Listeria monocytogenes* consisted of samples of different beef and pork products, of different dairy products and cheeses made from raw milk or pasteurised milk, several fishery products and some specific

products for infants. The results of these analyses are included in Table 16. At the processing plant, the percentage of contamination of some products, either by detecting presence in 25g or by enumeration, is at times high.

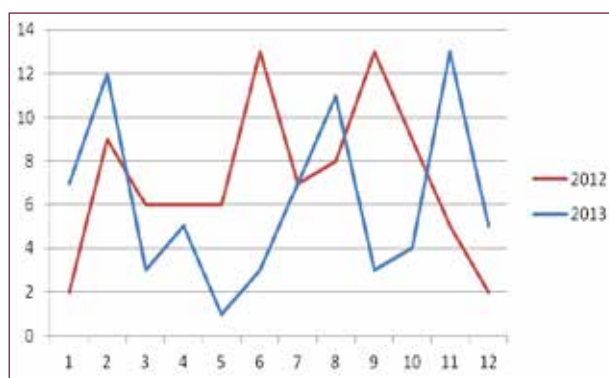
## Listeria monocytogenes in humans

### Data from the Sentinel Laboratory Network

In 2012, the Sentinel Laboratory Network reported 86 cases of listeriosis. In 2013, 74 cases of listeriosis were reported. This number presented a further decrease from the peak in 2011 (Figure 30). Cases are reported all over the year (Figure 31).



**Figure 30.** Annual number of *Listeria monocytogenes* infections in humans between 1994 and 2013. Sources: Sentinel Laboratory Network



**Figure 31.** Monthly number of *Listeria monocytogenes* infections in humans, 2012-2013. Sources: Sentinel Laboratory Network

In 2012, *Listeria monocytogenes* isolation rates were not equal for both genders; men (50.8%) and women (42%) in non-perinatal listeriosis. For 19% of the cases the gender is unknown, 39% of the cases are diagnosed in adults older than 64 year (Table 17). In 2013, *Listeria monocytogenes* isolation rates were almost equal between men (45%) and women (47%). 58% of cases are diagnosed in adults older than 64 year (Table 18). Elderly people and those with low immunity are more at risk of contracting 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.

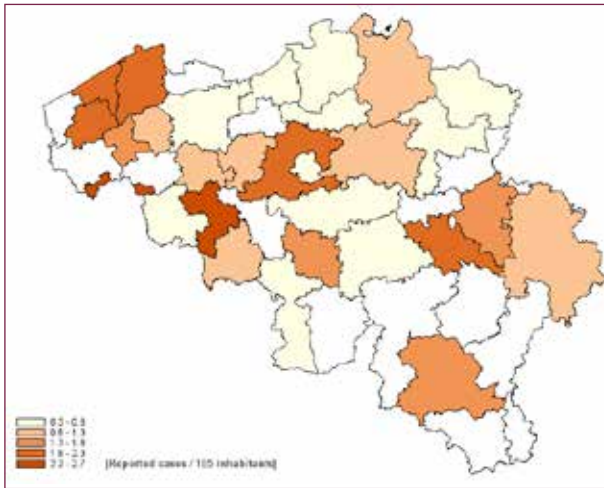
**Table 17.** Monthly number of *Listeria monocytogenes* infections in humans, 2012-2013. Sources: Sentinel Laboratory Network

AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	2	0.05	3	0.075	5	6.00
05-09	0	0.00	0	0	0	0.00
10-14	1	0.02	0	0	1	1.00
15-19	0	0.00	0	0	0	0.00
20-24	1	0.02	1	0.025	2	2.00
25-29	1	0.02	0	0	1	1.00
30-34	2	0.05	0	0	2	2.00
35-39	5	0.12	1	0.025	6	7.00
40-44	0	0.00	1	0.025	1	1.00
45-49	2	0.05	1	0.025	3	3.00
50-54	3	0.07	2	0.05	5	6.00
55-59	2	0.05	1	0.025	3	6.00
60-64	4	0.10	4	0.1	8	12.00
65-++	16	0.39	23	0.575	39	47.00

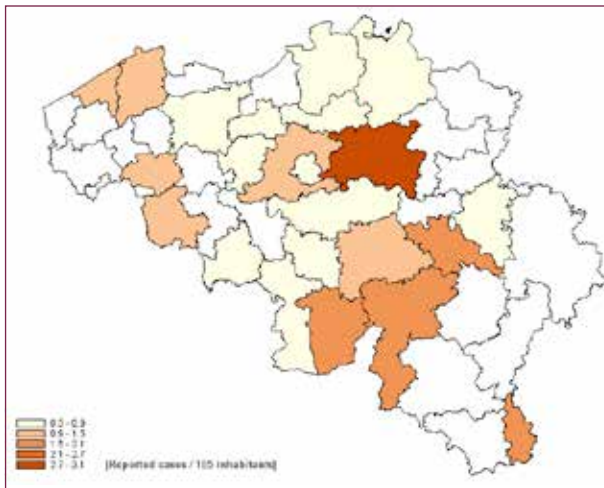
**Table 18.** Number of *Listeria monocytogenes* infections in humans by sex and by age group, 2013. Sources: Sentinel Laboratory Network

AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	1	0.03	1	0.03	2	3.00
05-09	0	0	0	0	0	0.00
10-14	0	0	0	0	0	0.00
15-19	0	0	0	0	0	0.00
20-24	0	0	0	0	0	0.00
25-29	0	0	0	0	0	0.00
30-34	0	0	2	0.06	2	3.00
35-39	2	0.05	0	0	2	3.00
40-44	1	0.03	0	0	1	1.00
45-49	0	0	0	0	0	0.00
50-54	4	0.11	0	0	4	5.00
55-59	2	0.05	2	0.06	4	7.00
60-64	2	0.05	4	0.12	6	8.00
65-++	12	0.3	19	0.58	31	46.00

The geographic distribution of listeriosis is partly due to the location of the sentinel labs, but for 2012 a remarkable high concentration of cases was found at the coast and in 2013 at the Leuven district. (Figure 32 and 33)



**Figure 32.** Incidence of *Listeria monocytogenes* infections in humans by district (N/105 inhab., 2012). Sources: Sentinel Laboratory Network



**Figure 33.** Incidence of *Listeria monocytogenes* infections in humans by district (N/105 inhab., 2013). Sources: Sentinel Laboratory Network

#### Data from the NRC

The completeness of the surveillance by the NRC is increasing. The number of cases reported in recent years is comparable to the number of other surveillance networks (2011; N=81, 2012; N=64, 2013; N=73). In 2013 47.7% of cases were males and 52.2 were females, 72.7% of non-perinatal listerioses occurred in adults aged 60+.

In 2012, the *Listeria* reference centre received 64 strains of *Listeria monocytogenes* of clinical human origin, representing a decrease of 21,7% with respect to 2011. Distribution of the serovars revealed the dominating position of 4b and 1/2a with 48.4% and 32.8% of the isolates respectively. Minor representations were seen for 1/2b (12.5%), 1/2c (3.1%) and 3a (1.6%). Seven strains were related to pregnancy. In 56% of the “none to pregnancy” related listeriosis cases presented a complementary pathology (linked with immunodepression).

In 2013, the *Listeria* reference centre received 73 strains of *Listeria monocytogenes* of clinical human origin, representing an increase of 14% with respect to 2012. Distribution of the serovars revealed the dominating position of 4b and 1/2a with 31.5% and 56.2% of the isolates, respectively. Minor representations were seen for 1/2b (6.8%), 1/2c (1.4%) and 3c (1.4%). Six strains were related to pregnancy (5 strains isolated from the mother and 3 strains from the newborn. In 57% of the “none to pregnancy” related listeriosis cases presented a complementary pathology (linked with immunodepression).

# Q-fever

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

## Coxiella burnetii

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Q-fever (Q for query) is a systemic disease caused by an obligate intracellular bacterium *Coxiella burnetii*. *Coxiella burnetii* occurs worldwide.

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

The biology and pathogenesis of *C. burnetii* are unusual. Of particular significance is the biphasic life cycle which generates a small cell variant form. This spore-like form is extremely resistant to environmental stress, particularly desiccation, which facilitates prolonged survival in the environment. Another important characteristic is the low minimum infectious dose to cause infection. Higher infectious doses appear to cause more extensive clinical disease in an important number of sensitive humans or animals.

*C. burnetii* is strongly related to pregnancy in both people and ruminants. The organism replicates to high levels in the trophoblasts of a mammalian placenta. Placental material following an abortion contains very high titres of organisms and this is considered to be the most important source of infection of other ruminants and people. It's also important to remember that acute infections in pregnancy often do not result in abortion and high titres of bacteria can be present in fluids at parturition and in the placenta of healthy offspring. Long after parturition, milk and vaginal secretions continue to contain considerable loads of bacteria. Similarly, in women, infection during pregnancy results in high titre infection within the placenta that can result in abortion, stillbirth, neonatal death, premature labour and low birthweight. Severity and outcomes of an infection may be related to difference in *C. burnetii* strains.

Almost all human Q fever infections are of zoonotic origin. Human-to-human transmission is very rare.

Most infections in people are asymptomatic or mild. Clinical infections typically present fever and pneumonia which can be severe and result in hospitalisation. A small proportion of human cases develop chronic infection usually manifesting endocarditis. Such chronic infections are difficult to treat and result in a substantial mortality.

## Q-fever in animals

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

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

Since 2010, the monitoring of *Coxiella burnetii* in ruminants was intensified. Tests on *Coxiella burnetii* were added to the abortion protocol of cattle, sheep and goats. Additionally, farms with milk producing goats and sheep were tested for the presence of *Coxiella burnetii* in bulk tankmilk. Both serology and RT-PCR were performed.

In the context of abortion, ELISA's were not performed on cattle samples in 2012 but 104 of the 9.121 RT-PCR analyses were positive. 154 ELISA's and 391 RT-PCR's of goats and sheep samples were performed in 2012, 5 of each tested positive.

In 2013, in the context of abortion, 2.761 ELISA's of cattle samples were performed of which 429 were positive, 15 ELISA's of sheep and 154 ELISA's of goats samples were performed of which one goat sample was positive. Of the 7.851 RT-PCR that were performed on cattle samples, 112 were positive, 1 was positive of the 75 sheep samples and 2 were positive of the 22 samples from goats.

The results of analyses of *Coxiella burnetii* in case of abortion are given in table 19.

**Table 19.** Results of analyses of *Coxiella burnetii* in the context of abortion, 2010 - 2013

Animal species	Year	ELISA		RT-PCR	
		Number of tests	Number of positives in percentage	Number of tests	Number of positives in percentage
Cattle	2010	2.191	14.4	5.254	9.5
Cattle	2011	337	11.3	7.120	1.5
Cattle	2012	0	0.0	9.121	1.1
Cattle	2013	2.761	15.5	7.851	1.4
Sheep	2010	55	0.0	76	2.6
Sheep	2011	12	0.0	143	0.6
Sheep	2013	15	0.0	75	1.3
Goats	2010	12	16.7	22	4.5
Goats	2011	6	0.0	39	5.1
Sheep and Goats	2012	154	3.2	391	1.3
Goats	2013	154	50.0	22	9.0

The results of the analyses in the framework of the bulk tank-milk monitoring on farms with dairy goats and/or dairy sheep are given in table 20.

**Table 20.** results of tankmilk monitoring on farms with milkgoats/milk-sheep, 2010 - 2013

Test	Year	Number of farms tested	Number of farms positive
ELISA	2010	118	17
	2011	115	17
	2012	108	23
	2013	110	26
RT-PCR	2010	118	15
	2011	115	9
	2012	108	12
	2013	110	12

Following recommendations are given or measures are taken on farms with positive RT-PCR results:

- Information to the farmer and his family on Q-fever disease, sources of infection, transmission, symptoms of infected people, ...;
- Advice to persons 'at risk', especially persons with pre-existing cardiac valvular disease or individuals with vascular grafts and pregnant women;
- Restricted access to barns housing potentially infected animals;
- 'Good-practice' recommendations in particular for dealing with animal births/abortions:
  - Stimulation of the mandatory declaration of abortions;
  - Put aborted animals in quarantine;
  - Analyses of placenta and aborted fetuses in case of any abortion;
  - Appropriate disposal of placenta, birth products, fetal membranes and aborted fetuses;
- Regular cleaning and disinfection of residences of animals and materials
- Pasteurization of milk and milk products from infected farms
- 'Good-practice' recommendations in particular for manure:
  - Store manure a minimum distance from human dwellings;
  - Compost manure of infected animals for a period of time;
  - Implement measures to prevent dust and airflow to residential areas when spreading manure;
  - Plough manure in immediately after spreading;
- Respect of sanitary measures regarding animal movements, control of animal gathering, no participation in exhibitions of infected farms
- Preventive vaccination of non-infected animals on seropositive farms with a phase-I vaccine containing inactivated *C. burnetii*.

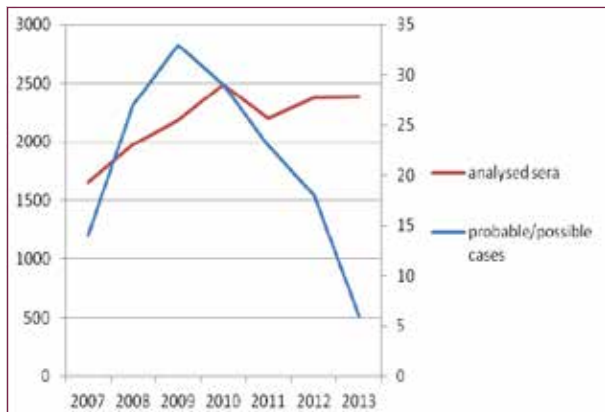
## Q-fever in humans

In 2012, at the Institute of Tropical Medicine (NRL), a total of 2,377 human sera have been examined for the presence of phase I and II IgM and IgG antibodies to *Coxiella burnetii*.

Seven confirmed/probable and eleven possible acute cases have been detected. An acute case is defined by a result of phase II IgM antibodies  $\geq 1/64$  as determined by IFA. The median age of the patients was 50.7 (range 21-67 years). Nine patients were male, 8 were female (one was unknown). One patient was probably infected in the Netherlands, four patients were probably infected in Belgium. The travel history and the probable country of infection of the other patients is not known.

In 2013, a total of 2384 human sera have been examined by the NRL.

Five confirmed/probable and one possible case have been detected. The median age of the patients was 46 (range 33-54 years). Four patients were male, two were female. The probable country of infection was unknown for 5 patients, for one patient this was Belgium.



**Figure 34.** Analyzed sera and probable/possible human cases of Q-fever between 2007-2013

# Salmonellosis

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Salmonella is an important zoonotic pathogen and a major cause of registered bacterial food-borne infections, both in individuals and in communities. The genus *Salmonella* is 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 the location 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 changes over time.

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

The common reservoir of *Salmonella* is the intestinal tract of a wide range of domestic and wild animals including cold blood animals and consequently a variety of foodstuffs covering both food of animal and plant origin become a source of infection for humans. Transmission often occurs when organisms are introduced in food preparation areas and are in favourable conditions able to multiply, e.g. due to inadequate storage temperatures, inadequate cooking or cross contamination of ready-to-eat food. The organism may also be transmitted through direct contact with infected animals or faecally contaminated environments and humans.

Food prepared with contaminated raw eggs, egg products or insufficiently heated poultry meat or pork is the major source of the human *Salmonella* infections. Therefore, surveillance programmes that in time detect *Salmonella* contaminations early in the 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 any clinical sign 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.

## Salmonellosis in feed

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

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

In 2012, 512 units of compound feedingstuffs were tested, of which 12 were positive for *Salmonella*: 1 duck feed, 1 for horses, 1 for laying hens, 3 for broilers, 5 dogfeeds and 1 feed for undetermined species. Regarding the five serotypes important for humans, one dogfeed contained *S. Enteritidis*. In addition, 321 feed materials were tested, and 17 were positive for *Salmonella* (15 of animal origin and 2 of plant origin). Regarding the five

serotypes important for humans 1 animal fat contained S. Typhimurium, 1 animal meal S. Enteritidis and 2 animal meals S. Infantis. In addition 18 dogchews were tested of which 1 was positive for S. Ohio. In total 30 different serotypes of Salmonella were identified in all types of animals feeds.

In 2013, 568 compound feedingstuffs were tested. Nine were found positive for one or more Salmonella serotypes. One S. Typhimurium was found in feed for cats. In 11 out of 327 units of feed materials, Salmonella was found: 9 in materials of animal origin and 2 of plant origin. Twice S. Infantis was identified in animal meal as one of the five serotypes important for humans. Finally, 11 dogchews were tested, with no positive results. In total 19 different serotypes were identified.

These data indicate no significant changes between 2012 and 2013. Feed remains one of the possible ways animals and animal products can become infected with Salmonella.

## Salmonella in poultry

### Salmonella in breeders and hatcheries

#### Control program in breeders

The regional animal health associations (i.e. "Association Régionale de Santé et d'Identification Animales" [ARSIA (<http://www.arsia.be/>)] and Dierengezondheidszorg Vlaanderen [DGZ Vlaanderen (<http://www.dgz.be/>)] organise the official sampling in the framework of the Belgian Salmonella control programme in breeders. Official samples are taken 2 weeks before entering the production unit and at the ages of 22, 46 and 56 weeks by technicians of DGZ or ARSIA. Routine samples are taken by the farmer at delivery of the day-old-chicks, at the age of 4 weeks and every 2 weeks during production. The same sampling protocol and analyses are used as in the previous years.

The hygiene level of hatcheries is also evaluated 4 times a year. These are done during visits of the technicians of the animal health services on non-hatching days and include various sites of the hatchery such as the 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 comparison. In addition, a specific Salmonella control is performed 4 times a year on pooled samples from dead-in-shell chicks and on fluff and meconium. These samples are taken by the owner and sent to the laboratory of the regional health service.

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

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

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

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

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

#### Epidemiological investigations and results of the 2012 – 2013 surveillance

In 2012, Salmonella was not found in one-day-old chicks (110 batches). During rearing (317 flocks), S. Agona was found in 2 flocks and S. Minnesota and S. O<sub>3,19</sub>:- were each found in 1 flock. In addition, 2 flocks were considered negative for S. Enteritidis after confirmation sampling. During production, of the 557 flocks (grandparent and parent flocks), 1 flock was positive for S. Enteritidis, 1 flock for S. Paratyphi B var. Java and 12 flocks were positive for serotypes not included in the programme. In addition, 1 flock was considered negative for S. Enteritidis after confirmation sampling, 1 flock for S. Paratyphi B var. Java and 4 flocks for S. Typhimurium. These flocks do not count as positive flocks.

In 2013, Salmonella was not found in one-day-old chicks (317 batches). During rearing (327 flocks), S. Agona, S. Cerro, S. Havana, S. Mbandaka, S. Meleagridis and S. Regent were each found in 1 flock. In addition, 3 flocks were considered negative for S. Typhimurium and one for S. Enteritidis after confirmation sampling. During production, of the 551 flocks (grandparent and parent flocks), 2 flocks were positive for S. Enteritidis and 10

flocks were positive for serotypes not included in the programme. In addition, 3 flocks were considered negative for *S. Typhimurium* after confirmation sampling. These flocks do not count as positive flocks.

During rearing, the number of positive flocks (all *Salmonella* spp.) decreased from 6 in 2008 to 3 in 2009, increased to 7 in 2010 after which a decrease is seen to 4 in 2011 and 2012. In 2013, an increase is seen to 6. During production, the number of *Salmonella* positive flocks for serotypes for which a target is set fluctuates between 0 and 3 in recent years. In 2013, 2 positive flocks were found. The source of infection could not be traced. The number of positive flocks of other serotypes has decreased slightly from 16 in 2011 to 12 in 2012 and 10 in 2013. A positive point is the decrease in the number of suspicious flocks where the presence of *Salmonella* could not be confirmed from 11 in 2011 to 6 in 2012 to 3 in 2013. All but one (*S. O6,8:Z10:-*) serotypes found in breeders were also found in broilers in 2012. Three serotypes found in breeders (*S. Havana*, *S. Isangi* and *S. Orientalis*) were not found in broilers in 2013.

### **Salmonella in layers**

#### Surveillance programme in commercial laying hen flocks

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

One flock of all farms with at least 200 laying hens is also sampled by the FASFC. The sampling protocols and analyses used were the same as the previous years.

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

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

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

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

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

#### Epidemiological investigations and results of 2012 - 2013 surveillance

In 2012, one batch of one-day-old chicks was positive for *S. Enteritidis*. During rearing, 445 flocks were sampled of which 4 were positive for *Salmonella* spp. (1 each for *S. Enteritidis*, *S. Agona*, *S. Livingstone* and *S. Senftenberg*). During production, 764 flocks were sampled of which 36 were positive for *Salmonella* spp. (15 for *S. Enteritidis* and 2 for *S. Typhimurium*).

In 2013, there were 221 different batches with one-day-old chicks of which 1 was positive for *S. Typhimurium* and one for *S. Agona*. During rearing, 279 flocks were sampled of which 1 was positive for *Salmonella* spp. (*S. Havana*). During production, 606 flocks were sampled of which 36 were positive for *Salmonella* spp. (10 for *S. Enteritidis* and 1 for *S. Typhimurium*).

The prevalence for all *Salmonella* serotypes decreased in 2012 compared to 2011 and increased from 4,94% in 2012 to 5,94% in 2013. However, after a status quo in 2012, the prevalence of *S. Enteritidis* and *S. Typhimurium* decreased in 2013 to 1,82%. This is the first time the EU-target of <2% *S. Enteritidis* or *Typhimurium* positive flocks is achieved in Belgium.

### **Salmonella in broilers**

#### Surveillance program in commercial broiler flocks

One-day-old-chicks are sampled on farms with a capacity of more than 5.000 birds. All broiler flocks with a capacity of 200 or more broilers are sampled by the holder in the last three weeks before slaughter. Ten percent of the holdings are also sampled by the FASFC. The sampling protocols and method of analyses have not changed.

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

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

As of 2009 on, it is forbidden to use antimicrobials as treatment for *Salmonella*. Before restocking the house, the house is cleaned, disinfected and the presence of *Salmonella* is checked by means of two samples each consisting of 25 swabs taken throughout the house. A hygienogram is performed. If *Salmonella* is found or if the hygienogram indicates that too many bacteria are present, the *Salmonella* control and/or the hygienogram have to be repeated after the next round. In addition

to the previous measures, the house has to be disinfected by an external company and thinning must be performed as last holding of the day if 2 consecutive flocks in 1 house are positive for the same serotype. If 3 consecutive flocks are positive for the same Salmonella serotype, an epidemiological investigation of the farm and optimizing the biosecurity and hygiene measures on the farm are added to the list of measures.

#### Epidemiological investigations and results of the 2012 – 2013 surveillance

In 2012, 5,593 batches of one-day-old chicks were sampled, 26 were positive for Salmonella spp. of which 11 for S. Enteritidis, 2 for S. Typhimurium and 9 for S. Minnesota. 8,739 flocks of broilers were sampled in the last 3 weeks of production. 301 flocks were positive for Salmonella spp. of which 33 for S. Typhimurium and 17 for S. Enteritidis. This is the highest number of S. Enteritidis positive flocks since the start of the programme. The main serotype found was the same as in 2011, Salmonella Paratyphi B (incl. var. Java).

In 2013, 5,507 batches of one-day-old chicks were sampled, 17 were positive for Salmonella spp. of which 8 for S. Enteritidis. 8,664 flocks of broilers were sampled in the last 3 weeks of production. 181 flocks were positive for Salmonella spp. of which 6 for S. Typhimurium and 7 for S. Enteritidis. The most common serotype found was S. Paratyphi B var. Java in 44 flocks, followed by S. Livingstone (19 flocks), S. Derby (17 flocks) and S. Minnesota (14 flocks).

In 2012, the prevalence of all serotypes in one-day-old chicks decreased compared to 2011. However there was a high number of batches of one-day-old chicks positive for S. Enteritidis. The number of S. Minnesota positive flocks due to positive breeders in 2011 was also very high. The prevalence in broiler flocks of S. Enteritidis and S. Typhimurium has increased compared with the results of 2011. The increase of the number of S. Paratyphi B (incl. var. Java) positive flocks continued in 2012.

In 2013, a further decrease in the prevalence of all serotypes in one-day-old chicks was seen. Even though S. Minnesota was no longer found in rearing breeders in 2013, 14 broilers flocks were still positive for S. Minnesota. The prevalence of Salmonella spp. in broiler flocks decreased significantly from 3,44% in 2012 to 2,09% in 2013.

#### **Salmonella serotypes of the surveillance in breeders, layers and broilers**

In 2012, the NRL analysed in total 863 Salmonella strains from poultry origin, which is about 9,3% less than in 2011 (n=952).

The proportion of serotype Typhimurium (9.8% and 6.2% in 2011 and 2012, respectively) tends to decrease, whereas that of serotype Enteritidis (6.8% in 2011; 13.2% in 2012) shows a major increase compared to the year before. No evolution was seen in the proportion of serotype Paratyphi B (24.7% and 23.6% in 2011 and 2012, respectively). It should be noticed that serotype 4,5,12:i:- took 6.1% of all strains serotyped.

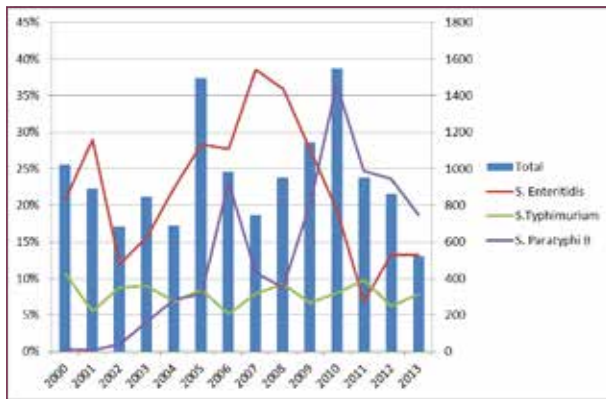
Some information on the origin of 532 Salmonella poultry isolates was available. Fifty-nine strains were from breeders of which 29 isolates belong to serotype Kottbus, 6 were S. Typhimurium, 5 S. Minnesota and 5 S. Agona. As in former years, the broiler isolates (n=400) belong to many serotypes and most were S. Minnesota (26.7%) and S. Paratyphi B (24.5%), but also S. Enteritidis (8.25%) and S. Typhimurium (7.7%) were frequently identified, followed by S. Rissen and serotypes Livingstone, Infantis and Mbandaka (all 14%). As for layer isolates (n=73) serotype Enteritidis (46.6%) was the most prevalent, but also S. Livingstone (11%) and S. Kottbus and S. Typhimurium (8.2%) were typed.

As for 2013, 522 Salmonella poultry isolates were analysed, which is about 39.5% less than in 2012. Compared to 2012, the proportion of both S. Enteritidis (13.2%) and S. Typhimurium (7.9%) remained at a similar level, whereas serotype Paratyphi B (18.8%) went somewhat down as compared to 2012.

Limited information on the origin of 522 Salmonella poultry isolates was available. Twenty-seven strains were from breeders of which 6 isolates belonged to serotype S. Enteritidis, 4 were S. Typhimurium, 3 S. Agona, 3 Mbandaka and 3 S. Paratyphi B var. Java. Eight isolates belonged to other serotypes. As in former years, the broiler isolates (n=323) belong to many serotypes and most were S. Paratyphi B var. Java (24.4%), but also S. Derby (7.1%), S. Livingstone (6.8%), S. Enteritidis (6.5%), S. Minnesota (6.2%), S. Typhimurium (5.9%), S. Infantis (5.6%) and S. 4,5,12:i:- (3.4%). As for layer isolates (n=83) serotype S. Enteritidis (38.6%) was the most prevalent, but also S. Typhimurium (15.7%), S. Infantis (9.6%) and S. Livingstone (6%) were found.

The number of poultry isolates yearly sent to the NRL varied between about 700 and 1 500. In the period 2007-2010 the volume of poultry strains nearly doubled, but in 2012 the number of isolates decreased to 863. The proportion of S. Enteritidis isolates fell down from more than 35% in 2007 and 2008 to less than 7% in 2011 and to 13.2% in 2012 and 2013. The proportion of S. Paratyphi B rose from about 10% in 2007 to more than 36% in 2010; in 2013 their proportion was about 18%. The ratio of S. Typhimurium strains remains since 2001 below 10% and reached 7.9% in 2013 (1,4,5,12:i:- not included).

During the years 2010, 2011 and 2012 the serotype Minnesota gained importance and reached a level of 17.6% in 2012 but in 2013 only 4% of the poultry isolates were found to be *S. Minnesota*.



**Figure 35.** Evolution of the percentages of the principal *Salmonella* serotypes isolated from poultry between 2000 and 2013. 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

### Surveillance program and sampling

There are no commercial turkey breeding flocks in Belgium. All meat turkey flocks with a capacity of 200 or more turkeys are sampled by the holder in the last three weeks before slaughter. The sample consists of two pair of overshoes. Of all flocks present at time of sampling, ten percent of the holdings are also sampled by the FASFC as part of an official control.

### Case definition, sanitary measures and vaccination policy

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

As of 2010, it is forbidden to use antimicrobials to treat *Salmonella*. Before restocking the house, the house is cleaned, disinfected and the presence of *Salmonella* is checked by means of two samples each consisting of 25 swabs taken throughout the house. A hygienogram is performed. If *Salmonella* is found or if the hygienogram indicates that too many germs are present, the *Salmonella* control and/or the hygienogram have to be repeated after the next round. In addition to the previous measures, the house has to be disinfected by an external company and thinning must be performed as last holding of the day if 2 consecutive flocks are positive for the same serotype.

If 3 consecutive flocks are positive for the same *Salmonella* serotype, an epidemiological investigation of the farm and optimizing the biosecurity and hygiene measures on the farm are added to the list of measures.

### Epidemiological investigations and results of the 2012 - 2013 surveillance

In 2012, 163 meat production flocks were tested, 191 in 2013. One flock tested positive for *S. O4,12:-:-* in 2012. In 2013 there was one flock positive for *S. Typhimurium*, one for *S. Havana* and 2 for *S. Paratyphi B* var. *Java*.

## Salmonella in other poultry

### Surveillance programme and sampling

Breeding flocks are at least sampled as day-old chicks, 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. On a voluntary basis, one-day-old chicks may also be sampled.

### Case definition, sanitary measures and vaccination policy

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

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

### Results of the investigation in 2012 - 2013 surveillance

In 2012, all 5 meat duck production flocks and 17 guinea fowl flocks sampled were negative for *Salmonella* spp. There were no breeding flocks.

In 2013, 4 guinea fowl flocks, 1 flock with meat ducks and 1 flock with patridge breeders were sampled. No *Salmonella* was found.

Seen the very low number of flocks of other poultry than chickens in Belgium, there is very little to no impact on human cases.

Only few *Salmonella* strains that originated from birds different from poultry were sent to the NRL. All but one pigeon strains analysed in 2012 (n=9) and all 18 pigeon *Salmonella* in 2013 were identified as *S. Typhimurium* var. *Copenhagen*, which is a common finding

## Salmonella in pigs

### Serology

#### Surveillance program in fattening pigs

As in previous years, the blood samples from fattening and growing pigs that were taken for the monitoring of Aujeszky's disease were in 2012 and 2013 also analysed for Salmonella. In 2012, 10 to 12 blood samples were taken in each farm every 4 months. In 2013 blood samples were taken only once a year. The analysis for Salmonella-specific antibodies was done in the veterinary laboratories of ARSIA and DGZ by means of a commercially available ELISA kit, following the manufacturer's instructions.

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

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

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

Pigs were not vaccinated since no vaccine was authorised/available in Belgium.

#### Results of the program

5.666 herds with fattening pigs were sampled in 2012. 1.998 herds had at least once a mean S/P ratio of more than 0,6. 106 herds were classified as Salmonella risk herds for the first time and 57 herds were classified as a Salmonella risk herd for a second or consecutive time.

5.332 herds with fattening pigs were sampled in 2013. 989 herds had at least once a mean S/P ratio of more than 0,6. 69 herds were classified as Salmonella risk herds for the first time and 27 herds were classified as a Salmonella risk herd for a second or consecutive time.

### Bacteriology

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

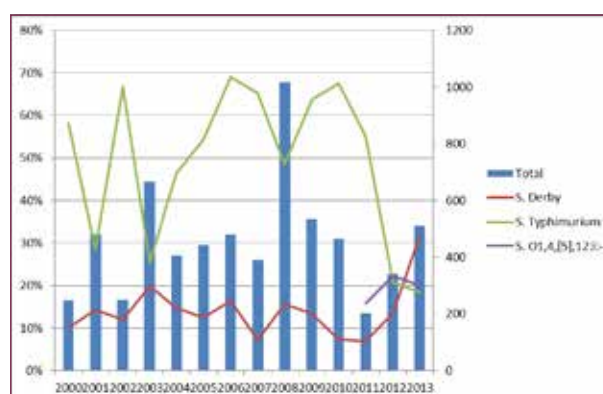
In the framework of bacteriological detection of Salmonella on risk herds, 417 samples were taken on 95 farms in 2012. Salmonella could be isolated on 40% of the farms. The main

serotypes found were S. Typhimurium (26 herds) and S. O4,(5),12:i:- (18 herds).

In 2013, 275 samples were taken on 65 farms. Salmonella could be isolated on 57% of the farms. The main serotypes found were S. Typhimurium (15 herds) and S. O4,(5),12:i:- (16 herds).

Data from the NRL demonstrate that the number of pig strains tested in 2012 was higher as compared to 2011 (n=341 and 203, respectively). Mostly S. Typhimurium isolates were found (30.8%), but also S. O4,[5],12:i:- (22.3%). As for 2013, significantly more Salmonella were tested as compared to 2012 (n=511). Derby (31.9%) and Typhimurium (18.4%) are the main serotypes, but also S. 1, 4,[5],12:i:- (20%) were found.

Until 2012, S. Typhimurium was absolutely the most prevalent serotype among pig isolates. A drop is seen in the percentage of S. Typhimurium starting from 2011 while the serotype 1,4,[5],12:i:- raised starting from 2011. Serotype Derby always is the second most important serotype except for 2012. In 2013 about 32% of the pigs isolated were found to be S. Derby.



**Figure 36.** Evolution of the percentage of the principal Salmonella serotypes isolated from pigs between 2000 and 2013.

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 2012 and 2013. In Belgium no Salmonella vaccine was authorized in cattle. Salmonella isolates were sent on a voluntary basis to the NRL for serotyping.

