Zoonoses and Zoonotic Agents in Humans, Food, Animals and Feed in the Netherlands 2003-2006

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PREFACE

It has been since 2002 that the last extended report on the occurrence of zoonoses in the Netherlands has been published. In many aspects this period has been a very turbulent one. In 2003 the world was shook by the advent of SARS, monkey pox made its way from West African forests into the suburbs of the USA and from 2004 on worldwide fears of pandemic flu due to H5N1 hit the global newspaper headlines. The Netherlands had its fair share of zoonotic misery as well. In 2003 the H7N7 avian flu virus struck and proved to be able to infect hundreds with the greatest ease and sadly claimed one fatal victim. Seemingly out of the blue, methicillin-resistant *Staphylococcus aureus* (MRSA) suddenly popped up in the livestock industry and put a heavy strain on the very strict Dutch MRSA policy. These cases clearly show that there is no room for complacency and calls for vigilance and preparedness. If there is one thing to expect it is the unexpected. On the bright side, these wake-up calls did have their positive impact. The fact that successful zoonoses control can only be achieved by close collaboration of all involved disciplines is now widely accepted.

Nationally, the Centre for Infectious Disease Control (CIb) officially commenced and the formation of the Food and Consumer Product Safety Authority (VWA) was finally completed. Both organisations underscore the need for effective zoonosis control and intend to collaborate closely to achieve this. Consequently, this report has been compiled under the auspices of CIb and VWA with substantial contributions by major players in the field of zoonoses.

This report is based on data that is reported annually to the European Commission, in accordance with the Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents. They are supplemented with data from Dutch surveillance, monitoring and control programmes and relevant research projects concerning zoonoses and zoonotic agents by the different institutions that have contributed to the preparation of this report. The report also includes information on recent research on the antibiotic resistance of micro-organisms derived from human and animal material. Specific documentation and reports regarding the described programmes and research projects are available from the authors mentioned in the editorial list. The extended dataset on antimicrobial resistance and trends in the Netherlands has been published recently as a report: Maran 2003, 2004 and 2005.

drs. André Kleinmeulman

Prof. dr. Roel Coutinho

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1 INTRODUCTION

1.1 Authorities

Zoonoses are diseases that are transmittable between vertebrate animals and humans. Both the Ministry of Public Health, Welfare and Sport (VWS) and the Ministry of Agriculture, Nature and Food Quality (LNV) in the Netherlands are responsible for the monitoring and the control of zoonotic diseases in the food production chain. The Dutch Food and Consumer Product Safety Authority (VWA) of the Ministry of LNV is responsible for inspection and supervision of the food production chain, including food, feed and animals. The VWA has a public health responsibility with regard to food-borne infections and zoonoses and is involved in meat inspections and in the registration and control of diseases, including zoonoses, in animals. Other institutions are also involved in the protection of animal health. By the request of the Ministry of LNV, the Animal Health Service (GD) is responsible for sampling in some animal disease surveillance programmes. In addition, the Product Boards for Livestock, Meat and Eggs (PVE) conduct the *Salmonella* and *Campylobacter* monitoring and control programme prescribed by European legislation under the responsibility of the Ministry of LNV.

The National Institute for Public Health and the Environment (RIVM) conducts research into public health and environmental issues in the Netherlands. RIVM conducts research commissioned by the ministries of Health, Welfare and Sport (VWS), Housing, Spatial Planning and the Environment (VROM) and Policymakers use RIVM research findings to develop, implement and enforce policy. RIVM not only conducts research itself, but gathers data from all over the world, which it then interprets and applies. To increase collaboration between local and national experts and policy officers that work in the area of communicable diseases, a new Centre for Infectious Disease Control (CIb) was established in 2005. The CIb is part of the RIVM and has the following tasks: strengthening infectious disease control; communication on behalf of the government, with both professionals (national and international) and the general public; support professionals; and the coordination of outbreak management. The CIb has expertise in the fields of epidemiology, microbiology as well as public health interventions. It assembles and disseminates national and international data and provides early warnings in case of a threat to public health. In addition, the CIb advises the Minister of Health and can instruct professionals and municipalities. Finally, the CIb promotes the quality of preparedness for, and in response to, infectious diseases in both normal and crisis situations.

In the Netherlands, notifiable human zoonotic diseases must be reported to the Municipal Health Services (GGD), whereas the registration of these diseases is the responsibility of the Inspectorate for Health Care (IGZ) of the Ministry of VWS. When a zoonotic disease is reported, the local GGD is responsible for the control of the disease. If more than one GGD is involved, the National Co-ordinating centre for Infectious Diseases (LCI) is responsible for the co-ordination of the control activities. IGZ can, often in

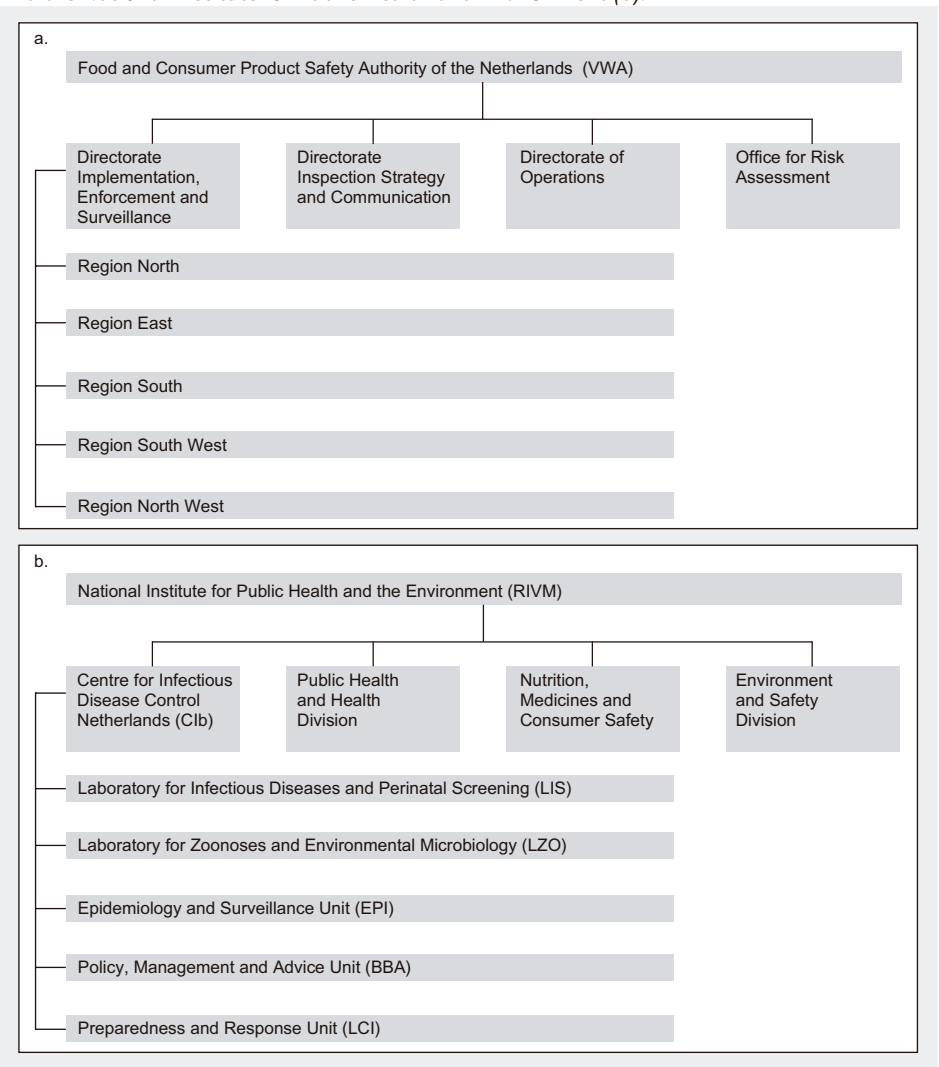
close collaboration with VWA, initiate monitoring and surveillance programmes for zoonotic agents and zoonotic diseases in humans. In the Netherlands, various laboratories are involved in the investigation of zoonoses in determining the presence of zoonotic agents in humans, food, animals and feed. VWA has several laboratories in which investigations are conducted on food and feed samples and animal waste products. Materials of human origin are examined for pathogens by the regional public health laboratories.

On behalf of VWA and IGZ, the RIVM conducts several monitoring and surveillance programmes regarding zoonoses and zoonotic agents (bacteria, viruses and parasites) in humans and animals or animal material. RIVM also houses the National and Community Reference Laboratory for *Salmonella*. Relevant research is also conducted at the Central Institute for Animal Disease Control (CIDC). The investigation of animals for the presence of rabies is a major activity of that institute. In collaboration with RIVM, this institute is also involved in a study on the risk factors concerning the development of campylobacteriosis in humans. The department of virology of the Erasmus Medical Centre (EMC) in Rotterdam plays an important role in studies on viral zoonoses, such as influenza. The National Influenza Centre, consisting of both EMC and RIVM, co-ordinates investigations on influenza in the Netherlands. Zoonotic agents can be transmitted from animals to humans in various ways. Foodstuffs of animal origin are the most important source of zoonoses. *Salmonella* and *Campylobacter* are the primary bacterial agents of food-borne zoonose. This report on zoonoses and zoonotic agents includes recent findings on antibiotic resistance from both humans and farm animals. An important development of that research is the detection of potential public health risks related to the use of antibiotics in animal husbandry.

Abbreviations of institutes and organisations involved:

AID	General Inspectorate	PDV	Product Board Animal Feed
ASG	Animal Science Group	PVE	Product Boards for Livestock, Meat and Eggs
CBS	Central Statistical Office		
CIDC	Central Institute for Animal Disease Control	RIVM	National Institute for Public Health and the Environment
EMC	Erasmus Medical Centre	UU	University of Utrecht
GD	Animal Health Service	VMDC	Veterinary Microbiological Diagnostic Centre
GGD	Municipal Health Service		
IGZ	Inspectorate for Health Care	VWA	Food and Consumer Product Safety Authority
LCI	National Coordination Structure for Infectious Disease Control	VWS	Ministry of Public health, Welfare and Sport
LNV	Ministry of Agriculture, Nature and Food Quality	WURC	Wageningen University and Research Centre
NRL	National Reference Laboratory		

Organization Charts of Food and Consumer Product Safety Authority of the Netherlands (a) And the National Institute for Public Health and Environment (b).



Notifiable diseases

Zoonosis	IZW	GWWD
Anthrax	X	X
Avian influenza	-	X
Botulism	X	-
Brucellosis	X	X
TSE's/(v)CJD	X	X
Glanders	-	X
Campylobacteriosis	-	X
Echinococcosis	-	X
EHEC/STEC	X	-
Leptospirosis	X	X

Zoonosis	IZW	GWWD
Listeriosis	–	X
Monkey pox	–	X
Psittacosis	X	X
Q-fever	X	–
Rabies	X	X
Salmonellosis	–	X
SIV	–	X
Toxoplasmosis	–	X
Trichinellosis	X	X
Tuberculosis	X	X
Tularemia	–	X
Viral haemorrhagic fever	X	X
Yellow Fever	X	–
Yersiniosis	–	X

IZW: Infectious Diseases Act (human)
GWWD: Animal Health and Welfare Act

1.2 Population data

Humans

Dutch population at 1 January 2006 (source: CBS)

Age distribution in years	Total
0-19	33,975,626
20-39	4,389,840
40-64	5,638,285
65-79	1,743,443
80 and older	587,016
Total	16,334,210
Sex distribution	**Total**
Male	8,077,407
Female	8,256,803

Dutch population over the last six years (source: CBS)

Year	2006	2005	2004	2003	2002	2001
Male	8,077,407	8,065,979	8,045,914	8,015,471	7,971,967	7,909,855
Female	8,256,803	8,239,547	8,212,118	8,177,101	8,133,318	8,077,220
Total	16,334,210	16,305,526	16,258,032	16,192,572	16,105,285	15,987,075
Growth +/-	+ 28,684	+ 47,494	+ 65,460	+ 87,287	+ 118,210	+ 123,125

Food/animals

Number of holding, related to animal species, and the number of animals per species as registered in 2006 (source: VWA)

Animal species	Number of holdings	Number of animals
Bovine animals	51,716	3,673,000
Dairy cows and heifers	27,089	2,546,428
Calves (< 1 year)	3,292	8,437,125
Sheep total	29,135	1,384,360
Milk ewes		651,485
Goats total	10,285	326,162
Pigs total	14,117	–
Fattening pigs	7,963	–
Chickens	2,662	91,782,254
Laying hens	1,612	41,641,960
Broilers	674	41,913,979
Turkeys	79	1,139,840
Ducks	95	1,043,349
Horses/ponies	16,945	128,473

Number of holdings, related to animal species, over the last eight years.

Animal species	2006	2005	2004	2003
Bovine animals including	51,716	57,361	38,358	39,191
Dairy cows	27,089	23,527	24,332	25,004
Veal calves	3,292	3,329	–	3,253
Pigs including	14,117	6,083	10,038	10,730
Fattening pigs	7,963	–	8,925	9,959
Breeding pigs	–	–	4,273	4,553
Sheep	29,135	28,997	14,396	14,731
Goats	10,282	10,104	4,532	4,709
Horses and Ponies	16,945	17,691	–	17,820
Poultry including	2,662	2,697	2,769	2,446
Broilers	674	740	771	777
Layers	1,612	1,245	1,540	1,223
Ducks and Turkeys	174	127	–	245

Number of animals (x 1000) over the last eight years (source: CBS)

Animal species	2006	2005	2004	2003
Bovine animals	3,673	3,798	–	3,759
including				
Dairy cows	2,546	1,433		1,478
Veal calves	523	533		732
Pigs		11,311	–	11,169
Sheep	1,384	1,362	–	1,185
Goats	326	292	–	274
Horses and Ponies	128	133	–	126
Poultry	91,782	91,850	–	79,235
including				
Broilers	41,913	42,679	–	42,289
Layers	41,641	29,932	–	30,498
Ducks and Turkeys	2,183,189	1,622	–	1,997

Number of slaughtered animals (x 1000), examined by the meat-inspection. (source: VWA)

Animal species	2006	2005	2004	2003
Bovine animals (incl. veal calves)	1,824	1,969	1,960	1,851
Pigs	13,846	14,376	14,340	13,893
Horses an Ponies		2	2	2
Sheep	240	633	620	450
Goat	–	–	20	22
Ducks and Turkeys	–	–	8,336	6,756**
Poultry	740,041	741,007	409,295	368,748**
including				
Broilers			397,046	359,100**
Hens			12,149	9,649**

** Due to the outbreak of Avian Influenza in 2003 the number of slaughtered poultry including ducks and turkeys was strongly reduced.

2 SURVEILLANCE AND CONTROL OF ZOONOSES IN HUMANS: GENERAL FEATURES

2.1 Risk analysis based monitoring and control

During the past ten years, significant progress has been made with the development of a risk-based and science-based framework for the management of food safety risks. The Codex Alimentarius Commission, and specifically the Codex Committee on Food Hygiene (CCFH), has played a pivotal role in this development, supported by the World Health Organization, the Food and Agricultural Organization of the United Nations, individual countries and experts. CCFH is now discussing a generic document to describe this risk management framework. In this framework, microbiological risk assessment (MRA) provides an essential input into the decision making process by linking measures in the food chain to control hazards with the health status of consumers. The application of MRA can be considered as a next step in the evolution of food safety management systems. This system is still based upon the application of hygienic practices (Good Manufacturing Practices, Good Hygienic Practices). Upon these, the Hazard Analysis Critical Control Points (HACCP) approach has been imposed that allows plant operators to target controls to risk factors that are specific to particular operations. HACCP has been widely accepted and is generally considered a major advance in food safety. HACCP does not, however, link the level of hazard control to public health objectives and consequently, the level of stringency of the food safety system is not based on objective criteria. This link is gaining more importance because food safety regulations are increasingly outcome based rather than rigidly describing the technology to be applied. This offers industry a greater degree of flexibility but requires from governments that they are more explicit about the public health targets to be met. Risk assessment has developed into a powerful tool to provide this link. The broader context of risk analysis (including also risk management and risk communication) is emerging as the new paradigm for decision making in food safety, allowing the gathering, evaluation and incorporation of a broad range of information (scientific, social, economic) into a decision making process that involves all relevant stakeholders.
The CCFH Risk Management Framework provides a systematic process, consisting of a series of steps to be taken consecutively or iteratively. These include:

- Preliminary microbiological risk management (MRM) activities;
- Identification and selection of MRM options;
- Implementation of MRM options;
- Monitoring and review of MRM options.