In 2012, the number of Salmonella isolates from cattle (n=47) that was sent to the NRL was similar as compared to 2011 (n=36). Most frequently found serotypes were S. Typhimurium (55.3%) and S. Dublin (23.4%). The proportion of S. Dublin isolates seemed to diminish as compared to former years. Also in 2013 a comparable number of strains was tested (n=43). Again, S. Typhimurium (60.5%) and S. Dublin (16.3) where the major serotypes, followed by S. 4,12:i:- (7%) and 4,5,12:i:- (4.7%).

In cattle, S. Dublin used to be the principal serotype between 2002 and 2010, but declined in 2010 and 2011 to the same low level as S. Typhimurium. In 2013 (as in 2012) S. Typhimurium (60.5%, S. 1,4,[5],12:i:- not included) was clearly the most prevalent isolated serotype from cattle samples.



**Figure 37.** Evolution of the percentage of the principal Salmonella serotypes isolated from cattle between 2000 and 2013.

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

In 2012 and 2013, sampling for Salmonella monitoring was done on the following matrices: carcasses, trimmings and raw ham of pork, minced meat and meat preparations of beef, carcasses, meat cuts and meat preparations of broilers, layer carcasses and minced meat and meat preparations of diverse species. Sampling of pork carcasses was done by means of swabs. The carcass samples of broilers and layers at slaughter consisted of neck skin. Different contamination levels were analysed: 25g, 600 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.

## Salmonella in meat and meat products

### Results of Salmonella surveillance in meat and meat products in 2012

**Table 21.** The results of the monitoring – Salmonella in minced meat, meat preparations and meat products of beef and pork - 2012

Species	Sample size	Prevalence 2012	Predominant serotype	Other serotypes (in decreasing order)
<b>Beef</b>				
Meat preparation (steak tartare) at retail (n=118)	25g	0.0%		
Meat preparation (steak tartare with herbs and sauce) at retail (n=118)	25g	0.0%		
<b>Pork</b>				
Carcasses at slaughter (n=534)	600cm <sup>2</sup>	10.9%	Typhimurium	Derby, Livingstone, London, Brandenburg, Infantis, Ohio, Isangi, AgonaMbandaka
Meat cuts at processing plant (n=151)	25g	2.6%		Derby, Enteritidis, Newport
Raw ham at retail (n=46)	25g	0.0%		
Raw ham at processing plant (n=42)	25g	0.0%		
Minced meat (steak tartare) at retail (n=40)	25g	5.0%	Derby	

Species	Sample size	Prevalence 2012	Predominant serotype	Other serotypes (in decreasing order)
<b>Broilers</b>				
Carcasses at slaughter (n=270)	1g	1.1%		Kentucky, Minnesota
Carcasses at slaughter (n=139)	25g (caeca)	2.9%	Paratyphi B	Infantis
Carcasses at retail (n=91)	25g	3.3%	Minnesota, Grampian, Kentucky, 6,7:-:-	
Meat cuts (skinned or with skin) at processing plant (n=590)	25g	3.7%	Paratyphi B	Typhimurium, Minnesota
Meat cuts (skinned or with skin) at retail (n=95)	25g	3.1%	Paratyphi B var Java	
<b>Layers</b>				
Carcasses at slaughter (n=444)	1g	11.3%	Enteritidis, Braenderup	Typhimurium, Infantis, Bareilly, Indiana, Derby
Carcasses at slaughter (n=183)	25g (caeca)	7.6%	Enteritidis, Paratyphi B	Braenderup, Hessarek
Carcasses at retail (n=118)	25g	7.6%	Braenderup	Infantis, Enteritidis, Indiana
<b>Poultry (other than Gallus gallus)</b>				
Meat cuts (with skin) at retail (n=69)	25g	4.3%	Stanleyville, Paratyphi B var. Java	
Meat cuts (without skin) at retail (n=72)	25g	5.5%	Paratyphi B, Copenhagen, 4:e:h:-	
Meat preparation at retail (n=59)	10g or 25g	1.7%	Derby	
Meat preparation at processing plant (n=59)	10g or 25g	10.2%	Paratyphi B, Paratyphi B var. Java, Derby	
Meat products intended to be eaten cooked at processing plant (n=58)	25g	1.7%	Paratyphi B, var. Java	
Meat products intended to be eaten cooked at retail (n=59)	25g	0.0%		
<b>Minced meat, meat preparations and meat products (beef and pork)</b>				
Minced meat intended to be eaten raw at retail (n=59)	25g	3.4%	Derby	
Minced meat intended to be eaten cooked at retail (n=58)	10g	0.0%		
Meat preparation intended to be eaten raw at retail (n=46)	25g	0.0%		
Meat preparation intended to be eaten cooked at retail (n=59)	10g	1.7%	Bovismorbificans	
Meat preparation intended to be eaten raw at processing plant (n=57)	25g	3.5%	SDerby	
Meat preparation intended to be eaten cooked at processing plant (n=58)	10g	1.7%	Derby	

#### Results of Salmonella surveillance in meat and meat products in 2013

**Table 22.** The results of the monitoring – Salmonella in meat, meat preparations and meat products of beef and pork - 2013

Species	Sample size	Prevalence 2013	Predominant serotype	Other serotypes (in decreasing order)
<b>Beef</b>				
Meat preparation (steak tartare) at retail (n=56)	25g	0.0%		
Meat preparation (steak tartare with herbs and sauce) at retail (n=59)	25g	0.0%		

Species	Sample size	Prevalence 2013	Predominant serotype	Other serotypes (in decreasing order)
<b>Pork</b>				
Carcasses at slaughter (n=379)	600cm <sup>2</sup>	8.2%	Typhimurium, Derby	Brandenburg, Infantis, Augustenborg
Meat cuts at processing plant (n=291)	25g	1.7%	Typhimurium	Derby
Raw ham at retail (n=46)	25g	0.0%		
Raw ham at processing plant (n=37)	25g	0.0%		
<b>Broilers</b>				
Carcasses at slaughter (n=232)	1g	2.2%	Paratyphi B	
Carcasses at slaughter (n=303)	25g (caeca)	2.6%	Paratyphi B	Typhimurium, Enteritidis, Infantis
Carcasses at retail (n=91)	25g	3.3%	Paratyphi B, Infantis, Paratyphi B var. Java	
Meat cuts (skinned or with skin) at processing plant (n=758)	25g	5.4%	Paratyphi B	Derby, Infantis, Enteritidis, Typhimurium, Stanley, Saintpaul, Braenderup Muenster, Brandenburg
Meat cuts (skinned or with skin) at retail (n=59)	25g	0.0%		
<b>Layers</b>				
Carcasses at slaughter (n=234)	1g	13.7%	Enteritidis	Infantis
Carcasses at slaughter (n=45)	25g (caeca)	2.2%	Infantis	
Carcasses at retail (n=118)	25g	5.9%	Enteritidis	Isangi, Infantis
<b>Poultry (other than Gallus gallus)</b>				
Meat cuts (with skin) at retail (n=33)	25g	3.0%	Albany	
Meat cuts (without skin) at retail (n=26)	25g	3.8%	Kottbus	
Meat preparation at retail (n=64)	25g	1.6%	Derby	
Meat preparation at processing plant (n=31)	25g	19.3%	Newport	
Meat products intended to be eaten cooked at processing plant (n=59)	25g	0.0%		
Meat products intended to be eaten cooked at retail (n=40)	25g	0.0%		
<b>Minced meat, meat preparations and meat products (beef and pork)</b>				
Minced meat intended to be eaten raw (all species) at retail (n=59)	25g	0.0%		
Minced meat intended to be eaten cooked at retail (all species) (n=58)	10g	0.0%		
Meat preparation intended to be eaten raw (all species) at retail (n=56)	25g	0.0%		
Meat preparation intended to be eaten cooked (all species) at retail (n=59)	10g	1.7%	Paratyphi var. Java	
Meat preparation intended to be eaten raw (all species) at processing plant (n=34)	25g	2.9%	Typhimurium O5-	
Meat preparation intended to be eaten cooked (all species) at processing plant (n=47)	10g	10.6%	Typhimurium, Derby	

The contamination rate of different products of pork, broilers and layers is mentioned in the next table 23 for the period 2005–2011.

**Table 23.** Evolution of the food Salmonella prevalence, 2005-2013

	Samples	Sampling level	2005	2006	2007	2008	2009	2010	2011	2012	2013
Pork	Carcasses	600cm2	9.3%	7.1%	16%	14.6%	13.4%	8.5%	6.8%	10.9%	8.2%
	Cutting meats	25g	7.3%	2.4%	4.1%	5.7%	3.4%	1.7%	2.1%	2.6%	1.7%
Broilers	Carcasses	1g	5.7%	1.4%	10.3%	7.0%	6.0%	3.0%	3.9%	1.1%	2.2%
	Cutting meats	25g	14.2%	13.3%	7.4%	7.2%	8.0%	6%	5.6%	3.7%	5.4%
Layers	Carcasses	1g	22.6%	35.6%	45.4%	23.4%	36.0%	33%	21.5%	11.3%	13.7%

The Salmonella contamination of pig carcasses increased in 2007 and decreased continuously since 2008 to fall under 10% in 2010 and 2011. This contamination increased slightly in 2012 to decrease again under the 10% in 2013.

The Salmonella contamination of broiler carcasses decreased since 2007 and remained at the same level over the last 2 years. The Salmonella contamination of layer carcasses varied a lot over the last years.

The trends in Salmonella contamination of pig-, broiler- and layer carcasses are shown in the next figures 38 - 40.

### Salmonella in other food of animal origin.

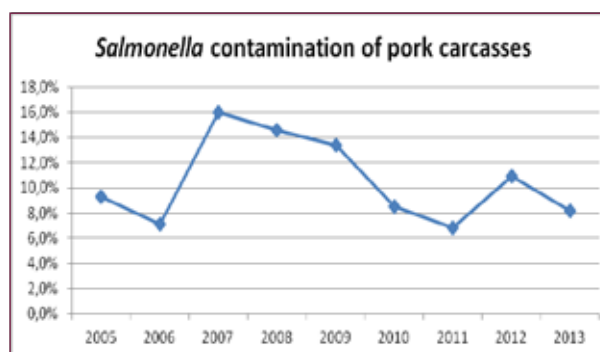
The results of the national monitoring program of milk and dairy products for 2012 are as follows:

- at retail: dried food for medical use for babies (n=59), infant formula (n=86), nursing bottles (n=110), cheese of pasteurised milk (n=235), cheese of raw milk (n=143), butter of raw milk (n=23), ice cream (n=47)
- at processing plant: cheese of pasteurised milk (n=113), milk powder (n=43), cheese of raw milk (n=71)
- at farm: cheese of raw milk (n=54), butter (n=35), cream (n=35), ice cream (n=48).

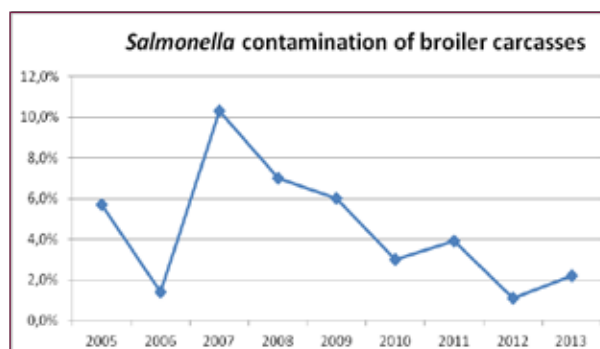
All results were negative, except for dried food for medical use for babies at retail (1.7% positive) and milk powder at processing (2.3% positive).

The results of the national monitoring program in other foods of animal origin and food from non animal origin for 2012 are as follows:

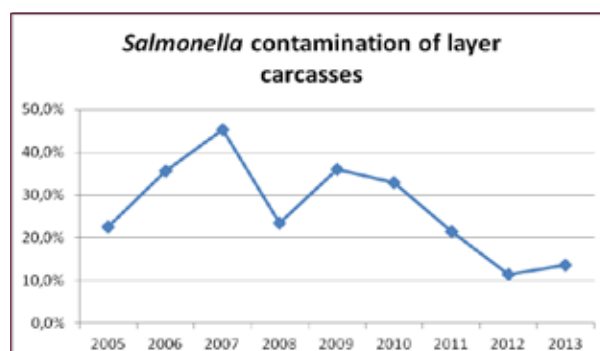
- at retail: fresh fish that could be eaten raw (n=90), cooked molluscs (n=45), cooked crustaceans (n=46), meat salad (n=46), chicken salad (n=47), crustacean salad (n=46), live bivalve molluscs (n=92), raw crustaceans (n=46), chocolate (n=46), confectionary with chocolate (n=46), table eggs



**Figure 38.** Salmonella contamination of pork carcasses, 2005 – 2013



**Figure 39.** Salmonella contamination of broiler carcasses, 2005 – 2013



**Figure 40.** Salmonella contamination of layer carcasses, 2005 – 2013

(n=118), liquid egg products (n=20), salty preparation based on raw eggs (n=24), dessert based on raw eggs (n=25), gelatine (n=87), melons (n=114), red berries (n=46), unpasteurised fruit juices (n=74), pre-cut fruits and vegetables (n=60), vegetables (n=359), dried spices and herbs (n=59), fresh herbs (88).

- at processing plant: gelatine (n=10), cooked crustaceans and molluscs (n=91), raw crustaceans (n=45), sandwich spreads (crustacean, meat, chicken) (n=139), egg products (n=114).

All results were negative, except for live bivalve molluscs at retail (1.1% positive) and raw crustaceans at processing (2.2%).

The results of the national monitoring program of milk and dairy products for 2013 are as follows:

- at retail: dried food for medical use for babies (n=59), infant formula (76), nursing bottles (n=110), cheese of pasteurised milk (n=215), cheese of raw milk (n=157), ice cream (n=49), raw milk (n=10)
- at processing plant: cheese of pasteurised milk (n=160), cheese of raw milk (n=81), milk powder (n=45)
- at farm: cheese of raw milk (n=123), butter of raw milk (n=92), cream of raw milk (n=42), ice cream (n=45), raw milk (n=39).

All results were negative.

The results of the national monitoring program in other foods of animal origin and food from non animal origin for 2013 are as follows:

- at retail: fresh fish that could be eaten raw (n=91), cooked crustaceans (n=46), meat salad (n=8), chicken salad (n=46), crustacean salad (n=46), fish salad (8), bakery products (n=60), live bivalve molluscs (n=46), raw crustaceans (n=46), table eggs (n=118), liquid egg products (n=19), salty preparation based on raw eggs (n=25), gelatine (n=41), desserts containing raw eggs (n=50), fruits (n=45), vegetables (n=662), pre-cut fruits and vegetables (n=57), dried spices and herbs (n=59), fresh herbs (n=10), unpasteurised fruit juices (n=76)
- at processing plant: gelatine (n=4), cooked crustaceans and molluscs (n=91), raw crustaceans (n=45), sandwich spreads (crustacean, fish, meat, chicken) (n=118), egg products (n=113), dried herbs and spices (n=59), vegetables (n=45).

All results were negative, except for table eggs (0.8% positive) at retail and cooked crustaceans at processing (6.1% positive).

## Salmonella in humans

### Surveillance programme and methods used

Data about human salmonellosis cases and human isolates were obtained from 165 clinical laboratories. In 2013, the NRC received 2874 Salmonella isolated from 2762 patients. In 2012, 3170 samples were received. 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 *S. Enteritidis*, *S. Typhimurium*, *S. Hadar* and *S. Virchow*. All isolates of *S. Brandenburg*, *S. Derby*, *S. Infantis*, *S. Newport*, *S. Typhi* and *S. Paratyphi* were selected and tested for their antimicrobial susceptibility.

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

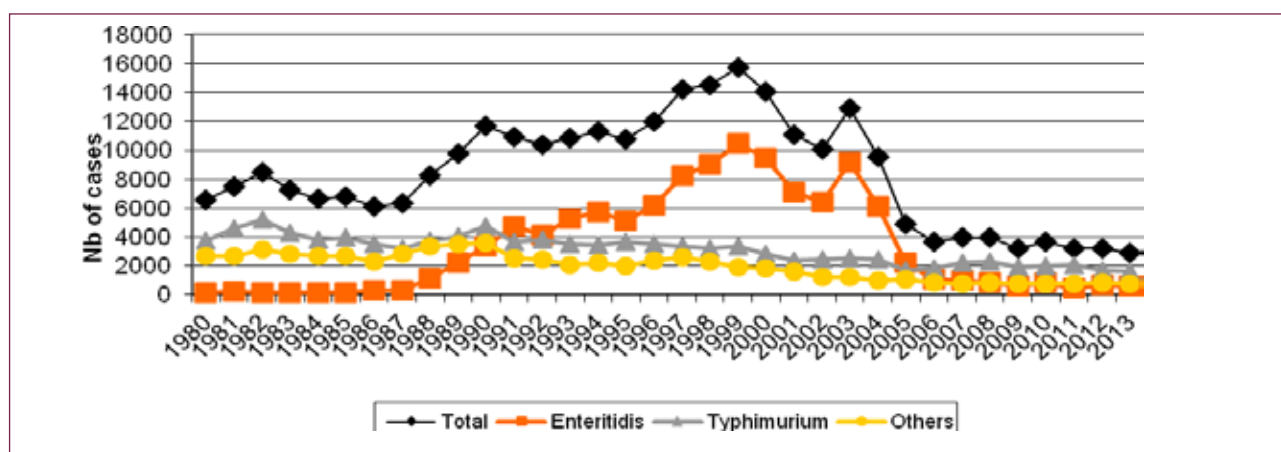
### Epidemiological investigations and results of 2012 - 2013 surveillance

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 xx, Table xx). In that year, the number of *S. Enteritidis* was exceptionally high. Between 2000 and 2011, the total number of laboratory-confirmed cases varied between 14,088 and 3,231 (Table xx). In 2003, the high number of salmonellosis cases mainly resulted from the increase of the serotype *Enteritidis*. These isolates exceeded for the first time 70% of the total number of Salmonella strains analysed. From 2005 a substantial decrease of *S. Enteritidis* infections compared with the annual number of cases in the period 2000-2004 was recorded. This decrease persisted in 2012-2013, with the total number of cases caused by *S. Enteritidis* decreasing to 663 in 2012 and 575 in 2013 cases, respectively.

In recent years, the number of *S. Typhimurium* isolates remained at a level of about 2 000 strains per year but decreased in 2013 to 1556 isolates (Table xx). After decreasing over several 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 38 cases in 2012 and 44 cases in 2013, respectively. Regarding *S. Virchow*, about 140 to 150 isolates were annually registered from 2000 to 2003, whereas since 2006 less than 50 strains were yearly reported. A remarkable drop of *S. Brandenburg* (322 in 2000 vs 16 in 2012 and 20 in 2013) cases was noted over the last years. Similarly, the number of *S. Derby* cases is shrinking since the beginning of 2000.

**Table 24.** Trends for the most prevalent *Salmonella* serotypes from 1995 to 2013

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
<b>Total</b>	10754	12008	14239	14514	15774	14088	11065	10075	12792	9543	4916	3693	3975	3944	3208	3660	3231	3170	2874
Enteritidis	5138	6145	8284	9003	10492	9503	7112	6398	9118	6075	2226	1052	987	824	587	823	481	663	575
Typhimurium	3623	3522	3347	3221	3348	2799	2370	2438	2486	2459	1659	1826	2233	2279	1862	1969	2030	1703	1556
Autres	1226	1564	1778	1559	1262	1028	956	793	818	684	765	633	596	685	668	744	627	703	623
Derby	107	118	157	162	138	169	158	92	100	64	67	52	64	44	42	25	31	34	36
Brandenburg	241	214	296	274	279	322	200	148	66	63	76	47	29	36	8	16	16	16	20
Virchow	245	178	114	115	86	147	143	132	152	91	65	46	28	29	18	24	14	13	20
Infantis	174	267	263	180	169	120	126	74	52	107	58	37	38	47	23	59	32	38	44



**Figure 41.** Trend of the human *Salmonella* isolates and of the two major serotypes Enteritidis and Typhimurium over the last thirty one 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 (21,8% of cases), with no significant gender difference.

**Table 25.** Human cases of *Salmonella*: Age and gender distribution 2011. Note that the gender of all salmonellosis cases is not known.

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

2013	Salmonella				Salmonella Enteritidis				Salmonella Typhimurium			
	Total	M	F	SR	Total	M	F	SR	Total	M	F	SR
< 1 yrs	204	93	111	0,8	34	18	16	1,1	73	30	43	0,7
1 - 4 yrs	900	456	444	1,0	173	98	75	1,3	507	243	264	0,9
5 - 14 yrs	629	316	313	1,0	122	70	52	1,3	350	171	179	1,0
15 - 24 yrs	190	82	108	0,8	36	13	23	0,6	69	35	34	1,0
25 - 44 yrs	237	125	112	1,1	68	35	33	1,1	55	27	28	1,0
44 - 64 yrs	258	130	128	1,0	58	29	29	1,0	83	42	41	1,0
>= 65 yrs	294	137	157	0,9	59	29	30	1,0	108	52	56	0,9
Unknown	21	12	9	1,3	3	2	1	2,0	9	6	3	2,0
<b>Total</b>	<b>2733</b>	<b>1351</b>	<b>1382</b>	<b>1,0</b>	<b>553</b>	<b>294</b>	<b>259</b>	<b>1,1</b>	<b>1254</b>	<b>606</b>	<b>648</b>	<b>0,9</b>

Regarding the seasonal distribution (Fig. 42), about 200 to 300 cases were monthly reported between July and September 2013. From October to December, the monthly number of isolates gradually decreased.

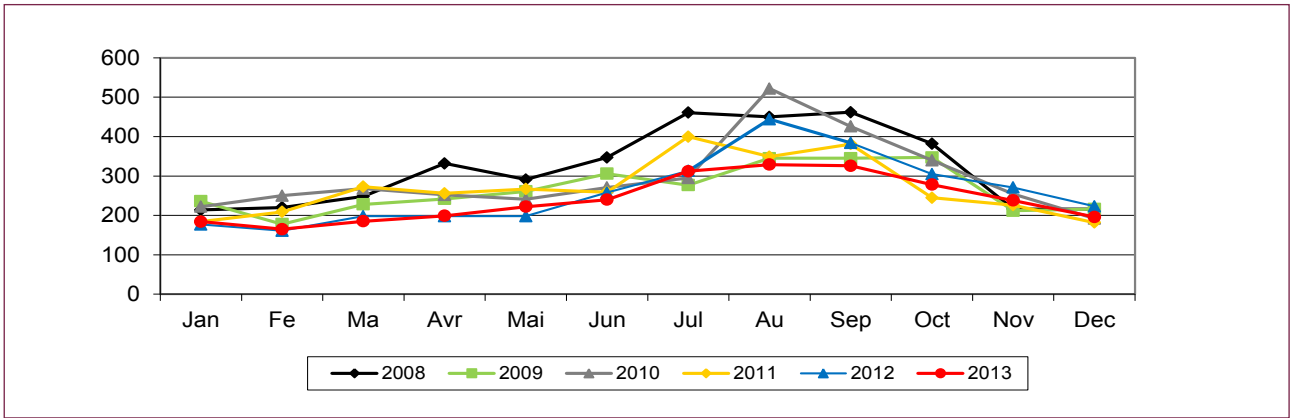


Figure 42. Seasonal distribution 2008–2013.

# Tuberculosis

Sophie Bertrand, David Fretin, Annick Linden, Vanessa Matthys, Luc Vanholme, Maryse Wanlin, Wouter Arrazola de Onate

## Zoonotic tuberculosis (*Mycobacterium bovis*)

Zoonotic tuberculosis in humans caused by *Mycobacterium bovis* is rare.

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

In developing countries, where *M. bovis* is largely prevalent among cattle, some studies reported that 3-6% of all human 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.

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 surveillance programme implies:

- Skin testing of animals at purchase (mandatory);
- Repeated skin testing of animals born or originating from a non-official tuberculosis free Member State or region or from a third country for 3 consecutive years during winter;
- Annual skin testing of all female animals on dairy herds with a direct sale to the consumer of raw milk or raw milk products;
- 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) or animals of contact herds;
- Follow-up tuberculin testing of breakdown herds for 5 consecutive years during winter;
- Systematic post mortem examinations at the slaughterhouse. In case a suspected lesion is identified, a sample is sent to the NRL for analysis

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

The FASFC is informed about any doubtful or positive result of the intradermal tuberculin skin testing and may decide to re-examine (additional tests e.g. comparative intradermal tuberculin test, interferon-gamma test for test & slaughter strategy) the animals or to kill them (slaughter & test strategy, with additional confirmatory tests e.g. culture and PCR). If *M. bovis* is isolated as a consequence of post mortem examinations or of mandatory slaughter & test strategy, 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.

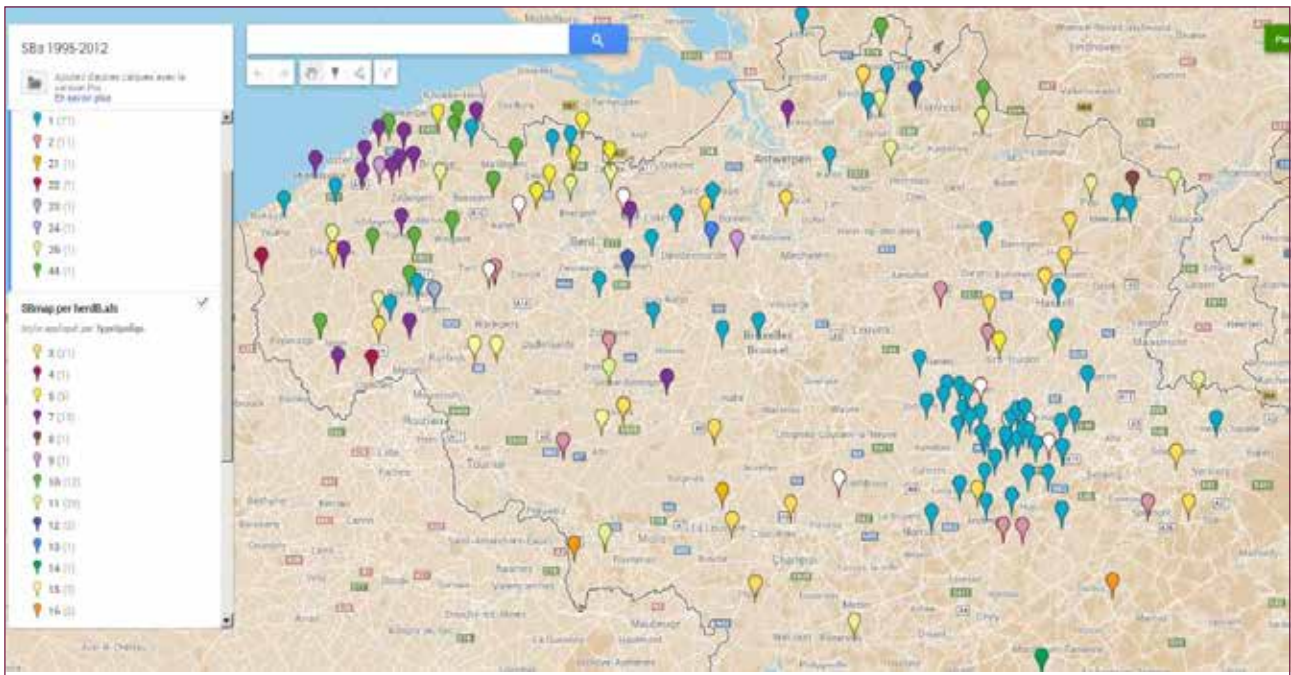
Isolation of *M. bovis* and biochemical testing is exclusively performed in the NRL where also IFN-gamma, PCR and molecular typing by means of IS6110 RFLP, spoligotyping or more recently MIRU-VNTR are done to support the epidemiological investigations and to prove eventually the link between different cases.

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 WIV-ISP. As a consequence, a comprehensive database of the vast majority of *M. bovis* types isolated in Belgium since 1995 is maintained. Analysis of molecular profiles of all isolates obtained from Belgian TB breakdowns allowed the identification of a predominant spoligotype (SB0162).

In Belgium, vaccination against tuberculosis is prohibited.

Next map (Figure 43) shows the spatial distribution of *M. bovis* spoligotypes from bTB breakdown herds in the period 1995 – 2012 (Research project Dynatub).



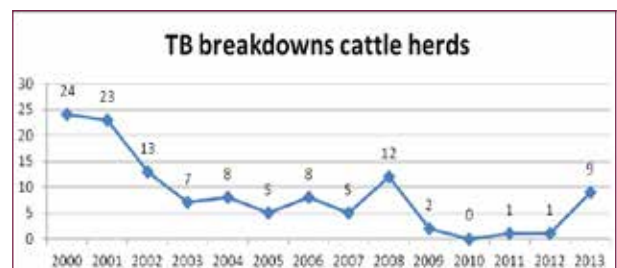
**Figure 43.** Spatial distribution of *M. bovis* spoligotypes from bTB breakdown herds in the period 1995 – 2012

### Epidemiological investigations and results of 2010-201 surveillance

The evolution of tuberculosis outbreaks in Belgian cattle herds over the last years is indicated in table 26 and figure 44.

**Table 26.** Evolution of bovine tuberculosis outbreaks in cattle herds in Belgium, period 2000-2013

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
TB Break-downs	24	23	13	7	8	5	8	5	12	2	0	1	1	9



**Figure 44.** Evolution of bovine tuberculosis breakdowns in cattle herds in Belgium, period 2000 – 2013

In December 2012 a bovine TB breakdown herd was detected after post-mortem examination at a slaughterhouse in the Netherlands of a bovine of Belgian origin and already staying for some months in a Dutch herd. Tuberculin testing at the Belgian herd of origin resulted in 95 positive and 34 doubtful results on a total of 190 bovines. By stamping-out of all animals, 53 out of 191 slaughtered animals had clearly bTB suspected lesions by post-mortem examination. Finally, after epidemiological investigations, all animals of 135 contact herds had to be followed-up by tuberculin testing.

The next photos are showing some results of the comparative tuberculin testing taken at this breakdown herd: photo 1 exsudation at the injection site of avian and bovine tuberculin, photo 2: extremely enlarged dermal nodule at the bovine tuberculin injection site, photo 3: necrosis (black points) of the skin at both injection sites of avian and bovine tuberculin.



In 2013, 9 bovine TB breakdown herds were detected: 3 by post mortem examination at Belgian slaughterhouses and 6 by an epidemiological link or contact with another breakdown herd. On these 9 breakdown herds respectively 149, 214, 263, 223, 411, 164, 250, 25 and 225 animals were present. All breakdown herds were totally stamped out with the exception of the 4th and the 6th breakdown herd were only partial slaughter of reactor animals to the tuberculin testing took place. Due to this bTB breakdowns respectively 185, 180, 79, 30, 20, 90, 270, 78 and 80 contact herds had to be followed-up by tuberculin testing.

In 2012 and 2013, respectively 272.160 and 183.903 bovines had a routine follow-up tuberculin testing due to breakdowns and 322.883 and 302.630 bovines had a tuberculin test carried out after the introduction into new herds.

The next photos 4 to 7 show some typical bTB lesions of a young animal (female, 10 months, dairy) with generalized bTB of one of the breakdown herds of 2013 by post-mortem examination at the slaughterhouse.



*Photo 4 : incised enlarged necrotic caseous bronchial lymph node*



*Photo 5: tubercles on the lung surface*

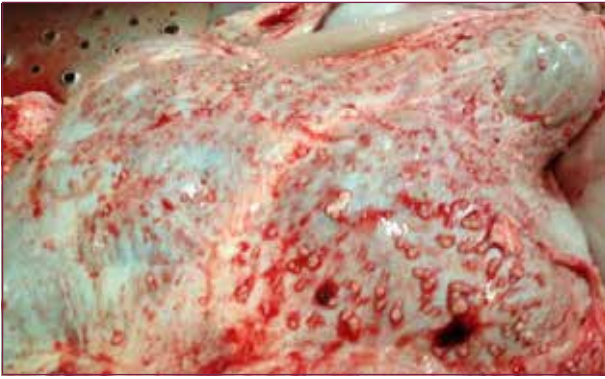


Photo 6 : tubercles on the rumen



Photo 7 : tubercles on diaphragm and pleura

### Mycobacterium bovis in wildlife

Between 2000 – 2013, all suspected lesions of wildlife were sent to the NRL for analysis of tuberculosis. This passive monitoring never detected a positive case of *M. bovis* by culture in wildlife until now. Only environmental Mycobacteria (non tuberculous e.g. *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *hominisuis* or co-infections) were detected in wildlife from time to time.

In 2012 and 2013, there is a targeted monitoring in wildlife of the surrounding areas of the bovine TB breakdown herds as this is also done in case of an outbreak of bovine brucellosis. The aim is an early detection of any presence of bovine TB in receptive wildlife species in Belgium as this is the case in some neighbouring countries. The species especially taken into consideration are deer, wildboar and badgers. During the period 2009 - 2012 deer (50), 53 wildboar and 77 badgers have been analysed by coloration, PCR (direct on organs or suspected lesions) and by culture. Bovine TB was not detected in these wildlife species.

### Mycobacterium bovis in humans

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

In 2012, 3 human cases of bovine tuberculosis were identified and confirmed by culture followed by molecular identification in the NRL, Scientific Institute of Public Health (WIV-ISP). Among the 3 patients, 1 coming from urine, 1 coming from matter and 1 lymphadenopathy were observed.

In 2013, 14 human cases of bovine tuberculosis were identified and confirmed by culture followed by molecular identification in the NRL. Among the 14 patients, 2 patients showed a pulmonary form of the disease, and 6 patients had lymphadenopathy.

All the bovis strains were genotyped by the spoligotyping technique.

Zoonosis due to *M. bovis* occurs infrequently in Belgium and was more often detected in patients from North African origin. Noteworthy, the number of cases in the older agegroup of the Belgian population slightly increased over the last years. This could be a reactivation of a previous disease. This justifies a special attention to the identification of the species *M. bovis* within the *Mycobacterium tuberculosis* complex, which is not possible with the most common molecular techniques used in the laboratories to identify positive cultures of mycobacteria. *M. bovis* being resistant to pyrazinamid. This first-line drug must not be included in the treatment of zoonosis.

## Human tuberculosis (Mycobacterium tuberculosis)

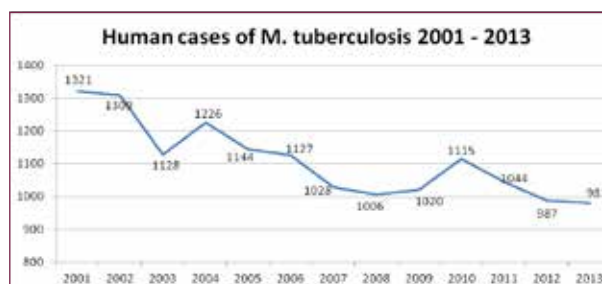
Mycobacterium tuberculosis is responsible for human tuberculosis but can also infect all kind of animal species. The reporting of a human tuberculosis-case is mandatory. The surveillance and follow-up of cases is organised by the regional public health authorities. Regional databases are combined in a national tuberculosis registry. Additional information can be found on VRGT/FARES website <http://www.vrgt.be/tuberculose>

The incidence of human tuberculosis shows little variation over the last years. From 2001 to 2013 respectively 1321, 1309, 1128, 1226, 1144, 1127, 1028, 1006, 1020, 1115, 1044, 987 and 981 new cases of active human tuberculosis were detected (Figure 45). The incidence was 8.9/100 000 in 2012 and 8,8 / 100.000 in 2013. This is lower than the incidence of 2011 9,5/100.000 and is actually the lowest incidence ever registered. (The regional incidences in 2013 were respectively 6.6/100.000 for Flanders, 7.2/100.000 for Wallonia and 26,2/100.000 for Brussels. As in previous years and over different countries the highest incidences are registered in big cities (Brussels, Antwerp, Liège, Charleroi). Poor living conditions, poverty and migration are known risk factors for tuberculosis. Two out of three cases reported in 2013 had no preceding occurrences (antecedents). For one in four it is unknown whether or not there were previous antecedents. Relevant risk factors for developing active disease after infection through exposure are conditions with an impaired immune system: HIV, diabetes, alcoholism, IV-drug use . . . . Poverty and stress impair the immune system as well.

The median age of the patients was 37 (37 as well in 2013) years. Almost two out of three patients is male (64,6 %). The incidence is higher among residents that do not have the Belgian nationality (43 /100 000) compared to Belgian citizens (4.7/100 000). Belgian patients differ from non-Belgian patients in age distribution. Belgian patients, show a linear increase over age, while for patients with another nationality the maximum incidence is in the age-group from 15-29 years.

Almost 70% (69,5 %) of patients suffer from the pulmonary form of tbc. This is important since this is the possible contagious form. The presence of multidrug resistant forms (resistant to isoniazide and rifampicine) is stable. In 2013, it was present in 1,8 % of cases for whom an antibiogram was available.

After one year of therapy the following conclusions could be drawn (so for cases reported in 2012 ), 77,7 % were cured after treatment, 11,9 % ended the treatment prematurely, multi-cause mortality remains high at 10,2 %.



**Figure 45.** Number of patients with human tuberculosis between 2001–2013 (source VRGT-FARES)

# Yersiniosis

Vera Cantaert, Michel Delmée, Lieven De Zutter, Luc Vanholme, Julie Wits

## Yersinia enterocolitica

The genus *Yersinia* comprises three main species that are known to cause human infections: *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* (plague which is believed to no longer exist in Europe). Specific types of *Y. enterocolitica* and *Y. pseudotuberculosis* cause food-borne infections. Since most human infections are caused by *Y. enterocolitica*, this chapter deals mainly with *Y. enterocolitica* infections.

Only certain biotypes of *Y. enterocolitica* strains 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 the clinical picture may be confused with appendicitis. In a small proportion of cases, complications such as skin rash, joint pains, and/or bacteraemia may occur.

Pigs are the primary reservoir for the human pathogenic *Y. enterocolitica* types. In infected pigs the bacteria are most likely to be found in the tonsils.

However other animal species, e.g. cattle, sheep, deer, small rodents, cats and dogs may also carry pathogenic biotypes. Clinical disease in animals is uncommon.

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

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

Infection caused by *Y. pseudotuberculosis* shows many similarities with the disease pattern of *Y. enterocolitica*. Infections are mainly caused by the ingestion of the bacteria present in raw vegetables.

## Yersinia enterocolitica in food

The FASFC organised a monitoring of meat since 1997, which showed a very low prevalence of *Yersinia enterocolitica* in pork, beef and poultry. In 2012 and 2013, monitoring of *Yersinia enterocolitica* was concentrated on pig carcasses and meat products of pork and beef.

**Table 27.** Monitoring *Yersinia enterocolitica* in pork carcasses, and meat products of pork and beef

Sample	Quantity analysed	Percentage of positive samples 2009	Percentage of positive samples 2010	Percentage of positive samples 2011	Percentage of positive samples 2012	Percentage of positive samples 2013
Pig carcasses at slaughter	600 cm <sup>2</sup>	-	-	2.3% (n=384)	0.0% (n=56)	13.0% (n=366)
Minced meat (containing pork) intended to be eaten raw at retail	1g	0.0% (n=184)	0.0% (n=55)	0.0% (n=44)	0.0% (n=13)	0.0% (n=10)
Minced meat (containing pork) intended to be eaten cooked at retail	1g	0.0% (n=179)	0.0% (n=55)	0.0% (n=43)	0.0% (n=33)	0.0% (n=25)
Minced meat (beef) intended to be eaten raw at retail	1g				0.0% (n=33)	0.0% (n=35)
Minced meat (beef) intended to be eaten cooked at retail	1g				0.0% (n=12)	0.0% (n=21)
Minced meat (beef) at processing	1g					0.0% (n=10)
Minced meat (containing pork) at processing	1g					0.0% (n=40)

## Yersiniosis in humans

In 2012, the Sentinel Laboratory Network registered 257 cases, corresponding to a national incidence estimated at 2,3 per 100,000 inhabitants.

In 2013, the Sentinel Laboratory Network registered 347 cases, corresponding to a national incidence estimated at 3,1 per 100,000 inhabitants.

Since 1986, when 1514 cases were reported by this network, the number of human infections in Belgium significantly decreased, in recent years however a new increase is observed (Figure 46).

Cases were observed all over the year (Figure 47).

Twenty-one percent to 27% of cases were children up to 4 years old (Table 29 and 30). In 2012, 54% of cases were male, 46% were female. In 2013, 48% were female and 46% were male (6% had an unknown gender).

As already reported in former years, the incidence in Flanders is higher than in Wallonia. Geographical distribution of cases is partly due to the location of the sentinel labs. (Figure 48 and 49).

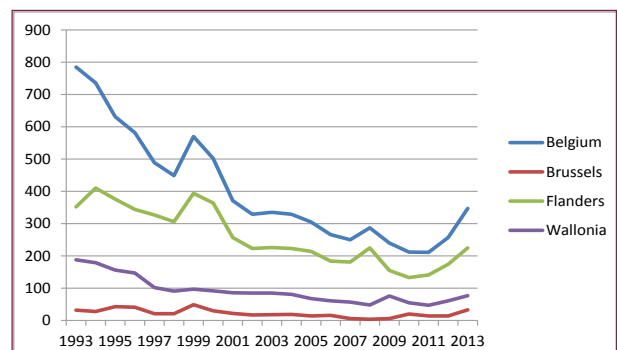
Bio-serotyping was performed by the National Reference Laboratories (KUL and UCL).

**Table 28.** Number of *Yersinia enterocolitica* infections in humans by sex and by age groups, 2010. Source: Sentinel Laboratory Network

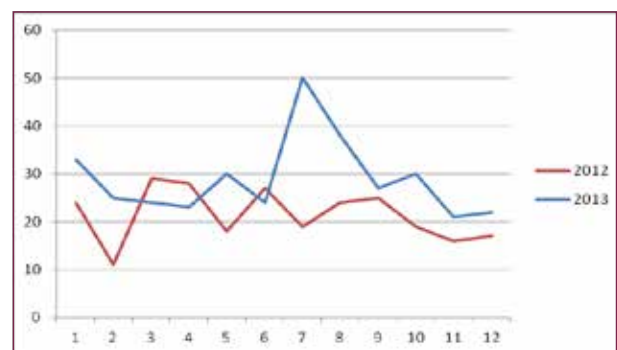
AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	44	31.88	28	23.73	72	28.00
05-09	14	10.14	21	17.8	35	14.00
10-14	7	5.07	12	10.17	19	7.00
15-19	4	2.9	8	6.78	12	5.00
20-24	9	6.52	3	2.54	12	5.00
25-29	1	0.72	1	0.85	2	1.00
30-34	4	2.9	4	3.39	8	3.00
35-39	6	4.35	2	1.69	8	3.00
40-44	3	2.17	6	5.08	9	4.00
45-49	3	2.17	6	5.08	9	4.00
50-54	5	3.62	4	3.39	9	4.00
55-59	6	4.35	3	2.54	9	4.00
60-64	7	5.07	4	3.39	11	4.00
65-++	22	15.94	13	11.02	35	14.00

**Table 29.** Number of *Yersinia enterocolitica* infections in humans by sex and by age groups, 2013. Source: Sentinel Laboratory Network

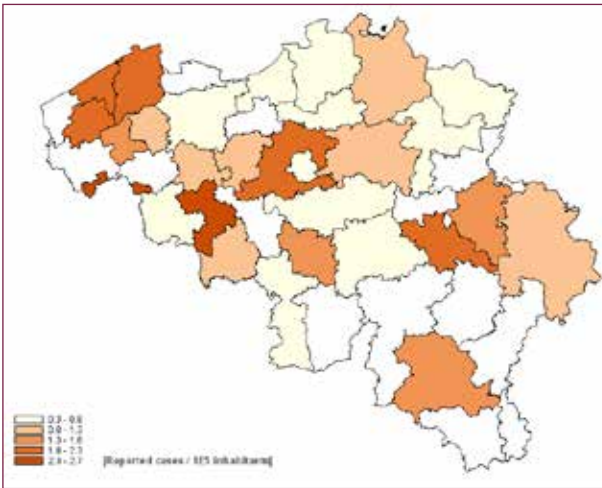
AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	43	24.86	50	30.12	93	27.00
05-09	26	15.03	20	12.05	46	13.00
10-14	15	8.67	25	15.06	40	14.00
15-19	10	5.78	8	4.82	18	5.00
20-24	11	6.36	6	3.61	17	5.00
25-29	9	5.2	3	1.81	12	3.00
30-34	3	1.73	10	6.02	13	4.00
35-39	5	2.89	3	1.81	8	3.00
40-44	8	4.62	1	0.6	9	3.00
45-49	3	1.73	3	1.81	6	3.00
50-54	6	3.47	4	2.41	10	3.00
55-59	6	3.47	11	6.63	17	5.00
60-64	5	2.89	2	1.2	7	2.00
65-++	19	10.98	19	11.45	38	11.00



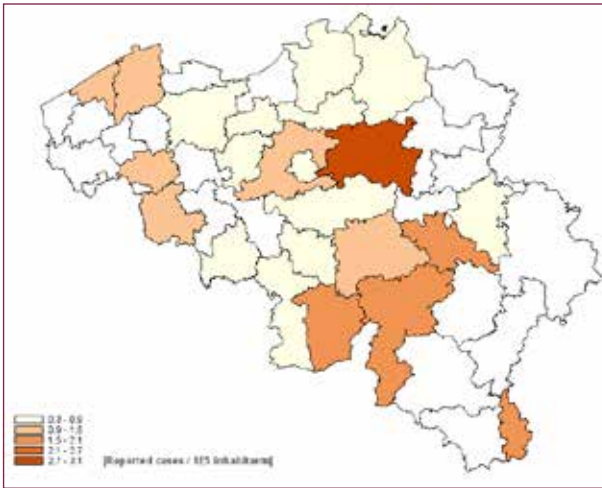
**Figure 46.** Total number of *Yersinia enterocolitica* infections in humans by year (1986-2013). Source: Sentinel Laboratory Network



**Figure 47.** Weekly number of *Yersinia enterocolitica* infections in humans, 2012-2013. Source: Sentinel Laboratory Network



**Figure 48.** Incidence of *Yersinia enterocolitica* infections in humans by district (N/105 inhab., 2012). Source: Sentinel Laboratory Network



**Figure 49.** Incidence of *Yersinia enterocolitica* infections in humans by district (N/105 inhab., 2013). Source: Sentinel Laboratory Network

# Viral diseases

## Hantaviruses

Toon Braeye, Paul Heyman

### Hanta disease

Wild (or laboratory) rodents are the reservoir for hantaviruses worldwide; humans are accidental hosts. In the last decade also insectivores and bats were found to carry hantaviruses but so far their pathogenicity to humans was not confirmed. The infection is chronic and apparently asymptomatic in host animals. A hantavirus serotype is hosted by a specific rodent, insectivore or bat species. According to the infectious agent and its region, hanta-viral diseases in humans present, with different level of severity, as mild infections to severe hemorrhagic fever with renal syndrome (HFRS) depending on the causal serotype. HFRS shows as an acute onset of fever, lower back pain, hemorrhagic manifestations and renal involvement. Hantavirus pulmonary syndrome (HPS)- predominantly occurring in the Americas- 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.

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

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

### Hantaviruses in animals

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

No official monitoring programme was realized during the reporting period.

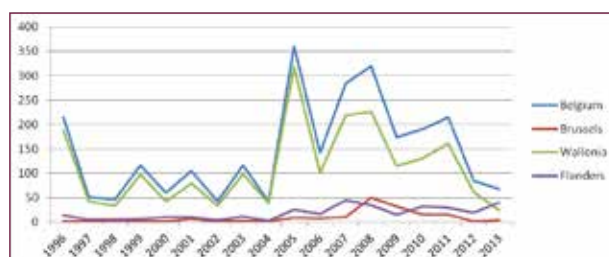
Scientific research programs to monitor the seroprevalence of various animal species are still running.

### Hantaviruses in humans

In 2012, the Sentinel Laboratory Network and the NRL reported 82 cases of hantavirus infection. In 2013, 68 cases of hantavirus were reported. This report indicates a further decrease since the peak in 2010/2011. Recently a high peak was observed in 2005 (N=372). 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-summers of 1996, 1999, 2001, 2003, and specially 2005, 2008 and 2011. The years between 2005 and 2011 were epidemic years (above the threshold of 150 cases/year) (Table 30 & Figure 50).

**Table 30.** Total number of Hantavirus infections in humans by year (1996-2013)

Year Diagnosis	brussels		flanders		wallonia	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
1996	2	1,18	14	4,09	188	9,31
1997	1	0,59	6	1,75	43	2,13
1998	3	1,78	5	1,46	34	1,68
1999	2	1,18	7	2,05	97	4,8
2000	2	1,18	10	2,92	43	2,13
2001	7	4,14	10	2,92	79	3,91
2002	2	1,18	4	1,17	33	1,63
2003	3	1,78	12	3,51	99	4,9
2004	2	1,18	3	0,88	38	1,88
2005	9	5,33	26	7,6	318	15,75
2006	8	4,73	17	4,97	101	5
2007	10	5,92	45	13,16	219	10,85
2008	50	29,59	35	10,23	227	11,24
2009	32	18,93	16	4,68	115	5,7
2010	16	9,47	32	9,36	130	6,44
2011	15	8,88	30	8,77	161	7,97
2012	1	0,59	20	5,85	61	3,02
2013	4	2,37	40	11,7	24	1,19



**Figure 50.** Distribution of Hantavirus infections in humans (months), 1996–2013. Sources: Sentinel Laboratory Network and NRL

Generally, the majority of cases are male adults over 19 years. In 2012, there were 74% males and 26% females (Table 31).

In 2013, there were 51% males and 49% females (Table 32).

**Table 31.** Number of cases of Hantavirus infections in humans by sex and age groups, 2012. Sources: Sentinel Laboratory Network and NRL

AgeGroup	female		male		total	
	Fre- quency	Percent	Fre- quency	Percent	Fre- quency	Percent
00-04	0	0	0	0	0	0,00
05-09	0	0	0	0	0	0,00
10-14	0	0	0	0	0	0,00
15-19	1	4,55	2	3,17	3	4,00
20-24	3	13,64	3	4,76	6	7,00
25-29	2	9,09	4	6,35	6	7,00
30-34	4	18,18	9	14,29	13	15,00
35-39	1	4,55	7	11,11	8	9,00
40-44	0	0	7	11,11	7	8,00
45-49	4	18,18	2	3,17	6	7,00
50-54	1	4,55	12	19,05	13	15,00
55-59	2	9,09	8	12,7	10	12,00
60-64	1	4,55	2	3,17	3	4,00
65-++	3	13,64	7	11,11	10	12,00

**Table 32.** Number of cases of Hantavirus infections in humans by sex and age groups, 2013. Sources: Sentinel Laboratory Network and NRL

AgeGroup	female		male		total	
	Fre- quency	Percent	Fre- quency	Percent	Fre- quency	Percent
00-04	0	0	0	0	0	0.00
05-09	0	0	2	6.06	2	3.00
10-14	0	0	2	6.06	2	3.00
15-19	2	5.71	1	3.03	3	4.00
20-24	0	0	3	9.09	3	4.00
25-29	3	8.57	2	6.06	5	7.00
30-34	2	5.71	3	9.09	5	7.00
35-39	2	5.71	0	0	2	3.00
40-44	1	2.86	6	18.18	7	10.00
45-49	1	2.86	2	6.06	3	4.00
50-54	5	14.29	4	12.12	9	13.00
55-59	3	8.57	1	3.03	4	6.00
60-64	3	8.57	1	3.03	4	6.00
65-++	5	14.29	5	15.15	10	15.00

Over the period 2012-2013, the highest incidence rate is reported in the district of Neufchâteau (21.5/105 inhabitants, 13 cases) and in Dinant (13.1/105 inhabitants, 14 cases). The same geographical spread has been observed in previous years. This is related to the distribution of the rodent population.

Part of the increase observed since 2004 could be due to a greater awareness among health professionals and to higher hantavirus testing. However, under-diagnosing of hantavirus infections remains a problem. Changes in the surveillance system (since the end of 2011 changes were made to the NRC system) make it harder to interpret the reported numbers.

More information on preventive measures to minimize the risk of getting infected by hantavirus can be found by the link <http://www.iph.fgov.be/epidemie/labore> on the informative folders section.



**Figure 51.** Incidence of Hantavirus infections in humans by district (N/105 inhab., 2012-2013). Source: Sentinel Laboratory Network

# Hepatitis E Virus

Annick Linden, Damien Thiry, Steven Van Gucht, Magali Wautier

## Introduction

Hepatitis E is a disease caused by the hepatitis E virus (HEV), a member of the genus *Hepevirus*, Familia *Hepeviridae*.

HEV is one of the most important causes of acute hepatitis in adults in developing countries. Contrary to the old dogma, however, the virus is not restricted to developing countries, and sporadic cases are increasingly recognized in Europe.

Thus far, four HEV genotypes that infect humans are recognized. Genotype 1 is found in Asia and Africa, genotype 2 in Mexico, genotype 3 in Europe and the United States and Genotype 4 in Asia. Genotype 1 and 2 infections have been identified exclusively in humans, whereas genotype 3 and 4 viruses have been isolated from swine, deer, mongoose, cattle and rabbits, in addition to humans. Genotype 3 and 4 might be well distributed among swine and raw or undercooked pork may be a source of zoonotic infections.

From its discovery in 1983, documented HEV transmission was linked almost exclusively to contaminated water, with typically large outbreaks in developing countries (genotype 1). In Western countries, genotype 1 infections are typically imported after traveling to endemic regions. That association changed abruptly with the discovery of sporadic, non travel-related, sporadic hepatitis E cases in Europe (genotype 3). Often these sporadic genotype 3 cases were associated with consumption of uncooked deer or pork meat. HEV transmission by blood transfusion has also been described. The seroprevalence in blood donors in developed countries ranges from 1 to 3%. In the USA, a higher prevalence has been seen in persons working with swine. There are more and more indications that genotype 3 HEV is an emerging food-borne infection in Western countries.

In developing countries, genotype 1 causes an oro-faecally transmitted, water-borne, acute and self-limiting hepatitis that does not progress to a chronic disease. The infection can however be lethal in pregnant women.

In Europe, infections with genotype 3 usually cause mild hepatitis, but chronic cases are also documented, for instance in immune compromised solid-organ transplants recipients and HIV-infected individuals. The first chronic case was reported in Belgium in 2012. Genotype 3 infection might cause serious complications in patients with underlying chronic liver disease.

## Hepatitis E Virus in animals

### Domestic pig (*Sus scrofa*)

A study performed on 420 pigs, sampled throughout Belgium in 2010, showed an individual seroprevalence of 73% ( $\pm 4$ ; 308/420). The individual seroprevalence was significantly different between the two regions ( $\text{Chi}^2 = 4.83$ ; 1 degree of freedom;  $P = 0.03$ ): 66% ( $\pm 8$ ; 79/120) in the Walloon Region and 76% ( $\pm 5$ ; 229/300) in the Flemish Region. Moreover, 93% of the tested herds were found to contain at least one seropositive pig. Serological assays cannot discriminate between different genotypes.

Four out of 420 pig sera were detected positive for HEV RNA by conventional nested RT-PCR. These 4 sera came from pigs aged between 3 and 4 months. All sequences from the 4 positive sera belonged to genotype 3, subtype f.

These results are in agreement with the major findings in other European countries, except for one genotype 4 virus found in Belgium. The relative low detection of HEV RNA (4 out of 420 pig sera) can be explained by the age of the sampled pigs, which were less than 6 months, but probably older than 2 to 4 months, when the peak of viraemia is usually observed.

### Wild boar (*Sus scrofa scrofa*)

An apparent seroprevalence of 33% ( $\pm 4.6$ ; 125/383) was obtained on a sample of 383 wild boar sera in 2010 in Wallonia. Five out of 61 livers and 4 out of 69 sera of young wild boars were detected viropositive and the sequences obtained belonged to genotype 3, subtype f. These data are in agreement with the situation observed in other European countries.

The link between HEV infection in pigs, wild boars and humans need to be further analyzed to support the hypothesis of a zoonotic transmission in Belgium (project SPF HEVEA).

### Red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*)

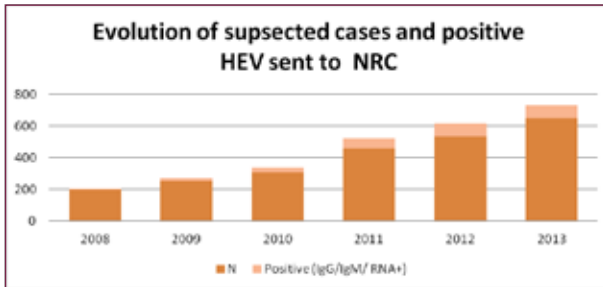
A sampling of the deer population was conducted in Wallonia during the 2012 hunting season: 189 sera from roe deer and 235 sera from red deer were used for serology; 84 sera from red deer and 68 sera from roe deer, aged of more than one year, were used for virology.

An apparent seroprevalence of 1% ( $\pm 1.4$ , 2/189) and 3% ( $\pm 2.2$ , 7/235) was obtained in red deer and roe deer respectively. Both positive red deer were between 1 and 2 years old and among HEV-positive roe deer, there were 3 less than 1 year and 4 over 1 year old. No serum was viropositive.

In contradiction to the prevalence detected in wild boars (33%) ( $\pm 4.6$ , 125/383) and pigs (66%) ( $\pm 8.4$ , 79/120) in the same region (Wallonia), deer do not seem to represent an epidemiologically significant reservoir host.

## Hepatitis E Virus in humans

Since 2008, the National Reference Centre for Hepatitis Viruses of the WIV-ISP, receives samples for HEV diagnosis. The number of suspected and confirmed cases increases year by year.



**Figure 52.** Evolution of suspected cases and laboratory-confirmed HEV cases analyzed at the National Reference Centre of Hepatitis Viruses of the WIV-ISP.

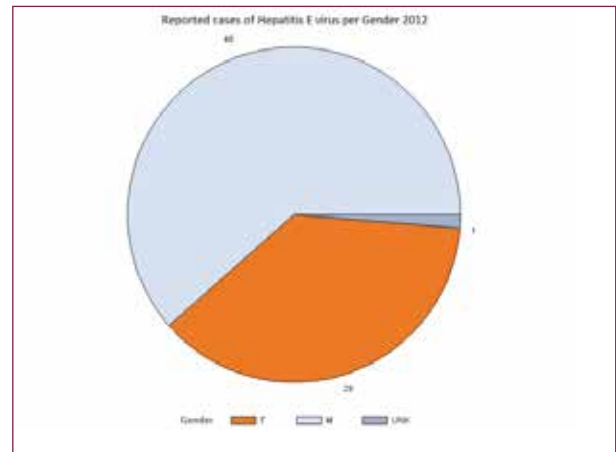
In 2012, the WIV-ISP received 614 suspected cases. Thirteen percent were IgG-positive. The seroprevalence was higher in men than in women, and in people older than 65 years. 32 cases with recent infection (presence of IgM and/or RNA in the serum) were detected. Twelve virus-positive samples were genotyped: 2 belonged to genotype 1 (imported cases) and 10 belonged to genotype 3 and probably represented locally acquired cases.

In 2013, 731 samples were tested for hepatitis E. 11.2% tested positive for IgG. Men were again more seropositive than women, and the average age of cases was between 50 and 54 years old. 35 recent infection cases (IgM and/or RNA-positive) were observed. The virus of 16 cases could be genotyped: all belonged to genotype 3.

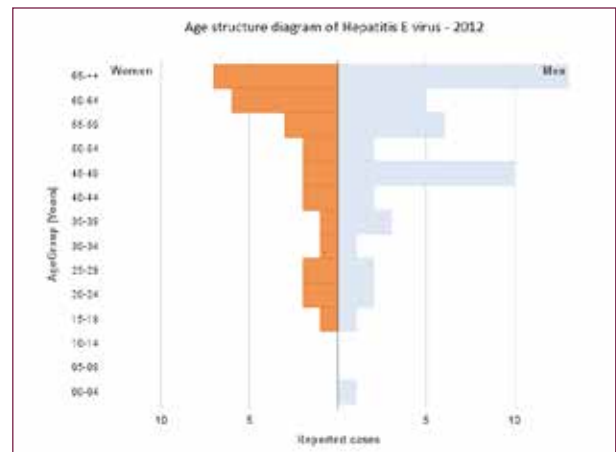
The phylogenetic analysis showed that most of the virus-positive samples of 2012-2013 belonged to genotype 3, with a predominance of subtype 3f and 3c, which are typical for European swine strains.

In Belgium, most clinical hepatitis E cases in humans are caused by genotype 3 strains, which are closely related to European swine strains. Our data are therefore indicative of zoonotic transmission from swine.

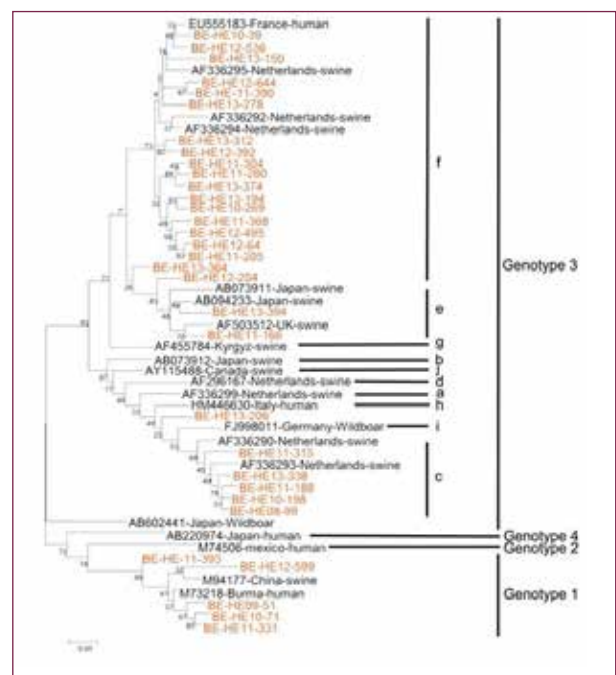
**Figure 55.** Consensus phylogenetic tree of ORF2 sequences of selected viruses (red color) typed in Belgium during 2008-2013. Most Belgian strains from human patients belong to genotype 3 and are closely related to European swine strains.



**Figure 53.** reported cases (IgG and/or IgM and/or RNA positive) of Hepatitis E virus per gender for 2012



**Figure 54.** reported cases (IgG and/or IgM and/or RNA positive) of Hepatitis E virus per gender and age for 2012



# Rabies

Bernard Brochier, Vanessa Suin, Steven Van Gucht, Raymond Vanhoof

## Rabies

Rabies is a zoonotic viral disease caused by a rhabdovirus of the genus *Lyssavirus*. The animal reservoirs are carnivores (typically foxes or dogs) 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:

- Rabies virus (RABV), worldwide spread
- Lagos bat virus (LBV), Africa
- Mokola virus (MOKV), Africa
- Duvenhage virus (DUVV), Africa
- European bat lyssavirus 1 (EBLV-1), Europe
- European bat lyssavirus 2 (EBLV-2), Europe
- Australian bat lyssavirus (ABLV), Australia
- Aravan virus (ARAV)
- Khujand virus (KHUV)
- Irkut virus (IRKV)
- Shimoni virus (SHIBV)
- West Caucasian bat virus (WCBV)

Classic rabies virus (RABV) 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. 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 European 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 lyssaviruses (EBLV-1 and EBLV-2), that are related to the Classical rabies virus. Some, but not all, 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 direct exposure to bats is highly recommended. Education and recommendations should be given to bat handlers 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 neglected.

### Officially-free rabies status.

In July 2001, Belgium obtained the official status of rabies-free country according to the OIE guidelines and the WHO recommendations. The last indigenously acquired case of rabies occurred in Belgium in a bovine in July 1999. Unfortunately, the official rabies-free status of Belgium was suspended at the end of October 2007 due to the detection of a rabid dog illegally imported from Morocco. The clinical diagnosis was confirmed by laboratory testing after euthanasia of the animal. Finally 32 persons and 18 pet owners with possible contact with the rabid animal were detected. Medical information and follow-up by experts of the WIV-ISP and Flemish Community of all contact persons was realised. Belgium regained its official rabies-free status on 28 October 2008. In April 2008, another case of rabies in a dog was diagnosed. The dog was imported from Gambia, stayed for 1 week in Brussels and was then moved to France. Symptoms only developed shortly after arrival in France, but most likely the dog was already infectious during its temporary stay in Belgium.

No indigenous cases of human rabies have been reported since 1922, although imported cases are diagnosed from time to time.

## Rabies in animals

### Surveillance program and methods used.

Food animals with central nervous symptoms are suspect for rabies and therefore should be notified to the FASFC. Affected animals are killed and their brain is examined by immunofluorescence and RT-PCR 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 wildlife has a suspected behaviour or present specific lesions, brain samples are examined at the NRL.

### Vaccination policy.

Because of elimination of sylvatic rabies in 2001, vaccination of foxes by baits (started in 1989) was stopped by the end of 2003.

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

### Movements of companion animals

Regulation<sup>1</sup> on international movements of companion animals

The fox rabies epizootic, that swept through Europe in the 1960s, prompted some European countries that had remained free of the disease, including the United Kingdom and Sweden, to introduce a six-month quarantine system to prevent the introduction of rabies. In the early 2000s, the improved epidemiological status of the entire European Union (EU) following a programme of oral vaccination of foxes led these countries to gradually abandon the quarantine system in favour of effective and less burdensome alternative animal health measures (Pet Travel Scheme). The improved situation also led the EU to regulate the non-commercial international movement of companion animals to prevent any risk of rabies being reintroduced into Member States. This EU regulation has been fully harmonised since the 1st of January 2012, when the United Kingdom, Ireland, Malta and Sweden aligned their rabies regulation with that of the other Member States.

This regulation applies to international movements of dogs, cats and ferrets and depends on the epidemiological status of the countries of origin and destination.

### Non-commercial movements between European Union Member States

Before dogs, cats and ferrets can be moved within the EU, they must have microchip identification, rabies vaccination and a European model passport. Vaccination is valid either up to 21 days following the primary vaccination protocol established by the manufacturer in the country where the vaccine is administered, or immediately in the case of a booster vaccination if the period of validity of the previous vaccination has not expired. The period of validity is that indicated in the manufacturer's instructions of the vaccine.

### Non-commercial movements from non-European Union countries.

The regulation governing non-commercial movements between EU Member States also applies to certain non-EU countries with a favourable epidemiological status. Animals from these countries must be identified, vaccinated after the age of three months and hold an official health certificate or a passport in line with an EU passport.

For entry into the EU of a dog, cat or ferret from a non-EU country with an unfavourable epidemiological status (including most African and Asian countries), the regulatory measures have been strengthened with a mandatory pre-entry check on the efficacy of the rabies vaccination. A blood test for rabies antibody titration must be performed by an authorised veterinarian at least 30 days following the animal's vaccination and three months before it is moved to the EU Member State. This three-month waiting period following the blood test reduces the risk of importing an animal that is incubating the disease, as vaccination can prevent the infection, but can not eliminate incubating virus.

Rabies antibody titration is one of the two variants of a serum neutralisation test and must be performed by an EU-accredited laboratory. Neutralising antibody levels at or above 0.5 International Units per millilitre of serum are indicative of post-inoculation immunity.

In 2012 and 2013, the EU approved WIV-ISP Rabies National Laboratory tested respectively 1899 and 2100 pets blood samples for anti-rabies antibody titration.

<sup>1</sup> Regulation (EU) No 576/2013 of the European Parliament and of the Council of 12 June 2013 on the non-commercial movement of pet animals and repealing Regulation (EC) No 998/2003.

### Epidemiological investigations and the results of the 2012 - 2013 surveillance.

In 2012, a total of 517 brain samples were examined for rabies virus at the NRL in the frame of the passive rabies surveillance program. The samples originated from wildlife (n=156), especially foxes (n=48) and dead-found bats (n=108) and domesticated animals (n=374), especially cattle (n=178) and sheep and goats (n=114 and 54). All analyses were negative.

In 2013, a total of 360 brain samples were examined for rabies virus at the NRL. The samples originated from wildlife (n=39), especially foxes (n=11) and dead found bats (n=27) and domesticated animals (n=324), especially cattle (n=138) and sheep and goats (n=125 and 39). All analyses were negative.

The high number of cattle and small ruminants analysed is a consequence of the surveillance system for transmissible spongiform encephalopathy (TSE). For these species, all suspected TSE cases are first examined for rabies. Rabies must be considered in the differential diagnosis of TSE, although the course of the disease is usually shorter.