The preliminary MRM activities are a key element in this process, and include the identification of a food safety issue, the establishment of a risk profile, the formulation of a risk assessment policy and, if necessary, the commissioning of a risk assessment. Many food safety problems will be handled based on existing legislation or guidelines, or can be addressed on the basis of the risk profile that provides a concise, systematic evaluation of all available information. If a risk assessment is deemed necessary, the

risk profile will provide the necessary background to define the statement of purpose and to guide the commissioning process. Here, "options" is a broad term describing all possible actions that risk managers may take. They include traditional measures such as microbiological criteria, inspection or auditing procedures, import certificates etc. but also new, risk based approaches. A recent FAO/WHO Expert Meeting has provided further guidance on these approaches. They include the direct application of MRA results or the formulation of intermediate targets.

Direct application of MRA results can now be considered an established approach that is increasingly being used at the national and international level. In this approach, the public health benefits of proposed measures are evaluated by comparing the results of the baseline risk assessment model with those simulating (several) intervention(s). The relative risk in these two scenarios is an indication of the public health gains to be expected. Such information can then be combined with additional information, e.g. economic evaluations or acceptance by stakeholders, to inform the decision making process. Direct application of MRA results may be problematic if there are many different food operations producing the same kind of food. This may necessitate the development of a series of MRAs to include the diversity between such operations. In import situations it may be difficult to obtain the necessary information for a MRA in the country of origin. Also, the direct application of MRA results offers a limited flexibility to industry. For these reasons, it is considered desirable to define intermediate targets such as performance objectives (PO) and food safety objectives (FSO). CCFH has provided definitions of these new concepts but there is currently no consensus on how these concepts are to be applied in practice. A key problem is that the current definitions are not appropriate for use in combination with probabilistic risk assessment, i.e. risk assessments that incorporate the variability in the food chain and the uncertainty about the available information. This is a major drawback that needs to be resolved before the new concepts of intermediate targets can be applied. Note that PO and FSO are not criteria that are directly controllable by food industry. They are design criteria that need to be translated into practical criteria such as performance criteria (PC) and process criteria (PrC) that can be implemented in practice.

Priority setting of food borne pathogens

Human health is threatened by a wide variety of pathogens transmitted by food. Effective and efficient policy-making on control, prevention and surveillance of these food borne pathogens must focus on the most relevant ones. Therefore a need was expressed by Dutch decision makers to develop methods for priority setting of existing and emerging food borne and zoonotic pathogens to provide an objective basis for decisions on future projects.

The relevance of the pathogens is assessed by various criteria. The project will focus on disease burden (in Disability Adjusted Life Years - DALYs) and cost of illness as key decision variables. As a first step, estimates were produced in 2005 for (thermophilic) *Campylobacter* spp., Shiga-toxin producing *Escherichia coli* O157 (STEC O157), *Listeria monocytogenes*, *Salmonella* spp., norovirus, rotavirus, (non-typhoidal) *Salmonella* spp. and *Toxoplasma gondii*. Estimates refer to all cases of illness, irrespective of transmission route. Later, for every pathogen the fraction attributable to food and the most important food products will be indicated. Among the pathogens that were evaluated, noroviruses and rotaviruses are the agents that cause most cases of gastro-enteritis in the general population. Yet, the disease burden is somewhat lower than that of *Salmonella* spp. and less than half of that of *Campylobacter* spp. This is related to the fact that most cases of viral gastroenteritis

are mild, of relatively short duration, and have a low case-fatality ratio. Also, in contrast to viral infections, bacterial gastroenteritis can result in more serious sequelae that are long-lasting and/or chronic, resulting in a considerable disease burden. For STEC O157, the disease burden in the population is relatively low, but per case it is the highest of all evaluated pathogens. This is mainly related to the relatively high mortality of young children. The disease burden per case of listeriosis and toxoplasmosis is even higher because of high case-fatality ratios. Even though the incidence of toxoplasmosis in the Dutch population is very uncertain, it has been shown that on a population basis *T. gondii* causes the highest disease burden of the seven evaluated pathogens.
Using cost of illness as the indicator, the impact of viral gastroenteritis is somewhat larger than that of *Campylobacter* spp.. *Salmonella* spp. has the lowest COI of the four pathogens considered. In all cases, the indirect health care costs (mainly temporary absence from work) were much higher than the direct health care costs. For chronic and long-lasting diseases, such as those associated with bacterial infections, the direct health care costs do contribute significantly to the total cost. Direct non-health care costs were very low for all four pathogens. These results show that costs associated with food borne pathogens may have an impact on very different sectors of the society, namely the public health sector, ill citizens and employers. The effects of discounting are limited because most costs relate to acute effects.
Thus, the relative societal impact of food borne pathogens differs according to the criteria chosen. For example, should all cases be considered or only cases searching medical services; what is the indicator chosen (e.g. incidence of illness, incidence of fatal cases, COI or DALYs); what is the perspective taken (for example the society (all costs) or the public health sector only); is the impact considered on the total population or on an individual basis?
The project also pays attention to other factors including expected effectiveness and efficiency of control measures and public perception. In October 2005, two groups of respondents (citizens and experts) were asked to evaluate the risk of four pathogens (*T. gondii*, *Salmonella* spp., norovirus and the BSE agent) using an Internet-based enquiry. In general, citizens expressed the opinion that chemical contaminants were the main food safety problem, but the difference with pathogens was small. Experts clearly indicated pathogens to be of most concern. Almost all respondents were familiar with BSE and *Salmonella* spp., approximately half of the citizens knew of *T. gondii* (clearly more women than men) whereas norovirus was known to only one out of six respondents. The risk of all four pathogens was considered similar by both groups of respondents, and there were no significant differences between citizens and experts. There was, however, strong inter-individual variability in the answers. Respondents also indicated which dimensions of risk they found most important. Experts indicated the three aspects of the classical risk paradigm: severity of the effect, probability and number affected. For citizens, the number affected was less important than the level of personal control.

2.2 Laboratory surveillance

National networks

A sentinel-based surveillance programme on bacterial pathogens called Laboratory Surveillance Infectious diseases (LSI) has been operative since 1989. Sixteen regional public health laboratories (PHLs) participate in this programme. All primary isolates of *Salmonella* of patients are sent to the National Reference Laboratory (NRL) at the RIVM for serotyping and phagetyping. A representative selection is tested for antibiotic susceptibility by CIDC. The coverage of the Salmonella surveillance is estimated to be 64%. Basic information from the patient is collected, such as age, gender, residence, country of infection and the possible source of infection. From 1995 onwards, on a weekly basis, the same laboratories also report the total number of detected *Campylobacter* spp. and the total number of stool samples examined. The coverage of the *Campylobacter* surveillance is estimated at 52%. From 2002 onwards, basic information from the patient is collected and species and antibiotic resistance of the pathogen is determined.

In 1996, *Escherichia coli* serotype O157 (sorbitol-negative isolates that agglutinate with *E. coli* O157 antiserum) that may produce shiga toxin, was added to the LSI programme. Primary isolates of *E. coli* O157 are now sent to the NRL for confirmation and further typing. Besides O and H typing, isolates are typed for the presence of shiga toxin genes (*stx*1 and *stx*2), the *E. coli* attaching and effacing gene (eae gene), and the enterohemolysin gene. They are also characterised by pulsed-field gel electrophoresis. From April of 1999, all Dutch medical microbiological laboratories have contributed to the surveillance of shiga toxin producing *E. coli* (STEC). In this enhanced STEC-surveillance programme, the municipal health services interview all diagnosed cases in order to obtain detailed information on risk factors and clinical aspects, using a standardized questionnaire. In 2005 and 2006 RIVM, in collaboration with eight medical microbiological laboratories, assessed the relative importance of non-O157 serogroups STEC in the Netherlands using a real-time PCR specifically developed for this purpose.

The surveillance of *Listeria* spp. is part of the LSI programme since 1989. It includes isolates sent to the RIVM for typing by the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) and it is considered to have coverage of almost 100% for the severe cases of listeriosis. In 2005, the existing surveillance was enhanced. Since then, all laboratories are requested to report positive cases to the public health services and submit *Listeria* isolates of patients with meningitis or septicaemia to the NRLBM. The NRLBM sends these strains to the RIVM for serotyping and PFGE. Isolates of cases with other clinical manifestations of listeriosis are sent directly to the RIVM for typing. The public health services collect clinical and risk factor information of patients, using a standardised questionnaire.

International networks

The results of the Dutch human laboratory surveillance for Salmonella, Campylobacter and STEC are reported on a monthly basis to the EU-network ENTERNET. Outbreaks of these pathogens that are of international importance are communicated within this network and may lead to collaboration with respect to (molecular) typing, trace back of food products and epidemiological analysis. On behalf of the WHO surveillance programme for the control of food borne infections and intoxications in Europe data on food borne infections are reported to WHO on a yearly basis. RIVM, for example, is involved in a number of international projects, including EVENT (Enteric Virus Emergence New Tools), DIVINE (Prevention of emerging (food-borne) enteric viral infections), ENIVD (European Network of imported Viral Disease) Echinorisk (risk assessment echinococcosis), Trichiporse (trichinella in pork and horse), COST 920 (foodborne zoonoses) and most work packages of the Med-Vet-Net network (medical-veterinary Network of excellence).

Epidemiological studies (NIVEL, SENSOR, CASA and CASAVE)

Gastrointestinal diseases, including zoonotic diseases caused by *Salmonella*, *Campylobacter* and STEC, have been he focus of several Dutch based epidemiological studies. These studies were performed on different levels of the surveillance pyramid (Figure 2.2.1.). They estimated the number of symptomatic cases in the general population,

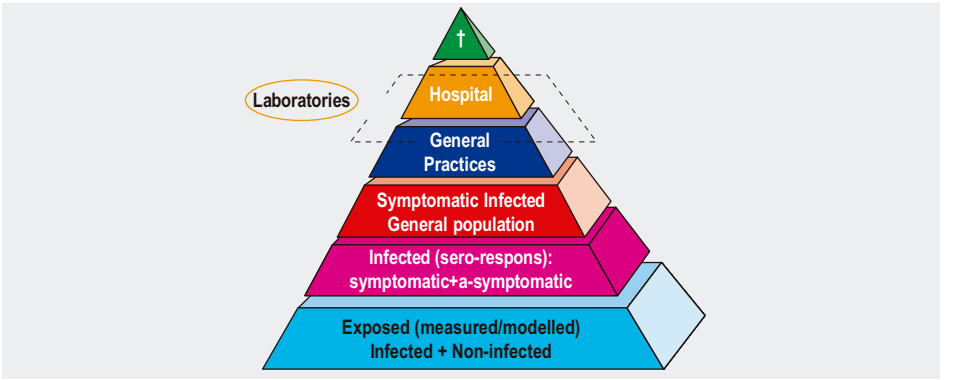


Figure 2.2.1. The Surveillance Pyramid

those presented to a physician, and how these numbers relate to real and reported numbers derived from laboratory testing. The NIVEL study (1996-1999) focused on general practices, and the SENSOR-study (1999) focused on the general population. Both studies revealed the relative importance of a large panel of pathogens (bacteria [and toxins], viruses and parasites). The studies also revealed the existence of a diagnostic deficit concerning the amount of acute gastroenteric disease occurring in the general population and the proportion that visited a doctor because of the disease.
With respect to *Campylobacter* and *Salmonella*, a large-scale laboratory driven case-control study was performed from April-2002 to May-2003 (CASA-study). The study was designed to establish the risk factors involved in acute gastroenteritic disease caused by these pathogens (see 4.3 and 4.19). Over ten thousand frequency matched controls (10,250) were approached, as well as 3169 patients with a laboratory confirmed *Campylobacter* and 1171 with a *Salmonella* infection. In a selection of cases and controls, a follow-up study has been performed (CASAVE-study: 2005-2006) into the chronic sequelae of the infections and into host factors characterised by DNA polymorphisms detected in collected mouth swabs. To reconstruct the whole pyramid (hospital uptake, chronic sequelae, and mortality), it was necessary to take into account additional available national data. The understanding of the Dutch surveillance pyramid allowed for the multipliers between the different layers of the pyramid to be assessed, and enabled the estimation of the true burden of disease and the cost of illness involved. These are mentioned in the introductions of the sections on *Salmonella* (4.19) and *Campylobacter* (4.3). In collaboration with other EU countries, studies are in progress that compare estimates of infection between countries based on serology and these studies extend the surveillance pyramid by yet another layer, a bottom one that assesses human exposure for the most important sources.

2.3 Early warning and outbreak response

Threats to public health caused by infectious diseases usually appear unexpected, but can have major consequences in a very short period of time. Recognition of these threats is essential. In the Netherlands, the “early warning committee” (EWC) has been

operating since 1999 under the authority of the Health Inspectorate. Its main task is to assess information from various sources, foreign as well as domestic, in order to timely recognise threats to public health caused by infectious diseases. If necessary, the EWC can initiate any further outbreak investigations, and can take measures to control any occuring outbreak. The weekly meeting of the early warning committee takes place at the National Institute of Public Health and the Environment (RIVM). Participants are microbiologists and epidemiologists from various departments of the RIVM, including the National Coordination Centre for Outbreak Management (LCI), as well as representatives from the Food Safety Authority (VWA). Prior to the meeting, each participant selects information items from various sources which, in his opinion, are important to discuss at the meeting ("signals"). There can be several reasons for selecting a signal. These are outlined in a protocol, and are based on experience. A sudden change in the incidence or prevalence of an infectious disease, the appearance of an infectious disease among certain groups of people or in certain places, or the emergence of a totally new or unknown disease are some of the reasons mentioned. During the meeting, the various signals are discussed and interpreted by the participants in order to estimate the threat to public health in the Netherlands. On the same day, the RIVM sends a report of the meeting to about 500 people engaged in the control of infectious diseases in the Netherlands: physicians and nurses of the municipal health services, microbiologists, specialists in infectious diseases, infection control practitioners, the Ministry of Health and the Inspectorate of Health. The report is formulated in such a way that signals are not deducible to persons, institutions or locations. Evaluation in 2004 showed that the early warning committee recognised nearly all threats posed by infectious diseases and outbreaks of infectious diseases and which were of national importance, and/ or published in various sources of literature.

2.4 Zoonoses and occupational health and safety regulations

In certain working situations employees may be subjected to various forms of contact with zoonotic agents. The actual risk of transmission is dependent on several variables such as frequency, intensity and the nature of contact between employee and the animals or vector organism involved. From the juridical point of view, the relation between employer and employee is a special one. That is the employer is obliged to take all necessary measures to protect the employee's health and is fully liable to compensate for any health damage in the case of negligence. Risks need to be combated at the source as much as possible. The employer's obligations are prescribed in the Dutch Occupational Health and Safety (OHS) Act (Arbeidsomstandighedenwet). Enforcement of the OHS Act is a matter of the labour inspectorate (Arbeidsinspectie), which resides under the Ministry of Social Affairs and Employment.