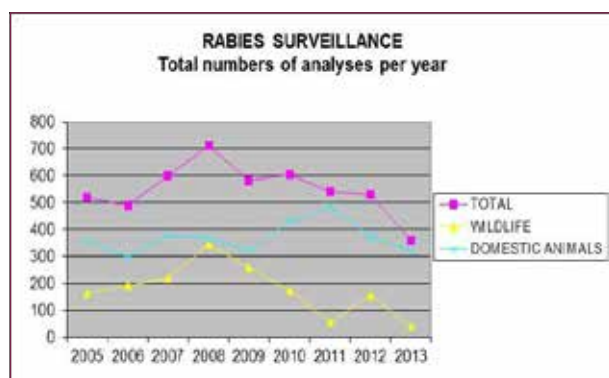


Figure 56. Total number of rabies analyses per year

The number of EBLV cases diagnosed in Europe seems to remain stable (Table 33). The number of requests for rabies prophylaxis upon close contact with a bat, received at the WIV-ISP, seems to have increased during the last years (Table 33). It is not clear whether this is due to an increased awareness of the possible risk of rabies in bats or due to an increase of contact incidents.

Table 33. Overview of the number of EBLV cases in bats in Europe (source: WHO Rabies Bulletin Europe) and Belgium (source: WIV-ISP, Belgium)

Number of EBLV-positive bats/total bats examined									
	2005	2006	2007	2008	2009	2010	2011	2012	2013
Europe	32/9416 (0,3%)	36/9398 (0,4%)	26/7933 (0,3%)	33/6226 (0,5%)	38/4125 (0,9%)	28/1803 (1,6%)	33/1484 (2,2%)	74/2184 (3%)	28/762 (3,6%)
Belgium	0/35	0/21	0/23	0/25	0/29	0/58	0/16	0/108	0/27

The next table 34 indicates the number of cases of bites or scratches from animals requiring post exposure prophylaxis and managed by the WIV-ISP, Belgium. Encounters with bats occurred mainly in Belgium, whereas most cases of suspicious bites from dogs, cats or monkeys were acquired abroad in travelers visiting rabies-endemic regions. Travelers were treated, or continued their treatment, upon return in Belgium.

Table 34. Number of cases of bites or scratches from bats or dogs requiring post exposure prophylaxis and managed by the WIV-ISP, Belgium.

Year	Number of persons requiring post exposure immunisation	Upon risk exposure to				
		Bats	Dogs	Monkeys	Cats	Other animals
2012	141	14	77	22	16	12
2013	230	23	114	61	23	9

# Tick-borne Encephalitis Virus

Bernard Brochier, Vanessa Suin, Steven Van Gucht

## Introduction

Tick-borne encephalitis virus (TBEV) is a highly pathogenic flavivirus and the most important arthropod-borne virus in Europe. Tick-borne encephalitis (TBE) has become a considerable public health risk in several European countries, with currently 3000 hospitalized cases per year. TBEV is transmitted by the most common European tick species, *I. ricinus* and occasionally also by unpasteurized ruminant milk. TBEV causes a neurotropic disease and clinical signs vary from mild fever to meningitis, encephalitis or myelitis. Patients who develop this syndrome require many years of treatment and rehabilitation due to long-term neurological symptoms. TBE can only be treated symptomatically but can easily be prevented by vaccination, which is strongly recommended for people of all ages who live or travel in endemic areas

The distribution of TBEV (3 subtypes) spans almost the entire Southern part of Eurasia, presently from Alsace-Lorraine/Southern Norway to North eastern China/Japan, and consists of up to 30,000 endemic foci. Worldwide, 10,000-12,000 cases of human TBE are reported annually, including more than 3,000 cases in Europe. European TBEV is endemic in 27 countries of which the Central European countries, the Baltic States and Russia are most severely affected. Recently, new endemic foci have appeared in France, Greece, Denmark, Norway, Italy, Sweden, Finland and Germany. Although infected foci exist in Germany near the Belgian south-eastern border, no autochthonous TBE case has been diagnosed in humans in Belgium so far.

Small rodents such as field mice (*Apodemus* spp.) show asymptomatic viraemia and constitute competent reservoirs for TBEV. Large mammals such as roe deer, wild boar and domestic ruminants mainly serve as tick amplifying hosts. They are unable to transmit the virus, as they only develop low virus titers. However, they do develop TBEV-specific antibodies rendering them into good sentinel animals. Dogs appear to be only occasional hosts for TBEV, though they may carry infected ticks from endemic to non-endemic areas.

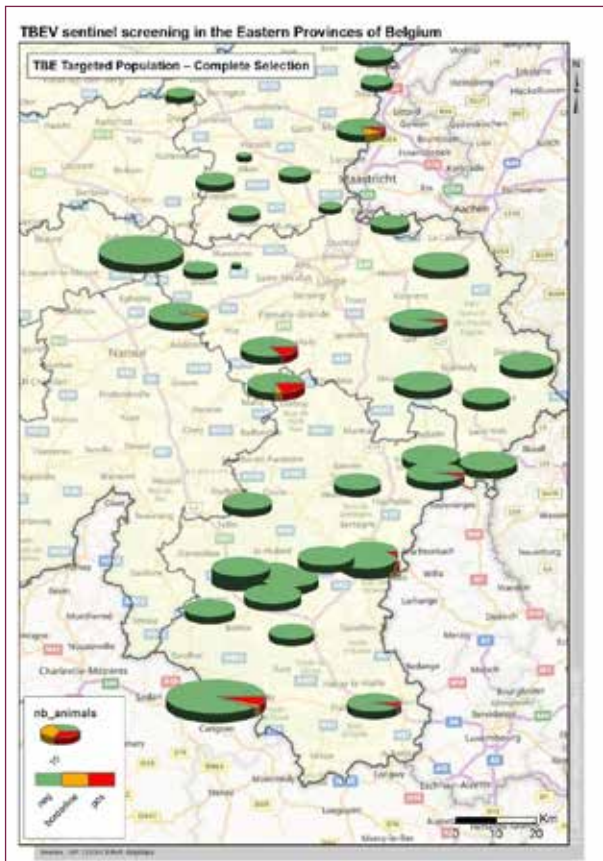
## Tick-borne Encephalitis Virus in animals and ticks in Belgium

TBE is emerging in Europe's canine population, and the numbers of clinical cases in dogs are expected to increase. In 2010, a seropositive dog was detected in Belgium but the exact origin of the infection (autochthonous or travel-related) could not be traced.

Between 2011 and 2013, the Network of Wildlife Diseases (Ulg, Liège) detected 2 seropositive roe deers in the South of Belgium (n=598) and the TBE Belgian national reference centre (NRC) (WIV-ISP, Brussels) detected 4 seropositive roe deers (n=127), but the specific localisations were not known.

TBEV-infected domestic ruminants (such as bovines) usually remain asymptomatic, but can transmit live virus in raw milk and unpasteurized milk products such as cheese. In 2014, the TBE NRC will publish a seroprevalence study on bovines. This serological screening was performed on Belgian cattle (n=650), selected from the 2010 Belgian national cattle surveillance serum bank. All samples were tested by the gold standard seroneutralisation test. Seventeen bovines were seropositive and six had borderline results. An apparent seroprevalence of 2.6% - 3.5% was found in older cattle located in the targeted Belgian provinces. Seropositive cattle were clustered in a few municipalities, demonstrating for the first time the presence of TBEV-infected foci in the targeted areas in south-east Belgium (Figure 57). Furthermore, a seroprevalence study in wild boars (n=320) is currently in progress within the TBE NRC (WIV-ISP, Brussels).

TBEV has not been detected or reported in rodents or ticks in Belgium so far.



**Figure 57.** Distribution of TBEV seropositive and doubtful (borderline) bovine cases in Belgium

## Tick-borne Encephalitis Virus in humans in Belgium

In 2010, a National Reference Centre for TBE was established within the WIV-ISP to confirm the diagnosis of TBE in humans. No autochthonous TBE case has been diagnosed in humans in Belgium so far. In 2012 and 2013, 75 and 208 human samples were analysed for TBE diagnosis. Test samples came from patients with the following symptoms: meningo-encephalitis and/or fever during the spring/summer/autumn, patients that returned within less than 1 month from an endemic region or with a tick bite or that consumed non-pasteurised milk products from an endemic TBE region.

From 2010 to 2014, 5 imported cases of human TBE were diagnosed, originating from Austria, Sweden, Norway and Kyrgystan.

The imported cases from Norway and Sweden were old TBE infections. Recent TBE infections were diagnosed in Belgian of unvaccinated tourists returning from Austria (summer flu-like syndrome, no tick bite reported) and from Kyrgystan (neurological symptoms and tick bite history).

An epidemiological study in patients suspected for neurological Lyme disease is currently in progress in collaboration with the Lyme disease NRC (UCL, Brussels).

# West Nile virus

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

## West Nile virus

West Nile virus (WNV) is the etiological agent of an emerging zoonotic disease whose impact on animal and public health is considerable as it is nowadays the most widespread arbovirus in the world (Kramer et al., 2008). WNV is indeed endemic in large parts of Africa, Australia, India, the Middle East, and, more recently, North America, while Europe and the Mediterranean basin countries are the scene of severe outbreaks (Jimenez-Clavero, 2012). WNV is mainly transmitted by mosquitoes, with wild birds being its natural reservoirs (Pfeffer and Dobler, 2010). Occasionally, WNV may also affect a wide range of other vertebrates, among which humans and horses (*Equus caballus*) can be particularly sensitive; those are nonetheless considered as dead-end hosts as they are not able to transmit the virus to feeding mosquitoes because of the low and short-term viremia they develop (Kramer et al., 2008) (figure 58).

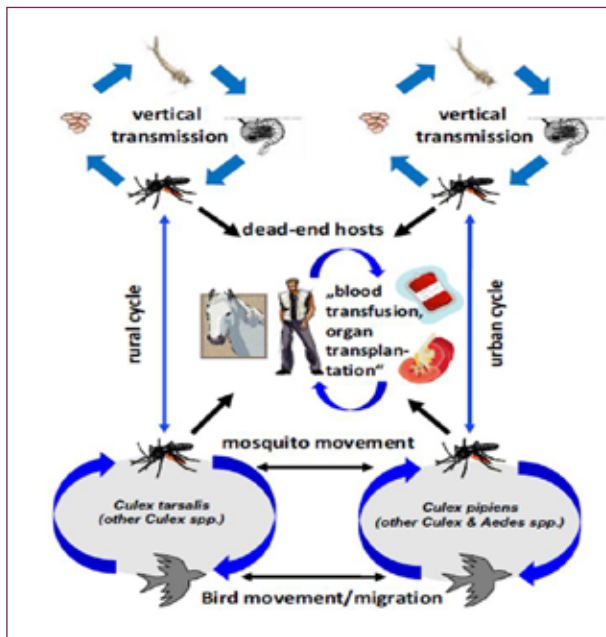


Figure 58. Transmission scheme of WNV

Migratory birds are involved in the transmission cycle of this virus as reservoir and amplifying hosts of the virus. Migratory birds overwintering 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 viraemia being weak and of short duration. These hosts do not contribute to the transmission cycle. In humans, the majority of WNV infections cause a non-symptomatic or a mild flu-like illness. However some infections can cause encephalitis which may lead to death, particularly in elder patients. The incubation period is 3-6 days. In human infections, transmission by direct contact does not occur. However, virus transmission by transfusion of blood and blood products as well as by organ transplantation and breastfeeding has been observed. Most equine infections are subclinical or unapparent, as approximately 10% of the infected horses develop clinical neuro-invasive disease. The fatality rate of clinically affected horses can reach 40%.

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

## West Nile virus in birds

Birds had been considered as less susceptible to WN disease until high mortality rates were recorded in flocks of young domestic geese in 1998 in Israel (Banet-Noach et al., 2003a; Malkinson et al., 2002), followed by mass die-offs amongst 326 species of birds since 1999 in the United States (De Filette et al., 2012). Data collected from natural and experimental infections of Nearctic bird species with American WNV isolates have shown that Passeriformes are the most represented order among the affected birds, followed respectively by the Pelecaniformes, Falconiformes and Columbiformes, whereas most of the Galliformes are refractory to WNV infection (Komar et al., 2003; van der Meulen et al., 2005; Wheeler et al., 2009). Among Passeriformes, the family Corvidae ranked as the most highly susceptible species to WNV (Wheeler et al., 2009). Indeed, dead corvids, particularly American Crows (*Corvus brachyrhynchos*), have been the hallmark of the North American WN epidemic and have been used to track its progress and distribution (Eidson et al., 2005; Julian et al., 2002).

Opposite to the North American observations, sporadic isolated death events and low mortality rates have been reported among European birds, even when those were associated to severe human and equine WNV outbreaks (Calistri et al., 2010; Dauphin et al., 2004; Jourdain et al., 2007; Valiakos et al., 2011). However, it should be taken into account that bird bodies might have been poorly detected because of their relative small size, their taking up by scavengers (Wobeser and Wobeser, 1992) or limited monitoring of wild bird mortality. It was also hypothesized that European birds, which have been exposed to WNV for decades throughout migratory contact with African infested zones (Hubalek and Halouzka, 1999) might have developed a herd immunity (Baitchman et al., 2007; Hahn et al., 2006; Kwan et al., 2012; Nemeth et al., 2008). Accordingly, experimental infections of European bird species with WNV strains circulating in Europe are necessary to assess the relative susceptibility of European bird species to local WNV isolates. In our laboratory (Service of Avian Virology and Immunology Virology Operational Direction, CODA-CERVA) experimental infection of Carrion Crows to evaluate their sensitivity to European lineage has been carried out and the validity of different avian matrices such as sera, oral swabs and feathers for viral detection has been also confirmed (Dridi et al. 2013).

### Early warning system

Since 2010, a programme based on passive surveillance of abnormal bird mortalities (corvids and raptors) as well as on active serological (free range poultry provided by the annual Avian influenza EU screening, Canadian geese, swans, corvids) and virological surveillance (corvids and raptors) has been realized. This programme is mandated by FASFC and is executed in close collaboration with the Royal Belgian Institute of Natural Science (RBIN). The collection of wild bird carcasses was done in collaboration with different revalidation centers. Specific baited trapping cages were built to catch Corvidae in rural areas as well as in the city centre of Brussels where samples were collected.

The serological tests included competition ELISA (ID Screen West Nile Competition, ID VET) and specific seroneutralisation assays to WNV and USUTU virus as confirmation tests for positive ELISA reactions. Virological analyses were performed using a RT-PCR targeting the NS2A region (in house diagnostic test)

For passive surveillance in 2012/2013, 203/62 brains and gut of corvids or raptors tested in pool by molecular test (RT-PCR) and all were negative.

For active surveillance, buccal (b) and cloacal swabs (c) from different Raptor species were tested and were negative for virological investigations (Table 35)



Figure 59. Bait trapping allowing corvid capture



Figure 60. Goshawk (*Accipiter gentilis*) chicks

Table 35. Active surveillance of WNV by buccal (b) and cloacal (c) swabs of Raptor species in 2012-2013

Year	2012	2013	2012-2013 Total
Species	b+c	b+c	
<i>Accipiter gentilis</i>	109	76	185
<i>Accipiter nisus</i>	101		101
<i>Bubo bubo</i>	94	119	213
<i>Buteo buteo</i>	65		65
<i>Falco peregrinus</i>	136	132	268
<i>Milvus milvus</i>	14		14
<b>Total</b>	<b>519</b>	<b>327</b>	<b>846</b>

The majority of serums and buccal swabs (b) from different corvid species were tested negative in serology and virology, respectively (Figure 36). In 2012 only serums from *Corvus corone* were positive by ELISA but not confirmed by the seroneutralisation assays (WNV and USUTU virus). Serum came from birds caught the 05/04/2012 in Mont Saint Guibert (Brabant Wallon). In 2013, two serums from free range poultry were tested positive by ELISA but again not confirmed by seroneutralisation test.

**Table 36.** Active surveillance of WNV by sera (s) and buccal (b) swabs of Corvid species in 2010-2011

Year	2012			2013			2012-2013	
Species	Type	b	s	Total	b	s	Total	
<i>Corvus corone</i>		375	465	840	225	474	699	1539
<i>Corvus monedula</i>		399	175	574	279	189	468	1042
<i>Corvus frugilegus</i>		1		1		2	2	3
<i>Pica pica</i>		6		6	9	9	18	24
<b>Total</b>		<b>781</b>	<b>640</b>	<b>1421</b>	<b>372</b>	<b>355</b>	<b>727</b>	<b>2581</b>

Other bird serological investigations on 3676 free range poultry were tested negative by ELISA.

To summarize, the results obtained in 2012 and 2013 indicated that there is no clear evidence of WNV circulation in Belgium to date. This monitoring will continue in 2012 as requested by the FASFC in close coordination with CODA-CERVA and RBIN.

### West Nile virus in humans

In 2012-2013, respectively 236 and 309 samples were analysed. In 2012 three WNV human cases were detected, no cases were detected in 2013.

More details can be found in a 2014 publication in epidemiology and infection on West Nile Virus and Chikungunya imported infections into Belgium, period 2007-2012. (<http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=9221040&fileId=S0950268814000685><http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=9221040&fileId=S0950268814000685>).

Abstract of the study: "Arboviral infections are emerging among tourists travelling to (sub)tropical regions. This study aims to describe the importation of chikungunya virus (CHIKV) and West Nile virus (WNV) into Belgium over a 6-year period from 2007 to 2012. Clinical samples were obtained from travellers presenting at the outpatient clinic of the Institute of Tropical Medicine (ITM), Antwerp, Belgium or submitted to the Central Laboratory for Clinical Biology of the ITM. Testing was performed by serology and/or by real-time reverse transcriptase-polymerase chain reaction. A total of 1288 returning travellers were investigated for CHIKV infection resulting in 34 confirmed and two probable diagnoses (2.80%). Out of 899 patients, four confirmed and one probable imported WNV infections were diagnosed (0.55%). No locally acquired cases have been registered in Belgium until now and the geographical origin of the imported infections reflects the global locations where the viruses are circulating".

# Parasitic diseases

## Cryptosporidiosis

Toon Braeye, Leen Claes, Lieselotte Cnops, Marjan Van Esbroeck, Luc Vanholme

### Cryptosporidiosis

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

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

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

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

Prevention can be done through good personal hygiene, avoiding unsafe water sources, washing hands carefully after going to the toilet or contacting stool, and before eating or preparing food. It is recommended to wash and peel all raw fruits and vegetables before eating. If possible, contact should be avoided with infected humans or infected animals.

Water from lakes, rivers, springs, ponds or streams should not be drunk. If safety of the drinking water is questionable, water should be boiled. Suspect water supplies can also be carefully filtered before drinking.

### Cryptosporidiosis in animals

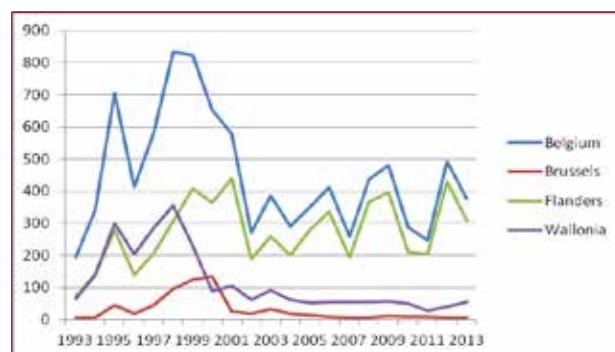
No official monitoring program of cryptosporidiosis in animals was organized in the reporting period. Laboratory diagnosis confirmed some clinical suspicions of cryptosporidiosis.

### Cryptosporidiosis in humans

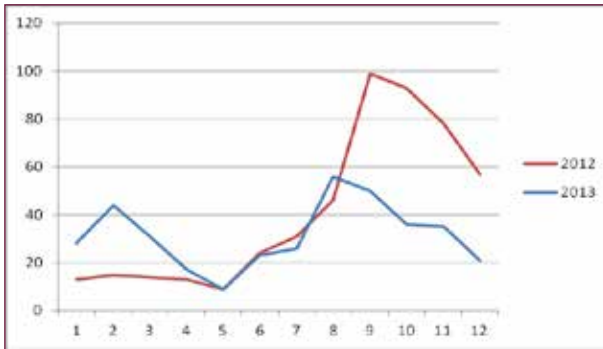
Infections with *Cryptosporidium parvum/hominis* occur worldwide. Most reports describe endemic outbreaks caused by contaminated water and food. The infectious oocysts may survive in the environment for months.

In 2012, the Sentinel Laboratory Network reported 492 cases of cryptosporidiosis, corresponding to a national incidence estimated at 4.46 per 100,000 inhabitants. In 2013, the Sentinel Laboratory Network reported 376 cases of cryptosporidiosis, corresponding to a national incidence estimated at 3.39 per 100,000 inhabitants. The incidence was very high in 1998 and 1999 (8.2 per 100,000 inhabitants) (Figure 61).

Cases were observed all over the year with a peak at the end of summer (Figure 62)



**Figure 61.** Total number of *Cryptosporidium* infections in humans by year, 1993-2013. Source: Sentinel Laboratory Network



**Figure 62.** Monthly number of *Cryptosporidium* infections in humans, 2012-2013. Source: Sentinel Laboratory Network

In 2012, 39% of all cases was reported in 1 to 4 year old children. In the adult population cases are equally distributed over the age groups (Table 37).

**Table 37.** Number of *Cryptosporidium* infections in humans by sex and by age groups, 2012. Source: Sentinel Laboratory Network

AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	73	30.04	117	47.18	190	39.00
05-09	31	12.76	32	12.9	63	13.00
10-14	10	4.12	13	5.24	23	5.00
15-19	9	3.7	7	2.82	16	3.00
20-24	12	4.94	12	4.84	24	5.00
25-29	17	7	9	3.63	26	5.00
30-34	27	11.11	19	7.66	46	9.00
35-39	22	9.05	11	4.44	33	7.00
40-44	10	4.12	2	0.81	12	2.00
45-49	8	3.29	8	3.23	16	3.00
50-54	2	0.82	7	2.82	9	2.00
55-59	3	1.23	0	0	3	1.00
60-64	4	1.65	0	0	4	1.00
65-++	7	2.88	6	2.42	13	3.00

In 2013, 33% of cases was reported in 1 to 4 year old children. As in 2012 the cases were evenly distributed in the adult population (Table 38)

**Table 38.** Number of *Cryptosporidium* infections in humans by sex and by age groups, 2013. Source: Sentinel Laboratory Network

AgeGroup	female		male		total	
	Fre-quency	Percent	Fre-quency	Percent	Fre-quency	Percent
00-04	49	25.93	70	39.11	123	33.00
05-09	16	8.47	25	13.97	42	11.00
10-14	13	6.88	9	5.03	22	6.00
15-19	11	5.82	7	3.91	18	5.00
20-24	19	10.05	5	2.79	25	7.00
25-29	14	7.41	8	4.47	22	6.00
30-34	20	10.58	10	5.59	30	8.00
35-39	11	5.82	12	6.7	24	6.00
40-44	6	3.17	7	3.91	13	3.00
45-49	3	1.59	5	2.79	8	2.00
50-54	5	2.65	3	1.68	8	2.00
55-59	4	2.12	5	2.79	9	2.00
60-64	3	1.59	3	1.68	6	2.00
65-++	11	5.82	2	1.12	14	4.00

In 2012, the incidence was 6.8 per 100,000 inhabitants in Flanders, 1.2 per 100,000 inhabitants in Wallonia and 0.6 per 100,000 inhabitants in Brussels-Capital Region. In 2013, the incidence was 4.9 per 100,000 inhabitants in Flanders, 1.6 per 100,000 inhabitants in Wallonia and 0.5 per 100,000 inhabitants in Brussels-Capital Region. The mean incidences in Wallonia and in Brussels-Capital Region between 2005-2010 were 1.56 and 0.96 per 100,000 inhabitants respectively. In Flanders the mean incidence in the period 2005-2010 was 5.0 per 100,000 inhabitants. The incidences observed over the different districts vary, part of this is due to the locations of the sentinel labs.

# Cysticercosis

Leen Claes, Luc Vanholme

## Cysticercosis

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

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

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

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

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

## Cysticercosis in cattle

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

In 2012 and 2013, respectively 512.503 and 480.009 carcasses of adult cattle and 312.430 and 328.066 veal calves were examined.

Figures 63 and 64 from the FASFC show that in 2012 and 2013, 9 and 16 carcasses of adult cattle were rejected for generalised cysticercosis. In addition, the carcasses of respectively 1.205 and 978 adult cattle were treated by a 10 days freezing period at -18°C before release for human consumption.

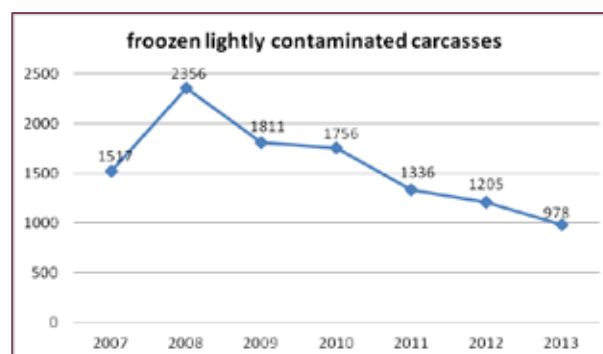


Figure 63. Cysticercosis; detection of lightly contaminated bovine carcasses at slaughterhouse, period 2007 - 2013

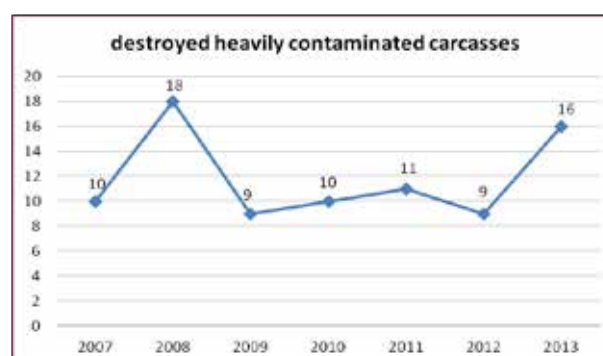


Figure 64. Cysticercosis; detection of heavily contaminated bovine carcasses at slaughterhouse, period 2007 - 2013

# Echinococcosis

Yves Carlier, Leen Claes, Annick Linden, Carine Truyens, Steven Van Gucht, Luc Vanholme

## Echinococcosis

Human echinococcosis is caused by the larval stage of the small tapeworm *Echinococcus* spp. Two main species are responsible for the human disease : *Echinococcus granulosus* and *Echinococcus multilocularis*.

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

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

*Echinococcus multilocularis* has a similar life cycle as *E. granulosus*. Foxes and to a lesser extent dogs are the definitive hosts of this parasite. Small rodents and voles are the intermediate hosts. Foxes are infected by ingestion of contaminated rodents. *E. multilocularis* is the agent of highly pathogenic alveolar (multilocular) echinococcosis in humans. Humans can be infected by ingestion of eggs. Alveolar echinococcosis is of particular public health relevance as it results in a chronic cancer-like liver disease. Ingestion of the eggs by humans can result in the development of invasive cysts in the liver.

Most untreated cases in humans are fatal. In the intermediate hosts, the larval form of the parasite remains in the invasive proliferative stage in the liver, thus invading the surrounding tissues.

Risk factors for human contamination include contact with dogs having access to the outdoors, contact with foxes 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°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 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.

No data are available for the reporting period.

## Echinococcus in wildlife

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

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

According to a study performed in 2007-2008, the parasite remained rare in Flanders and Brussels. There was thus no proof of an increase in prevalence. In 2007-2008, 187 foxes from the regions of Brussels (n = 56) and Flanders (n = 131) were examined by the WIV-ISP with the intestinal scraping technique. In Flanders, most foxes were healthy specimens that were shot during the hunting season. Storage of cadavers and sampling of the intestines was done by the Research Institute for Nature and Forest (INBO). In Brussels, most examined foxes were traffic victims that were deposited by police officers. Sampling was intensified in the south of Flanders and the southwest of Brussels. Indeed, in the southwest of Brussels 3 positive foxes were found during a former study in 1996-1999 (Vervaeke et al., 2003). None of the examined foxes from 2007-2008 carried *E. multilocularis* cestodes. The possible reasons for the difference in prevalence between the north and south are not known, but may include differences in altitude, climate, fox diet or low abundance of suitable intermediate hosts.

### Echinococcus in humans

The NRL performs around 300 serological analyses per year for antibodies against *Echinococcus granulosus*. An average of 8% of the samples gives positive results (20 to 30 positive cases / year). Amongst these positive cases, on average 45% are new diagnosed cases of hydatidosis, the others are patients in serological follow-up after surgical and/or etiological treatment.

The NRL also realizes between 250 and 300 serological analyses/year for antibodies against *Echinococcus multilocularis*. In screening tests using a crude antigenic extract, an average of 5% of the samples is positive, i.e. 7 to 21 patients/year. More specific tests (realized in the NRL or in collaboration either with the Institute for Parasitology of the Faculty of Medicine, Berne, Switzerland, or the Centre National de Référence Echinococcose alvéolaire, Besançon, France) show that most of these positive cases correspond to cross reactions between *Echinococcus multilocularis* and *Echinococcus granulosus*, i.e. most of these patients suffer from hydatidosis produced by *E. granulosus*.

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

By the end of 2011, the NRL has diagnosed 11 cases of alveolar echinococcosis (8 cases before 2003, 4 cases between 2004 and 2011). They concerned patients between 36 and 90 years old (7 males and 4 females). Two cases presented a rapid evolution due to immunosuppression. Two other cases were lethal due to surgical complications.

Surprisingly, five new serologically positive cases were detected in 2012 and 2013 (two in 2012 and three in 2013; 3 males of 30, 31 and 77 years old and 2 females of 29 and 54 years old). One of them (F29) is a woman living in Belgian Ardennes who has adopted and takes care of a fox cub but does at the moment not presents any hepatic lesion. The four other patients harbour hepatic lesions: 3 of them also live in the Belgian Ardennes while the last one has contracted echinococcosis in Tibet.

We don't know at the moment if this apparent increase of detected cases of alveolar echinococcosis is significant, if it reflects enhanced awareness of healthcare providers, or a possible increased prevalence level in natural hosts as suggested by Davidson RK et al (Trends Immunol 2012).

# Toxoplasmosis

Stéphane De Craeye

*Toxoplasma gondii* is an obligate intracellular protozoan that can be found worldwide. The final hosts are the felidea (more commonly house cats); humans and almost all warm-blooded animals are intermediate hosts. The sexual cycle takes place exclusively in the intestines of the felidea within the first two weeks after infection, resulting in the shedding of millions of oocysts. Once in the environment, these oocysts sporulate and stay infectious for a long time, depending on the climate. Ingestion of oocysts by a seronegative host leads to toxoplasmosis. The infection has an acute and a chronic phase, the latter characterised by the presence of persistent tissue cysts in the host (in muscles, brain, heart ...). Ingestion of infected tissues by a seronegative host (final or intermediate) will also lead to the development of the disease.

## Toxoplasmosis in animals

The majority of warm blooded animals are indiscernible carriers of tissue cysts. The prevalence depends on many factors, like for example the feeding habit of the animal: herbivores are more exposed to oocysts and carnivores more exposed to tissue cysts. Other factors include the age of the animals, the geographical location, the type of farm management and even the hygienic conditions of the farm.

There is a need for efficient serological and molecular biological methods for both indirect and direct detection of *T. gondii* in livestock and food. Animals can be screened serologically to see if they have been infected, but a positive serological result does not prove that infectious cysts are present in all their tissues. The presence of tissue cysts in meat can be detected by PCR, but bio-assay is still more sensitive. Furthermore, only bio-assay can demonstrate the presence of viable and thus infectious cysts in meat products. Screening farm animals is

not routinely done in Belgium and therefore no data on the status of toxoplasmosis in Belgian livestock is available. The European Food Safety Authority has recommended the initiation of monitoring programs in the pre-harvest sector on sheep, goats, pigs and game.

The clinical consequences of veterinary toxoplasmosis depend on the animal species, its feeding habit and the infecting *T. gondii* strain. In sheep and goats, a primary infection during gestation leads to abortion, stillbirth or weak lambs causing important economic losses. In general, seroprevalence in sheep and goats is high and can be up to 70% or more. The outcome of toxoplasmosis in cattle is less clear: although seroprevalence can be high, there is less association with transmission to humans. The prevalence in pigs and poultry merely depends on the production system, with a higher infection rate in animal friendly farms ("free range") because the animals have out-door access.

In general, animals with outdoor access have a higher prevalence of toxoplasmosis, and the presence of cats and rodents on the farm is also an important risk factor. Limitation of all these factors is important as preventive measures.

A recent seroprevalence study performed on 3170 sera collected from Belgian domestic sheep showed the presence of specific anti- *T. gondii* IgG in 87.4% of the animals, corresponding to 96.2% of the tested flocks. The tested samples originated from animals older than one year, from flocks originating from East-Flanders, West- Flanders, Antwerp, Limburg, Flemish Brabant and Wallonia and were collected by the Federal Agency for the Safety of the Food Chain (FAVV-AFSCA) in the frame of the Visna-Maedi/ CAE screening program. Table 39 gives an overview of the seroprevalences per region.

**Table 39.** Seroprevalence of *T. gondii* found in Belgian domestic sheep.

Geographical Origin		Flocks		Sheep		True Prevalence (95% CI)
		# Tested	# Positive (%)	# Tested	# Positive (%)	
Flanders	Antwerp	23	23 (100%)	402	240 (59.7%)	65.2% (58.5% - 62.6%)
	East-Flanders	48	48 (100%)	563	479 (85.1%)	96.6% (92.1% - 99.6%)
	Flemisch Brabant	31	31 (100%)	370	323 (87.3%)	97.8% (93.8% - 99.8%)
	Limburg	20	19 (95%)	256	222 (86.7%)	97.3% (92.5% - 99.7%)
	West Flanders	36	36 (100%)	667	569 (85.3%)	96.8% (92.8% - 99.7%)
Wallonia		51	44 (86.3%)	912	567 (62.2%)	68.6% (63.0% - 74.8%)
<b>Total</b>		<b>209</b>	<b>201 (96.2%)</b>	<b>3170</b>	<b>2400 (75.7%)</b>	<b>87.4% (82.0% - 93.4%)</b>

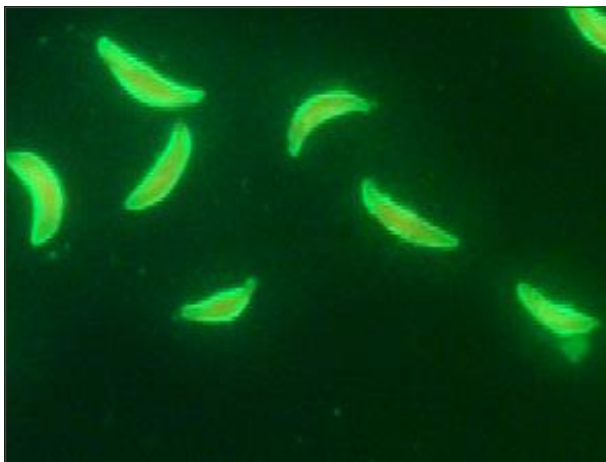
The seroprevalence in domestic cats varies between 25 to 70% and is higher in stray and feral cats. A serological survey of Belgian domestic cats demonstrated an overall chance of 6% for a seronegative cat to become infected within the next life-year and actively shed oocysts, with the highest infection rate during its first life-year (source: NRL for Toxoplasmosis at WIV-ISP)

**Table 40.** Animal sera tested for the diagnosis of toxoplasmosis during 2012 and 2013 at the NRL for Toxoplasmosis at IPH

Serology				
	IgM positive		IgG positive	
	2012	2013	2012	2013
Sheep*	124/136	12/13	130/136	11/13
Goat*	8/33	1/3	20/33	2/3
Cats**	76/311	54/260	179/311	128/260
Dogs**	196/365	66/239	199/365	107/239
Bovine*	0/1	5/16	1/1	5/16
PCR				
	2012		2013	
Sheep*	5/58		1/7	
Goat*	2/23		0/3	

\*Samples tested in the context of abortions;

\*\* samples tested in the context of veterinary diagnosis.



**Figure 65.** *T. gondii* tachyzoites (Fluorescence microscopy (FITC), source WIV-ISP)

## Toxoplasmosis in humans

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

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

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

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

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

During the period 2012-2013, the NRC center at the WIV-ISP isolated 17 *T. gondii* strains from amniotic fluid, placenta or blood samples tested in the context of a suspected congenital toxoplasmosis.

Only a very limited number of drugs can be used to control the infection. In addition, these are only active on the free form of the parasite. The treatment takes a long time, does not prevent the formation of tissue cysts. For this reason, preventive measures are very important for high-risk patients. Efforts are made to avoid a primary *T. gondii* infection during pregnancy. The mode of acquiring toxoplasmosis from meat, cat faeces and contaminated soil is so circumscribed that simple but effective measures should be recommended during pregnancy: regular hand-washing, especially after contact with cats, meat, soil and water. Freezing meat (at -20°C for minimum 48 hours) before consumption and adequate heating of fresh meat (internal temperature of minimum 60°C) during preparation are other effective measures. Cleaning the cat litter should be avoided during pregnancy.

## Trichinellosis

Leen Claes, Luc Vanholme, Julie Wits

### Trichinella

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

The clinical signs of acute trichinellosis in humans are characterised in a first phase by nausea, vomiting, diarrhea, 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, diarrhea or constipation. In more severe cases, difficulties with coordination of movements as well as heart and breathing problems may occur.

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

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

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

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

In Belgium, since 1979, all fattening pigs intended for the intracommunity market or for the export are systematically examined for the presence of *Trichinella*. Belgium never detected a single positive carcass. A request to obtain the status "Region with negligible risk of *Trichinella*" was introduced in March 2010 to the European Commission. This status allows a MS to apply under specific conditions a risk-based surveillance program instead of systematic analysis of all fattening pigs. Belgium was granted the status of "Region with negligible risk of *Trichinella*" by the European Commission in December 2010. Based on this status, but only for fattening pigs held under controlled housing conditions, the *Trichinella* analysis does not have to be performed if the meat is intended for the EU market. With this status, *Trichinella* analysis could only be performed on sows and boars and also on fattening pigs held under free-ranging conditions. Analysis of almost all fattening pigs held under controlled housing conditions was still performed in 2013 because the final destination of the meat is not always known at the moment of slaughter.

## Trichinella in animals

### Surveillance programme and methods used

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

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

Notification to the FASFC is compulsory.

### Results of the 2012-2013 surveillance

In 2012 and 2013, a total of respectively 11.377.705 and 10.379.381 pigs were examined.

In 2012 and 2013, respectively 9.529 and 8.511 solipeds (mainly horses) and 14.519 and 9.610 wild boars were examined. One larva of *Trichinella* spp. was detected in a wild boar and in a pool of 11 wild boars in 2012. No larvae of *Trichinella* spp. were detected in 2013.

## Trichinella in wildlife

During the winter of 2011-2012, 524 wild animals were examined (507 foxes, 11 badgers, 2 cats, 1 raccoon and 3 marten). One larva was recovered from a pool of 20 animals (18 foxes and 2 badgers). Unfortunately the larva could not be identified to the *Trichinella* species level by PCR.

During the winter of 2012-2013, 540 wild animals were examined (511 foxes, 15 badgers, 1 wild cat, 8 raccoons, 4 beech marten and 1 European polecat). Three larvae were recovered from two pools of 20 foxes each.

A measure to prevent spreading of trichinellosis among wildlife is not to leave offal of hunted animals in the field. Of course, the most important way of spreading *Trichinella* among wildlife is by the ingestion of infected preys and carcasses.

# Food-borne outbreaks

Nadine Botteldoorn, Laurence Delbrassinne, Sarah Denayer, Katelijne Dierick

In Belgium different authorities share competences related to foodborne outbreaks (FBO).

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

The Communities (Flemish, French and German speaking Community) deal with person related matters such as human health and infectious diseases, and are entitled to perform epidemiological investigation by its medical Public Health Inspectors in case of a FBO.

The Institute of Public Health (WIV-ISP), the Scientific Service of Foodborne Pathogens, which is National Reference Laboratory for Foodborne Outbreaks (NRL FBO), analyses the suspected food samples, coordinates the analyses of human samples, collects all data on FBOs and gives scientific support to the FASFC officers and the Public Health Inspectors.

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

## Food-borne 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 the FASFC, the Communities (French, Flemish and German speaking), the sentinel laboratories network for human clinical microbiology, the National Reference Laboratory for FBOs, and the National Reference Centers for Salmonella and Shigella, Listeria and C. botulinum.

The reporting includes both general and household outbreaks.

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

## Major etiological agents

### Food-borne bacteria

#### Salmonella enterica

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

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

Since 2005, *Campylobacter* is the most frequently reported illness of foodborne origin in humans in Belgium. *Campylobacter jejuni* and *C. coli* infections cause diarrhea, which may be watery or sticky and can contain blood. Other symptoms often observed are fever, severe abdominal pain, nausea, headache, malaise and muscle pain. Rarely, it can cause constipation leading to a misdiagnosis of appendicitis. The illness usually occurs 2-5 days after ingestion of the contaminated food or water and generally lasts 7-10 days, but relapses are not uncommon (about 25% of cases). Some rare but feared complications are acute colitis, reactive arthritis and Guillain-Barré syndrome.

Raw poultry meat and raw pork is frequently contaminated by *Campylobacter*. Raw milk and cheeses made from raw milk are also sources of infections.

### **Yersinia enterocolitica**

Yersiniosis is frequently characterized by symptoms such as gastroenteritis with diarrhea and/or vomiting; however, fever and abdominal pain are typical symptoms. *Yersinia* infections can also cause pseudo-appendicitis and reactive arthritis. Illness onset is usually between 24 and 48 hours after ingestion of food or water, which are the usual vehicles of *Yersinia*. Contaminated and undercooked pork is the most common source of infection.

### **Clostridium perfringens**

The common form of *Clostridium perfringens* poisoning is characterized by intense abdominal cramps and diarrhea which appear 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-limiting within 24 hours but less severe symptoms may persist in some individuals for 1 or 2 weeks. In most instances, the cause of poisoning by *C. perfringens* is temperature abuse of prepared foods. Indeed, small numbers of spores are often present after cooking and can multiply to toxic levels during cooling and storage of prepared foods at abuse temperatures under anaerobic conditions (e.g. fat layer on stock). Meat, meat products, and gravy are the foods most frequently implicated.

### **Staphylococcus aureus**

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

### **Bacillus cereus**

Two types of food poisoning, an emetic and a diarrheal type, are described. 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 diarrheal 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 diarrheal type is often associated with cooked meat and meat products.

### **Food-borne viruses**

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

Although numerous fecal-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, fruit and food handler contamination are also reported. Increased awareness towards viral infections is raised thanks to improved molecular detection methods. In particular real-time polymerase chain reaction (RT-PCR) allows detection of viral ribonucleic

acids (RNA) and has made diagnosis and outbreak management easier.

Noroviruses are among the most important causes of gastroenteritis in adults and often occur as outbreaks which may be foodborne. They are the most common cause of non-bacterial FBOs recognised in Europe and United States and have been diagnosed worldwide. Noroviruses can be transmitted from person-to-person, or indirectly via food or water contaminated with feces or vomit. They cause mild and usually self-limiting gastroenteritis. Considering the high attack rates and the fact that Norovirus can cause disease in all age groups, the socio-economic burden of Norovirus infection is very important.

### Marine biotoxins

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

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

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

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

## Parasites

### Giardia lamblia

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

## Food-borne outbreaks

### Reported outbreaks

During 2012, a total of 327 outbreaks of collective food-borne infections and intoxications were recorded in Belgium. More than 1469 people were ill and at least 59 persons were hospitalized. One death was reported but the causative agent for that case could not be confirmed. As only one person was involved, this outbreak was not reported to EFSA.

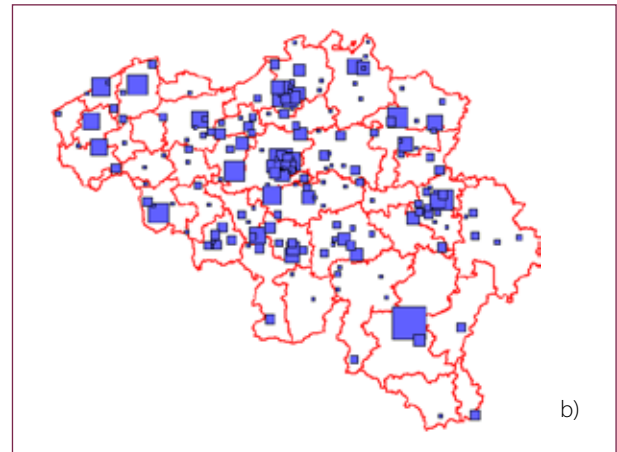
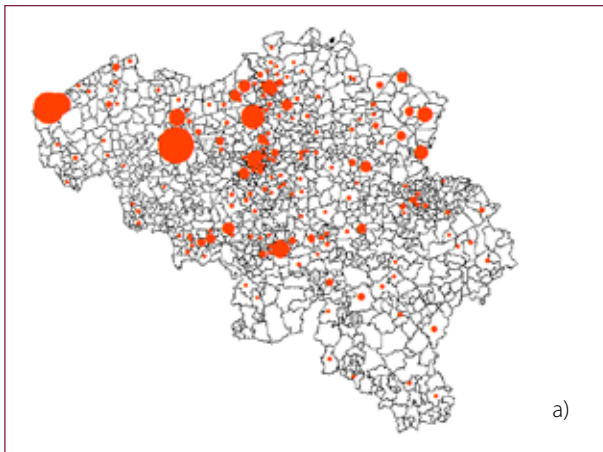
During 2013, a total of 311 outbreaks of food-borne infections and intoxications were recorded in Belgium. More than 1312 people were ill among whom at least 94 persons were hospitalized. None of the human cases died due to the consumption of contaminated food in any of the reported food-borne outbreaks.

The geographic distribution of all reported foodborne outbreaks in 2012-2013 is shown in figure 66.

### Causative agents

In total 31 and 23 verified outbreaks were reported in 2012 and 2013, respectively. In these outbreaks the causative agent was detected in the implicated food and/or was clearly pointed by descriptive epidemiology. All other outbreaks were classified as weak evidence outbreaks where the agent was unknown, no samples were sent for analysis or the agent could be only detected at human level where the link between human cases and the consumption of a specific foodstuff was missing.

In 2012, Norovirus was the most frequently detected food-borne pathogen in nine collective food-borne outbreaks and 94 persons became ill (table 41). The next most reported agents were Salmonella and E. coli O157:H7 being at the origin of 6 and 3 outbreaks, respectively. In total 30 persons were ill and 19



**Figure 66.** Geographical distribution of FBOs in Belgium 2012 (a) -2013 (b). Size of labels indicates number of human cases involved in the outbreak.

were hospitalized due to an *E. coli* O157:H7 infection. Consumption of raw bovine meat was at the origin of these outbreaks. Twenty-six disseminated cases of *Salmonella* Stanley were reported in different regions of the country and was a part of an international outbreak in Europe. Turkey meat was suspected but the origin of the outbreak could not be confirmed. In the other 4 *Salmonella* outbreaks, 12 persons became ill and 3 of them were hospitalized. *Salmonella* was also isolated in a co-infection with *Campylobacter* where 2 cases were reported.

Histamine was responsible for 4 outbreaks causing 28 ill people and 2 hospitalizations. High levels of histamine were quantified in tuna fish.

In one outbreak, an enterotoxin producing *Bacillus cereus* could be confirmed in the food and in another outbreak an emetic toxin producing strain could be isolated, which corresponded with the rapid onset of the vomiting symptoms observed in the patients.

For one outbreak, enterotoxin producing *Bacillus cereus* was isolated from nutmeg whereas enterotoxin A producing *Staphylococcus aureus* could be isolated from sausages. Enterotoxin A producing *Staphylococcus aureus* was also detected in 3 other outbreaks. For one of those outbreaks 30 human cases were reported and enterotoxin producing *Clostridium perfringens* was isolated from a human case.

Diarrheic Shellfish poisoning (DSP) was at the origin of 2 outbreaks resulting in 110 human cases and was due to the consumption of contaminated mussels.

Thermotolerant *Campylobacter* was detected in a single outbreak.

In 2013, bacterial toxins of *Bacillus cereus* and coagulase positive *Staphylococcus* (CPS) were the most frequently detected food borne pathogens in four food borne outbreaks each, in

which 30 and 59 persons became ill, respectively (table 41).

Both pathogens were at the origin of a co-infection where 5 toddlers started vomiting shortly after the consumption of mashed carrots. Enterotoxin producing *Bacillus cereus* and enterotoxin A producing CPS were isolated from leftovers. Enterotoxin A positive CPS was also isolated from the human cases and the food handler. The third most reported agent was *Campylobacter* being at the origin of 3 outbreaks. In total 20 persons became ill and 2 were hospitalized. Consumption of poultry meat or mixed meals containing chicken meat was at the origin of these outbreaks.

Histamine was also responsible for 3 outbreaks causing 7 ill people and 3 hospitalizations. High levels of histamine were quantified in tuna fish for two of these outbreaks and pitta meat is probably linked to the third outbreak.

Two outbreaks were caused by enterotoxinogenic *Clostridium perfringens* and caused diarrhea in 88 human cases. For one of these outbreaks, the pathogenic strain could be confirmed in both the implicated foodstuff (stew) and the human cases (stool). In the other outbreak, the strain could only be isolated from human cases, but the outbreak was epidemiologically linked to a common food source.

Eighteen disseminated cases of *E. coli* O157:H7 could be linked to the consumption of raw bovine minced meat.

Norovirus was detected in one outbreak that occurred in a residential institution and caused 20 ill persons among which 5 were hospitalized. Norovirus was detected in the witness meals.

Three family members got ill due to the presence of *Salmonella* Enteritidis in tiramisù. Two of them were hospitalized. The isolated strain from the food had the same phagetype and MLVA profile as the human isolates.

For one outbreak, no causative agent was detected in the suspected food. However, the ten children in a nursery started vomiting or presented fever within 10 minutes following the consumption of cauliflower puree with chicken. Therefore, there was a strong epidemiological evidence that the food was at the origin of the outbreak.

**Table 41.** FBOs in humans in Belgium in 2012 -2013

Causative agent	Outbreaks 2012				Outbreaks 2013			
	N	Human cases	Deaths	Hospitalisations	N	Human cases	Deaths	Hospitalisations
Bacillus cereus	2	24			4	30		
Staphylococcus	2	3		1	4	45		
Clostridium perfringens					2	88		
Campylobacter	1	2			9	45		11
Verotoxinogenic E. coli	3	30		19	10	41		25
Hepatitis A Virus								
Norovirus	9	94		4	1	20		5
Listeria					2	4		
Shigella								
Other agents	2	110			2	5		2
Parasites								
Salmonella	6	40		3	10	33		15
Histamine	4	28		2	4	9		3
Co-infections	3	37			3	31		2
Unknown	295	1101	1	30	260	947		31
<b>Total</b>	<b>327</b>	<b>1469</b>	<b>1</b>	<b>59</b>	<b>311</b>	<b>1312</b>		<b>94</b>

In 2012, no causative agent could be identified in 90% of the reported food-borne outbreaks (N=295 out of 327) and in 2013 this percentage was 84% (N=260 out of 311). An important reason for the low identification rate is the absence of leftovers of the suspected meal for most of these outbreaks or late reporting by the consumer. Therefore, the analysed food products do not always represent the consumed food that might have been at the origin of illness. In addition, for many outbreaks no samples were sent for analysis. In 2012, only in 40% (N=132 out of 327) of the outbreaks, samples (human and/or food) were sent for analysis of which 24% (N=32) resulted in the detection of a causative pathogen. Some of the latter outbreaks have been categorized as weak evidence outbreaks because the reported symptoms did not coincide with the isolated pathogen. In 2013, in 38% (N=119 out of 311) of the outbreaks, samples (human and/or food) were sent for analysis of which 43% (N=51) resulted in the detection of a pathogen. Some of the latter outbreaks (N=29) have also been categorized as a weak evidence outbreak.

### Source of the food-borne outbreaks

Most FBOs were due to the consumption of meals composed of different ingredients, namely 31% and 43% for 2012 and 2013, respectively (Figure 67 on the next page). Meat and meat-based products were responsible for 17 % (2012) and 23% (2013) of the outbreaks. In 4.2% (2012) and 8% (2013) of the outbreaks the involved foodstuff was not reported.

### Setting of the food-borne outbreaks

The places of exposure reported to the NRL FBO were almost identical during the last years.

In 2012, restaurants and take away or fast food outlets were the most important locations of exposure, being the setting of 63,9 % and 8,5 %, respectively, of food-borne outbreaks in Belgium (Figure 68). Catering at work or in community kitchens were reported in respectively 3,3 % and 0,6 % of the food-borne outbreaks. 16,2 % of the outbreaks happened at home.

In 2013, restaurants and take away or fast food outlets were the most important location of exposure, being the setting of 48.6 % and 18.3 %, respectively, of food-borne outbreaks in Belgium. Catering at work or community kitchens are reported in respectively 2.6 % and 3.9 % of the food-borne outbreaks. 16,7 % of the outbreaks happened at home.

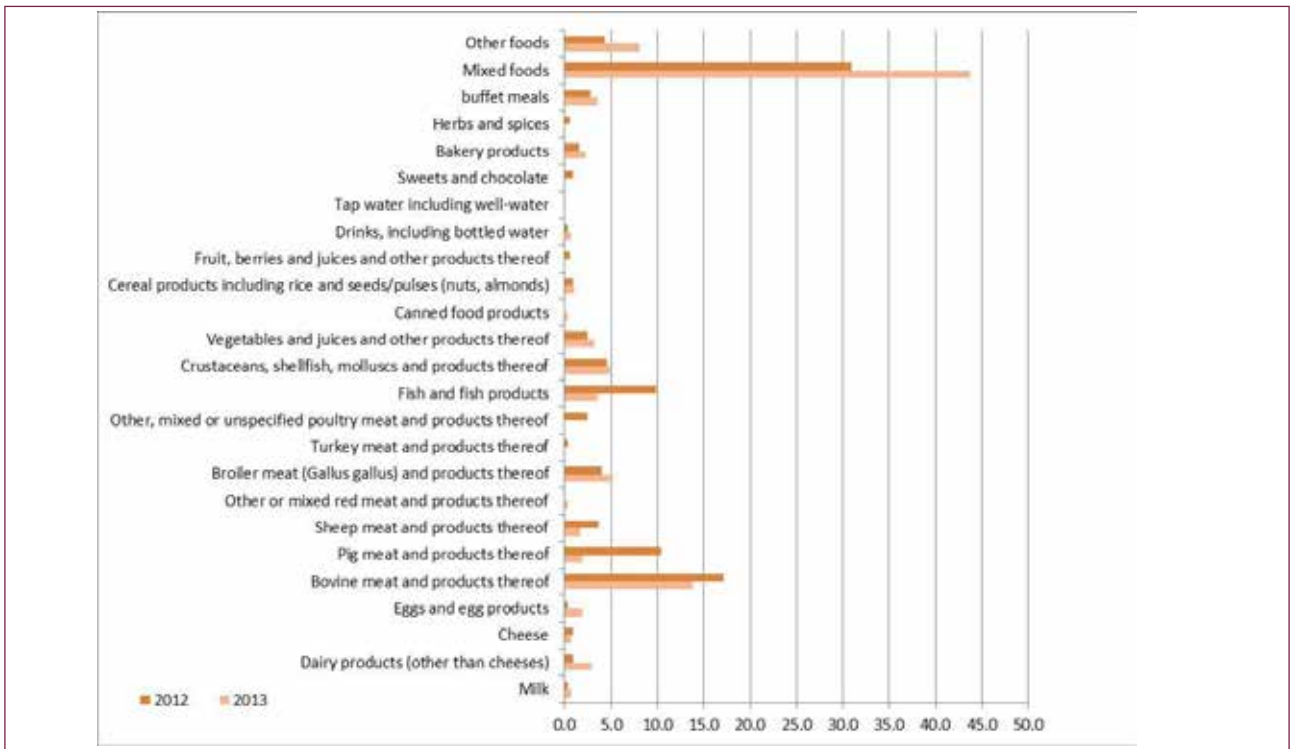


Figure 67. food vehicles responsible for FBO in 2012 and 2013

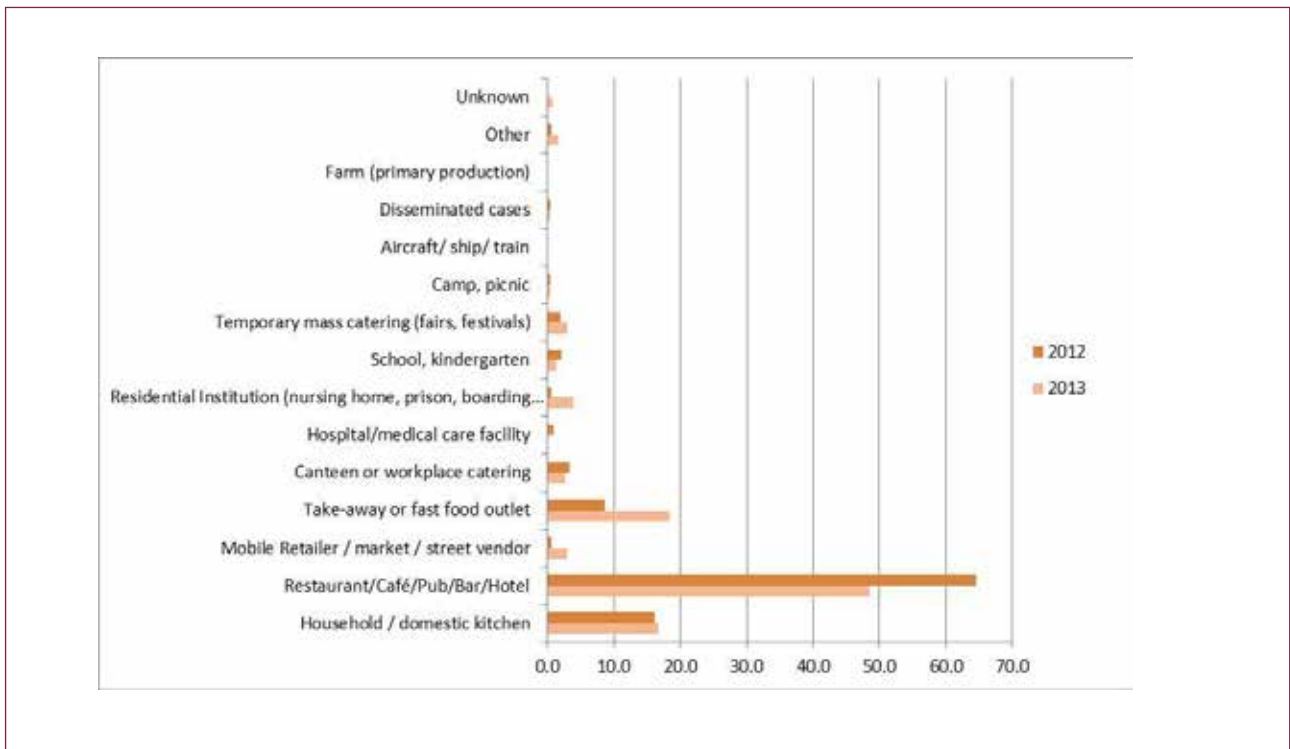
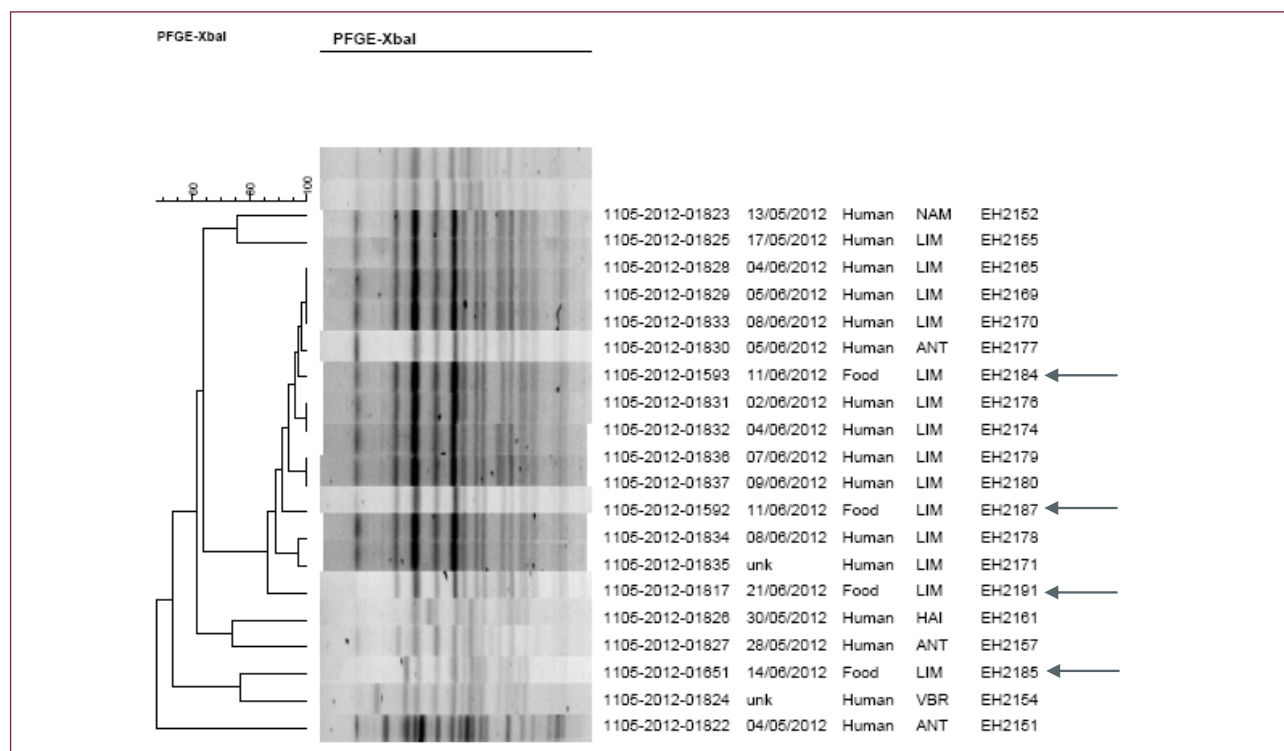


Figure 68. Place of exposure in FBOs in 2012-2013

## Description of food-borne outbreaks of special interest

In June 2012, an outbreak of bloody diarrhea and hemolytic-uremic syndrome (HUS) caused by *Escherichia coli* O157:H7 (vt2 eae positive) occurred in North-East Limburg, Belgium. On the 4th of July, the outbreak involved 24 cases among which 17 were laboratory-confirmed. Four patients (two children and two middle-aged women) developed HUS. The source of the

outbreak could be traced back to the slaughterhouse thanks to efficient sampling, exploratory interviews and a case-control study. The link between human and food isolates was demonstrated using PFGE and IS629-typing (Figure 69). The patients were most frequently infected through the consumption of raw bovine meat products such as “steak tartare”.



**Figure 69.** PFGE cluster analysis of *E. coli* O157 strains isolated from human cases and food samples during an outbreak in June 2012 in Limburg, Belgium. Human strains were kindly provided by the NRC *E. coli* (UZ Brussels)

One year later, in June-July 2013, another outbreak of bloody diarrhoea and haemolytic-uremic syndrome (HUS) caused by *Escherichia coli* O157:H7 (vt2 eae positive) occurred in Flanders, Belgium. This outbreak involved 18 disseminated cases and all were laboratory-confirmed as well as they could be linked using molecular typing techniques such as IS629-typing. The source of the outbreak could be traced back to the processing plant. The patients in this outbreak were also infected through the consumption of raw bovine meat products such as “steak tartare”.

In 2012, 20 out of 22 children from a kindergarten started vomiting within 30 minutes after the consumption of rice with cucumber and chicory. The rice was stored during 24 hours before preparation of the meal. High levels of *Bacillus cereus* (107 cfu/g) containing the gene encoding the emetic toxin could be isolated from leftovers of the meal. Interestingly, the level of cereulide was quantified using LC-MS and was between 0.35-

4.2 µg/g. These concentrations were moderate compared to previously described cases in literature. Though, the detected levels in this outbreak were critical for toddlers whereas one of the adults that also consumed the contaminated meal did not become ill. The lower ingested dose of cereulide per kg body weight can explain the absence of symptoms in the adult.

In 2013, enterotoxinogenic *Clostridium perfringens* was found at levels up to 6 log cfu/g in leftovers of meat stew, that was at the origin of an outbreak that occurred in a residential institution and led to an outbreak that resulted in 70 cases. The pathogenic strain was also isolated from human cases. After preparation, the stew was stored refrigerated for 24 hours and reheated just before consumption. Insufficient cooling of the stew before refrigerated storage probably caused perfect growth conditions for *C. perfringens* to reach such high levels.

## Trends in food-borne outbreaks 2006 - 2013

From 1999, food-borne outbreaks data have been collected at the Institute Public Health (WIV-ISP). Between 1999 and 2010, 39 up to 116 food-borne outbreaks have been reported each year (Figure 70).

In 2011, an increase in the number of reported food-borne outbreaks was observed. The increase was probably due to an adapted Outbreak investigation procedure at the FASFC in 2011 and/or an increased awareness by consumers after the German E. coli O104:H4 outbreak. Since 2011, the number of reported food borne outbreaks remains the same.

Between 1999 and 2013, the number of human cases involved in outbreaks ranged from 500 to 1500, with an exception in 2010 when more than 4000 persons became ill (Figure 71).

In 2010, the number of ill people was much higher compared to other years, which was due to a large water borne outbreak in Flanders where more than 12000 persons were exposed and at least 3000 became ill. The average number of ill persons per outbreak for this period ranged from 4 to 11 human cases per outbreak, except for 2010 where the average was 39.7 ill cases/outbreak as previously explained (Figure 72).

The average number of ill people decreased since 2011, probably because of the increased number of reported outbreaks with a small number of human cases.

For many of the reported outbreaks no causative agent could be identified (Figure 73). An important reason for this is the late notification of the food intoxication or absence of leftovers of the meal in many of those outbreaks. Together with the increase of the number of reported food-borne outbreaks in 2011, the number of food-borne outbreaks for which no samples were sent for analysis was subjected to a 3-fold increase as well.

Causative agents are mainly detected in the outbreaks where a questionnaire was completed and/or a collaboration between the Food Safety Agency and the Health Inspection (FASFC-HI) existed (Figure 74). It is mainly for the so-called complaints that no samples are analysed, mostly because of a late notification by the consumer and when sampling was considered not to be relevant anymore.

Regarding the causative agents, Salmonella together with Norovirus were the most prevalent causative agents in food borne outbreaks between 2006 and 2013 (table 42).