Occupational Health and Safety Legislation

Dutch OHS legislation is derived from European legislation for the most part. Rules concerning biological agents are prescribed in the OHS legislation as well as in the Envi-

ronmental Protection act (Wet Milieubeheer). The OHS Act, the OHS Order (Arbobesluit) and the Environmental Protection Act are equally important in regard to working conditions. The European Directive 2000/54/EC forms the legal basis for the risk group classification of bacteria, viruses, moulds and parasites. The Directive governs the protection of workers from risks related to the exposure to biological agents. In the Netherlands, the directive has been implemented in the OHS legislation. Dutch OHS legislation has a stratified structure. The framework consists of the OHS Act, the OHS Order as well as the OHS Policy Regulation (Arboregeling), all of which comprise binding requirements. Furthermore, OHS Policy Rules (Arbo beleidsregels), OHS information leaflets (Arbo informatiebladen) and OHS standards (Arbo normen) set non-binding requirements. The OHS Act deals with general obligations of employers and employees, whereas the OHS Order describes more specific technical and organizational measures that have to be complied with when working with dangerous substances or biological agents. Section 9 of the Order specifically addresses biological agents. Parts of the OHS Act and the OHS Order form the juridical basis to elaborate on certain OHS topics by Ministerial Order. Finally, specific rules regarding the combat of risks related to the exposure to biological agents may be formulated in the OHS Policy Rules.

Compulsory risk assessment is of great importance to the framework of OHS legislation. This implies that all possible risks need to be mapped, weighed and reduced to acceptable levels by means of a scheme of approach. If there is a reasonable chance that an employee may be exposed to biological agents, the nature, extent and duration of the exposure have to be assessed within the frame work of risk inventory and evaluation (RI&E) in order to determine the specific risk posed to the employee.

Division of risk groups

Micro-organisms are divided into risk groups on the basis of the following criteria:

- Ability to cause human disease
- Risk of spreading to the community
- Availability of effective prophylaxis or treatment

Table 2.4.1. Division of risk groups

Risk Group	Pathogenicity	Risk of spread to community	Prophylaxis/Treatment
1	Negligible	n.a.	n.a.
2	Moderate	Unlikely	Available
3	High	Possible	Available
4	High	Highly Possible	Unavailable

This division mainly relates to infectious risks due to occupational exposure. Corresponding physical containment levels have been defined for biological agents belonging to group 2, 3 or 4. Examples for agents belonging to group 2 are *Campylobacter spp*., MRSA and the Measles virus. *Bacillus anthracis*, *Mycobacterium bovis* and the Monkeypox virus represent group 3 organisms. Group 4 organisms are exclusively found

among viruses, the Ebola-, Marburg and Lassaviruses being prominent examples for this group. OHS legislation distinguishes between situations where micro organisms may be used intentionally (e.g. biotechnology, diagnostics, research) and where micro organisms may be present inadvertently (e.g. in patients or clinical specimens). Furthermore, OHS legislation distinguishes between industrial processes and laboratories, especially with regard to management measures.

Vaccination

If there is any possibility that an employee may contract an infectious disease during his work the employer is obliged to offer vaccination free of charge. This may, however, never be mandatory, while at the same time the employer can demand that only vaccinated employees may commence certain activities. An employee's refusal of vaccination is no valid reason for dismissal; suitable work has to be offered in stead. Nonetheless, upon appointment of new personnel compliance with vaccination schemes may be stipulated as a condition.

High-Risk Groups

High-risk groups comprise employees who have an increased risk of contracting a zoonosis. Transmission may either occur through

- direct contact with living animals (e.g. touch, bite, scratch)
- contact with contaminated animal products
- vectors (e.g. ticks, flies)
- contact with animal waste

Three groups of employees explicitly mentioned in the Arbowet are especially vulnerable when working with infectious micro-organisms, they are

- youths
- pregnant women
- elderly persons

Persons with congenital or acquired immunodeficiency are equally at risk. Younger persons (under the age of 18) are not allowed to work with class 3 or 4 agents. The special vulnerability of pregnant women results from the damage that several infections can cause in the unborn child. Thus, the OHS order prescribes that pregnant women should not work with *Toxoplasma* or rubella virus unless their immunity has been proven.

Zoonoses

Although the OHS act does not specifically address zoonoses, it is quite clear that the entire framework of OHS legislation is applicable to this issue as well. The provisions of the general OHS legislation, and the OHS Order in particular, cover all work-related possibilities of contracting a zoonosis. Extensive obligations with regard to risk mapping (RI&E), health monitoring and taking all conceivable preventive measures are listed. The labour inspectorate supervises compliance with these obligations. Company medical doctors and/or OHS services must report work-related zoonoses to the Dutch

Centre for Occupational Diseases (Nederlands Centrum voor Beroepsziekten). Generally, occupational diseases are underreported to a large extent and this is all the more true for work related zoonoses. The reporting system has been active for 5 years.

Table 2.4.2. Number of work-related zoonoses 2002 – 2006 (1st 6 months)

Zoonosis	Number of reports
Lyme disease	41
Malaria	27
Cutaneous leishmaniasis	14
Dengue	7
Psittacosis	5
Fungal skin infections (e.g. trichophytosis)	3
Weil's disease	4
Myasis, larva migrans (incl 1 case of tumbu fly larvae)	4
Streptococcus suis infection	2
Avian flu related conjunctivitis	1
Pruritic rash due to rodent mites	1
Rickettsia conorii infection	1
Toxoplasmosis	1
Amoebiasis (incl *Entamoeba histolytica*)	2
Non human primate bite incident	1
Bilharziosis	2
Erysipelas	1
Histoplasmosis	1
Mycobacterium marinum	1
SARS	1
Phlegmona of the hand	1
Campylobacteriosis	1
Other, non-specified zoonoses	7

Table 2.4.3. Number of reported zoonoses per year

Year	Number of reports
2002	21
2003	28
2004	35
2005	40
2006 (1st 6 months)	8
Total	132

Developments

The number of reports of zoonotic occupational diseases is low. However, it is a reasonable assumption to ascribe this to professional inattention rather than implying a truly low incidence. In order to improve the situation and heighten awareness among professionals, several initiatives have started recently. The Ministries of Social Affairs

and Public Health have agreed to work together more closely on the matter, protocols specifically addressing zoonotic occupational diseases are formulated, a knowledge information system for OHS professionals (Kennisinformatiesysteem Infectieziekten, KIZA) has been aired and an OHS medical officer as well as an OHS hygienist have been appointed at the National Coordinating Body for Infectious Diseases (LCI).

3 MONITORING AND CONTROL OF ZOONOTIC AGENTS IN FEED, ANIMALS AND FOOD PRODUCTS: GENERAL FEATURES

3.1 Monitoring programme for *Salmonella* in feed

Within the framework of the EU Directive 2003/99/EG, samples of feed ingredients and compound feed have been taken regularly for several years in the Netherlands. In line with the directive, the feed sector has implemented a national monitoring programme for *Salmonella* in compound feed and feed ingredients. This programme is disclosed in the 'Regulation PDV Zoonoses and zoonotic agents animal feed sector 2006'. Standards and the necessary control measures are prescribed in detail in the GMP+-regulation of the feed chain sector for the purpose of controlling *Salmonella* in (poultry) feed. The aim of the programme is to minimise the introduction of salmonella into the poultry chain through animal feed. The programme started seven years ago, but has intensified during the past five years, particularely, the monitoring of feed ingredients.

The GMP+ standards (maximum *Salmonella* incidence and process standards for enterobacteriaceae) for 2005 and 2006 are presented in table 3.1.1.

Table 3.1.1 Product standards Salmonella

Product norms 2002	Maximum salmonella contamination % in batches to be delivered	Maximum % with S. Enteritidis / S. Typhimurium in batches to be delivered
Poultry compound feeds and animal feed for single delivery to poultry companies, for:		
Top breeding	0^+ %	0^+ %
Raising parent stock	0^+ %	0^+ %
Parent stock	0^+ %	0^+ %
Rearing hens laying sector	1 %	0^+ %
Laying hens	1 %	0^+ %
Consumption turkeys	0^+ %	0^+ %
Broilers	0^+ %	0^+ %
Process norms	Maximum cfu enterobacteriaceae per gram	
	Target value	Action limit
Poultry compound feeds for:		
Top breeding		100
Raising parent stock		100
Parent stock		100
Other poultry compound feeds if given heat treatment, for:	<100	1.000
Breeding hens laying sector	<100	1.000
Laying hens	<100	1.000
Meat turkeys	<100	1.000
Broilers		

Control measures are mostly aimed at processing during the production of poultry feed and at the supply of salmonella-critical feed ingredients, which are used in feed. In addition, there are general control measures in the GMP+ standard for animal feed (other than poultry) in order to minimise the introduction of salmonella by way of feed. For these kinds of feed so far only standards for enterobacteriaceae after heat treatment have been determined: the target value is '< 100 cfu' enterobacteriaceae per gram, the action threshold is 1,000 enterobacteriaceae per gram.

Monitoring is performed to verify the effectiveness of the control measures for both *Salmonella*-critical feed ingredients and poultry feed. The following feed ingredients have been assessed as being salmonella-critical for 2005/2006: South-American extracted soy beans and expeller, untreated fishmeal, extracted rapeseed and expeller, toasted soy beans, European sunflower meal and expeller and eggshells. In addition, the programme monitors feed for other animal species apart from poultry and of non-Salmonella-critical feed materials, to avoid unexpected contamination from a formerly unsuspected source. The monitoring is primarily done by the companies involved as part of their GMP+ programmes. An extra national, independent verification is done by the Product Board Animal Feed.

3.2 Farm animals

3.2.1. Monitoring and control programmes for Salmonella and Campylobacter in poultry

In 1997, the poultry sector (PVE: Product Boards for Livestock, Meat and Eggs) started an eradication programme for *Salmonella* and *Campylobacter*, called the "Plan of Approach for *Salmonella* (and *Campylobacter*)". The rules of this programme are based on five principles: hygiene requirements, cleaning and disinfection, incoming and outgoing inspections, reporting results and measures to be taken after infection occurred. The objectives of the plan of approach were to achieve a reduction, in the infection level of the broiler flocks with *Salmonella* at the end of the slaughter process, to less than 10% infected and a reduction in the infection level of flocks of layer hens to less than 5% with *Salmonella enteritidis* and *S. typhimurium*. In 2000, the objectives were not attained and additional measurements were considered necessary. These adjustments resulted in the control programme *Salmonella* and *Campylobacter* in poultry meat 2000^{+} and the control programme in the egg sector 2001^{+}. New objectives were set along with these new programmes. The main objective for the poultry meat sector was set at a maximum *Salmonella* contamination level of produced meat of 10%. The objective in the egg sector was tightened to 0^{+}% *S. enteritidis*/*S. typhimurium* infected eggs in the consumption channel. For both sectors the end target in 2010 is a contamination degree with *Salmonella* of 0^{+}%.

Table 3.2.1. Monitoring for Salmonella in poultry flocks. Campylobacter is monitored only at the broiler farm and at slaughter.

Production chain	Incoming	Outgoing
Breeding flocks		
Grand parent rearing	*day of arrival*: box paper (40 pieces) *4 weeks of age*: cloacal samples (2×30)	*max. 14 days before transfer*: faecal samples (6×25)
Grand parent stock	*22 –24 weeks of age*: faecal samples (2x150) or 5 pair of boot swabs (2 pools)	*every 2 weeks* (≥age 24 weeks): faecal samples (2x150) or 5 pair of boot swabs (2 pools)
Hatchery*	*Every two weeks*:* One composite sample containing 5 hatcher basket liners, or 10 g broken eggshells from 25 hatcher baskets. 25 g sample must be tested.	every hatching entity is sampled once: fluff (5x5 g)
Parent rearing	*day of arrival*: box paper (40 pieces) *4 weeks of age*: cloacal samples (2×30) or 5 pair of boot swabs	*max. 14 days before transfer*: faecal samples (6x25) or 5 pair of boot swabs
Parent stock	*22-24 weeks of age*: faecal samples (2x150) or 5 pair of boot swabs (2 pools)	*from 24 weeks of age*, *every 2 weeks*: faecal samples (2x150) or 5 pair boot swabs (2 pools)
Hatchery*	As described in "Hatchery" below "Grand parent rearing/stock"	*meat*: *every hatching entity is sampled once*: fluff (5x5 g). *laying*: *every 2 weeks one hatching entity is sampled*: fluff (5x5 g)
Meat production		
Broiler farm**	*day of arrival*: box paper (40 pieces)	*from 21 days onwards:* faecal samples (2×15 samples or 2 pair of plastic shoes)
Slaughterhouse**	faecal samples (small intestine) (1×30)	breastskin sample (25 g), every batch filet surface samples (25 g), 1 sample/day
Egg production		
Layer at rearing age		*max. 21 days before transfer*: blood samples (0,5% of the animals in a flock with a min. of 24 and a max. of 60 samples)
Layers		*Every 15 weeks (≥ age of 24 weeks ± 2 weeks):* samples of faecal material and dust.

*Sampling at the hatchery is only compulsory when the operator managing the breeder flocks prefers monitoring in that phase and is in agreement with the hatchery.
** Campylobacter is tested for in 1 out of 4 samples at the broiler farm and at slaughter.

Developments in the control programme Salmonella and Campylobacter poultry meat 2000⁺ and eggs 2001⁺

As the new control programme is focused on *Salmonella* contamination of the end product, alterations were introduced in the monitoring system at slaughterhouses, and a monitoring commitment was added for the cutting plants. In slaughterhouses samples are taken from every batch of end product and these are then analysed for

Table 3.2.2. Control measures in poultry flocks in case of Salmonella infection. Campylobacter is not controlled for.

Production chain	Measures
Grand parent rearing/stock	In case of SE/ST: destruction of the flock; any other serotype*: tracing (supervision of veterinarian), cleaning and desinfection and swab test afterwards. New flock can be placed when the swab test was negative.
Hatchery	After verification at the poultry house, SE/ST infected eggs are eradicated. When necessary for reaching the specified target of the programme PPE can prescribe that Salmonella infected eggs, including serotypes SH, SV and SI*, are hatched logistically.
Parent rearing/stock	As described in "Grand parent rearing/stock"
Hatchery	As described in hatchery below "Grand parent rearing/stock"
Meat production	
Broiler farm**	Tracing in case of Salmonella (supervision of veterinarian); cleaning and disinfection and swab test afterwards.
Slaughterhouse**	Logistical slaughter of Salmonella infected flocks.
Egg Production	
Layers rearing	If SE/ST: eradication of the flock or the eggs to the processing industry; tracing (supervision of veterinarian), cleaning and disinfection, and swab test and hygiene check afterwards.
Layers	If SE/ST: eggs to the processing industry; tracing (supervision of veterinarian), cleaning and disinfection, and swab test afterwards. Vaccination of the following flocks in the house.

* In the laying sector only in the case of the recently emerging serotypes Hadar, Virchow and Infantis.
** There are no control measures related to positive Campylobacter findings

Salmonella (about 550 flocks per week) and occasionally for *Campylobacter* (about 150 flocks per week). Based on these monitoring results, contamination percentages can be calculated. Slaughterhouses that deliver more than 10% *Salmonella*-contaminated poultry meat, are obliged to formulate a plan of action to improve the situation. The slaughterhouses receive certificates from the Commodity Board, which they can offer for publication, on which the results of contamination percentages for a period of three months (quarterly) are presented. An essential change in the control programme concerns the start of tracing attempts in the poultry chain. In case of a *Salmonella* infection in a flock, the *Salmonella* serotype that caused the infection must always be identified. Furthermore, it is mandatory to carry out serotyping of *Salmonella* isolates from Salmonella contaminated end products. In this way, the kind of *Salmonella* serotypes circulating in the poultry meat chain can be known at any time. In addition, since 1 January 2002, the destruction of *S.enteritidis/S.typhimurium* positive breeding and production flocks and hatching eggs has been made mandatory. Since 2007, in certain situations but not all, it is obligatory to cull *S.* Virchow*, S.* Hadar or *S.* Infantis positive breeding and production flocks. The monitoring and control activities for *Salmonella* and *Campylobacter* are summarized in the Tables 3.2.1. and 3.2.2. Monitoring results for *Salmonella* are described in section 4.19 and for *Campylobacter* in section 4.3.