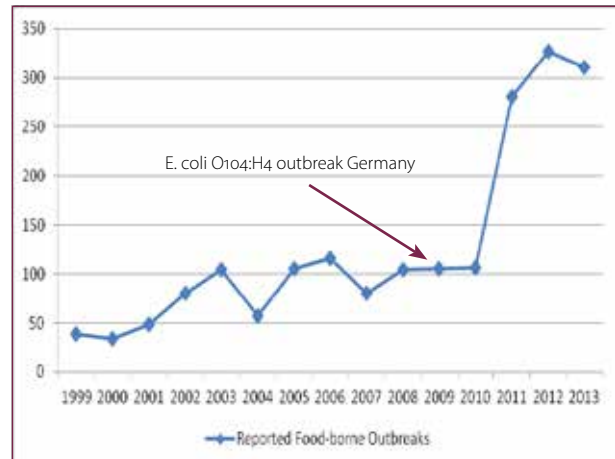


Figure 70. Trends in food borne outbreaks 1999-2013

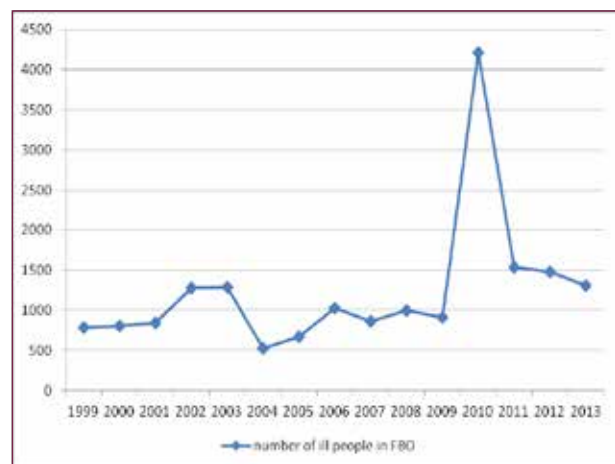


Figure 71. Trends in human cases involved in food borne outbreaks 1999-2013

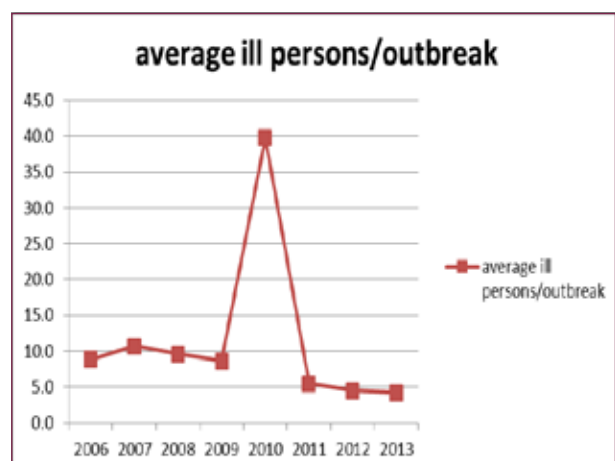
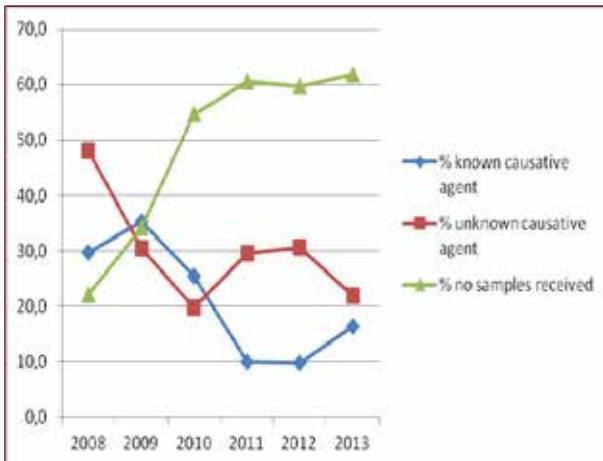
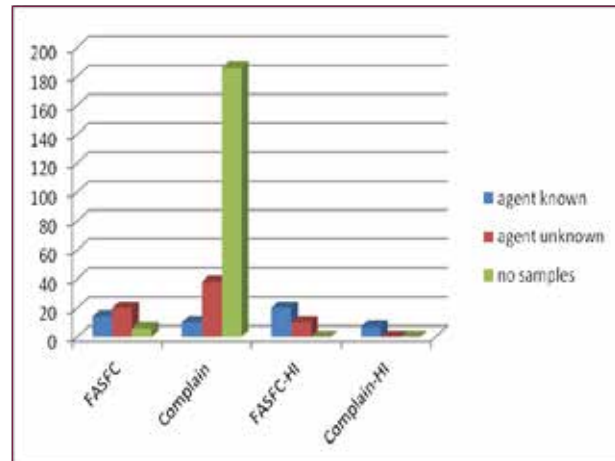


Figure 72. Trends in the average number of human cases per outbreak between 2006-2013



**Figure 73.** Distribution of pathogen detection in food-borne outbreaks 2008-2013



**Figure 74.** Numbers of known agent outbreaks according to the involved actor and investigation effort in 2013 (FASFC: Federal Agency for Safety of the Food Chain; HI: Health Inspection)

**Table 42.** Evolution of number of outbreaks per causative agent and affected persons 2006-2013

agent	FBO (number)									persons affected (Number)							
	2006	2007	2008	2009	2010	2011	2012	2013	2006	2007	2008	2009	2010	2011	2012	2013	
Salmonella	14	8	3	5	5	2	6	10	134	99	39	68	55	7	38	33	
Staphylococcus	7	5	2	2	0	2	2	4	48	69	32	24	0	7	3	59	
Bacillus cereus	6	7	2	4	4	8	2	4	175	57	10	53	88	87	24	30	
Campylobacter	5	2	6	4	3	5	1	9	48	10	31	8	4	103	2	45	
Norovirus	4	10	7	7	7	2	9	1	154	348	439	91	429	13	94	20	
E. coli O157/STEC	1	2	3	1	2	3	3	10	2	16	11	4	6	8	30	41	
Listeria	3	5	1	2	0	1	0	2	3	5	2	4	0	11	0	4	
C. perfringens	0	0	1	4	0	0	0	2	0	0	100	43	0	0	0	88	
other	10	1	6	8	6	5	9	9	32	17	5	27	3058	229	192	45	
no samples			23	36	58	170	195	192			105	169	305	521	544	575	
unknown	66	40	50	32	21	83	100	68	436	230	67	364	137	553	557	372	
<b>Total</b>	<b>116</b>	<b>80</b>	<b>104</b>	<b>105</b>	<b>106</b>	<b>281</b>	<b>327</b>	<b>311</b>	<b>1032</b>	<b>851</b>	<b>841</b>	<b>855</b>	<b>4211</b>	<b>1539</b>	<b>1484</b>	<b>1312</b>	

It is noticed that the number of food-borne outbreaks due to Salmonella decreased after the vaccination strategy for poultry in Belgium since 2006. The number of outbreaks involving Norovirus is important each year and involves each time many human cases per outbreak. In 2010, the number of ill people was much higher compared to other years, which was due to a large water borne outbreak in Flanders.

During 2013, a high number of VTEC/EHEC outbreaks but also Campylobacter outbreaks were reported compared to previous years, implicating an important number of human cases. Other causative agents included Diarrhetic Shellfish Poisoning, Hepatitis A, Giardia, Cryptosporidium, histamine and co-infections implicating more than one causative agent.

## Prevention of food-borne outbreaks

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

## Working group on food-borne outbreaks

The working group was created in 1995 by the Institute of Public Health (WIV-ISP) and brings together, on a voluntary basis, the main actors in the field of foodborne infections and intoxications in Belgium.

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

The group is composed of delegates representing

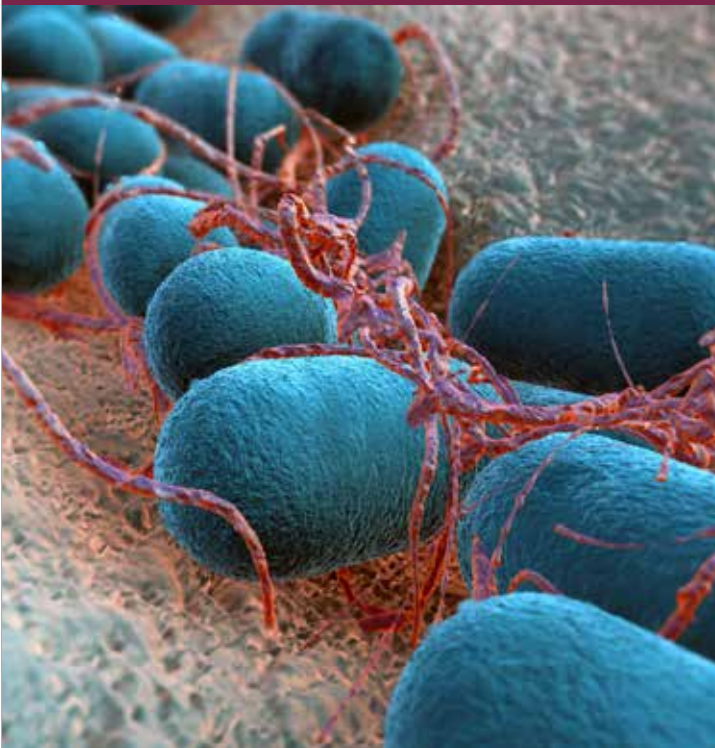
- The FPS - Public Health, Food Chain Safety and Environment,
- The NRL-Foodborne outbreaks (WIV-ISP)
- The NRL Food Microbiology (WIV-ISP)
- The human NRC for Salmonella, Shigella and Listeria
- The Scientific Service Epidemiology of infectious diseases(WIV-ISP)
- The FAVV-AFSCA,
- The Health Inspection Services of the Communities,
- The Brussels Community Coordination Commission,
- The poison Centre,
- The Food microbiology laboratory of the University of Ghent,
- The Food microbiology laboratory at the University of Liège,
- The CODA-CERVA.

The main goals of the working group are to exchange field information on epidemiological and microbiological investigations during outbreaks, on trends and evolutions in both microbiological and legal affairs, on prevention of other outbreaks 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.

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.

# Part II

## Report on antimicrobial resistance



trends and sources 20102- 2013

As already mentioned in the preface, the second part of this Report on Zoonotic agents in Belgium will be for the second time focussed on antimicrobial resistance. This part will mainly deal with the analysis of AMR of *Campylobacter* and *Salmonella* from food in 2012 and 2013 and the analysis of AMR of *Salmonella*, indicator *E. coli* and indicator Enterococci isolates from animals in 2011- 2013 and also on the MRSA surveillance of cattle in 2012 and pigs in 2013.

The antibiotics used for antimicrobial resistance (AMR) or antimicrobial sensitivity testing (AST) and their abbreviations are mentioned in the next table.

**Table 43.** Abbreviations of antibiotics used

Abbreviation	
AMP	Ampicillin
CHL	Chloramphenicol
CIP	Ciprofloxacin
COL	Colistin
FFN	Florfenicol
FOT	Cefotaxime
GEN	Gentamicin
KAN	Kanamycin
NAL	Nalidixic acid
SMX	Sulphonamide
STR	Streptomycin
TAZ	Ceftazidime
TET	Tetracycline
TMP	Trimethoprim

# Antimicrobial resistance of Campylobacter

Nadine Botteldoorn, Sarah Denayer, Katelijne Dierick, Cristina Garcia Graells, Olivier Vandenberg

## AMR of Campylobacter strains isolated from meat and meat products

### Surveillance programme and method used

In 2012 and 2013, 621 and 490 Campylobacter strains respectively, isolated in the frame of the zoonoses monitoring programme and originating from poultry, (carcasses of broilers, filets, entrails, meat preparation and carcasses of spent hens) and pork were sent for antimicrobial susceptibility testing. Of these, 376 in 2012 and 343 in 2013, were actually included for antimicrobial susceptibility testing. A non-negligible number of strains were lost because of several reasons intrinsically related to the fragility of this organisms (inability to resuscitate the strain or contamination upon arrival), and administrative reasons such as incomplete information of the isolates upon arrival (mainly source of isolation).

The number of the isolates tested for AST per matrix is indicated in the table below.

Matrix	2012	2013
Poultry (n)	344	289
Pork (n)	32	54

In poultry, 344 and 289 strains were isolated in 2012 and 2013 respectively, which included broiler meat or carcasses and spent hens. In poultry meat, *C. jejuni* was the dominant species in both analysed years (242 out of 344 (70.3 %), in 2012 and 233 out of 289 (80.62%) in 2013), followed by *C. coli* (102 out of 344 (29.6 %), in 2012 and 56 out of 289 (19.37%) in 2013).

Thirty two and fifty four, of the analysed strains were isolated from pork meat or carcasses in 2012 and 2013 respectively. *C. coli* was, except for one isolate, the only species found in pork carcasses in 2013.

Minimum Inhibitory Concentrations (MIC) were determined by using broth microdilution method (Sensititre EUCAMP panel). From 2014, a new European Decision on the harmonization of the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria is adopted which specifies new interpretative threshold for resistance for *C. jejuni* and *C. coli*. Therefore, the antimicrobials tested and the epidemio-

logical cut-off values (ECOFF) used are listed in the table 44 below following the official Journal of the European Union (L303/26 14.11.2013). The European Decision has not included the monitoring of chloramphenicol any more. In order to compared results in an accurate way, recalculation of resistance to the antibiotics using the new breakpoint established in the European decision was done for the last four years, from 2010 to 2013 included.

**Table 44.** *Campylobacter* in meat and meat products: list of antimicrobials tested and breakpoints used.

Antimicrobial	Breakpoints (µg / ml)	
	<i>C. jejuni</i>	<i>C. coli</i>
Chloramphenicol	16*	16*
Tetracycline	1	2
Nalidixic acid	16	16
Ciprofloxacin	0.5	0.5
Erythromycin	4	8
Gentamicin	2	2
Streptomycin	4	4

\* Breakpoint value of 2012

Data from 2012 and 2013 were analysed according to the new breakpoints established in the European Decision.

The term multi-resistance is used for isolates with phenotypes identified as resistant to three or more antimicrobial classes. This implies for example, that resistance to ciprofloxacin and nalidixic acid represents resistance to one class of antimicrobials.

The percentage of resistant strains of Campylobacter in food during 2012 and 2013 is reported in the table 45. Resistance varied according to the Campylobacter species and the matrices. For those isolates recovered from poultry meat, the highest resistance detected in *C. jejuni* and *C. coli* was to tetracycline and quinolones while in *C. coli* isolated from pork the highest resistance detected was for tetracycline followed by streptomycin and quinolones. A detailed description of the resistance for each matrices is done in the following sections.

**Table 45.** Antimicrobial susceptibility testing of *Campylobacter* in food: Percentage of resistant strains

	Poultry meat				Pork	
	2012	2013	2012	2013	2012	2013
	<i>C. jejuni</i> (n=242)	<i>C. jejuni</i> (n=233)	<i>C.coli</i> (n=102)	<i>C.coli</i> (n=56)	<i>C.coli</i> (n=32)	<i>C.coli</i> (n=54)
Chloramphenicol	0	0.85	0	0	0	1.85
Tetracycline	50	39	71	71	87	82
Ciprofloxacin	45	39	71	62	43	48
Nalidixic acid	44	39	71	62	43	50
Gentamicin	0	0	3	0	3	0
Erythromycin	1	1.71	20	12	16	16
Streptomycin	0.82	1.71	22	14	78	60

## Antimicrobial resistance in *Campylobacter* from poultry meat

In 2012 and 2013, 344 and 289 *Campylobacter* strains respectively, were isolated in poultry meat and carcasses and tested for antimicrobial susceptibility.

Overall antibiotic resistance was more prevalent in *C. coli* than in *C. jejuni*, with only 16 and 17% (17 out of 102 in 2012, and 10 out of 56 in 2013 respectively) of *C. coli* strains sensitive to all antibiotics, similar to 2011. The number of *C. coli* multi-resistant strains, resistant to 3 or more classes of antibiotics was 30.39% in 2012 and decreased to 14.28% in 2013. The most common phenotype found was resistance to Cip-Nal-Tet (32 out of 102 in 2012 and 23 out of 56 in 2013). For the multi-resistance phenotype the most predominant one was Cip-Nal-Ery-Tet (11 out of 102 in 2012 and 2 out of 56 in 2013). A high resistance was observed in 2012 and 2013 for tetracycline (71%), ciprofloxacin (71-62%) and nalidixic acid (71-62%), very similar to values found in 2011. To mention the increase observed for nalidixic acid in 2012 and 2013, which was 18% and 9% higher respectively, than in 2011 (Fig 75).

For *C. jejuni*, 38% and 44% of all strains were sensitive to all antibiotics tested, in 2012 and 2013 respectively. The multi-resistance found in 2012 and 2013 was very low 2.48 and 1.28% respectively. Although the resistance against tetracycline and quinolones remained high, a decreased was found in 2013 respected to 2012, tetracycline (39% vs 50%), ciprofloxacin (39% vs 45%) and nalidixic acid (39% vs 44%). Resistance to streptomycin decreased from 5% in 2011 to below 2% in 2012 and 2013 (Fig 75). Similar to *C. coli* the resistance phenotype most commonly found in *C. jejuni* was Cip-Nal-Tet (74 out of 242 in 2012 and 52 out of 233 in 2013)

The overall resistance of *Campylobacter* is detailed in the following tables 46 and 47.

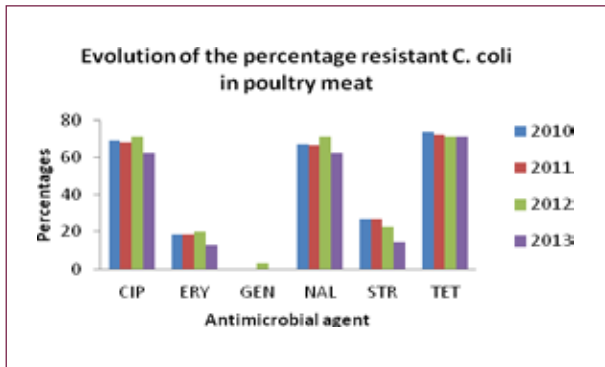
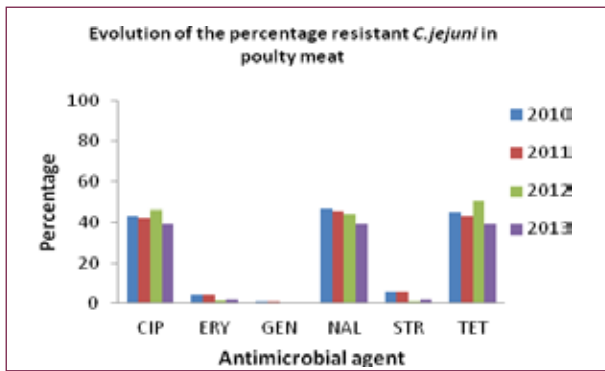
**Table 46.** *C. jejuni* in poultry meat

	2012		2013	
	n	%	n	%
sensible	92	38.02	102	43.77
1	63	26.03	72	30.90
2	81	33.47	56	24.03
3	4	1.65	3	1.28
4	2	0.83	0	0
5	0	0	0	0

**Table 47.** *C. coli* in poultry meat

	2012		2013	
	n	%	n	%
sensible	17	16.67	10	17.86
1	16	15.69	12	21.43
2	38	37.25	26	46.43
3	25	24.51	6	10.71
4	6	5.88	2	3.57
5	0	0	0	0

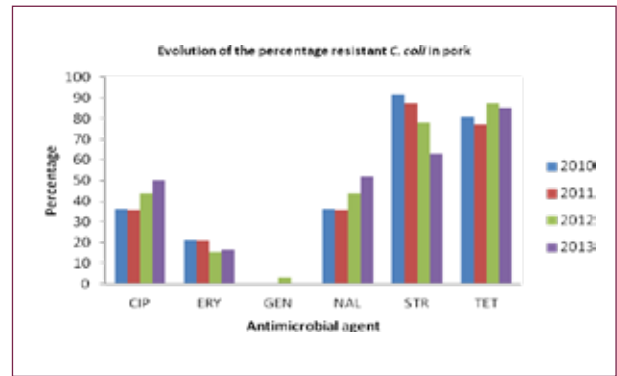
In the following graph (Figure 75) the trend in resistance is given for *Campylobacter* strains isolated from poultry during the last 4 years. The trends in antimicrobial resistance are stable since 2010, no resistance against gentamicin has been observed and very low resistance against erythromycin followed by streptomycin. The highest resistance found is, as previous years, against tetracycline, followed closely by ciprofloxacin and nalidixic acid.



**Figure 75.** Evolution of the percentage of antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry meat

**Table 48.** *C. coli* in pork

	2012		2013	
	n	%	n	%
sensible	1	3.13	3	5.56
1	3	9.38	7	12.96
2	15	46.88	26	48.15
3	10	31.25	14	25.93
4	3	9.38	4	7.41
5	0	0.00	0	0



**Figure 76.** Evolution of the percentage of antimicrobial resistance of *Campylobacter coli* isolates from pork meat

## Antimicrobial resistance in *Campylobacter* from pork

In total, 32 and 54 *Campylobacter* isolates were analysed, in 2012 and 2013 respectively, which belonged to *C. coli*.

The number of isolates that were sensitive to all tested antibiotics was still low, 3-5% which is however an increase compared to last year (2%). The highest level of resistance was found for tetracycline, 87-82% of the isolates in 2012-2013 were resistant. The resistance against streptomycin decreased (60%) in 2013 compared to the very high resistance found between 2010-2012 (91-78%). Resistance to quinolones continued to increase for both ciprofloxacin and nalidixic acid, in comparison with the period 2010-2011.

Related to the isolates from pork, the number of susceptible isolates to all antibiotics was still very low, corresponding to 3.1-5.5% for 2012 and 2013. Also the multidrug resistance, resistance to three or more classes of antibiotics was higher in 2012 (40.63%) than in 2013 (33.34%). The resistance more frequently found in isolates from pork in 2012 and 2013 were Str-Tet (13/32 and 14/54), followed by Cip-Nal-Tet (3/32 and 8/54)

## AMR of *Campylobacter* strains isolated from humans

### Methods used

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

### Results of 2001-2013 surveillance

The results of resistance to ampicillin, erythromycin and ciprofloxacin are presented in Tables 49 to 52. These results point out the need to monitor antibiotic resistance in *Campylobacter* from human and food.

**Table 49.** Resistance of *Campylobacter* in Belgium human fecal isolates, trend from 2001 till 2013, based on data from the NRL (Laboratory for Microbiology, St.-Peter University Hospital, Brussels)

Antimicrobial agent	2001 N = 280 % resistant	2002 N = 266 % resistant	2003 N = 212 % resistant	2004 N = 291 % resistant	2005 N = 260 % resistant	2006 N = 246 % resistant	2007 N = 263 % resistant	2008 N = 284 % resistant	2009 N = 327 % resistant	2010 N = 354 % resistant	2011 N = 174 % resistant	2012 N = 184 % resistant	2013 N = 196 % resistant
Ampicillin	13,9	15,8	16,0	29,2	32,3	51,6	57,4	50,7	50,2	57,6	84,48	59,78	50,24
Erythromycin	3,2	0,0	9,4	6,5	6,9	8,1	5,2	3,5	2,4	3,1	7,47	3,8	2,9
Ciprofloxacin	18,9	22,6	24,5	28,1	33,1	50,4	49,8	40,8	37,6	52,5	59,19	63,59	58,45

**Table 50.** CHU Saint-Pierre data 2011 on *Campylobacter* antibiotic resistance (activity of CHU Saint-Pierre and Jules Bordet Institute):

Microorganism		AMP (S)	AMC (S)	ERY (S)	CIP (S)
<i>Campylobacter jejuni</i>	%	14	79	95	43
	n	23	128	154	69
	<b>Total</b>	<b>162</b>			
<i>Campylobacter coli</i>	%	33	75	58	17
	n	4	9	7	2
	<b>Total</b>	<b>12</b>			

**Table 51.** CHU Saint-Pierre data for 2012 on *Campylobacter* antibiotic resistance.

Microorganism		AMP (S)	AMC (S)	ERY (S)	CIP (S)
<i>Campylobacter jejuni</i>	%	37	98	97	38
	n	62	164	162	63
	<b>Total</b>	<b>167</b>			
<i>Campylobacter coli</i>	%	71	94	88	24
	n	12	16	15	4
	<b>Total</b>	<b>17</b>			

**Table 52.** CHU Saint-Pierre data for 2013 on *Campylobacter* antibiotic resistance.

Microorganism		AMP (S)	AMC (S)	ERY (S)	CIP (S)
<i>Campylobacter jejuni</i>	%	43,3	96,6	96,6	42
	n	78	175	178	74
	<b>Total</b>	<b>180</b>			
<i>Campylobacter coli</i>	%	43,75	87,5	87,5	31,25
	n	7	14	14	5
	<b>Total</b>	<b>16</b>			

# Antimicrobial resistance of Salmonella

## AMR of Salmonella strains isolated from food

Nadine Botteldoorn, Sarah Denayer, Katelijne Dierick, Cristina Garcia Graells

### AMR of Salmonella strains isolated from meat and meat products

#### Surveillance programme and method used

During 2012 and 2013 respectively 286 and 331 strains of *Salmonella enterica* isolated during the zoonosis monitoring program were sent to the Scientific Institute of Public Health for serotyping and determination of antimicrobial resistance. Different food matrices were sampled, mainly poultry (carcasses from broilers and spent hens, chicken parts and meat preparations) and pork (carcasses and meat cuts). Other matrices where *Salmonella* was isolated were ready-to-eat meals, meat, meat preparations, frog's legs, pudding, liquid egg product, ham and dry sausage. Since 2011, the AMR was performed only on the ten most prevalent serotypes.

Minimum Inhibitory Concentrations (MIC) were determined by the broth dilution method using Sensititre EUMVS2 panel, as recommended by the EU RL antimicrobial resistance. Quality control was performed by using an *Escherichia coli* ATCC 25922 strain. The antimicrobials reported are listed in the table 53 and were adapted to the new European Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/653/EU). The breakpoints used for the interpretation of the results were adapted to the EU Decision as well and results were recalculated retrospectively since 2010 based on the new breakpoints. From 2014 on, the EU Directive does not request the monitoring of resistance of *Salmonella* to florfenicol, kanamycin and streptomycin, so we are now in a transitional period. In this report, we interpreted these results according to CLSI and using epidemiological cut-off values from EUCAST 2012. Similarly, no cut-off value has been established in the new Decision for sulfamethoxazole. Therefore the results represented here were interpreted according to the breakpoint of EUCAST 2012.

**Table 53.** *Salmonella* from food: list of antimicrobials tested with their breakpoints

	ECOFF* (R> mg/l)
Ampicillin	8
Cefotaxim	0.5
ceftazidim	2
Nalidixic acid	16
Ciprofloxacin	0.064
Tetracycline	8
Colistin	2
Florfenicol	16**
Gentamycin	2
Kanamycin	8**
Streptomycin	32**
Trimethoprim	2
Sulfamethoxazole	ND***
Chloramphenicol	16

\*EUCAST Epidemiological cut-off values. Following the EU decision of 14.11.2013

\*\* Interpretation was according to CLSI and using epidemiological cut-off values from EUCAST 2012.

\*\*\* cut-off R> 256mg/l according to EUCAST 2012

The level of resistance of *Salmonella* isolates from poultry and pork differs depending on antibiotic classes. Details of resistance is shown in the following table 54, for each single antibiotic in both years. In general in poultry, the resistance for ampicillin, cephalosporin's, chloramphenicol, gentamycin and tetracycline remains as previous years. However, a progressively increasing resistance was noticed for the quinolones, streptomycin and trimethoprim over the period 2012-2013. Of note was the increased resistance to kanamycin, from 1% in 2012 to 15% in 2013. In pork the highest resistance was observed for sulfamethoxazole and ampicillin in 2012 and 2013 followed by streptomycin, tetracycline and trimethoprim. More detailed descriptions for each particular matrix can be found in the next section.

**Table 54.** Percentage of resistance in *Salmonella* isolates from poultry and pork in 2012-2013

	Poultry		Pork	
	2012 % (n=91)	2013 % (n=99)	2012 % (n=157)	2013 % (n=139)
Ampicilline	23.08	22.22	51.59	39.57
Cefotaxime	3.30	3.03	1.91	0.00
Ceftazidime	3.30	3.03	1.91	0.00
Chloramphenicol	1.10	2.02	10.19	5.04
Ciprofloxacin	21.98	28.28	1.27	2.88
Colistin	26.37	15.15	4.46	0.72
Florfenicol	0.00	1.01	3.82	2.15
Gentamicin	0.00	0.00	0	0.71
Kanamycin	1.09	15.15	3	1.43
Nalidixic acid	21.97	28.28	2	2.15
Streptomycin	5.49	22.22	47.77	31.65
Sulfamethoxazole	13.18	30.30	54.14	41
Tetracycline	3.29	5.05	40.76	34.53
Trimethoprim	24.17	40.4	25.47	35

The serotype distribution varies depending on the matrix. The detailed distribution is shown in the table 55. *Salmonella* Typhimurium was the most prevalent serotype in pork (105 out of 157, 67% in 2012 and 60 out of 139, 43.16% in 2013). On poultry, *Salmonella* Enteritidis (44 out of 91, 48.35% in 2012 and 46 out of 99, 46.46% in 2013) was the predominant one, followed by *Salmonella* Paratyphi B var Java (18 out of 91, 19.78% in 2012 and 22 out of 99, 22.22% in 2013) and *Salmonella* Infantis (10 out of 91, 10.98% in 2012 and 10 out of 99, 10.10% in 2013).

**Table 55.** Serotype distribution with the number of *Salmonella* isolated from poultry and pork

	Poultry		Pork	
	2012 (n=91)	2013 (n=99)	2012 (n=157)	2013 (n=139)
Typhimurium*	7	1	105	60
Derby	2	1	20	34
Enteritidis	44	46	5	0
Newport	0	0	0	0
Infantis	10	10	0	6
Agona	6	1	0	1
Brandenburg	0	1	15	11
Paratyphi B	3	15	0	0
Paratyphi B var Java	18	22	0	0
Mbandaka	0	1	1	3
Livingstone	0	0	8	4
others	1	1	3	20**

\*included Typhimurium, Typhimurium 1.4.[5].12:1-, Typhimurium var. O:5-, Typhimurium var Copenhagen

\*\* subspecies I (enterica) I 4:l, Subspecies I

The percentage of isolates recovered from poultry and pork resistant to one or more antibiotic classes are summarized in the table 56. In general, the susceptibility to all antibiotics in poultry has slightly decreased from 43% in 2012 to 33.3% in 2013. In pork, however an increase of 7% of susceptibility to all antibiotics tested was found in 2013 compared to 2012. The resistance to three or more antibiotics increased in poultry, and in 2013, 38% of the isolates were multidrug resistant, being Amp-Cip-Nal-Tmp and Kan-Str-Tet-Tmp, the most common phenotypes. In pork, an important decrease in multidrug resistance was observed in 2013. The predominant phenotypes detected were Amp-Str-Smx-Tet and Amp-Str-Smx-Tet-Tmp.

**Table 56.** The percentage of *Salmonella* isolates which are susceptible and multi resistant from the different food matrices.

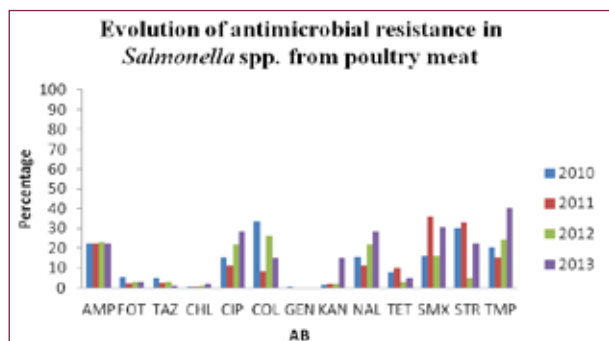
	Poultry		Pork	
	2012	2013	2012	2013
Susceptible	42.86	33.33	31.21	38.13
1	27.47	21.21	14.01	19.42
2	3.30	7.07	2.55	6.47
>3	26.37	38.38	52.23	35.97

## AMR of *Salmonella* strains isolated from poultry meat

Antimicrobial resistance in strains isolated from poultry meat

In 2012 and 2013, 91 and 99 *Salmonella* isolates respectively, from poultry meats were tested for their antimicrobial susceptibility (Figure 77). In 2012 a total of 43% were susceptible to all tested antimicrobials, which was comparable to 2011. However in 2013 this trend decreased and only 33.3% were susceptible to all antibiotics tested. Some variations have been observed the last year. While resistance to ampicillin, gentamicin, cefotaxime, ceftazidime, chloramphenicol remained stable as in 2011, resistance to ciprofloxacin, nalidixic acid, colistin and trimethoprim has increased strongly in 2012 and remain high in 2013. Except for colistin a decreased of 10% has been noticed in 2013. Of note is the high resistance to trimethoprim in 2013, it increased 16% since 2012 and compared to 2011 with 25%. A remarkable increase of resistance was found for the quinolones, ciprofloxacin and nalidixic acid, over the last two years (22% and 28% vs. 11% in 2011). An important fact observed was the high increase in kanamycin resistance (15.15%) observed in 2013 compared to a susceptible phenotype in the previous years. The resistance was almost only detected in the *Salmonella* Paratyphi B and Paratyphi B var Java serotypes. Of the in total 37 Paratyphi

B isolates, 15 were resistant to kanamycin (40.54%). For colistin an increase was noticed in 2012 compared to 2011 (26% vs. 8%) however in 2013 resistance decreased to 15%. The strains resistant to ciprofloxacin were isolated mainly from broilers and poultry meat products and belonged to the serotype Paratyphi B. The resistant strains to colistin were isolated mainly from spent hens and belonged to the serotype Enteritidis in line with the results reported in 2011.



**Figure 77.** Evolution of the last four years of the percentage of antimicrobial resistance in *Salmonella* isolates from poultry meat

In 2012 and 2013, 44 and 46, respectively *Salmonella* Enteritidis isolates, were analysed for their antibiotic susceptibility. This serotype showed very low level of resistance, 43% and 61% of the isolates were susceptible to all antibiotics tested in 2012 and 2013 respectively. None of the isolates except one in 2013 showed resistance to three or more antibiotic classes during the last two years. Resistance to colistin was the only exception worthy to mention. Although in 2012 an increase of resistant isolates up to 50% was noticed, in 2013 only 32% of the isolates tested were resistant to this antibiotic reaching again the same level as in 2011.

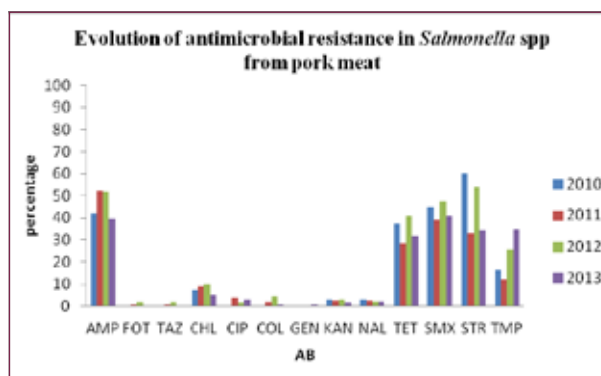
In total, 21 and 37 *Salmonella* Paratyphi B (including Paratyphi B var Java) isolates were recovered in 2012 and 2013, respectively, from poultry-derived food products and were tested for their antibiotic susceptibility. In 2012 and 2013, this serotype showed a strong resistance to three or more antibiotics, 83.33% and 92% were multidrug resistance, respectively and none of the isolates were susceptible to any antibiotic tested. In 2012, 76% of the isolates were resistant to ampicillin, ciprofloxacin, nalidixic acid and trimethoprim. Together with those resistances, two isolates were resistant to cephalosporins as well, cefotaxime and ceftazidime, which represent 9% of the isolates in total. Those isolates were recovered from poultry meat preparations. In 2013, resistance to ampicillin, ciprofloxacin and nalidixic acid decreased to 46% and 54% respectively, whereas the trimethoprim resistance reached 100%. In addition, three

isolates (8%) were resistant to all the antibiotics mentioned before together with cefotaxime and/or ceftazidime. All these three *Salmonella* Paratyphi B strains were isolated from poultry skin. As we mentioned in the previous paragraph, resistance to kanamycin has strongly increased in 2013, up to 40% for this particular serotype. It is a pity it is no longer recommended to analyse the resistance from 2014 on.

### AMR of *Salmonella* strains isolated from pork

In total 157 and 139 *Salmonella* strains, isolated in 2012 and 2013 respectively, from pork were tested for their antimicrobial susceptibility (Figure 78).

This includes strains from carcasses and meat cuts. The resistance to cefotaxime, ceftazidime, and gentamycin remains below 2% since 2010. Resistance to quinolones varies between 1-3% and remains stable since 2010 without important variations. However, resistance to ampicillin has decreased from 52% in 2011 and 2012 to 40% in 2013. Unlikely, tetracycline resistance increased in 2012 from 28% in 2011 to 41% in 2012 and remains 36% in 2013. This represents an increase of 13% and a subsequent decrease of 4% compared to the previous years. For trimethoprim an important increase of 13% was observed in 2012 vs 2011 and remains stable in 2013, 26% of the isolates were resistant to this antibiotic. The percentage of strains susceptible to all antibiotics was 38% which represents an increase of 7% compared to 2012. The resistance of the isolates to three or more antibiotics decreased to 35.97% in 2013 vs 52.23% in 2012.



**Figure 78.** Evolution during the last 4 years of the percentage of antimicrobial resistance in *Salmonella* isolates from pork meat

In total, 105 and 60 *Salmonella* Typhimurium were analysed for antimicrobial susceptibility in 2012 and 2013 respectively. The highest resistance was found for ampicillin, followed by tetracycline and trimethoprim. Resistance to ampicillin decreased

from 73% in 2012 to 62% in 2013. Resistance to tetracycline had some fluctuations in the last two years, from 2011 to 2012 the resistance increased from 42% to 53%, however in 2013 a slight decrease was observed from 52% to 45%. The level of resistance to trimethoprim from the last two years remains stable, (31%) however a remarkable increase of 15% was noticed in 2012 vs 2011.

Of note was the decrease in the number of isolates resistant to three or more antibiotics in 2013 48.33% vs 68.57% in 2012. The number of isolates susceptible to all tested antibiotics increased in 2013, 20% vs 14.28% in 2012.

## AMR of Salmonella strains of animals

Patrick Butaye, Hein Imberechts, Vicky Jasson

Salmonella is one of the most important bacterial zoonotic agents. Though in general, infections in humans do not require treatment, in some severe cases it may be lifesaving. Therefore, surveillance for resistance in Salmonella from animals is of importance. Besides direct resistance transfer, indirect resistance transfer may occur: resistance genes on mobile genetic elements may be transmitted from Salmonella to commensal human bacteria and eventually to human pathogens. Salmonella may also cause health problems in animals as has been shown in bovines and to a lesser extent in pigs.

In general, all Salmonella strains that were transferred to CODA/CERVA for serotyping in the NRL Salmonella for animal health were passed on to the NRL Antimicrobial Resistance for susceptibility testing. Strains from bovines were mainly obtained from ARSIA and were from infections. Care was taken to include in the analyses only one strain per farm/animal/other per sampling point. An official epidemiological surveillance programme was only established for poultry in 2012 and 2013. Strains were plated on blood agar, suspended colonies were inoculated in buffered broth and susceptibility was tested using a micro broth dilution method (Trek Diagnostics). The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected.

Detailed reports of the Salmonella surveillance programmes in 2012 and 2013 can be found on [www.coda-cerva.be](http://www.coda-cerva.be).

### Results

The results on all Salmonella isolated (Table 57) largely depend on the prevalence of the different serotypes. Some serotypes do not seem to acquire resistance easily, as opposed to others. Most resistance was found against sulphonamides (40%) and ampicillin (34.7%). Slightly lower is streptomycin resistance and significantly lower are the other antibiotics. Resistance against trimethoprim and tetracycline is approximately 30%, while

resistance against chloramphenicol, ciprofloxacin and nalidixic acid is between 10 and 20% (respectively 11%, 18% and 15.5%). High level of ciprofloxacin resistance (MIC > 4 µg/ml) is seen in 1 strain only. Other resistances are below 5% except for colistin resistance which was approximately 7%. It should be noted however that some serotypes have a natural lower susceptibility for this antibiotic (such as *S. Enteritidis* and *S. Dublin*) and the high prevalence should be abstracted from the prevalence of these serotypes in the sample. The more *S. Enteritidis* and *S. Dublin* present, the higher the prevalence of resistance against this antibiotic.

The combination of sulphonamide and trimethoprim resistance, as calculated from the data obtained by the separate compounds was 23.8%, which is quite similar to what is obtained with trimethoprim (29.5%).

The number of cephalosporin resistance strains is much lower than before with only 24 strains being resistant, most of them originating from poultry and some from pigs. One strain was isolated from feed. The serotype most involved was *S. Paratyphi B* (16 strains). Two *S. Typhimurium* and two monophasic variant isolates were also cephalosporin resistant. The other serotypes involved were *S. Enteritidis*, *S. Anatum*, *S. Derby* and *S. Agona* (one strain each).

High level ciprofloxacin resistance (MIC > 4 µg/ml) is limited to one strain. Twenty-six strains were resistant to ciprofloxacin while nalidixic acid susceptible. This may be explained by the presence of plasmid mediated quinolone resistance. Frequently, the plasmids on which these genes are located are carrying multiple resistances. In this sample this was not evident. A large diversity of serotypes was involved of which *S. Typhimurium* and its monophasic variant were the most frequent encountered serotypes. Further studies on this resistance should bring more clarity into the situation.

Chloramphenicol resistance remains relatively high, with approximately 11% of the strains being resistant. It should be noted that florfenicol resistance was at a little more than 3% and this type of resistance gives cross resistance to chloramphenicol. However, 8% is still attributed to the "old gene". Florfenicol resistance is found mainly in *S. Typhimurium* and its monophasic variant which can be due to the presence of the Salmonella Genomic Island. Aminoglycoside resistance, with the exception of streptomycin, remains low.

As for antibiotic resistance per serotype, *S. Typhimurium*, *S. Paratyphi B* and *S. O4,[5],12:i:-* are amongst the most resistant serotypes with highest resistance against ampicillin and by large also against sulphonamides, streptomycin and tetracyclines. Striking is the difference for resistance against (fluoro)quinolones and trimethoprim which are extremely high in *S. Paratyphi B*. Cephalosporin resistance in *S. Typhimurium* was a little more than 1%, which is substantially lower than in 2012. Comparing *S. Typhimurium* and *S. O4,[5],12:i:-*, resistance in the monophasic *S. Typhimurium* is higher. Striking is the resistance to ciprofloxacin and nalidixic acid in *S. Paratyphi B*. The clonal spread of this type of resistance shows that it is very well integrated in the population and that it will only disappear by eliminating the strains rather than by other means. It should be noted however that none of the strains had high level of quinolone resistance (MICs were  $\leq 2 \mu\text{g/ml}$ ) indicating that there are no strains having the two typical mutations associated with high level resistance. The higher number of resistance against ciprofloxacin compared to nalidixic acid may be explained by the presence of plasmid mediated quinolone resistance. Also trimethoprim resistance is extremely high; all but two strains were resistant. It is also the serotype in which cephalosporin resistance is highest.

While in 2012 *S. Minnesota* was quite a prevalent serotype, this was not the case in 2013. *S. Derby* is now the third most prevalent serotype. Contrary to *S. Minnesota*, *S. Derby* originates mainly from pigs. This serotype is quite susceptible to most antibiotics with the notable exceptions of sulphonamides and streptomycin. Of notice is also the rise of *S. Livingstone*, also associated with both poultry and pigs. It is already the third consecutive year that this serotype increases in number. Resistance in this serotype is of a similar level as *S. Enteritidis*: very few resistances are observed. It should be noted that the high level of colistin resistance in *S. Enteritidis* as well as in *S. Dublin* is because their normal susceptibility towards this antibiotic is lower than for other Salmonella serotypes; as such this should not be regarded as acquired resistance. In *S. Infantis*, cephalosporin resistance has disappeared completely.

The antibiotic resistance of Salmonella isolates is determined by the spread of the serotypes within the animal species. In poultry there is a Salmonella control programme based on isolation. In pigs, the second largest group, control programmes are mainly based on serology. Resistance is highest in strains from pigs, though this may be explained mainly by the fact that the serotypes present in pigs are the ones most associated with resistance (*S. Typhimurium* and monophasic *S. Typhimurium*). The significantly higher resistance against fluoroquinolones in poultry is to be noted. Also cephalosporin resistance is higher in poultry, albeit non-significantly. Few strains are from pigeons and cattle and these are mainly isolated for diagnostic reasons.

Overall, more than 40% of the Salmonella strains remained susceptible to all antibiotics tested (Table 58), similar to what has been found in earlier years. Multi-resistance (defined as resistant to 3 or more antibiotics) was observed in approximately 35% of the strains. Taking into account the *S. Enteritidis* and *S. Dublin* strains should not be regarded as resistant, 45.9% of the strains are fully susceptible, but the multi-resistance did not change (due to the fact that this was mostly the only resistance in these strains). The isolate resistant to eleven of the antibiotics tested was a monophasic *S. Typhimurium* and of the 4 strains resistant to 10 antibiotics, 3 were *S. Paratyphi B* and one was a *S. Typhimurium O5-*. Typically these strains were susceptible to colistin. The 12 strains resistant to 9 antibiotics were all but two *S. Paratyphi B* and all were susceptible to gentamicin. One strain was colistin resistant and most were resistant to cephalosporins. Most of these strains were from poultry origin.

### Comparison between resistance data from 2011, 2012 and 2013

Table 59 summarizes the data of overall resistance observed in Salmonella from 2011 to 2013. In 2013 the number of strains analysed has decreased by approximately 20%. Seen also the differences in prevalence of serotypes amongst the tested strains, the table should be interpreted with care. Generally resistance prevalence remained the same. The difference seen for colistin may be explained by the fact that in 2012 quite more *S. Dublin* strains were tested. The decline in cephalosporin resistance may be explained by the fact that fewer *S. Paratyphi B* strains were tested. The large variability in (fluoro)quinolone resistance may be influenced by the fact that this resistance is not transferable and spreads clonally. The large changes in the population of Salmonella present, as well as the fact that the sample that each year is different (as demonstrated by the multitude of serotypes and different resistance profiles), may cause such variations.

As for the resistance data in the most frequently isolated serotypes from 2011 to 2013, numbers of strains tested are sometimes low causing large confidence intervals and complicating the interpretation of the results. There are also large variations between years, especially for *S. Derby* of which the numbers tested increased substantially in 2013 and *S. Livingstone* and monophasic *S. Typhimurium* that are gradually increasing since 2011. Serotypes that are decreasing are *S. Paratyphi B*, *S. Rissen*, *S. Minnesota* and *S. Typhimurium*. Some striking features were the disappearance of fluoroquinolone resistance in *S. Enteritidis*, indicating that other clones are present in the population. In this serotype also, the resistance against cephalosporins has diminished albeit non-significantly ( $p = 0.0732$ ). Also other resistances seem to have diminished albeit also not significantly. In *S. Infantis*, fluoroquinolone resistance seems to increase. This may be due to the spread of a specific clone, as has been shown before for *S. Infantis*. The numbers of *S. Livingstone* increased over the years, but resistance in this serotype remains low. The numbers of *S. Minnesota* dropped substantially and this does not allow to observe eventual differences in resistance prevalence in this species. Looking at the data of *S. Paratyphi B* where the number of strains dropped dramatically in 2013, there are quite some differences. Resistance against ampicillin dropped substantially concomitant with cephalosporin resistance (for the latter, there was no significant difference). Chloramphenicol resistance increased from 2% to nearly 37%. This increase was not caused by an increase in florfenicol resistance (this resistance is cross resistant with chloramphenicol resistance, but the other way round is not true). It should be noted that this antibiotic is not used since a long time, and thus there should be a co-resistance selection involved. This was not clear from the database. However, it should be noted that strains resistant to chloramphenicol are in general resistant to at least 4 different antibiotics. Also streptomycin and tetracycline resistance increased over the 3 years. Trimethoprim resistance remains at an extremely high prevalence with nearly all the strains being resistant. The number of monophasic *S. Typhimurium* nearly doubled in the last 3 years. There are no striking events, resistance remains high and the differences seen are not significant. Contrary to the monophasic *S. Typhimurium*, *S. Typhimurium* (both O5+ and O5-) decreased; also in these serotypes resistance remained largely the same with only non-significant differences.

Looking at the differences according to the animal species (data not shown),  $\beta$ -lactam resistance in strains from poultry decreased while chloramphenicol resistance increased. This is mainly caused by the evolution of *S. Paratyphi B*. The evolution of colistin resistance should be disregarded since this is mainly caused by the proportion of *S. Enteritidis*, which is intrinsically less susceptible to colistin. No striking other evolutions were registered. As in poultry, ampicillin resistance declined in pigs but this was without an increase of chloramphenicol resistance. The number of strains from bovines is too low to make any conclusion. Strains from pigeons, albeit the numbers are also low, remain susceptible to most antibiotics. The strains from feed, important since they can bring in new resistance genes in a stable, are at the low side with no obvious evolution apart from a decrease in ampicillin resistance.

### Conclusions of the report

The analysis of the evolution of resistance in *Salmonella* is hampered by the distribution of the various serotypes among the animal populations over time. There are some major trends in prevalence of the different serotypes which influences the overall analysis of the data.

Overall, it seems that  $\beta$ -lactam resistance has diminished, which may be due to the fact that cephalosporin use in poultry has stopped. On the other hand, this type of resistance reduced also among pig strains. Striking is that  $\beta$ -lactam resistance reduction in poultry was concomitant with an increase in chloramphenicol resistance, an antibiotic not used anymore for more than 20 years. This was not observed in pigs.

The highest levels of resistance were observed in strains from poultry and pigs, though from other animal species few strains were tested and care should be taken with the interpretation.

The interaction of the different resistances and the complex epidemiology of the different serotypes make the analysis very complicated and a better interpretation of the data can only be obtained by more in depth molecular epidemiological studies.

**Table 57.** Antimicrobial susceptibility of *Salmonella* serotypes of all origins, data 2013

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	STR	TAZ	TET	TMP
<=0.008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	170	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	553	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	40	0	0	384	0	0	0	0	0	0	0	0
0.12	0	0	12	0	0	447	0	0	0	0	0	0	0	0
0.25	0	0	72	0	0	67	261	0	0	0	0	442	0	0
0.5	2	0	55	0	0	9	627	0	0	0	0	445	0	640
1	326	0	21	0	0	0	32	0	0	0	0	19	73	14
2	262	10	7	863	30	0	1	0	0	0	21	1	550	2
4	18	110	0	51	489	0	1	875	713	0	61	2	28	0
8	1	640	1	17	328	24	1	12	56	6	325	3	9	2
16	1	69	0	0	54	0	3	6	18	15	135	0	4	1
32	4	10	0	0	12	0	2	0	3	55	86	19	15	3
64	317	24	0	0	10	0	3	1	4	275	44	0	20	269
128	0	68	0	0	8	0	0	4	137	166	48	0	231	0
256	0	0	0	0	0	0	0	33	0	43	211	0	0	0
512	0	0	0	0	0	0	0	0	0	16	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	6	0	0	0	0
>1024	0	0	0	0	0	0	0	0	0	349	0	0	0	0
Total tested	931	931	931	931	931	931	931	931	931	931	931	931	930	931
NR	323	102	168	68	30	24	10	44	144	371	303	24	270	275
%R	34.7	11.0	18.0	7.3	3.2	2.6	1.1	4.7	15.5	39.8	32.5	2.6	29.0	29.5
CI	31.6-38	9-13	15.6-21	5.7-9	2.2-5	1.7-4	0.5-2	3.5-6	13.2-18	36.7-43	29.5-36	1.7-4	26.1-32	26.6-33

Grey colour: concentrations tested; Line: breakpoint; N: Number; NR: Number resistant; %R per cent resistant; CI: confidence interval

**Table 58.** Multi-resistance in *Salmonella* isolates, data 2013.

N antimicrobials	N strains	N strains cumulative	% strains	% strains cumulative
0	394	394	42.3	42.3
1	106	500	11.4	53.7
2	50	550	5.4	59.1
3	60	610	6.4	65.5
4	104	714	11.2	76.7
5	85	799	9.1	85.8
6	57	856	6.1	91.9
7	36	892	3.9	95.8
8	22	914	2.4	98.2
9	12	926	1.3	99.5
10	4	930	0.4	99.9
11	1	931	0.1	100.0
12	0	931	0.0	100.0
13	0	931	0.0	100.0
14	0	931	0.0	100.0

**Table 59.** Comparison of antimicrobial susceptibility of *Salmonella* serotypes of all origins (data 2011, 2012 and 2013)

	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	STR	TAZ	TET	TMP
<b>2011</b>														
N	1159	1160	1160	1158	1160	1158	1160	1158	1158	1158	1158	1157	1160	1156
NR	471	69	298	64	36	111	24	33	297	446	306	85	262	386
%R	40.6	5.9	25.7	5.5	3.1	9.6	2.1	2.8	25.6	38.5	26.4	7.3	22.6	33.4
CI	37.8-44	4.7-7	23.2-28	4.3-7	2.2-4	7.9-11	1.3-3	2-4	23.2-28	35.7-41	23.9-29	5.9-9	20.2-25	30.6-36
<b>2012</b>														
N	1163	1163	1163	1163	1163	1163	1163	1163	1163	1163	1163	1163	1163	1162
NR	513	139	393	241	50	161	40	62	374	490	371	147	339	418
%R	44.1	12.0	33.8	20.7	4.3	13.8	3.4	5.3	32.2	42.1	31.9	12.6	29.1	36.0
CI	41.2-47	10.1-14	31.1-37	18.4-23	3.2-6	11.9-16	2.5-5	4.1-7	29.5-35	39.3-45	29.2-35	10.8-15	26.5-32	33.2-39
<b>2013</b>														
N	931	931	931	931	931	931	931	931	931	931	931	931	930	931
NR	323	102	168	68	30	24	10	44	144	371	303	24	270	275
%R	34.7	11.0	18.0	7.3	3.2	2.6	1.1	4.7	15.5	39.8	32.5	2.6	29.0	29.5
CI	31.6-38	9-13	15.6-21	5.7-9	2.2-5	1.7-4	0.5-2	3.5-6	13.2-18	36.7-43	29.5-36	1.7-4	26.1-32	26.6-33

# Antimicrobial resistance of indicator commensal bacteria

Marc Aerts, Patrick Butaye, Jean-Baptiste Hanon, Hein Imberechts, Stijn Jaspers, Yves Van der Stede, Pierre Wattiau

## Introduction

Antimicrobial resistance (AMR) has become a major public health concern leading to the set-up of national and European surveillance programs to follow-up AMR evolution. Non pathogenic bacteria of the intestinal flora that are present in most animals and in humans are considered as good indicators to monitor antimicrobial resistance. In this study we performed a trend analysis of the prevalence of antimicrobial resistance in commensal *Escherichia coli* and *Enterococcus* spp. (*E. faecium* and *E. faecalis*) based on data of three consecutive years (2011-2012-2013) in Belgium. We also evaluated the level of multi-resistance (resistance against at least three antibiotics by the same strain) and its trend over the same period.

## Sampling and laboratory testing

Representative faecal samples from cattle (veal calves and young beef cattle), slaughter pigs and broiler chickens were taken at the farm or at the slaughterhouse within a nationwide surveillance program of the Federal Agency for Safety of the Food Chain (AFSCA-FAVV). Isolates of *E. coli* (n=2504) and *Enterococcal* strains (n=1380) were obtained at the two Regional laboratories ARSIA and DGZ and sent to the National Reference Laboratory (CODA-CERVA) for identification and susceptibility testing using a micro-dilution technique (Sensititer®). A panel of 14 antimicrobials specified by the European commission was used for *E. coli* and a custom panel including 12 antibiotics was used for *Enterococci* isolates. For each strain and each antimicrobial substance, the Minimal Inhibitory Concentration (MIC) was read and recorded in a database. For each bacterial species and each antimicrobial separately, the percentage (with confidence intervals) of observed resistant strains was calculated per year and per animal category. Quantitative MIC values were converted in binary qualitative values (Resistant / Susceptible) based on the susceptibility breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).



Figure 79. Laboratory testing

## Statistical analysis

Several statistical methods were used to model the observed trends: (i) univariate models based upon categorical data (logistic regression, generalized logit models) or upon continuous data (models for interval-censored data, mixture models), (ii) multivariate model (Generalised Estimating Equation models or GEE). In addition, diversity indexes (entropy and weighted entropy) were calculated to describe the degree of diversity of multi-resistance: a higher weighted entropy index reflecting a shift to multi-resistance against a larger number of antibiotics.

## Prevalence of resistance in *E. coli*.

High levels of resistance against several antimicrobials were observed for the three consecutive years in all animal categories except in beef cattle. Decreasing trends were observed in veal calves and slaughter pigs for antimicrobials for which there was a low to moderate resistance prevalence. Increasing trends were observed in beef cattle for two antimicrobials (gentamicin, kanamycin) for which there was a low to moderate resistance prevalence.

The situation is worrying in broiler chickens: in this animal category, we observed a high resistance prevalence ( $\geq 60\%$ ) for half of the tested antimicrobials (7/14). Moreover, in this animal category, increasing trends of resistance were observed, with the multivariate analysis models, for 5 different antimicrobials (chloramphenicol, ciprofloxacin, colistin, florfenicol, nalidixic acid), including two substances for which there was high resistance prevalence (ciprofloxacin, nalidixic acid) (Figure 80).

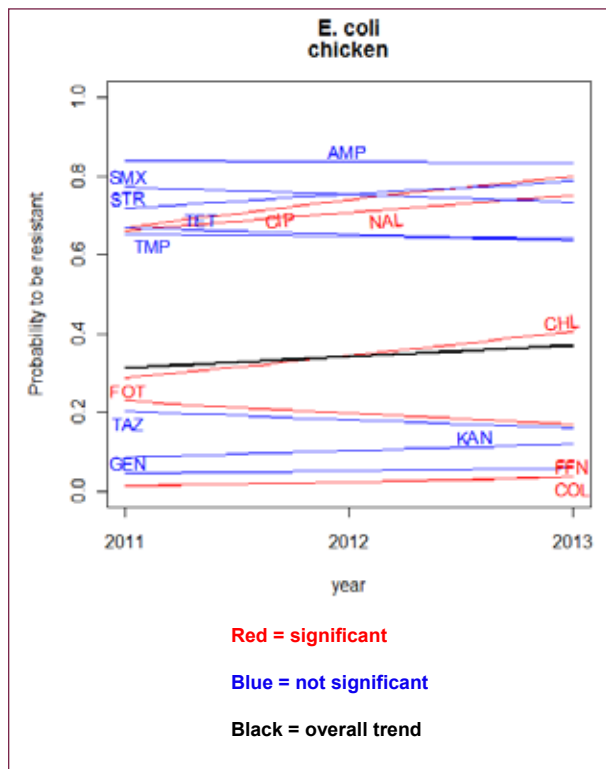


Figure 80. Probability to be resistant

## prevalence of resistance in Enterococci spp.

For these bacteria species, the number of tested isolates was sometimes insufficient to obtain significant trends. This was especially the case for the year 2011.

For *E. faecalis* resistance prevalence was high for 3 antimicrobials (erythromycin, streptomycin and tetracycline) in all animal categories except in slaughter pigs for which only the resistance against tetracycline was high. Increasing resistance was observed mostly in strains from veal calves, including against 2 antimicrobials for which there was a high prevalence of resistance (erythromycin and tetracycline).

For *E. faecium* the resistance prevalence was generally low

to moderate except for synergid for which there was a very high prevalence of resistance ( $> 80\%$ ) in all animal categories. Decreasing trends were observed for several antimicrobials in all animal categories except in veal calves for which there was an increasing resistance to 2 substances (erythromycin and tetracycline).

## Multiresistance in commensal flora

In broiler chickens, the percentage of multi-resistant strains remained very high ( $>80\%$ ) during the three consecutive years both for *E. coli* and for Enterococci (tables 60 to 62). In veal calves this figure for *E. coli* was very high ( $>70\%$ ) during the three years and was increasing for Enterococci although the lack of data in 2011 makes such trend questionable. In slaughter pigs multi-resistance was high for *E. coli* and decreasing for *E. faecium*. In beef cattle the level of multi-resistance was moderate.

The comparison of diversity indices (weighted entropy) shows that for *E. coli* strains, multi-resistance against a high number of antibiotics was higher in isolates from the veal calves animal category. The indices were lower for Enterococci isolates in all animal categories meaning that resistance to high number of antimicrobials was less frequent compared to *E. coli*.

## Conclusion

The models show that trends were sometimes diverging within the same animal species, with an increasing resistance for some antibiotics and decreasing for others without obvious explanation (e.g. florfenicol and cefotaxime in chickens) and the trends observed in Enterococci were sometimes conflicting with the trends observed in *E. coli*. (e.g. chloramphenicol in veal calves).

Nevertheless some of the results were more conclusive. A high to very high prevalence of resistance was observed for some of the tested antibiotics during the three consecutive years. Such high levels of resistance have already been observed previously both in pathogenic and non-pathogenic bacteria and results are comparable to those from other surveillance programmes in some other European countries such as in the Netherlands. The use of such antimicrobials should be carefully monitored especially in livestock species with intensive practices (veal calves, broiler chickens, slaughter pigs), for which the highest resistance prevalence were observed in our study.

The situation is particularly worrying for *E. coli* isolated from broiler chickens with a high prevalence of resistance for several antimicrobials and yet, an increasing trend for some of them. It is also in this animal category that the level of multi-resistance was the highest, both in *E. coli* and Enterococci isolates. The prevalence of multi-resistance was also high for slaughter pigs and veal calves in *E. coli* isolates but no significant increasing/decreasing trend was detected in these animal species.

To conclude, the methodology and statistical tools applied in this study led to the observation of some trends and high prevalences of resistance after three years of monitoring; these results need to be confirmed by data covering a longer period. It is therefore recommended to continue the surveillance program to advise the public health authorities, based on objective data, in the measures to be taken to reduce antimicrobial resistance.

**Table 60.** *E. coli*

	Veal Calves	Beef Cattle	Chickens	Slaughter Pigs
Ampicillin	++		++	++
Chloramphenicol	↓		↑	
Ciprofloxacin	↓		++ ↑	↓
Colistin			↑	
Florphenicol			↑	
Cefotaxime			↓	↓
Gentamicin		↑		
Kanamycin		↑		
Nalidixic acid	↓		++ ↑	↓
Sulphonamide	++		++	++
Streptomycin	++		++ ↑	++
Ceftazidime				
Tetracycline	++		++	++
Trimethoprim	↓		++	++

**Table 61.** *E. faecalis*

Table xx2 : <i>E. faecalis</i>	Veal Calves	Beef Cattle	Chickens	Slaughter Pigs
Ampicillin				
Chloramphenicol	↑	↑		
Ciprofloxacin				
Erythromycin	++ ↑	++	++	
Florfenicol				
Gentamicin				
Linezolid			↓	
Salinomycin				
Streptomycin	++	++	++	
Synercid				
Tetracycline	++ ↑	++	++	++
Vancomycin				

**Table 62.** *E. faecium*

	Veal Calves	Beef Cattle	Chickens	Slaughter Pigs
Ampicillin				
Chloramphenicol		↓	↓	↓
Ciprofloxacin				
Erythromycin	↑	↓		
Florfenicol				
Gentamicin				
Linezolid			↓	
Salinomycin				
Streptomycin		↓		
Synercid	++	++	++	++
Tetracycline	↑	↓	↓	
Vancomycin			↓	

# MRSA surveillance

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## 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 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.

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

In 2008, 2009 and 2010 no official monitoring programme was in place for MRSA.

In 2011, the FASFC organised a MRSA surveillance in poultry. In 2012 and 2013, the FASFC has realised a MRSA surveillance in cattle (dairy cattle, cattle for meat production, veal calves) and pigs, respectively.

## MRSA in cattle, surveillance 2012.

A specific FAVV-AFSCA surveillance programme on the prevalence and diversity of MRSA in bovines was executed for the year 2012. For this surveillance, the European Food Safety Authority (EFSA) proposed standardized protocol for the isolation of MRSA from dust samples was used in order to obtain comparable prevalence results all over Europe. However, this protocol was estimated not to be very sensitive during the previous survey in poultry in 2011. Since bovines and especially veal calves were expected to have a higher prevalence, we compared the single broth enrichment method with the double broth enrichment method to determine whether there were differences between the two methods in this higher prevalence population. This work was supported in part by the EMIDA-ERA NET project on MRSA (2011-2014).

A detailed report of the MRSA surveillance in 2012 can be found on [www.coda-cerva.be](http://www.coda-cerva.be).

### Methods

Four hundred and thirty-two farms were sampled during 2012. Of these, 141 were dairy farms, 187 farms reared beef cattle and 104 reared veal calf. Twenty animals per farm were sampled using nasal swabs and these were pooled per farm. Incubation was done in Mueller-Hinton broth supplemented with NaCl (6.5%). Subsequently, one ml of this broth was added to Tryptic Soy Broth supplemented with cefoxitin (3,5mg/l) and aztreonam (75mg/l) and after incubation this culture was plated on MRSA-ID to recover suspected MRSA colonies. In addition to this double broth enrichment method, an alternative, single broth enrichment method was applied on samples from 106 farms in which the second enrichment in antibiotic supplemented broth was omitted. PCR was performed on extracted DNA of selected colonies to identify MRSA (Maes et al. 2002). All MRSA isolates were spa-typed by sequencing the repetitive region of the spa gene encoding for the staphylococcal protein A. The CC398 PCR was performed on all MRSA following protocol described by Stegger et al. 2011. This method allows the rapid detection of the *S. aureus* sequence type ST398. Finally, Multi Locus Sequence Typing (Enright et al., 2000) was done on MRSA isolates that were negative in the CC398 PCR and/or showed a spa-type that has never been associated with CC398

before. The antimicrobial resistance of MRSA strains was performed by micro-dilution (Sensititre®). The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected.

## Prevalence

By using the official double broth enrichment method, 82 samples (19.0%, 95% CI [15.3% - 22.7%]) were positive for MRSA. Among these farms, 14 (9.9%, 95% CI [5.0% - 14.9%]) were dairy farms, 19 (10.2%, 95% CI [5.8% - 14.5%]) were farms holding meat cows and 49 (47.1%, 95% CI [37.5% - 56.7%]) were farms raising veal calves. Some differences, although not significant, were observed between the two isolation methods (see full report on [www.coda-cerva.be](http://www.coda-cerva.be)). Since one sample has been lost after identification, antimicrobial resistance and molecular characterization is given for 81 samples.

## Antimicrobial resistance

As expected, all strains were resistant to ceftiofur and penicillin. More than 90% of the strains were resistant to tetracycline (96.3%) and trimethoprim (95.1%). A high prevalence of resistance was also observed to clindamycin (86.4%), erythromycin (86.4%), kanamycin (80.2%) and gentamicin (76.5%). More than half of the strains were also resistant to streptomycin (58.0%). Lower resistance levels were detected to fusidic acid (27.2%),

sulfamethoxazole (25.9%), quinupristin/dalfopristin (23.5%), tiamulin (17.3%), ciprofloxacin (16.0%), rifampicin (13.6%) chloramphenicol (12.3%) and mupirocin (9.9%). No resistance were observed to linezolid and vancomycin (Table 63).

Multi-resistance was calculated and results are shown in table 64. It should be noted that the strains are resistant to at least 2 antibiotics, ceftiofur and penicillin since they are a selection of MRSA. As such 50% of the strains are at least resistant to 9 different antibiotics. Two strains had resistance to as much as 16 different antibiotics, remaining susceptible only to ciprofloxacin, linezolid and vancomycin. The strains resistant to 15 or 16 antibiotics were all t011, they originated from 2 veal calves and 3 meat type bovines. The one strain resistant to 14 antibiotics was a t6228 strain.

The one strain resistant only to 4 antibiotics was a t1456 strain and originated from a dairy cow. The strains resistant to 4, 5 or 6 antibiotics were t011 (1 dairy cow, 2 veal calves), t1456 (meat type cow) and t1985 (veal calf and meat type cow). The MLST 30 strain was resistant to 9 different antibiotics and the MLST 8 strain was resistant to 7 different antibiotics. Seen the low number of isolates from meat cows and dairy cows, comparison between the different groups is not relevant. However, results were similar: when there was a high prevalence of resistance in one group, this was also the case for the other groups and the same is true for the lower prevalences.

**Table 63.** MICs of 81 methicillin-resistant *S. aureus* from bovines for antimicrobial tested with micro-broth dilution. Value indicated in light blue cells means that the MIC was lower than the lowest concentration tested. Values in darker blue cells mean that the MIC was higher than the highest concentration tested. Epidemiological cut-off value is indicated with the line in bold.

Antimicrobial	% strains at concentration															%R	Confidence interval
	≤0.0016	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512			
CHL							4.9	46.9	35.8	2.5	8.6	1.2			12.3	6.1-22	
CIP			18.5	27.2	9.9	2.5	2.5	11.1	28.4						16.0	33-56	
CLI		12.3	1.2	1.2	1.2	0.0	0.0	84.0							86.4	77-93	
ERY			3.7	7.4	2.5	0.0	2.5	2.5	81.5						86.4	77-93	
FOX				0.0	0.0	0.0	0.0	3.7	18.5	77.8					100.0	95.5-100	
FUS				72.8	12.3	2.5	9.9	2.5							27.2	17.9-38	
GEN					22.2	1.2	3.7	7.4	21.0	44.4					76.5	65.8-85	
KAN							16.0	3.7	2.5	2.5	9.9	65.4			80.2	69.7-88	
LZD					23.5	75.3	1.2	0.0							0.0	0-4	
MUP				86.4	3.7	3.7	0.0	0.0	0.0	0.0	0.0	0.0	6.2		9.9	4.4-19	
PEN		0.0	0.0	0.0	1.2	8.6	90.1								100.0	95.5-100	
RIF	86.4	2.5	0.0	1.2	9.9										13.6	5.2-20	
SMX											70.4	3.7	11.1	14.8	25.9	16.8-37	
STR							14.8	22.2	4.9	9.9	48.1				58.0	46.5-69	
SYN				32.1	44.4	8.6	8.6	6.2							23.5	14.8-34	
TET				2.5	1.2	0.0	0.0	0.0	1.2	95.1					96.3	86.6-99	
TIA				75.3	7.4	0.0	0.0	17.3							17.3	9.8-27	
TMP						4.9	3.7	1.2	0.0	1.2	88.9				95.1	87.8-99	
VAN					87.7	12.3	0.0	0.0	0.0						0.0	0-4	

**Table 64.** Total number of MRSA isolates corresponding to the different genotypes recovered and separated by farm types.