3.2.2 Surveillance of production animal health by the Animal Health Service

Since 2003, the Animal Health Service (GD) has implemented a national system for the surveillance of production animal health. This was done on request of the major stakeholders of the Dutch agricultural industry, i.e. the Dutch Dairy Board, the Product Boards for Livestock, Meat and Eggs and the Ministry of Agriculture, Nature and Food Quality. These parties need information on animal health to help them safeguard consumers and human health, give warrants to other countries, evaluate policies and to enable them to act instantly in problem situations. The three objectives of this surveillance programme are the following:

- Monitoring of well known exotic OIE list diseases;
- Detection of new or emerging diseases
- Description and analysis of trends and developments of various aspects of animal health.

The animal health surveillance of the GD consists of a number of complementary components with which information on the animal health situation is collected.

VeeKijker (Livestock-scope)

The purpose of the VeeKijker is to detect exotic and emerging diseases. It does so by combining a second line consultancy desk with the task of collecting and evaluating information. Private veterinarians are especially motivated to contact the "VeeKijker" through a nationally advocated telephone number at the GD in cases of incidents or herd health problems that are unfamiliar to them. Calls are handled by a consistent group of veterinary specialists from the GD. When deemed necessary, these specialists visit the farms to assess the problem. To further motivate farmers and veterinarians to contact the "VeeKijker", regular feedback of information is provided by means of publications on GD websites and in magazines, presentations and newsletters. About 10.000 consultations are handled annually.

Diagnostic pathology

Information is also collected from diagnostic pathology records at the GD. Annually farmers and veterinarians submit about 6000 cadavers for post-mortem investigation. A team of six veterinary pathologists at the GD conducts over 95% of post-mortem examinations on large animals in the Netherlands. Diagnostic pathology is a useful instrument for detection of emerging diseases. In addition to establishing the cause of death or disease, information is collected on resistance of pathogens against antimicrobial drugs.

Veterinary toxicology

In addition to the "VeeKijker" and the diagnostic pathology unit, the surveillance system incorporates veterinary toxicology. The consequences of intoxications in production animals are not necessarily restricted to individual cases only, but can involve a

cluster of farms in one neighbourhood or with a common supplier, and so, incidentally a part of the food chain. For this surveillance component, all relevant fields of expertise (clinical investigation of living animals, post mortem examinations, environmental analyses and toxicological investigations) are present within the GD.

Prevalence studies

In various animal populations, prevalence studies are performed regularly. In the cattle population for example, prevalence studies on a number of infectious diseases are conducted. Farms are selected by means of random sampling, and examined serologically either in a bulk milk sample (dairy herds) or a fixed number of animal sera. The choice of diseases is made by the stakeholders and is mainly based on the zoonotic aspects (*Leptospira hardjo, Salmonella* Dublin and *Salmonella* typhimurium) or economical aspects of infectious pathogens (Bovine Viral Diarrhoea virus (BVDV), Infectious Bovine Rhinotracheitis (BHV1), Neospora caninum). This surveillance component is pro-active and serves to describe prevalence and trends of infectious diseases over time.

Data analysis on census data

In this surveillance component for cattle-health, key monitoring indicators (KMI) have been developed based on census data from five different data sources. These KMI allow monitoring and analysing trends and changes of aspects of cattle health over time. The data sources are:

- Identification and Registration (I&R) with information on all cattle (date and place of birth, previous and current place of residence, on-farm and off-farm movements),
- The national rendering plant "Rendac" with information on cadavers collected on-farm
- Milk Control Station with information on bulk milk quality,
- Dutch Cattle Improvement Organization with information on milk production and cow somatic cell counts,
- Animal Health Service with information on certified free status of BHV1, BVDV, leptospiroses and salmonellosis.

By use of the unique farm identity number (UBN), data is aggregated by herd on a quarterly basis. Within six main herd types, herds are characterised by herd size, open/closed farm management system, certification of disease status, province and milk production level.

Interpretation and aggregation of information

Information from all surveillance components is combined and interpreted regularly in relation to the three goals that are set for monitoring. The collection of information, and aggregation and interpretation of data is done by specialists in various fields of expertise (veterinary medicine, pathology, laboratory, epidemiology and statistics). They meet on a regular basis to discuss the results of the information collected from different sources in mutual relationship. If, as a result of these discussions, certain information indicates an unknown disease or a threat to human or general animal health, further investigation is instigated. Initially, this is done by designing a small-

scale research project (pilot study). Such a pilot study can involve farmer or veterinarian participation, questionnaires, sampling of selected animals and/or post mortem investigation. Whenever useful, GD-specialists cooperate with specialists from institutes in other fields of expertise to obtain a broader view on specific issues. With regard to zoonotic diseases for example, cooperation is often entered into with RIVM. Results and findings are reported to the stakeholders quarterly or, in case of emergency, instantly. In addition, the GD advises stakeholders on possible actions.

3.2.3 Food chain information at slaughterhouses

With the implementation of the hygiene regulations EC 852/2004, EC 853/2004 and EC 854/2004, the possibility for the application of a differentiated inspection regime for fattening pigs under certain conditions was created by which one or more incisions can be omitted (henceforward to be referred to as "visual inspection"). The verbatim text is as follows:

'The competent authority may decide, on the basis of epidemiological or other data from the holding, that fattening pigs, housed under controlled housing conditions in integrated production systems since weaning, need, in some or all of the cases referred to in paragraph 1, only undergo visual inspection.'

The condition 'epidemiological or other data' mentioned above is supplementary to food chain information, which has to be provided with animals destined for slaughter and has become mandatory on January 1, 2006. Food chain information comprises information on the health status of the animals, such as treatments, the occurrence of diseases or results of analyses to diagnose diseases.

Based on this new legislation, VWA together with a major player in the meat industry started a pilot in 2005 in which a regime for visual inspection was applied in one abattoir (Helmond). Under the legislation (EC directive 64/433) in force in 2005, incisions were still mandatory. Therefore, the pilot was a combination of visual inspection and traditional inspection. The objective of the pilot was to gain insight into a couple of issues. It was not known whether the system of visual inspection could guarantee that the correct food chain information would be provided in the right manner. Furthermore, it was not clear if visual inspection could warrant the same level of food safety as the traditional inspection regime. In order to translate these issues into verifiable working procedures, the following three procedures were developed by the abattoir during the initial phase:

- Procedure Control of Mycobacterium avium in fattening pigs
- Procedure Food chain information
- Procedure Visual inspection

Conclusion

The pilot showed that the level of compliance with the written procedures was high to very high. It was found that meat inspection could be performed on an adequate level during the pilot. Some operational issues, however, emerged which need to be addressed. Also, the fine-tuning of the tasks of the VWA and official auxiliaries working under the auspices of an independent foundation had not been elaborated. This item has to be dealt with in a follow-up, which should involve auxiliaries as well. Finally, the matter of enforcement has to be developed. Based on the data generated, it was concluded that food chain information had been supplied reliably. In order to assess the impact on food safety, several effects had to be assessed and weighed.

Detrimental effects on of food safety:

- The number of condemnations in visual inspection is reduced, 0.005% of the carcasses are not condemned as compared to the regular regime. The relevance of this for food safety is disputable. Therefore it was concluded that there is a very limited loss of food safety.
- Two out of six endocartitis cases were detected with visual inspection. This is concluded to be is a very limited loss of food safety as well.

Beneficial effects on food safety:

- Risk-based testing of antibiotic residues (risk defined on the basis of provided food chain information) provided an improvement of food safety.
- Omitting of incision of the mandibular lymph nodes accomplished a substantial reduction of *Salmonella* cross contamination in that region. It is concluded that this provides a considerable improvement of food safety.
- A control system for *Mycobacterium avium* based on antibody testing and defining a herd status has the potential to improve food safety. Exact quantification of the food safety effect is, however, not yet possible. Therefore, further research is required.

Finally, the effects on food safety of two aspects remained equivocal:

- Incision of lymph nodes as a method for the detection of *R. equi* does not seem very meaningful; cutting lymph nodes could even provoke the spread of the bacterium through other parts of the carcass.
- Incision of lymph nodes as a method for the detection of *M. avium* does not seem very meaningful either, also with respect to calculated prevalence within the population of pigs.

With regard to the demonstrated spread of *Salmonella* as a result of the cutting of mandibular lymph nodes, it has been concluded that the omission of incision of the mandibular lymph nodes together with an alternative system to control M. avium, results in an improvement of food safety. The outcome of the pilot has been relevant for the further implementation of a pork supply chain inspection regime.

Granulomatous lesions in lymph nodes of slaughter pigs bacteriologically negative for Mycobacterium avium subsp. avium and positive for Rhodococcus equi

Mycobacterium avium subsp. avium (MAA) is a potential zoonotic pathogen, which belongs to *M. avium* complex bacteria (MAC). Genotyping of MAA strains isolated from humans and pigs revealed that these strains have a high homology. This could indicate that pigs are a source of infection for humans or that pigs and humans share common sources of infection, e.g. the environment. In pigs, infections with MAA are usually limited to the lymph nodes. Especially the sub-maxillary and mesenteric lymph nodes are affected. In accordance to European Union legislation (Regulation 2004/854/EC), infections caused by Mycobacteria in pigs are diagnosed presumptively in slaughterhouses by meat inspectors. The sub-maxillary lymph nodes of slaughter pigs are incised and examined at post-mortem inspection for granulomatous lesions. Futhermore, the mesenteric lymph nodes are inspected for granulomatous lesions visually, by palpation and, if necessary, by incision. The prevalence of granulomatous lesions in lymph nodes of pigs was studied from January to August 2004 in two slaughterhouses in the Netherlands. In this period 2,116,536 pigs were examined for the presence of granulomatous lesions in the sub-maxillary nodes. In 15,900 (0.75%) of these pigs, lesions could be detected.
Nine farms with the highest incidence of lesions at post-mortem meat inspection, registrated at one of the slaughterhouses for the period September until December 2003, were selected for a more detailed pathological and bacteriological examination. On these farms, the prevalence of lesions in sub-maxillary lymph nodes ranged from 2.3 to 5.7% with a mean of 3.0%. The results of the pathological examination showed that 98 (7.7%) out of 1276 pigs had granulomatous lesions in the sub-maxillary nodes and one (0.1%) pig showed lesions in its mesenteric lymph node. *Mycobacterium avium* subsp. *avium* could not be isolated from the lymph nodes of these 99 pigs with lesions and from a selection of lymph nodes (n=61) of pigs without lesions, whereas, *Rhodococcus equi* was isolated from 44 out of 98 (44.9 %) of the sub-maxillary lymph nodes with granulomatous lesions.
In 1996, the prevalence of granulomatous lesions in lymph nodes of slaughter pigs was 0.5% and in 54.2% of the cases MAA was isolated. The results of this study showed that the prevalence of granulomatous lesions in lymph nodes in 2004 was 0.75%, an increase in comparison to the results of 1996. However, in contrast to the results of the study in 1996, in 2004 no MAA bacteria could be detected in lymph nodes after bacterial examination. *Rhodococcus equi* was frequently isolated from granulomatous lesions in sub-maxillary lymph nodes. In this survey *R. equi* was the most important bacterium causing lymphadenitis in pigs.
The results of this study show that detection of granulomatous lesions in pig lymph nodes by eye is not a reliable diagnostic test to determine an infection with MAA. Futhermore, additional examinations by culture methods appear to be necessary to estimate the true prevalence of MAA infections in pigs. However, this approach is time-consuming and laborious. Therefore, other more fast and reliable tests for the detection of MAA infections in pigs are strongly needed. Finally, the high occurrence of *R. equi* in lymph nodes of pigs provokes the question as to the risk of *R. equi* transmission from pigs to the human population.

3.2.4 Salmonella-monitoring in fattening pigs and at slaughterhouses in the Netherlands

The obligation to monitor *Salmonella* for farms with fattening pigs and slaughterhouses is prescribed in a Regulation of the Product Board for Livestock and Meat (PVV). This regulation has been enforced since 1 February 2005

Fattening pig farms

All farms with at least 30 fattening pigs must monitor for *Salmonella*. Within a period of 4 months, 12 blood samples have to be collected, either on the farm itself or at the slaughterhouse, and tested for antibodies against *Salmonella*. Antibody titres > 40% OD are regarded as positive. The score (1–3) of a farm depends on the number of positive results. Scores of the last three periods of four months (1 year) are added up to cat-

egorize the pig farm. The results of more than one year of monitoring (4 periods of 4 months) are: Category 1: 69% of the farms, Category 2: 26% and Category 3: 5%.

Score criteria of a pig farm during a 4 months period		*Pig farm Category: sum of score of 3 consecutive 4 month periods score (1 year)*	
< 20% of the blood samples positive	Score 1 (good)	Score sum: 3 or 4	Category 1
20% - 40% of the blood samples positive	Score 2 (reasonable)	Score sum: 5, 6 or 7	Category 2
> 40% of the blood samples positive	Score 3 (attention)	Score sum: 8 or 9	Category 3

The Product Board has advised category 3 farms to reduce their risk of *Salmonella* infection. This is in anticipation of future obligations or even sanctions that might follow when a farm has been shown to belong to an unfavourable category. Moreover farmers are made aware that category and production have been shown to be related. Taking suitable measures, especially on category 3 farms, can thereby have a positive influence on the results of a pig farm. The Product Board advises the farmers about such measures and advises them to contact their veterinarian.

Slaughterhouses

The number of pigs slaughtered annually determines the number of samples that have to be taken at the slaughterhouse. Slaughterhouses slaughtering 10.000-150.000 pigs a year have to sample 10 carcasses every 2 weeks. Sampled carcasses have to originate from different pig farms and every sample is tested individually. Slaughterhouses slaughtering more than 150.000 pigs a year have to sample 5 carcasses every working day. These samples are being tested as one pool sample. Sampling is done according to the regulations of Decision 2001/471/EG. Instead of the sponge sampling method, slaughterhouses can use the cork drill method. Most of the slaughterhouses use this method. In 2005, the average *Salmonella* contamination of all participating slaughterhouses, calculated for the sponge sampling method was, 0.8%.

3.2.5 Surveillance of zoonotic bacteria in farm animals in the Netherlands

Adequate control of zoonotic bacteria and other zoonotic agents in the food production chain largely depends upon the availability of reliable data of the occurrence of these agents both at farm animal level and at retail level. In view of this need, the Zoonosis Directive (2003/99/EC) issued by the European Commission in 2003 to replace Directive 92/117/EEC, obliges all Member States to report on the occurrence of zoonoses and zoonotic agents annually. In this context, since 1997, the Food and Consumer Product Safety Authority (VWA) in collaboration with the National Institute for Public Health and the Environment (RIVM) and the Animal Health Service (GD) has run a surveillance programme in farm animals in the Netherlands with the main objective of monitoring trends in the occurrence of zoonotic bacteria. In the period 1997-2002, the

programme focussed on *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157 in laying hens, broilers, finishing pigs, dairy cattle and veal calves. In 2003, no samples were collected due to the epidemic of avian influenza in the Netherlands. In 2004 and 2005 the programme focussed on *Salmonella* spp. in finishing pigs and *E. coli* O157 in dairy cattle and veal calves. In 2006, fewer samples were taken due to blue tongue, which was found for the first time in the Netherlands.

A two-stage sampling scheme is used to accurately estimate the annual prevalences of the target bacteria at flock/herd level. Each year, the number of flocks/herds to be sampled is calculated in concordance with the estimated prevalences of an accuracy of 5% at a 90% confidence level. On each farm, one flock/herd is randomly selected for sampling. From the selected flock, 60 fresh faecal samples are taken, enabling detection of at least 5% shedding animals at a 95% confidence level. For microbiological examination the samples are aggregated into five pooled samples. Yearly, approximately 100 to 200 flocks/herds of each animal category are sampled. In addition to monitoring trends, the occurrence of certain types of bacteria prevailing in animals is related to those causing disease in humans. Also, essential data on farm and flock/herd characteristics are gathered to perform risk factor analyses (RFAs). Results are used to develop intervention strategies.

Salmonella spp. in fattening pigs

In 2003, no samples were collected due to the epidemic of avian influenza in the Netherlands.
Table 1 shows the prevalence of *Salmonella* in fattening pigs in the first half year and the second half year of 2005. The lower prevalence figure found in the second half of 2005 may be due to changes that have been applied since July 2005 with respect to the execution of the programma and the detection method used (only MSRV was used in stead of MSRV and RV). *Salmonella* Typhimurium DT104 was found in 20% of the salmonella-positive flocks.

Table 1. Percentage of salmonella positive herds of fattening pigs

Year	Quarter 1&2 (%)	Quarter 3&4 (%)
2000	30.2	39.8
2001	38.5	26.3
2002	33.6	21.8
2004	25.7	32.8
2005	25.8	13.6

In 2006, 22% of a total 100 sampled herds were salmonella positive. *Salmonella* Typhimurium was found in 41 % of the salmonella-positive flocks.

Table 2. Frequency distribution of salmonella serotypes in fattening pigs
N = no. of positive herds; n = no. of isolates.

2004 (N=65)			2005 (N=34)		
Serotype	*n*	%	Serotype	*n*	%
Typhimurium	34	52	Typhimurium	20	49
Derby	11	17	Derby	6	15
Brandenburg	4	6	Brandenburg	4	10
Panama	4	6	Others	11	27
Others	12	19			

3.2.6 Hygiene and zoonotic agents on petting farms, care farms and farmyard campsites

In three successive years, the Food and Consumer Product Safety Authority (VWA) visited petting farms, care farms and farmyard campsites. Their objectives were to determine the hygiene status of these farms and to describe hygienic measures that were implemented to reduce the risk of transmission of zoonotic agents from animals to humans. For this purpose, a questionnaire was completed for every farm. Additionally, samples of freshly voided faeces were collected to determine the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) O157, *Salmonella* spp. and *Campylobacter* spp.

Results and hygiene measures

For at least 85% of the farms visited, the overall impression of the hygiene was recorded to be good, and at only one petting farm and two care farms was the status judged as poor. The VWA distributes information signs to petting farms for free (see below). Petting farms can collect these information signs at the regional VWA offices. Information signs were present at 90 of the 132 visited petting farms (68%). The code of hygienic practices was present at 84% of the petting farms. The target of the VWA, regarding the presence of the information sign and the hygienic code is 100%. So, this result is tolerable but still not good enough. The code of hygienic practices at petting farms being present on almost half of the care farms and farmyard campsites was a pleasant surprise, because it had only been distributed among petting farms. The code must have been downloaded from the VWA website on the farm managers' own initiative.

The VWA deems the presence of a well-equipped hand-washing facility important. Ninety-two percent of the visited petting farms had a hand-washing facility of which 92% were equipped with a soap dispenser and only 54% with paper towels. Ninety-five percent of the visited care farms contained a hand-washing facility. Of these, 85% were equipped with a soap dispenser and just 24% with paper towels. For farmyard campsites, these percentages were respectively 95, 35 and 10%. A well-equipped hand-washing facility is of great importance for good hygienic management. In the future, the number of hand-washing facilities present and their equipment has to be improved.

Although the overall impression of hygiene at the petting farms visited in 2002 was good, a number of points for improvement stood out, e.g. informing visitors on hygiene and hand-washing, the equipment of the hand-washing facility, a facility to clean footwear, a specifically designated area where visitors can eat that is strictly separated from the animals, and a quarantine ward with distinct clothing and boots. The inspections carried out in 2004, three years after the code of hygienic practices had been issued, showed a slight improvement in respect to these points of interest, with the exception of the equipment of the quarantine ward.

The importance of the creation of a safe farm environment for both visitors and employees is being underlined by the results of the bacteriological examination: at almost two-third (64.9%) of the petting farms, and around half of the care farms (56%) and farmyard campsites (45.2%), STEC O157 and/or *Salmonella* spp. and/or *Campylobacter* spp. were detected. As could be expected, the highest isolation rates for STEC O157 were found in cattle and small ruminants. *Salmonella* spp. and *Campylobacter*

The VWA information sign for petting farms

The sign says:	Wash Hands with Water and Soap – after having petted animals – before eating – before touching your face or mouth Please note: eating is allowed only in the designated areas and not in the meadows Please contact the manager for further inquiries. Just like humans, animals may harbour pathogenic micro-organisms. In the same way that one may contract an infection from another person this can happen via contact with animals. An infection can be transmitted via petting, cuddling or contact with manure. Although the actual risk of infection is low, young children and elderly persons are especially susceptible to certain infections.

spp. were found in fecal samples of several animal species, with the highest prevalence of *Salmonella* spp. in poultry and pigs and of *Campylobacter* spp. in pigs, poultry, cattle and small ruminants.

It is known that animals can carry micro-organisms and can excrete these with milk, urine and faeces, without being ill themselves. In this way, the environment of the animal can become contaminated. Visitors and employees who proceed among, touch, pet, and cuddle the animals, and walk in paddocks where contaminated animals are present, are at real risk to become infected with these micro-organisms.

Finally, there is a responsibility for visitors and employees themselves. It is important that they are aware of the risk associated with animal contact and know how to act to reduce the risk. If the farm manager makes every effort to minimize the spread of pathogens possibly present by maintaining good hygienic farm management procedures and good hygienic facilities, it is then up to visitors and employees to utilize proper hygienic practices.

The data provided by this study highlights the need for control measures at public and private farms to reduce human exposure to livestock faeces and thus the risk of the transmission of zoonotic diseases. Although the overall impression of hygiene at the farms enrolled in this study was good, there is still a need for improvement. Public awareness of the risk associated with handling animals or fecal material should be increased.

3.3 Food products

Poultry meat

A monitoring programme on the prevalence of *Salmonella* and *Campylobacter* in poultry meat has been operational during the last decennium. In this programme chicken carcasses and chicken parts, including legs, wings, breasts and fillets were randomly sampled at retail by VWA inspectors. Samples were taken only from refrigerated products without added ingredients. The sample size was 750 gram of meat per product group per shop and was based on the quantity of chicken bought by the average consumer at a time. The samples were taken depending on the market share at supermarkets, poultry seller (shops and markets) and butchers. In 2005 a total of 1506 samples of chicken meat were taken and analyzed for the presence of *Salmonella* en *Campylobacter*. The results of this monitoring programme are presented in sections 4.3 (*Campylobacter*) and 4.19 (*Salmonella*).

A total of 276 samples, caeca of broilers collected in slaughterhouses, were tested for the presence of *Salmonella* and *Campylobacter* following the “technical specifications for an EU monitoring scheme for *Campylobacter* in broiler chickens”. The main objective of this monitoring scheme is to provide data on the prevalence of thermophilic campylobacters in broiler chickens in the member states. The results of the testing in 2005 are presented in section 4.3 (*Campylobacter*).

Surveillance programmes, on the prevalence of *Salmonella* and *Campylobacter* in turkey meat, meat of other poultry and meat from chickens originating from organic production farms, were conducted in 2005. A total of 958 samples of turkey meat, 159 samples of other poultry meat and 211 samples of “organic” chicken meat were extracted from retail outlets. The results of these surveillance programmes are presented in sections 4.3 (*Campylobacter*) and 4.19 (*Salmonella*).

In order to get a picture of contamination levels of broiler meat in the EU member states and to enable the consideration of Community measures to combat *Salmonella* and *Campylobacter* in these foodstuffs, a coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU will start in 2007. The prevalence

of these pathogens will be measured in broiler meat carcasses produced in EU slaughterhouses and in fresh broiler meat and meat preparations on sale in retail outlets in the member states.

Red meats

In a surveillance programme for raw red meat, samples of beef, pork, lamb and veal were taken from the retail trade and analyzed for the presence of *Salmonella*, *Campylobacter*, *E. coli* O157 (STEC) and *Listeria monocytogenes*. The results of this surveillance programme are presented in sections 4.3 (*Campylobacter*), 4.14 (*Listeria monocytogenes*), 4.19 (*Salmonella*), and 4.8 (STEC).

Eggs and egg products

Although, there were no specific studies in 2005, a surveillance programme on the prevalence of *Salmonella* in table eggs, which includes the analysis of 45.000 eggs for the presence of *Salmonella,* was conducted from January 2006 to April 2007. This is a repeat of a survey carried out in 1999, in which 14 (0,03%) of 46.200 eggs were found positive for *Salmonella* and a survey carried out in 2003 in which 10160 eggs, from chickens originating from organic farms and/or receiving special feed, proved to be negative for *Salmonella*.

Dairy products

As part of a EU coordinated programme for the official control of foodstuffs, the bacteriological safety of cheeses made from pasteurised milk was assessed. A total of 789 samples were taken at both production and retail levels and tested for the presence and/or numbers of some pathogenic bacteria. *Salmonella* and *Listeria monocytogenes* were not detected in the tested samples. The results for one sample of ripened soft cheese tested for *Staphylococcus aureus* were unsatisfactory and this was the case for two samples of ripened soft cheese regarding the numbers of *Escherichia coli*.

3.4 Water

The most important sources for contamination of surface water with zoonotic pathogens are direct faecal waste from humans and animals, (un)treated wastewaters and run-off of animal manure. Animal faeces originating from wild life, livestock and pets may contaminate surface waters directly or by run-off. Animals both symptomatically and asymptomatically infected with pathogenic micro-organisms will excrete these in high levels specifically in faeces. Animals infected with zoonotic pathogens often experience infections without any specific symptoms that hinder their daily routine. The levels of zoonotic pathogens excreted by infected animals amount to 10^9 per gram of faeces. These concentrations of pathogens originating from faeces will decrease in surface waters by dilution in waters with lower levels of pathogens, inactivation and removal by attachment to other particles or by aggregation to other pathogens and subsequent sedimentation of aggregates. The UV component of sunlight and high temperature are important factors in the inactivation of pathogens in surface waters.

On the other hand, a range of zoonotic agents such as *Campylobacter*, *Cryptosporidium*, *Clostridium perfringens* and hepatitis E viruses can survive surface waters without loss of infectivity and pathogenicity. Animals that are exposed to these contaminated waters by for instance drinking may contract zoonotic infections with zoonotic pathoges and in turn sustain the infectious cycle. Of importance is the possibility of pathogen transmission to species that do not live in close proximity. Susceptible humans may become infected with zoonotic pathogens through the ingestion of contaminated surface water. This can occur during bathing or other activities in recreational waters such as diving. Insufficient or failing treatment of surface waters used for drinking water production may lead to concentrations of infectious zoonotic pathogens in drinking water which, in turn may lead to infections and subsequently to diseases in the exposed population. Unconfined groundwaters in rural areas may also lead to contaminated drinking water.

Drinking water

One of the most important objectives of the European Drinking Water Directive is to protect the health of the consumers in the European Union and to make sure the water is wholesome and clean. The Directive is translated into Dutch legislation with quality requirements such as absence of E. *coli* and enterococci in 100 ml of drinking water. Also, tap water provided by the owner to third parties should not contain micro-organisms, parasites or substances to such numbers per volume or concentrations that these may comprise detrimental public health effects. In addition, a risk assessment for *Cryptosporidium*, *Giardia* and (entero)viruses is required by Dutch legislation to derive an acceptable level of less than one infection in 10,000 persons per year. Since these levels can not be measured in tap water, the safety of drinking water should be demonstrated based on data regarding source water quality and treatment efficiency. Since other zoonotic agents should also be considered as possible health hazards via drinking water several studies have been done in the Netherlands. Surface and ground waters for drinking water production are monitored for the zoonotic pathogens *Cryptosporidium* and *Giardia*. These protozoan parasites were detected in for instance Meuse river water. In another study monitoring small ground water supplies were studied for the presence of E. *coli* O157:H7 which were isolated from some ground waters that otherwise met the drinking water standards.

Recreational water

The European Bathing Water Directive is based on the detection of faecal indicator bacteria and is designed to ensure the protection of people exposed to recreational waters from pathogens and therefore also from zoonotic infections. Though enteric infections may be largely prevented, skin complaints, for example from the zoonotic parasite *Trichobilharzia*, may still occur. Recreational waters are occasionally monitored for zoonotic agents such as *Cryptosporidium* and *Giardia, Campylobacter* and *Salmonella*. A small survey revealed the presence of hepatitis E viruses in the river Meuse, which is used for both recreation and drinking water production. Two outbreaks of cercarial dermatitis (one with 10 and another with 30 cases of skin complaints) were

associated with recreational lakes in the Netherlands, which was tested positive for *Trichobilharzia cercariae* by microscopy and by a novel PCR method

3.5 Wildlife

Zoonoses that are transmitted by direct contact with wildlife are present, endemic or can emerge in future. There is no formal wildlife monitoring or surveillance programme except for the serosurveillance of wild boar for Classical swine fever, Swine vesicular disease and Trichinellosis and wild birds for avian influenza. Furthermore, some wildlife populations are periodically investigated for specific purposes such as the identification of new pathogens. Examples are: the seals for phocine distemper, foxes for *Echinococcus multilocularis*, rodents for Hantavirus infection and *Leptospira*, and bats for rabies and other viral pathogens (see specific sections). Since several reports indicate that wildlife can pose a risk for human health because of the introduction and spread of new pathogens and the lack of coordinated monitoring and surveillance systems, there is a specific need to invest more in wildlife surveillance for infectious disease and for the collaboration of wildlife ecologists, biologists and other experts in the field of wildlife research.

3.6 Arthropods

Infections that are transmitted to humans from vertebrate animals by blood-sucking arthropods such as mosquitoes, sandflies, ticks and fleas are called arthropod-borne zoonoses. Arthropod-borne pathogens, including arboviruses, rickettsiae, bacteria, protozoa and filarial parasites, spend part of their life-cycle in cold-blooded arthropod vectors. Arthropod-borne zoonses already present or endemic in Europa and with a potential to emerge include West Nile virus, Crimean Congo Haemorrhaggic Fever, tick-borne encephaltitis, ehrlichiosis, bartonellosis, rickettiosis, Lyme borreliosis, babesiosis and leishmaniosis.

Since 2000, tick densities in different habitats and the presence of different pathogenic *Borrelia*, *Anaplasma* and *Ehrlichia* species found in these ticks are being studied by PCR and subsequent reverse line blot (RLB) analysis by collaboration between RIVM and WURC (ASG/Alterra). Since 2006, seasonal variations of tick populations at 25 locations in the Netherlands and the *Borrelia* spp. infection rate of the most important tick, *Ixodes ricinus,* have been monitored by the WURC and Association for Environmental Education (IVN).

Since 2006, the presence and/or establishment of the Asian tiger mosquito in the Netherlands have been monitored by Dutch Plant Protection Service (PD), WURC and RIVM. This surveillance was initiated after the discovery of specimen in a Dutch greenhouse that imports ornamental plants from Southeast China in 2005. Although this mosquito species is a known vector for several zoonotic pathogens, the main reason for concern is the introduction of dengue virus, a human pathogen with no animal reservoir. Further, following an outbreak of blue tongue, a viral disease of ruminants transmitted

by *Culicoides* species, in the Netherlands in 2006, surveillance for this genus of biting midges by the PD was initiated. Despite the importance of the latter two surveillances for veterinary and public health, they will not be discussed in this report as they do not concern zoonotic pathogens.