	MLST			spa-types										SCCmec				
	8	239	398	t011	t037	t121	t388	t1451	t1456	t1985	t3423	t6228	NT	III (3A)	IV (2B)	IV (2B&5)	V (5C2)	NT
Dairy farms	0	2	12	8	0	0	1	0	1	0	0	2	1	1	4	0	6	3
Beef farms	1	0	18	16	0	1	0	0	1	1	0	0	0	1	12	0	3	3
Veal farms	0	0	48	40	1	0	0	3	1	3	1	0	0	0	30	11	7	0
Total	1	2	78	64		1	1	3	3	4	1	2	1	2	46	11	16	6

## Discussion

The overall MRSA prevalence in bovines was 19.7%. This is much higher than the prevalence observed in poultry in 2011, but lower than the surveillance in pigs in 2007 and 2009. It should be noted however that for the surveillance in pigs, the methodology was quite different. As for the 2007 surveillance, 30 individual samples were taken per farm, and as for the 2009 surveillance (organised by EFSA), pooled dust samples were used. It was shown that the last method was not as sensitive as animal testing. In this study, 20 nasal samples were taken and pooled for analysis at the laboratory. Pooling of samples is interesting, though might have a lowered sensitivity when farm level prevalence is low. There are however few data available on the prevalence of MRSA in the nose of cows at farm level (Vandendriessche et al., 2013).

A first study in Belgium gave some indication of the high prevalence of MRSA in veal calves (Vandendriessche et al., 2013). In the Netherlands the high prevalence of resistance in veal calves was clearly demonstrated (Graveland et al, 2010). Here 88% of the farms were proven positive. The lower prevalence in our study may be explained by the differences in sampling. In the Dutch study, a more sensitive sampling was applied. Ten to 25 individual samples per farm were analysed. Lower prevalences were found in dairy cattle. Before in Belgium, MRSA CC398 was shown to be implicated in mastitis (Vanderhaegen et al., 2010) and in the nose of dairy cattle, however the latter study implicated only few farms (Vandendriessche et al., 2013). In this study, a low prevalence was found (1%). Today, the prevalence is at nearly 10%. However, care should be taken since in the actual study, a less sensitive method was used, but a larger sample. As such one cannot state that there is a difference with the former study. The presence of MRSA CC398 in dairy cattle is however of extreme importance seen that they may cause mastitis (Vanderhaegen et al., 2010). On positive farms, measures should be taken to eliminate as much as possible these MRSA strains to avoid they cause mastitis. The prevalence in beef cattle is the same as for dairy cattle. It would be interesting to investigate possible age related prevalence, as was seen in pigs (Crombé et al., 2012).

Also in this study we compared different isolation methods. In the study in a low prevalence population (poultry), we have demonstrated that there was no improvement in isolation rate using 2 different enrichments (Némeгаire et al., accepted). In this study, in a population with a higher MRSA prevalence there was also no significant difference between the two methods. This indicates that the second enrichment method is not adding any value to the obtained results and may be omitted.

Most isolates were resistant to tetracycline and trimethoprim. In this study two CC398 isolates were found to be susceptible to tetracycline; tetracycline susceptible strains are only very rarely found. Also trimethoprim susceptible strains are rarely observed. It has been shown that the gene in these strains is still present but not functional anymore (Verhegghe et al., unpublished results). The prevalence of erythromycin, clindamycin, kanamycin and gentamicin in this collection is extremely high compared to what has been found in strains from other origins in Belgium (mainly pig strains). Also the level of multi-resistance is extremely high. On 19 antimicrobials tested, not one was susceptible to all, but that is of course due to the fact that these are already selected for methicillin resistance, rendering them automatically resistant to the two  $\beta$ -lactam antibiotics tested. Nevertheless the strains with the lowest level of multi-resistance were resistant to 2-3 additional antibiotics (and this was only 2 strains). Two isolates were resistant to sixteen antimicrobials out of nineteen tested excluding ciprofloxacin, linezolid and vancomycin, three antibiotics that are last resort antibiotics in the treatment of MRSA infections in humans. If these strains would spread in mastitis in bovines, these animals would not be treatable anymore.

Most isolates were typical LA-MRSA ST398, spa-type t011. Other less prevalent spa-types associated to ST398 were also recovered. However, MRSA ST8 spa-type t121 and MRSA ST239 spa-type t037 and t388 are classified among hospital-acquired (HA)-MRSA. MRSA spa-type t121 was previously recovered in a Belgian hospital in 2002 and 2007 (Wildemaуwe et al. 2009) and is commonly found in Europe and in the United States (<http://spa.ridom.de>). This spa-type has also been found in bulk tank milk in the United States (Haran et al. 2011). MRSA

ST239 spa-type t388 and t037 are widespread HA-MRSA found in Europe, Asia, and America (Campanile et al. 20108). MRSA ST239 t037 was recovered also among MRSA in poultry during the previous Belgian survey in poultry in 2011. This interesting finding confirms the possible spread of HA-MRSA to livestock, as was hypothesized in 2011. This situation should also be followed up closely since it may be an indication of a new animal adapted MRSA strain originating from humans. Also CC398 strains originated from humans and adapted to animals (Price et al., 2012).

The diversity of spa-types in bovines was larger than what has been found in pigs during surveys in 2007 (Crombé et al, 2012) and during the EFSA baseline surveillance in pigs in 2009 (EFSA report, <http://www.efsa.europa.eu/en/efsajournal/pub/1597.htm>). In both studies, only spa types t011 and t034 were found. Here at least 7 CC398 spa-types were found. The spa gene codes for a surface protein that is under the selective pressure of host immunity. Differences in this immunity might be the indication that MRSA CC398 is changing its profile according to the host and that some host adaptations are underway. In 2013, a new surveillance in pigs is organised and it will be interesting to see whether the diversity has changed also in pigs.

As expected, SCCmec type IV was the most common type in veal calves (Cavaco et al. 2010) followed by SCCmec type V. In pigs, this was the other way round, being type V as the most prevalent cassette. Two isolates were carrying SCCmec type III(3A) and six isolates carried a non-typable SCCmec cassette. The type III(3A) SCCmec found here is not the classical type III found in HA-MRSA. It might also be an aberrant type V.

## Conclusions

In conclusion, MRSA has been found at lower prevalences in the nares of dairy and meat cows, but at elevated levels in veal calves. The diversity of strains was larger than what was seen in pigs, including also some more typically hospital associated MRSA. The diversity in SCCmec cassettes in CC398 is not anymore limited to the types IV and V but includes now also the type III cassette. Converse to pigs, the most prevalent SCCmec type was IV while in pigs it was type V, associated with zinc resistance.

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## MRSA in pigs – surveillance 2013

In the framework of the FAVV-AFSCA surveillance on antimicrobial resistance in commensal *E. coli* and *Salmonella*, a surveillance of MRSA in pigs was organised in 2013 in order to determine the prevalence and diversity of MRSA in pigs. To this end, the proposed European Food Safety Authority (EFSA) standardized protocol for the isolation of MRSA from dust samples was used in order to obtain comparable prevalence results all over Europe.

A detailed report of the MRSA surveillance in 2013 can be found on [www.coda-cerva.be](http://www.coda-cerva.be).

Twenty nasal swabs each were taken on 328 farms, pooled per farm and incubated in Mueller-Hinton broth supplemented with NaCl (6.5%). Subsequent selective enrichment was done in Tryptic Soy Broth supplemented with ceftiofur (3.5mg/l) and aztreonam (75mg/l) and plated on MRSA-ID. For characterization of the isolates, PCR was performed on extracted DNA to identify MRSA specific sequences (Maes et al. 2002), i.e. staphylococcal specific 16S rDNA gene, nuc1 gene specific for *S. aureus* and mecA gene responsible for methicillin resistance. All MRSA isolates were spa-typed by sequencing the repetitive region of the spa gene encoding for the staphylococcal protein A. The CC398 PCR was performed on all MRSA following protocol described by Stegger et al. 2011. This method allows the rapid detection of the *S. aureus* sequence type ST398. Finally, Multi Locus Sequence Typing (Enright et al., 2000) was done on MRSA isolates that were negative in the CC398 PCR and/or showed a spa-type that has never been associated with CC398 before. The antimicrobial resistance of MRSA strains was performed by micro-dilution (Sensititre®). The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected.

Two-hundred fifteen samples were found positive for MRSA, which corresponds to 65.6% (95% CI [60.1% - 71%]). Of these, antimicrobial susceptibility was tested on 211 strains (Table 65). As expected, all strains were resistant to penicillin. One strain is susceptible to ceftiofur, but this should be regarded as a methodological deviation since the presence of the mecA gene was demonstrated. It is known that MIC testing for MRSA is not 100% sensitive. More than 90% of the strains were resistant to tetracycline (98.1%) and to trimethoprim (95.7%). A high prevalence of resistance was also observed to ciprofloxacin (61.1%), clindamycin (66.4%) and erythromycin (57.3%). Kanamycin (43.1%) and gentamicin (45.5%) resistance was present in a little less than half of the strains.

Lower resistance levels were detected to streptomycin (30.8%), fusidic acid (20.4%), sulfamethoxazole (27.5%), quinupristin/dalfopristin (27.5%), tiamulin (32.7%) and rifampicin (19.0%). Resistance to chloramphenicol (10.9%) and mupirocin (10.0%) were around 10%. No resistance was observed to vancomycin. One strain was classified as resistant to linezolid, however, the MIC was close to the breakpoint and this should be confirmed by other methods.

Multi-resistance was calculated and results are shown in Table 66. It should be noted that all but one strain are resistant to at least 2 antibiotics, i.e. ceftiofur and penicillin, because they were included in the selective enrichment broth. Fifty percent of the strains are at least resistant to 7 different antibiotics. Seven strains have resistance to as much as 17 different antibiotics, remaining susceptible only to linezolid and vancomycin.

These strains are all MRSA CC398 t011. The strains resistant to 15 or 16 antibiotics are all but two t011, one strain is a t034 and another strain is t2370; all are CC398. The strains are additionally susceptible to chloramphenicol and/or ciprofloxacin and/or erythromycin and/or mupirocin. Of the 5 strains resistant to 14 antibiotics one is a t1456 strain and the other strains are t011.

The one strain resistant to 3 antibiotics is additionally resistant to tetracycline and is a t011 strain. Among the 5 strains resistant only to 4 antibiotics is a t4150 strain, a strain that has been detected before in poultry and bovines and was found to be a ST239, a hospital associated MRSA. This strain is phenotypically classified as susceptible. Three other strains resistant against 4 antibiotics are CC398 t011 and one strain is a t044 strain of which the sequence type was not determined.

**Table 65.** MICs of 211 methicillin-resistant *S. aureus* tested with micro-broth dilution. Values in grey cells mean that the MIC was higher than the highest concentration tested. Epidemiological cut-off value is indicated with the line in bold.

Dilution	Antibiotic and number of strains with MIC																		
	FUS	CHL	CIP	ERY	FOX	GEN	LZD	STR	SYN	TET	VAN	KAN	MUP	PEN	RIF	SMX	CLI	TIA	TMP
<=0.016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	171	0	0	0	0
0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	63	0	0
0.25	0	0	38	34	0	0	0	0	0	0	0	0	0	2	0	0	8	0	0
0.5	168	0	26	51	0	0	0	0	109	3	0	0	170	1	2	0	5	136	0
1	2	0	18	5	0	95	23	0	44	1	207	0	20	0	33	0	2	5	0
2	8	0	23	8	0	20	184	0	21	0	4	0	6	3	0	0	6	1	9
4	28	4	9	0	1	14	3	61	22	0	0	103	0	205	0	0	5	5	2
8	5	146	56	1	57	24	1	62	15	0	0	17	0	0	0	0	122	64	1
16	0	38	41	112	89	27	0	23	0	0	0	7	0	0	0	0	0	0	1
32	0	7	0	0	64	31	0	13	0	207	0	1	0	0	0	0	0	0	0
64	0	15	0	0	0	0	0	52	0	0	0	14	0	0	0	138	0	0	198
128	0	1	0	0	0	0	0	0	0	0	0	69	0	0	0	15	0	0	0
256	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	7	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
1024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49	0	0	0
%R	20.4	10.9	61.1	57.3	99.5	45.5	0.5	30.8	27.5	98.1	0.0	43.1	10.0	100.0	19.0	27.5	66.4	32.7	95.7
CI	15.2-26	17.0-16.0	54.2-68	50.4-64	97.4-100	38.6-52	0-3	24.6-38	21.6-34	95.2-99	0-2	36.3-50	6.3-15	98.3-100	13.9-25	21.6-34	59.5-73	26.4-39	92.1-98

**Table 66.** Multi-resistance in MRSA from pigs.

N antibiotics	N strains	% of strains	Cumulative % of strains
0	0	0.0	0.0
1	0	0.0	0.0
2	0	0.0	0.0
3	1	0.5	0.5
4	5	2.4	2.8
5	37	17.5	20.4
6	31	14.7	35.1
7	36	17.1	52.1
8	26	12.3	64.5
9	22	10.4	74.9
10	7	3.3	78.2
11	7	3.3	81.5
12	4	1.9	83.4
13	2	0.9	84.4
14	5	2.4	86.7
15	14	6.6	93.4
16	7	3.3	96.7
17	7	3.3	100.0
18	0	0.0	100.0
19	0	0.0	100.0

Among the 215 MRSA isolates recovered, 11 strains were excluded due to incomplete typing, not allowing a concise conclusion. Two of these had an invalid spa-type. Six were excluded because of the absence of a sequence type associated. The other three isolates were excluded because the CC398 PCR did not match the spa-type. The results of the 204 strains are summarised in Table 67.

**Table 67.** MRSA strain types

Spa-type	MLST	Number of strains
t011	398	180
t034	398	6
t044	80	2
t1451	398	1
t1456	398	2
t1580	398	2
t1985	398	2
t2123	398	1
t2370	398	2
t3171	398	1
t4150	239	1
t4432	398	1
t4872	398	1
t6228	398	1
t8100	398	1

Four strains that were negative in the CC398 PCR were a t034 strain (confirmed ST398 by MLST), a t4150 strain that has been shown to be associated with ST239 and two t044 strains that are associated with ST80 as determined by MLST. It can thus be concluded that of the 204 fully typed strains, three were not CC398. The ST239 strain has been found before in the surveillance in poultry in 2011, however, that isolate was a spa-type t037-ST239. ST239 is a HA-MRSA and the spa-type t4150 has rarely been reported, and this mainly in Asia and New Zealand. ST80 belongs to the community acquired MRSA and is considered the European CA-MRSA clone, but has been found also in the Middle East and North Africa. ST80, like ST239, has been associated with multiple spa-types.

Amongst the CC398 strains, 13 different spa-types were found. The vast majority were however the commonly isolated t011 and t034 types.

## Conclusions

Compared to former studies on the prevalence of MRSA in pigs in Belgium (2007 and 2009), there are no significant changes in prevalence, when you take into account that the 2009 European study by EFSA was less sensitive as the current study.

While in these earlier studies few spa-types were found, a larger diversity was detected. However, it should be noted also that in this study, the number of strains isolated was far higher than before due to the larger sample, allowing to have a better view on the diversity.

As in poultry and bovines, also in pigs, human associated sequence types were detected. In pigs, both a HA-MRSA and a CA-MRSA were detected. This indicates that we vigilance for the acquisition of virulence genes by MRSA CC398. If this clone is becoming more virulent, it may have major implications for both human and animal health.

Finally, and as has been demonstrated before, the CC398 clones are highly multi-resistant. The only antibiotic for which no resistance has been found was the glycopeptide antibiotic vancomycin.

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## MRSA surveillance in humans

The National Reference Centre for *Staphylococcus aureus* (NRCS) of the Université Libre de Bruxelles provides specialized analysis:

- Identification, characterisation and antimicrobial susceptibility testing of *Staphylococcal* isolates by means of
  - Phenotypic methods such as colony morphology, biochemical tests, minimal inhibiting concentration (MIC) determination, penicillin binding protein 2a (PBP2a) detection, glycopeptide susceptibility (population analysis), Mass spectrometry identification (Maldi-Tof)
  - Genotypic methods: identification of *Staphylococci* species by sequence analysis of *rpoB* gene, detection by PCR for *nuc* gene (for *S. aureus* identification), *mecA* gene (encoding oxacillin resistance), *mecC* gene (*mecA* homologue), *mupA* gene (encoding high-level mupirocin resistance), PCR for resistance genes to macrolides-lincosamides-streptogramins (MLS), tetracyclines, aminoglycosides and linezolid.
- Detection of toxin genes: PCRs for genes encoding exfoliative toxins A, B and D, Panton Valentine Leucocidin (PVL), Toxic Shock Syndrome Toxin-1 (TSST-1) and staphylococcal enterotoxins (se) including *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq*, *selr*. Detection by PCR for the presence of the *arcA* gene as marker of the pathogenic locus of the Arginine Catabolic Mobile Element (ACME).
- Molecular typing: Pulsed Field Gel Electrophoresis (PFGE) after *SmaI* macrorestriction, *spa* typing, multilocus sequence typing (MLST) and *Staphylococcal* Chromosome Cassette *mec* (SCC*mec*) typing, determination of the *agr* group.

The main activities of the NRCS are 1) susceptibility testing and detection of virulence genes for clinical strains and 2) characterization (genotyping and susceptibility testing) of staphylococcal strains isolated during local outbreaks or collected during epidemiological studies.

Since 1992, the NRCS organises microbiological surveillance of *S. aureus* every two or three years in collaboration with the ISP-WIV and the Belgian Infection Control Society (BICS). All hospital –affiliated laboratories are invited to collect five non-duplicate *S. aureus* isolates from hospitalized patients (two MSSA and three MRSA per hospitals). The objectives of this program are to follow the evolution of genotypes and the trends of antimicrobial resistance.

Since 1999, the NRCS participates in the European Antimicrobial Resistance Surveillance Network (EARS-net) in collaboration with the epidemiology section at ISP-WIV. Information about the protocol and the data of the EARS-net programme is available at the address <http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx>.

## Conclusions

Since 2002, the NLR has followed the epidemiology of community-associated (CA-) MRSA isolates for detection of exotoxin genes including the PVL. Of 1193 MRSA strains sent for toxin detection from 2002 to 2013, forty-two (n = 498) percent were PVL positive. Most of PVL-positive isolates were recovered from previously healthy children and young adults presenting skin and soft tissue infections, but severe infections, including meningitis and endocarditis, were also reported. Since 2008, the number of reported CA-MRSA infections has significantly increased. A large proportion of these infections (36%, 130/364) are caused by USA300 MRSA strains which replaced the European clone ST80. The emergence of USA300 MRSA is of great concern in view of severe infections recently described.

Since 2003, MRSA strains isolated from food-producing animals (FPA; livestock-associated MRSA, LA-MRSA) and belonging to the ST398 clone are regularly isolated from human populations in contact with livestock. In Belgium, especially veal calf and swine farmers, as well as veterinarians working with living FPA are at increased risk for colonization by LA-MRSA ST398. This clone was also recovered from a number of other animal species including horses, poultry and pets.

These LA-MRSA strains are mainly associated with asymptomatic colonization, but in a minority of cases they caused active diseases in humans. Of 93 LA-MRSA ST398 sent to NRCS between 2008 and 2013, 44 were from screening, the remaining were mainly isolated from SSTI (n = 19), the respiratory tract (n = 13) and bacteraemia (n = 9). Furthermore, MRSA ST398 strains represent a small proportion of the burden of MRSA strains in the population of patients admitted in acute-care hospitals (4.7 % of MRSA belonged to ST398 in the last *S. aureus* survey of 2013, of which 64.3% was from screening).

In 2010, MRSA strains carrying a novel *mecA* homologue gene, called *mecC*, were discovered in bovines. Meanwhile, *mecC*-MRSA have been detected in humans and diverse animal species across Europe. So far in Belgium, *mecC*-MRSA have been detected in humans (10 *mecC*-MRSA strains were sent to NRCS between 2006 and 2013), in dairy cattle, beef cattle, brown rats and rabbits. The presence of *mecC*-MRSA is worrisome as the *mecC* gene is difficult to detect using conventional diagnostic methods.

The national survey conducted in 2011 showed that over 90% of MRSA strains belonged to four epidemic clones: *spa* CC38 (formerly PFGE type B2) ST45-SCCmec IV (42.8%); *spa* CC8 (formerly PFGE type A20) ST8-SCCmec IV (23.6%), *spa* CC2 (formerly PFGE type G10) ST5-SCCmec II (12.8%), *spa* CC2 (formerly PFGE type C3) ST5-SCCmec IV (10.5%), which were found in 77 (72.0%), 49 (45.8%), 29 (27.1%), 26 (24.3%) hospitals, respectively. Only few isolates belonged to the PVL positive CA-MRSA USA300 clone and to the LA-MRSA ST398 clone. The final report of the last survey conducted in 2011 is available on the website (<http://www.mrsa.be>) of the NRL. Data from the last national survey performed in 2013 are being analysed at the moment. A detailed report will later be available on <http://www.mrsa.be>.

# Beta-lactam resistant phenotypes for E. coli

Nadine Botteldoorn, Patrick Butaye, Boudewijn Catry, Olivier Denis, Katelijne Dierick, Cristina Garcia Graells, Hein Imberechts, Béa Jans, Youri Glupczynski.

Bacteria that produce enzymes called extended-spectrum beta-lactamases (ESBLs) are resistant to many penicillin and cephalosporin antibiotics and often to other classes of antibiotics.

The 2 main bacteria that produce ESBLs are *Escherichia coli* (*E. coli*) and *Klebsiella* species. The ESBLs that *E. coli* most often produce are called CTX-M enzymes.

*E. coli* with ESBLs may cause urinary tract infections (UTIs) that can sometimes progress to more serious infections like blood poisoning, which can be life threatening. Resistance makes these infections more difficult to treat.

## ESBL producing E. coli in animals

Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins and cephalosporins but are inhibited by clavulanic acid. In Chapter 3 on the Antimicrobial resistance of indicator commensal bacteria, the evolution of resistance in the period 2011 – 2013 is described. Among the antibiotics tested were cefotaxime (CEF) and ceftazidime (TAZ), indicators for identifying ESBL resistance.

Whereas CEF and TAZ resistant *E. coli* in veal calves, beef cattle and pigs were at relatively low levels in the period studied (approximately 0% to 10%), significantly higher figures were observed in the chicken *E. coli* population, i.e. between 10% and more than 25%. For this reason, ESBL producing *E. coli* in this animal species is described in more detail.

It has been shown in the past that the prevalence of ESBL-strains of *E. coli* isolated from broiler chickens in Belgium was high (Smet et al., 2008; Persoons et al., 2010). Such a high prevalence might be caused by the off-label use of cephalosporines in one-day chicks.

The annual mean resistance against cefotaxime and ceftazidime in chickens increased significantly in 2012 compared to 2011 and then decreased significantly in 2013 compared to 2012. (Fig 81).

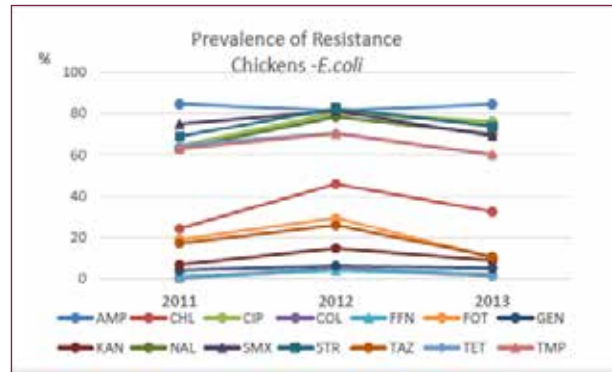


Figure 81. Prevalence of resistance of *E. coli* in chickens

Although a three year period is short to identify trends, the present results suggest that ESBL producing *E. coli* in chickens are more often present as compared to other animal species. In addition, detailed univariate and multivariate analysis (not shown) indicate that at least cefotaxime resistance in *E. coli* from chickens seems to have a tendency to diminish over the time period. Speculatively, this may be due to a diminishment of *ctx-M* genes, which have a specific activity on cefotaxime. These sequences frequently occur in *E. coli* from poultry and a retrospective study may confirm this tendency. The presence of *AmpC* enzymes may also be a cause of the differences seen. For these reasons, care must be taken before drawing definite conclusion; additional data from the coming years are needed to confirm the current observed trends.

## ESBL producing E. coli in food

### Enumeration of *E. coli* and *E. coli* ESBL in poultry carcasses, 2012-2013

In 2012 and 2013, an enumeration of ESBL producing *E. coli* was carried out on respectively 396 and 398 samples of poultry meat. The samples were taken in the framework of the monitoring program of the Federal Agency for the Safety of the Food Chain.

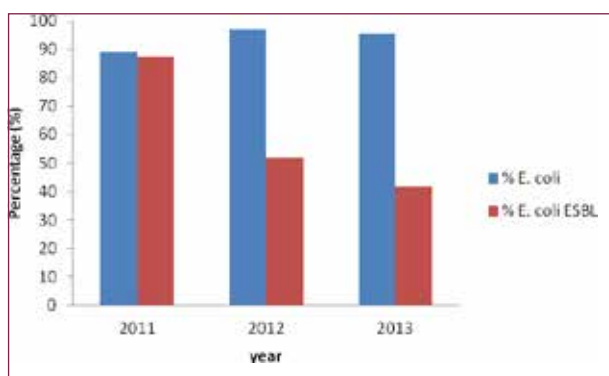
The method for the enumeration of the *E. coli* was based on the ISO-standard 16649-2. Two culture media were used; Tryptone Bile X-glucuronide (TBX) for the quantification of the total

number of *E. coli* and TBX supplemented with cefotaxime (CTX, 1 mg / l) for counting the *E. coli* that are resistant to cephalosporins of the third generation. Two colonies per sample were isolated as potential producers of  $\beta$ -lactamase. They were confirmed using the double disk method as recommended by the CLSI. The isolates were identified as producers of ESBL or AmpC.

A selection of *E. coli* that were confirmed as producers of ESBL / AmpC was tested for the presence of the resistance genes CTX-M, TEM, SHV, OXA, CMY according to the method that was described by the EURL-AMR. First the detection of the gene was performed by PCR to identify the gene family and then the sequencing was carried out to detect the variant.

In 2012 *E. coli* was recovered in 97% of the samples analyzed. *E. coli* that were resistant to cefotaxime were found in 52% of the samples positive for *E. coli*. In 2013 *E. coli* was recovered in 94% of the samples analyzed and *E. coli* which were resistant to cefotaxime were found in 42% of the samples positive for *E. coli*.

The isolates of *E. coli* isolated from TBX + CTX were considered presumptive ESBL producers. A comparison of the ratio of ESBL *E. coli* relative to all *E. coli* found on poultry carcasses, is proposed in Figure 82 for the control period 2011-2013.

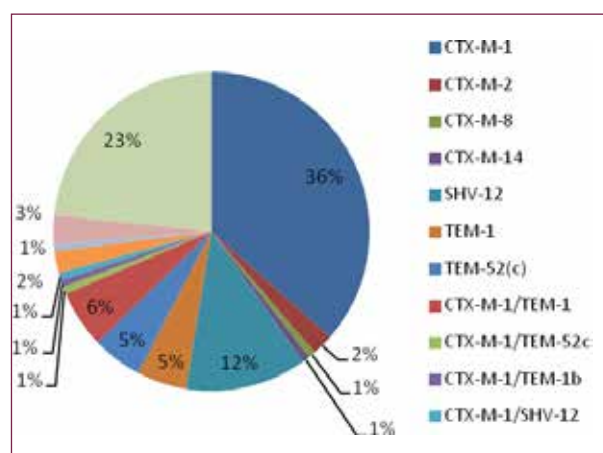


**Figure 82.** Ratio of ESBL-producing *E. coli* in relation to all *E. coli* that were found on poultry carcasses between 2011-2013.

The genotypic distribution of the isolates of 2013 is represented in Figure 83. Of the genotyped isolates 77.20% (105/136), carried genes that are specific to the type of ESBL. A much lower proportion of isolates (22.6%) (31/136), carried genes that are specific to the type of AmpC. The vast majority (52.38%) (55/105) of the isolates were carriers of genes belonging to the family CTX-M alone or in combination with other genes such as TEM or SHV (14.28%) (15/105)

The other isolates of the ESBL-phenotype were carriers of genes from the type TEM 13.3% (14/105) and SHV 16.20% (17/105) and 3.8%, a carrier of a combination of the two genes (4/105).

In the isolates with a confirmed AmpC- phenotype, the gene CMY-2 was found. In small number of poultry samples (n = 8) an isolate with an ESBL phenotype as well as an isolate with an AmpC phenotype were confirmed by genotyping.



**Figure 83.** Genotypic distribution of ESBL / AmpC-producing *E. coli* (n = 136)-2013

### Detection of ESBL producing *E. coli* in beef, pork and veal, 2013

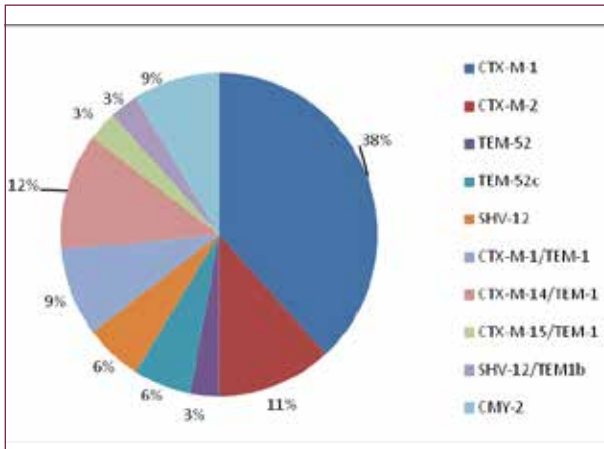
In 2013 a total of 44 *E. coli* isolates suspected to produce ESBL were isolated from pork, beef and veal. ESBL production was confirmed by the double disc method as described for poultry isolates. Results of the phenotypes detailed per matrix are listed in table 68. Thirty seven of 44 (84.10%) of the isolates have been confirmed as phenotype ESBL, 6 (18.18%) as phenotype AmpC and 1 (2.2%) could not be identified as ESBL nor AmpC

**Table 68.** Description of the origin of *E. coli* BLSE and AmpC isolates from beef, pork and veal

Reference	Description	ESBL	AmpC
		n	n
TRA 303	Pork minced meat	6	2
TRA 305	Beef cutting meat	2	0
TRA 306	Pork cutting meat	2	0
TRA 312	Preparation pork, beef, calf	1	0
DIS 815	Beef meat preparation (tartare)	3	0
DIS 823	RTE, minced meat, pork, beef, veal	6	2
DIS 874	RTE, Beef meat preparation (tartare), pork, beef, veal	3	1
DIS 888	NRTE, minced meat, pork, beef, veal	14	1

### Genotypic characterisation of E. coli isolates

A selection of 34 isolates have been characterised. The genotypic distribution of the different  $\beta$ -lactamase families are represented in the figure 84



**Figure 84.** Genotypic distribution of ESBL/AmpC E. coli isolated from pork, beef and veal (n=34)

### References

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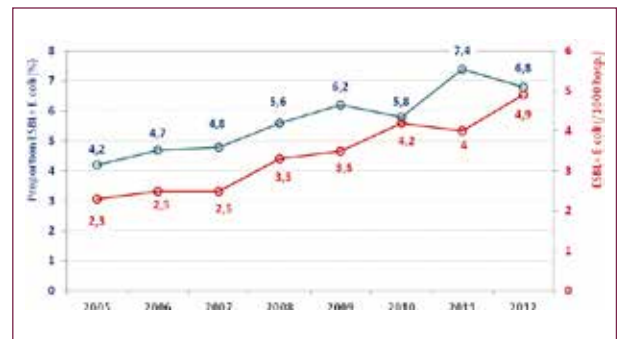
## ESBL producing bacteria in humans

At the end of the nineties, a rise in the antibiotic resistance of gram-negative bacteria, especially enterobacteria, was seen. Enterobacter aerogenes strains were found that produced extended spectrum beta-lactamases (ESBL). Beta-lactam antibiotics could no longer be used for the treatment of infections with these strains. A surveillance system was set up for multiresistant E. aerogenes (start 2000) and ESBL+ E. aerogenes (start 2002) in acute care hospitals. From 2005 on, the surveillance was expanded; ESBL+ Escherichia coli and Klebsiella pneumonia (2005), ESBL+ Enterobacter cloacae multiresistant (cefta-R + aminoglycoside-R + fluoroquinolone-R) Pseudomonas aeruginosa and Acinetobacter baumannii (2009) were added.

In 2012 national surveillance was organised by the WIV-ISP (in collaboration with the NRC for ESBL/CPE, UCL, Mont-Godinne) for E. aerogenes, E. cloacae, E. coli, K. pneumonia, A. baumannii and P. aeruginosa. Surveillance data was collected from 72 Belgian care facilities.

For the cohort of care facilities who participated at least three times to the surveillance since 2005, a positive evolution for ESBL+ E. aerogenes is seen (incidence per 1000 hospitalizations went from 2.5 to 1). Because of this positive evolution, data collection was stopped.

For ESBL+ E. coli and K. pneumoniae a negative evolution was seen. For E. coli, the incidence per 1000 hospitalizations went from 2.3 (2005) to 4.9 (2012). For K. pneumoniae the incidence went from 0.66 to 1.44. (Figure 85 and 86)



**Figure 85.** Evolution of the mean proportions and incidence numbers for ESBL+ E. coli (only facilities with 3 or more participations to the surveillance since 2005)



**Figure 86.** Evolution of the mean proportions and incidence numbers for ESBL+ K.pneumoniae (only facilities with 3 or more participations to the surveillance since 2005)

Though the surveillance of ESBL+ *Enterobacter cloacae* is still recent (started in 2009) the evolution also looks negative. In the first three years of surveillance the incidence per 1000 hospitalizations dropped from 0.94 to 0.74, in 2012 the incidence is estimated at 1.15.

The surveillance of *A. baumannii* and *P. aeruginosa* also started in 2009. The use of new case definitions for multiresistance in 2012 impede the interpretation of the surveillance data.

Overall a higher alertness is recommended for ESBL+ gram-negative Enterobacteriaceae. This is especially true for *E. coli* and *K. pneumoniae*. The first is a common cause of urinary tract infections, the latter is a renowned nosocomial pathogen. The incidence of both pathogens doubled in eight years. Multiresistant *Pseudomonas aeruginosa* incidence also went up.

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## trends and sources 2012 - 2013



- 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)