3.7 Antibiotic resistance

Antibiotic consumption in farm animals

Since 1990, the therapeutic use of antibiotics in animal husbandry has been monitored, based on total sales provided by the manufacturers and importers of veterinary medicines in the Netherlands (FIDIN).

The total sales of antibiotics increased in 2005 by 55.000 kg to 508.000 kg (+12%) (Table 3.6.1). Expressed in percentages, the sales of penicillines/cephalosporines (+24%), aminoglycosides (+22%) and macrolides (+21%) increased most rapidly.

Table 3.6.1. Total sales of antimicrobials in 2005 in the Netherlands.

Therapeutic group	kg of active substance in 2005 (x1000)	Difference with 2004
Penicillins/cephalosporins	54	24 %
Tetracyclines	307	14 %
Macrolides	29	21%
Aminoglycosides	11	22 %
Quinolones and fluoroquinolones	8	14 %
Trimethoprim/sulphonamides	93	0 %
Other	6	0 %
Total	508	12 %

Source: FIDIN.

Since 1997, the total sale of antibiotics for therapeutic use increased from 332.000 kg to 508.000 kg in 2005 (+53 %) (Figure 3.6.1.) an average increase of 6 % per year. In 1998, the live weight produced in veal calves, pigs and poultry amounted to about 3.500 million kilogram. From 1998-2005, the total live weight production of pigs, veal calves and broilers decreased by 10,8% (Figure 3.6.2.). Antibiotic usage per 100.000 kg live weight production in 1998 was 9,4 mg, this gradually increased to 16,4 mg in 2005 (Figure 3.6.2.).

Factors influencing live weight production:
- February 1997: outbreak of swine fever
- February 2001: outbreak of feet and mouth disease
- February 2003: outbreak of avian influenza

Particurlary in recent years and in general over the last decade, sales of antibiotics for therapeutic use have increased much more than the total weight of produc-

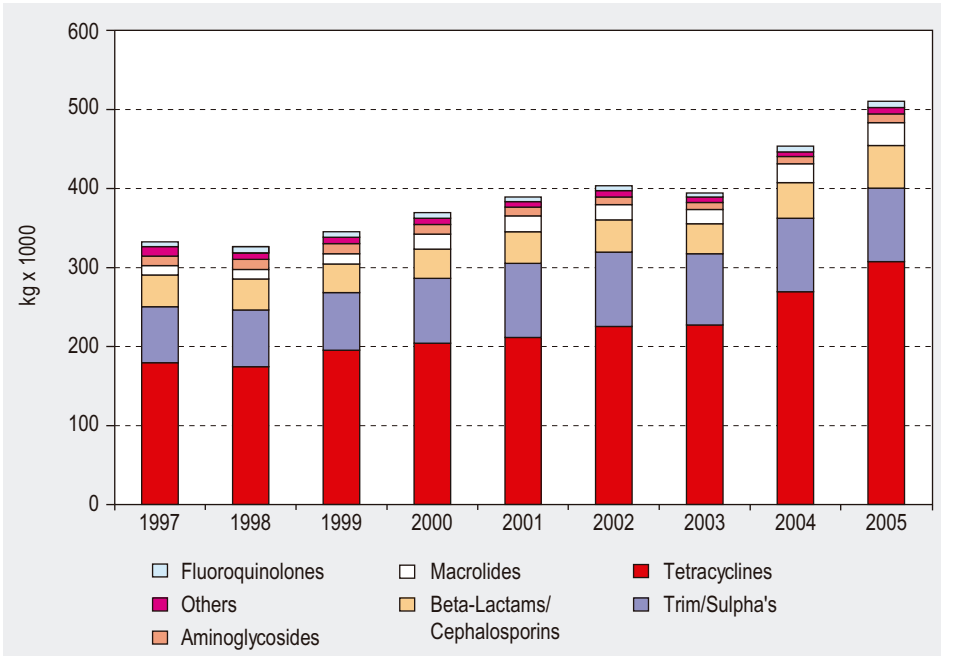


Figure 3.6.1. Usage of antibiotics for therapeutic use (active ingredient x 1000 kg) in the Netherlands from 1997-2005.

Source: FIDIN

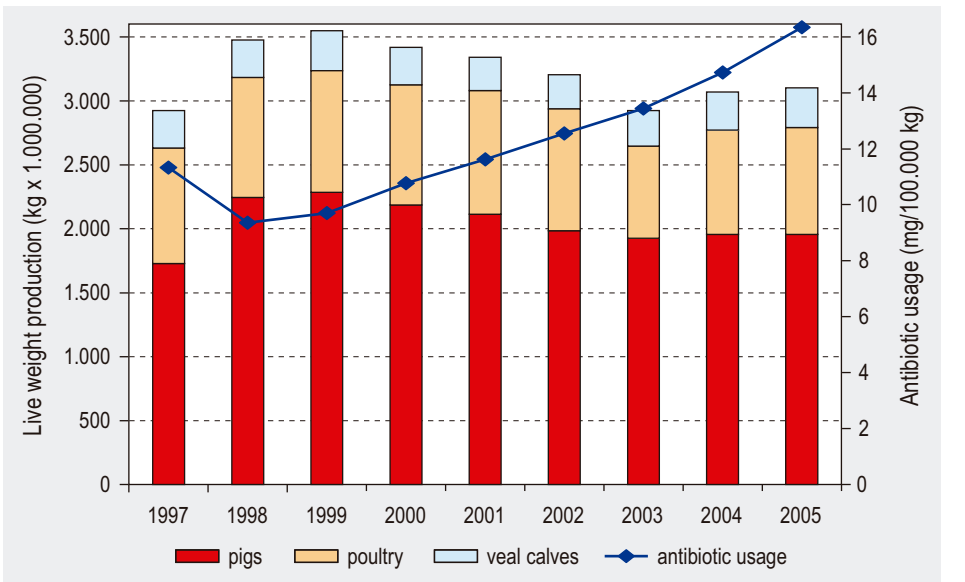


Figure 3.6.2. Live weight production in the Netherlands and antibiotic usage (mg) per kg live weight production (all farm animals) 1997-2005.

tion from livestock, whereas sales of antimicrobial growth promoters have gradually decreased. As the relative contribution for each therapeutic group remained practically unchanged, potency differences of molecules can only account for a small part of the growth in antibiotic consumption. This occurs, for example, when doxycycline is replaced by tetracycline. The result would be that more kilograms of active ingredients would be necessary to medicate the same number of animals. Sales of quinolones and macrolides (two classes of antibiotics of which the usage in food animals is under debate because of potential public health risks) rose substantially in 2005.
An explanation used to justify the growth of the antibiotic sales was the emerging of new infectious diseases in pigs (PIA and circo-virus). However, the presence of these diseases does not fully explain the 2005 rise in antibiotic sales. Other causes have to be considered as well. As in other countries, in the Netherlands there are few economic incentives for restricted antibiotic usage. On the contrary, high usage of antibiotics may be rewarded with sales (industry, wholesaler and veterinarian) or with better economic results (farmer). Being that antibiotics are cheap, investments in housing and preventive measures may be discouraged. Furthermore, there is no obligation to justify the use of antibiotics to the authorities and the general public.
The Dutch professional association for veterinarians (KNMvD) has an active antibiotic policy to promote restrictive and selective use of antibiotics. The continuous rise in sales of antibiotics demonstrates that this goal is not achieved. Obviously, self-regulation in this competitive market is failing. At the moment Directive 2004/28/EU, on the community code relating to veterinary medicinal products, is implemented in Dutch law. This process could be used to implement measures that stimulate more selective and restrictive use of antibiotics.

Breakpoints
In 2005, for the first year, epidemiological cut-off values for the wild-type distributions were used for the MIC-data analysis for food-borne pathogens and indicator organisms, instead of the previously used clinical breakpoints (e.g. CLSI). The effect of using the new cut-off values will result in a more sensitive detection of acquired resistance in these organisms.

3.8 Zoo animals / exotics

Like many other animals zoo animals may potentially harbour zoonotic agents. However, special attention is required because zoo animals may be host to agents not commonly seen in Europe or may carry pathogens that are closely related to human pathogens, as is the case with primates. At present, zoonotic disease in zoo animals is mostly recognized on an anecdotal base rather than through targeted surveillance. Recent incidents with tuberculosis in bonobo primates and other species, however, clearly demonstrate the need of the implementation of a more targeted approach. An approved zoo scheme, introduced by the European legislation, explicitly addresses the zoonoses issue and is likely to bring about change. Approved zoos are obliged to implement an annual disease surveillance plan that must include appropriate control zoonoses in the animals. Also, records pertaining to the results of diagnostic proce-

dures, among other things, have to be kept and made available to the appropriate authority.

Diseases, once related to specific geographical areas, now have the possibility to be introduced to the Netherlands/EU through international trade. Therefore, trade in live exotic animals is allowed only between officially recognised establishments such as institutes and zoological gardens. Only exotic animals with a proper and valid official health certificate with all required vaccinations and diagnostic tests are permitted for entry into the Netherlands. A separate certificate has to be provided for each consignment and the original must accompany the animals to the Border Inspection Post at the point of entrance into The European Community. The criteria in health certificate related to animal and public health are prescribed in European legislation Decision 91/496/EC, Directive 92/65/EC and Decision 79/542/EC. These are implemented in national legislation.

Present Controls of wild/exotic animals entering the European Community (EC) from third countries (outside the EC)

According to the health requirements all exotic live animals are divided into the following groups:
Exotic animals belonging to the species from taxa Proboscidea and Artiodactyla (elephant, hippopotamus, camel, llama, gazelle, giraffe, deer etc.) must originate from farms free from brucellosis for at least 42 days (certain ungulates 12 months), free from anthrax for at least 30 days, free from rabies for six months and free from Tuberculosis. They must also have not had contact with other animals lacking the same health status. Animals from certain countries may require some extra treatment against ecto- and endoparasites and Leptospira spp. Birds from the order Struthioniformes (the families nandoes, ostrich, emu, cassowaries and kiwis) can be imported from countries/regions free from avian influenza. Bird flu must be absent during a 30-day period prior to shipment to EU. Animals from these two groups may be imported only from the countries/regions having EU export permission and being on the list from which import is allowed.

All other exotic animals can be imported from all third countries with a proper health certificate. Live monkeys (Simiae and Prosimiae) must be kept isolated for a period of 30 days before exportation and during this time they must be tested for tuberculosis, Ebola and Monkey pox with negative results. The animals must not have had contact with animals from a holding in which rabies is present or is suspected or have been established during the last six months. For other animals including mammalians such as marsupials, rodents, lagomorphs, different wild carnivores the following rules apply. The importation of prairie dogs (*Cynomis* spp.) from USA is prohibited. The importation of rodents of non-domesticated species and squirrels originating from third countries from Sub-Saharan Africa is also not allowed. The animals must not come from or have been in contact with animals from a holding, in which rabies is present or is suspected or have been present within the last six months. In the case of fruit bats of the genus *Pteropus* from Malaysia and Australia the animals must comply with the following conditions: originate from captive colonies, be isolated in quarantined premises for at least 60 days, and test negative to Hendra and Nipah virus in an approved laboratory. Reptiles and amphibian are also in this group, but they do not need specific requirements regarding health certificate.

In one part of the Health Certificate, an official veterinarian declares/certifies that the consignment of animals meets these certain "general requirements", which are important in the prevention of zoonotic diseases:
- The animals were examined within 24 hours before loading on the holding of origin and during that examination no clinical symptoms of disease were determined,
- The animals have remained for 30 days prior to shipment on premises where they have not been in contact with animals of the same species, which do not meet the same health requirements,
- The animals have been sent in containers that are easy to clean and disinfect. The containers have been cleaned and disinfected with a registered disinfectant immediately before loading and dispatch.
- Birds from the family Psittacidae must be negative for psittacosis 60 days prior to shipment.

The health certificate is in general valid for 10 days from the date of shipment.

4 ZOONOTIC PATHOGENS

In the following, the most important zoonotic pathogens are discussed in alphabetical order.

4.1 *Bartonella henselae*

Although the clinical profile of cat-scratch disease (CSD) was described in 1950, the primary pathogen was not identified until 1993, 43 years later. That pathogen, formerly assigned to the genus *Rochalimaea*, is now designated as *Bartonella henselae*. *Bartonella* is a genus of aerobic gram-negative bacilli. The domestic cat is the only known reservoir of the *B. henselae* with a confirmed link to disease in humans. Despite the name, not all patients with CSD have been scratched or bitten by a cat. About 30% of the patients do not recall traumatic cat contact. The majority (60%) of patients with CSD are under the age of 20. Infection results in clinical symptoms 3 to 6 days post exposure, when a small papule (2-3mm) develops near the site of the scratch. Two to three weeks later proximal lymphadenopathy develops, often accompanied by myalgias, malaise and fatigue. About one third of the patients have low grade fever (<39°C). The majority of the patients with CSD have localized disease with mild systemic symptoms that resolve spontaneously within several months (on average 6 weeks). Symptoms in the late stages of the disease probably reflect an immune response to the infection as opposed to ongoing infection. The infection does not respond to antibiotics. In a small proportion (1 to 3%) of usually immunocompromised patients the infection can spread to many different sites in the body including the central nervous system (CNS), eyes, liver, spleen and bone. These aspecific forms of infection usually respond well to antibiotics (tetracycline, erythromycin, rifampin, ciprofloxacin are effective).

Animals

Bartonella henselae appears to have a perfect host-parasite relationship in the cat: infected animals are known to be able to carry high numbers of living bacteria in their blood ($1x10^6$/ml). Cats can be infected for months or even years with *B. henselae* without apparent clinical symptoms. Bacteraemia is most often detected in kittens and young animals (<24 months). There is clear evidence that the cat flea is a vector for *B. henselae* transmission from cat to cat. Polymerase chain reaction (PCR) testing of fleas has confirmed the presence of the organism. Worldwide, 4 to 89% of the cat population seem to carry *B. henselae* in their blood. These differences reflect geographic and climate differences that influence the breeding patterns of the cat flea. The bacterium has been detected in the blood of 22% of the Dutch cats.

Humans

Because of the non-specific nature of the presentation of CSD (lymphadenopathy) a wide differential diagnosis is required. Reports of a cat scratch or contact especially with flea infested kittens must be considered a risk factor. Most cases are transmitted

from young cats less than one year old. Culture of *B. henselae* directly from patient material or blood is very difficult because only 2% reported positive from blood cultures and the bacteria are only visible microscopically in the early stages of disease. PCR is more useful with reported sensitivity between 58 and 96%, but is not often used because this method requires a biopsy specimen. Laboratory confirmation of the diagnosis can also be provided by serology. The currently used serological assays have a high specificity (95-100%), but suboptimal sensitivity (40-100% for IgG and 60-74% for IgM), indicating that the number of diagnosed cases is likely to be an underestimate. The exact prevalence of CSD in the Netherlands is unknown but is estimated to be between 300 and 1000 new cases per year. In 2005, a total of 320 patients were identified at the RIVM based on a positive IgM response.

4.2 *Brucella* spp.

Bovine brucellosis used to be endemic in the Netherlands. Since the Dutch national *Brucella abortus* control programme for cattle started in 1959 its prevalence has dropped from about 30% to 1% in 1964. Sporadic cases occurred annually until 1995. The last infected herd was eradicated in 1996. In August 1999 by Decision 99/466/EC amended by Decision 00/69/EC the Netherlands was declared officially free of bovine brucellosis by the European Commission. *B. melitensis* has never been reported in animals in the Netherlands. By order 93/52/EC the Netherlands were also declared officially free of *B. melitensis*. The Netherlands is also free of *B. suis*. Only two cases were reported in the late sixties and two in 1973. Three of these cases were imported infected pigs and one was caused by feeding infected offal from imported hares.

Farm animals

Brucella abortus

From October 1999 the VWA has been responsible for controlling outbreaks of brucellosis. Monitoring is still undertaken by the Animal Health Service (GD), under responsibility of the Ministry of LNV. According to Directive 98/46 all animals older than 24 months in 20% of the present cattle herds were examined once a year from 1999 until 2005. In those years all herds examined proved to be negative. After five years of monitoring with negative results this monitoring has stopped in 2005, according Directive 64/432/EU. As prescribed in Directive 64/432/EU the official Brucella free status of the Netherlands is maintained by examining blood samples of all abortions in cows. Blood of cows that have aborted is first examined with a microagglutination test. Positive samples are re-examined by ELISA and Complement Fixation test. During several years now, no cases of bovine brucellosis have been diagnosed

Bulls are serologically examined for brucellosis according to Directive 88/407/EC before entering cattle sperm centres and later on a yearly base. In addition for export to some non-member states cattle is serologically checked for Brucellosis.

Table 4.2.1. Results of blood sampling of cows with an abortion 2005 -2006.

Number of	2005	2006
animals tested with serological blood test	4,176	2,336
suspended herds	74	79
animals retested positive serological	27	16
animals examined microbiologically	27	16
animals positive microbiologically	0	0

Brucella melitensis
To maintain the official free status an annual screening programme under Directive 94/953/EEC has been set up to prove with a confidence of 95% that less than 0.2% of sheep and goat herds are infected with *B. melitensis.* In 2005 and 2006 ovine and caprine samples were taken from about 10 % of all herds (1902 of 18562 and 1529 of 19932 herds, respectively). All samples gathered in this screening programme since 1994 have proved to be negative.

Brucella suis
The free status is maintained by the notification system. According to Directive 90/429/EEC all boars are examined before entering and leaving the semen centre. Sometimes breeding animals exported to non-member states have to be tested for brucellosis. In the case of certain fertility problems, breeding herds are tested for *B. suis* as well. All samples have been tested negative for *B. suis* thus far.

Humans
As cattle, sheep, goats and pigs in the Netherlands are free of brucellosis, the relevance of brucellosis as a zoonosis is virtually zero. In all reported human cases in the Netherlands, brucellosis was acquired by travelling abroad or through the consumption of contaminated imported products.

4.3 *Campylobacter* spp.

Thermophilic *Campylobacter* spp. (predominantly *Campylobacter jejuni* and *C. coli*) are the most frequently identified bacterial causal agents of gastroenteritis in the Netherlands and they pose a serious public health problem. Combining data of recent epidemiological studies performed in the Netherlands, in the general population and in general practices with data from laboratory surveillance, results in an estimate of approximately 59,000 cases of campylobacteriosis occurring in the general population in 2004, of whom about 14,000 consulted a general practitioner. In addition, there are about 60 cases of Guillain-Barre syndrome annually, more than a thousand cases of reactive arthritis, a few dozen other complications and about 25 deaths related to this disease. The disease burden for 2004 is estimated at 1200 (range 800-1600) Disability Adjusted Life Years (DALYs) per year, and an estimated cost-of-illness of € 20 million (14-35) per year.

Farm animals

PVE monitoring in the poultry meat production chain

Within the PVE control programme of the poultry meat production chain, additional screening has been performed since the end of 1997. Targets were set for reduction of *Salmonella* (not *Campylobacter*) contamination in the "end product" for 2000, again for 2002 and an end target of 0+% contaminations in 2010. This was thought to be achieved through additional hygiene measures at different levels in the poultry meat production chain as well as through measures. The monitoring results show that the *Salmonella* control programme in poultry has hardly been successful with regard to the reduction of *Campylobacter* contamination (Table 4.3.1). In fact the contamination levels have increased since 2003. However, the monitoring of poultry products by the VWA at retail (Table 4.3.2.), does indicate an improvement in 2005 and especially in 2006.

Table 4.3.1. Percentage of Campylobacter–positive broiler flock/batches from different links in the chain. At slaughter about 150 flocks are tested each week (Monitoring programme PVE)

Matrix	Link	1998	1999	2000	2001	2002	2003	2004	2005	2006
Faeces	broiler farm	16.8	13.3	11.0	12.8	18.0	14.3	10.0	14.0	13.3
Caecum	slaughterhouse	48.3	41.3	35.5	34.3	32.5	28.8	29.0	34.8	30.5
Neck skin	slaughterhouse	41.5	41.5	25.8	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Breast skin	slaughterhouse	n.i.	n.i.	25	11.3	15.3	11.5	30.3	38.5	41.5

n.i. no investigation

In the 2000+ programme, breast skins are tested as a more realistic proxy for the "end product". However, compared to the results of the monitoring of poultry at retail the contamination levels were much lower in breast skins until 2003 and much higher between 2005 and 2006. Except for 2005 and 2006, the retail contamination levels are closer to that found in caeca at slaughter; for *Salmonella* breast skin seems to be a better proxy for the findings at retail. (section 3.2.1). Figure 4.3.1 shows the quarterly change of contamination at the farm and at the slaughterhouse; both trends show a strong seasonality.

Food

During the years of 1996 – 2006, approximately 15.000 poultry meat samples were taken at retail by the VWA. The numbers examined varied yearly from 859 in 1999 to 1.600 in 2002. The isolation rates for *Campylobacter* show a decrease from 36.2% in 1996 to 23.5% in 1999, subsequently the rate went up again to a level above 30% and then down again to 22.1% in 2005 and further decreased considerably to 14.2% in 2006 (Table 4.3.2). Levels of contamination are still much higher in organically reared broilers than in regularly reared ones; although improvement have been seen in the past three years in organically reared broilers as well. The improvement at retail level in recent years is however not paralleled by the results of the PVE control programme at the slaughterhouse (Table 4.3.1. and Figure 4.3.1). The reason for this is not clear.

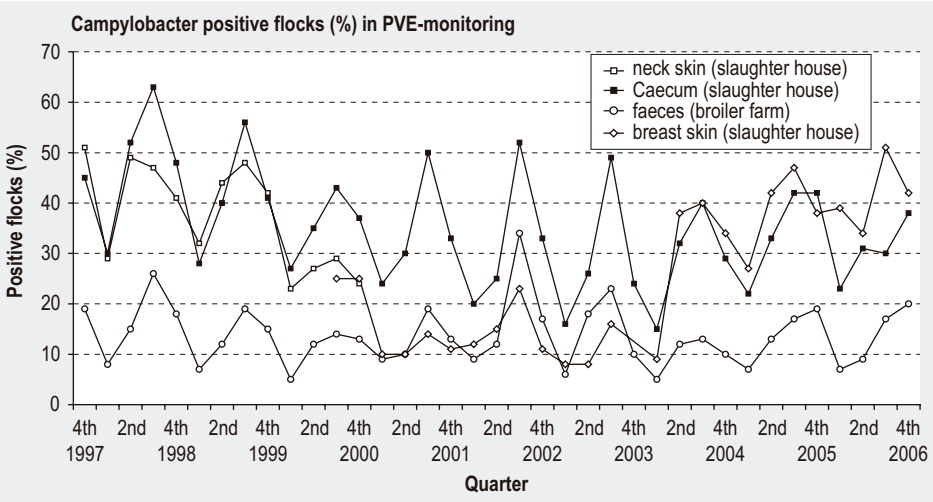


Figure 4.3.1. Percentage of Campylobacter-positive flocks from hatchery to the end of the slaughter line

Table 4.3.2. Campylobacter spp. in poultry meat (+/- 12% organic), fresh and chilled (Monitoring programme VWA)

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Poultry meat sample at retail	1325	1314	1077	859	1454	1581	1604	1431	1477	1404	1473
% *Campylobacter* spp. (organic)	36.2	31.8	26.9	23.5	30.5	32.5	31.3	25.9 (36.3)	29.3 (43.9)	22.1 (33.3)	14.2 (29.8)

Contamination levels of raw beef and pork are considerably lower than in poultry. Nonetheless *Campylobacter* has been shown to be present in beef intended to be eaten raw. *Campylobacter* is more prevalent in oysters and mutton.

Table 4.3.3. Campylobacter in 25g of raw meat at retail (Monitoring programme VWA)

	2003		2004		2005		2006	
	N	%+	N	%+	N	%+	N	%+
Beef and veal	678	0.1	847	0,8	463	1	936	0,4
Consumed raw							924	0.3
Pork	227	0	287	1	389	0	397	3
Mutton					106	5	53	11
Oysters	57	11						
Vegetables	233	1						

Humans

In 2006, 3,401 cases of campylobacteriosis were found (Table 4.3.3). The recorded cases correspond to primary bacterial isolates from 15 regional public health laboratories

(coverage: 52% of the Dutch population) from relevant patient material. This corresponds to an incidence of 40.0 per 100,000 inhabitants in 2006. Dutch epidemiological studies show that in order to arrive at the true incidence of patients with campylobacteriosis visiting a GP or in the general population the number of laboratory confirmed cases should be multiplied by about 2.2 and 9.2, i.e. 14,500 and 60,000 cases in 2006 respectively.

Table 4.3.4. Confirmed human cases of Campylobacter spp. and faeces tested for various reasons (Laboratory surveillance RIVM)

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Laboratory confirmed cases*	3741	3641	3427	3175	3474	3682	3421	2805	3383	3716	3401
Campylobacter spp. cases/ 100,000	46.4	45.0	41.7	38.7	42.1	44.3	40.8	33.3	40.0	43.8	**40.0**
Faeces tested / 100,000	1285	1278	1236	1222	1169	1113	1070	1088	1050	1028	**1128**
Explosions(#cases), IGZ						9(48)	15(98)	10(70)	8(30)	10(63)	5(13)

* Primary isolates from the 15 PHLs with an estimated coverage of +/-50% of the Dutch population for Campy lobacter

The number of human cases of campylobacteriosis decreased between 1996 and 1999 and then increased again until 2001, decreased by 25% up to 2003, increased again up to 2005 and decreased in 2006 to the level of 2004 (Table 4.3.4). Since 2001 this trend more or less follows that of poultry caeca at slaughter (Table 4.3.1); the recent reduction at retail (Table 4.3.2), however, is not reflected by the trend in human infections.
In the Netherlands, routine surveillance of *Campylobacter* has been restricted to laboratory-specific weekly frequencies only until 2002. From 2002 on, information about age, gender, residence, travel, species and antibiotic resistance has been registered as well. Between 2002 and 2006, 92,8% of the laboratory confirmed *Campylobacter* infections were due to *C. jejuni*, 5.9% were due to *C. coli*, 0.9% due to *C. lari* and 0.4% due to other species. Between 2002 and 2006, hospital admission or consultation of a medical specialist was involved in 17-24% of the *Campylobacter* infected patients. Cases involving travel varied between 7-11%, however this is underreported by the laboratories and is twice as high if individual patients are questioned about recent travel.

Seasonal evolution and year trend of campylobacteriosis
Similar to the data of the occurrence of salmonellosis (section 4.19), the incidence of campylobacteriosis in humans shows a seasonal variation. The number of human *Campylobacter* isolates strongly increases in May, has a 1st summer peak at the beginning of June and a 2nd larger summer peak from the end of August to early September (Figure 4.3.2). In contrast to *Salmonella* (Figure 4.19.6), the number of *Campylobacter*-positive poultry flocks at slaughter (caeca) shows an explicit seasonal fluctuation as well, paralleling the situation in humans, but lacking the 2nd summer peak. The upward trend in humans until 2001 is different from the gradually decreasing secular trend in poultry meat, however the dip in 2003 and upward trend afterwards up to

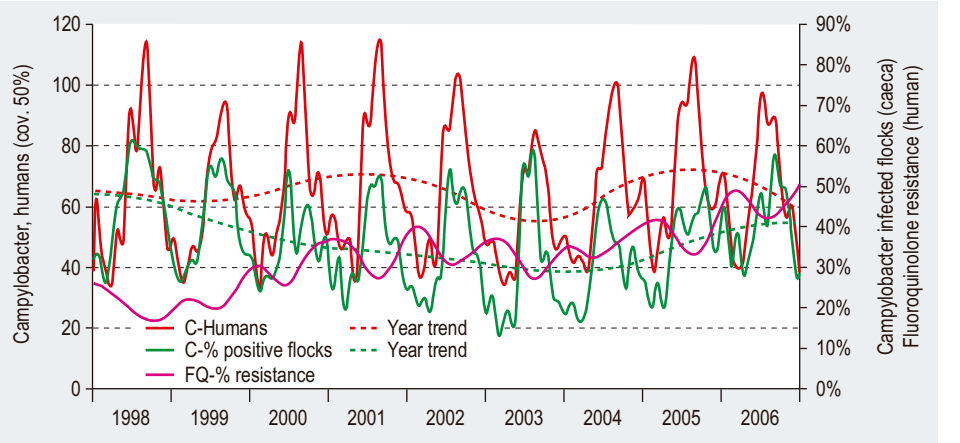


Figure 4.3.2. Seasonal and secular trends of the weekly occurrence of human cases of campylobacteriosis and the percentage of (caeca) positive flocks in the slaughterhouses (RIVM - PVE). The change in fluoroquinolone resistance concerned isolates derived from humans.

2005 is seen in both human surveillance and poultry monitoring. Resistance to fluoroquinolones increased from 25% in 1998 to 45% in 2006. Each year during the spring rise of *Campylobacter* in humans and poultry, fluoroquinolone resistance decreases 15%, gradually increasing again after the 1st summer peak.
The dip in 2003 is probably related to the avian influenza outbreak in poultry (Figure 4.3.3), when sales of poultry meat were significantly lower than in the years before and after 2003. Figure 4.3.3 shows that during the avian influenza outbreak the incidence of human cases were lower than experienced in former years and then returned to expected levels again in 2004.

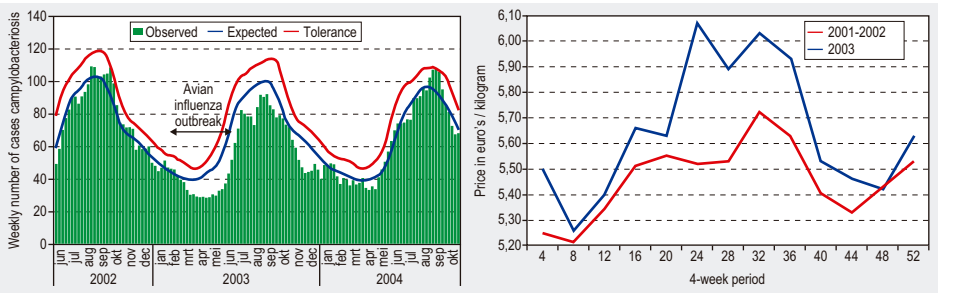


Figure 4.3.3. Seasonal course of human cases showing a dip in 2003 during the avian influenza outbreak below expected (left figure: based on trends in time series five years before); sales and prices of poultry meat were significantly lower in that period as well (right figure).

Antibiotic resistance

The resistance levels of isolates from humans and food producing animals showed a general tendency to increase, except for erythromycin resistance in *C. coli* from pigs which decreased after the ban of the growth promoter tylosine in 1999.

Farm animals

In 2005, for the first time *Campylobacter* spp. from dairy cows and veal calves were included in the surveillance. Isolates from veal calves showed the highest levels of resistance and multi drug resistance, whereas isolates from dairy cows were mostly susceptible. Also for the first time, isolates from raw poultry meat products imported from South America and from organically reared poultry and pigs were included. Resistance to erythromycin, representing the first choice drug for therapy of human campylobacteriosis, occurred more frequently in isolates from imported products, than in isolates from Dutch food animals. Surprisingly, in isolates from organically reared poultry and pigs resistance levels were similar to those of conventionally reared animals. Colonisation of biological animals with resistant campylobacters from the environment may be an explanation.

Humans

Between 2002 and 2006 resistance against fluoroquinolones (mainly ciprofloxacine) increased (Table 4.3.5) to about 45% in 2006 (cf. Figure 4.3.2), against tetracycline to about 22% and resistence to macrolides (erythromycine) doubled to more than 2%. Resistance to fluoroquinolons is clearly higher in travel-related infections (53-55%) than in endemic ones (36-45%). As fluoroquinolones are the medication of first choice in severe (traveller's) diarrhoea, this is of concern. The doubling of resistance to erythromycin within four years, antibiotic of first choice in case of a *Campylobacte*r infection, requires close monitoring as well.

Table 4.3.5. Antibiotic resistance in isolates of endemic acquired and travel-related infections with C. jejuni and C. coli, 2002-2006.

	Endemic (2002-2006)				Travel-related (2002-2006)				*C.* spp., total			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*		2006	2005	2004	2002/3
	N	R%	N	R%	N	R%	N	R%	R%	R%	R%	R%
Fluoroquinolone	9138	35.5	555	38.2	746	55.2	74	52.7	45.1	36.9	35.1	33.2
Tetracyclin	6654	19.1	489	22.1	518	27.4	65	15.4	21.7	22.2	19.6	18.5
Erythromycin	7555	1.4	522	3.3	625	1.8	69	2.9	2.2	2.1	1.3	1.2

Conclusions

Data from the public health laboratories indicate that the incidence of campylobacteriosis fluctuated considerably between 1996 and 2006. This is in contrast to trends seen in other industrialised countries in which the incidence steadily increased up to 2001 and decreased afterwards. A dip in the incidence in 2003 was probably related to the avian influenza outbreak in poultry when sales of poultry meat were significantly lower than in the years before and after 2003. The reasons for the variability in incidence are not understood, neither is its rather loose relationship with the contamination of poultry meat. As shown, food and probably the environment, including the domestic environment undergo continuous contamination from the reservoirs of *Campylobacter*. These, including farm animals, wild animals and pets, create many pathways by which humans (and poultry) can come into contact with *Campylobacter*. Between April 2002

CASA-study. Risk factors for endemic *Campylobacter jejuni* and *Campylobacter coli* infections in the Netherlands

Control of campylobacteriosis as the most common cause of bacterial gastroenteritis in the Netherlands, is hampered because the epidemiology is incompletely understood. Therefore, a case-control study of risk factors for campylobacteriosis was conducted during 2002-2003. Cases were laboratory-confirmed patients with campylobacteriosis. Controls were selected from the population registries of 25 municipalities by frequency matching according to the expected number of cases by age, sex, degree of urbanization and season. 1292 (46%) of the 2833 *Campylobacter jejuni* cases, 121 (47%) of the 256 *Campylobacter coli* cases and 3409 controls completed a questionnaire on risk factors. In multiple logistic regression, the consumption of chicken, undercooked meat, meat prepared at a barbecue and undercooked seafood, eating in a restaurant, ownership of cats and dogs, especially puppies, occupational exposure to raw meat as a cook or butcher, contact with persons with gastroenteritis symptoms outside the household and the recent use of gastric anti-secretory drugs were associated with endemic *C. jejuni* infection. For endemic *C. coli* infections, consumption of undercooked meat, meat prepared at a barbecue, tripe and foods brought at a stall, recent use of proton-pump inhibitors and swimming were identified as risk factors.
This study illustrates that important differences exist in sources of exposure and the impact of exposure for different *Campylobacter* species. Because the incidence of *C. jejuni* infections is higher than *C. coli* infections the level of exposure to *C. jejuni* is assumed to be higher as well and may lead to a better development of partial immunity explaining some of the differences.

Table Risk factors for endemic infections with *Campylobacter jejuni* and *C. coli* in their final multivariate models. (ns. not significant)

	Controls	*C. jejuni* cases		*C. coli* cases	
Total N (response %)	3106 (33)	1013 (46)		79 (47)	
Risk factors for endemic infections	N(%)	N(%)	PAR(%) (95%CI)	N(%)	PAR(%) (95%CI)
Chicken	2196 (70)	773 (77)	23 (10-33)		ns
Undercooked meat	316 (10)	188 (19)	10 (8-12)	26 (32)	4.9 (2.3-10.5)
Game	94 (3)	-	ns	7 (8)	5 (0-7)
Tripe or organ meat	42 (1)	-	ns	4 (5)	4 (1-5)
Meat BBQ/grill/microwave	626 (20)	292 (29)	12 (8-15)	27 (35)	2.3 (1.3-4.1)
Undercooked seafood	202 (6)	83 (8)	4 (2-5)	-	ns
Eating in a restaurant	1257 (40)	459 (46)	11 (4-16)	-	ns
Food bought at a stall	350 (11)	-	ns	14 (18)	10 (3-14)
Ownership of dogs	674 (22)	277 (27)	6 (0-11)	-	ns
Ownership of cats	698 (22)	268 (27)	7 (3-10)	-	ns
Occupational expos. raw meat	60 (2)	41 (4)	2 (0-2)	-	ns
Swimming	632 (20)	-	ns	23 (29)	17 (7-22)
Contact symptomatic persons outside the household	328 (11)	120 (12)	4 (1-6)	-	ns
Use of proton pump inhibitors	69 (2)	101 (10)	8 (7-9)	16 (20)	9.7 (4.6-20.6)
Use of H2-antagonists	23 (1)	16 (2)	1 (0.4-1)	-	ns
Total risk factors			51		66

ns: not significant

and April 2003, the laboratory–driven case-control study (CaSa project), was conducted to indicate the relative importance of the transmission routes involved and to quantify the role of possible risk factors. The results show that only slightly more than 50% of the cases can be attributed to known risk factors, with a significant portion attributed to poultry meat, pets and ant-acid drugs. The low number of cases explained some puzzling differences in the relative importance of foods and risk factors between *C. jejuni* and *C. coli* and are suggestive of a significant role of the development of partial immunity in persons regularly exposed to *Campylobacter*.

The CARMA-project: Integrating epidemiology, risk assessment and economics

When evaluating an intervention or policy, decision-makers need to determine its ability to positively impact public health (effectiveness), at a reasonable cost (efficiency) in a fair manner for all affected parties (equity). Risk assessment, epidemiology and economic analysis are all tools that can be used to aid risk management in such decisions. In the Netherlands this interdisciplinary approach involving different scientific institutes was applied to the control of *Campylobacter* on broiler meat. The CARMA project is a collaboration between the RIVM, the ASG, the Agricultural Economics Research Institute (LEI), the VWA and RIKILT - Institute of Food Safety, all in the Netherlands. Estimates were made of the potential benefits (Figure 1) and costs (Figure 2) of a large number of possible interventions to decrease human exposure to *Campylobacter* by consumption of chicken meat. Chicken meat consumption accounts for 20-40% of all cases of illness in the Netherlands. For this purpose, a farm to fork risk assessment model was combined with economic analysis and epidemiological data. Reduction of contamination at broiler farms could be efficient in theory. However, it is unclear which hygienic measures need to be taken and the costs can be very high. The experimental treatment of colonized broiler flocks with bacteriophages has proven to be effective and could also be cost-efficient, if confirmed in practice. Since a major decrease of infections at the broiler farm is not expected in the short term, additional measures in the processing plant were also considered. At this moment, guaranteed *Campylobacter*-free chicken meat at the retail level is not realistic. The most promising interventions in the processing plant are: limiting fecal leakage during processing, and the separation of contaminated and non-contaminated flocks (scheduling), followed by decontamination of the contaminated flock. New (faster and more sensitive) test methods to detect *Campylobacter* colonization in broiler flocks are a prerequisite for successful scheduling scenarios. Other methods to decrease the contamination of meat of colonized flocks such as freezing and heat treatment are more expensive and/or less effective than chemical decontamination. Based on the risk models developed for the project, quantitative criteria for *Campylobacter* on broiler meat are being developed and related to the Appropriate Level of Protection, as defined by Codex Alimentarius.

4.4 *Chlamydophila* spp.

Chlamydophila psittaci

Chlamydophila psittaci, an intracellular bacterium, is the causal agent of avian and human psittacosis, the latter also known as parrot fever or ornithosis. Notably birds of the psittacine family have been known to transmit the disease to humans, hence the designation psittacosis or parrot fever.

The term ornithosis relates to the possibility that members of almost any bird family may transmit the disease as well. The organism is usually transmitted by the inhalation of aerosolized dried faeces or respiratory discharge of infected birds. In the human host, psittacosis usually presents itself with influenza-like symptoms that, if left untreated, can result in serious health problems.

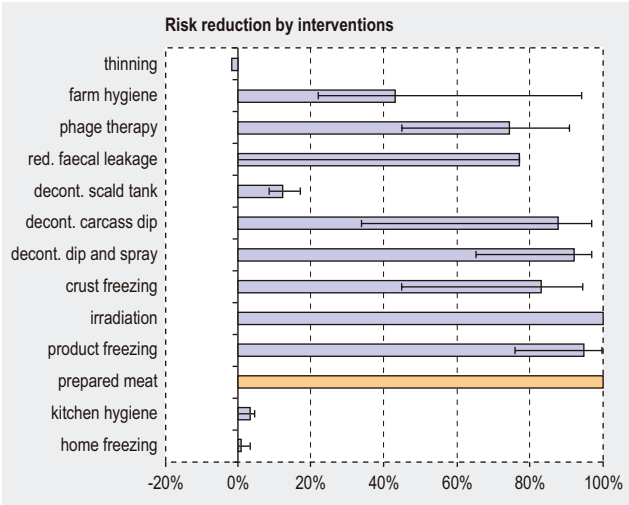


Figure 1. Risk reduction of campylobacteriosis by interventions in the broiler meat chain.

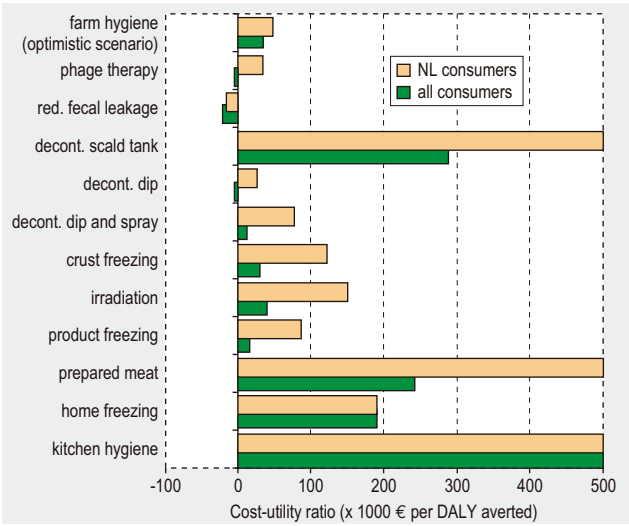


Figure 2. ***Cost-utility ratio of different interventions to reduce contamination of broiler meat with Campylobacter.*** *Data are presented when effect on Dutch consumers only are considered and when effects on all consumers (including export from the Netherlands) are considered. Only(products from) flocks that are tested positive (PCR-test) are treated*

Animals

Avian psittacosis is a notifiable animal disease. Registration of cases of avian psittacosis, however, has been fragmentary. With the formation of VWA in 2003, consistency of registration gradually increased and by 2006, the year of the fusion of RVV and KvW becoming effective, has become conclusive, although it is not known to what exact extent veterinary practitioners actually do notify avian cases. From 2003 to 2005, 15, 19 and 43 suspicions of avian psittacosis were reported to VWA of which 9, 6 and 12, respectively, were confirmed. The figures do not necessarily reflect an actual increase

of incidence, as they may well be the result of heightened awareness and more consistent case registration. A study in 2005 in Amsterdam revealed a prevalence of *C. psittaci* in feral pigeons of 5 to 10 %.

Several serotypes of *C. psittaci* are known, all of which have a certain preference for specific avian families and exhibit varying degrees of pathogenicity. *C. psittaci* is known to be widespread in pet birds, poultry and wild birds with psittacine birds and pigeons having the highest infection rates. Persistent infections appear to be common. Clinical features in birds, if any, are indicative of a systemic infection and are comprised of respiratory signs, diarrhoea and dullness. The organism is excreted in droppings and nasal discharge, often intermittently. Bacterial excretion may be triggered by various stress factors. It seems that *C. psittaci* is capable of true latency in which multiplication does not occur and sensitivity to antibiotic treatment ceases. Contrary to expectation, prophylactic sub-therapeutic antibiotic medication, as seen frequently in the psittacine bird trade, may actually induce latency, thus rendering the practice futile and hampering diagnostic procedures that require the agent to be shed. The organism remains viable in the environment for extended periods of time, giving rise to the possibility of indirect transmission without obvious bird contact. Today, official avian psittacosis testing entirely relies on PCR.

Humans

Like its avian counterpart, human psittacosis is a notifiable infectious disease. In contrast to the veterinary situation, case registration has been consistent over the years. From this, the picture emerges that the human incidence, which has been around 50 to 70 cases annually at the beginning of the nineties, halved in 1996 and remained stable until 2005 when a steep increase was noticed (Figure 4.4.1). The increased incidence was accompanied by an increase in the number of clusters (7 in 2005 compared to 1

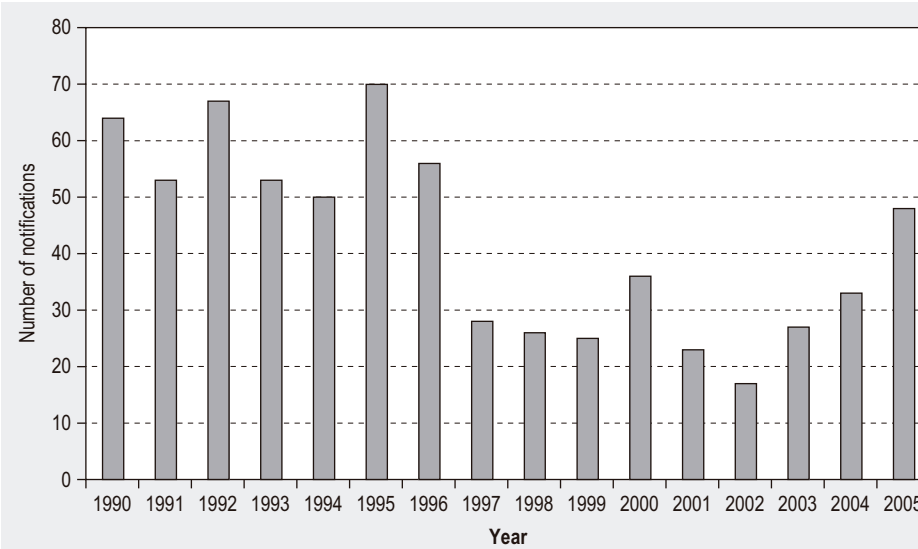


Figure 4.4.1. Annual number of psittacosis notifications, 1990 – 2005

in 2004). The reason for the increased overall incidence and the increase in number of related cases remains elusive.
Serologic testing is still the gold standard of laboratory psittacosis diagnosis. The specificity of serological tests, however, is doubtful, as cross-reacting antibodies, due to infection with other chlamydial species, may be present in the patient. PCR has become available in the last couple of years.

Conclusion

At present, no control programme for avian psittacosis is in place and the actual prevalence of psittacosis in caged and wild birds is not known. Also, it is not known whether the increased human incidence of psittacosis might indeed reflect an apparent increase of avian incidence, as the scarce figures seem to suggest. Clearly, it would be important to gain more insight in the prevalence of avian psittacosis in caged birds in order

Psittacosis in quarantine facilities

The VWA regularly receives reports of human patients with psittacosis with the request to start a source finding investigation. In many cases human infections can be related to the bird trade. A bird fancier had been admitted to intensive care with severe lung problems. Several of his birds at home had died unsuspectedly within a short period of time. Some birds showed signs of shortage of breath and had a ruffled appearance. Investigation of two dead birds confirmed the suspicion of psittacosis. The patient's family reported that new birds, in particular from bird exhibitions, had been added to the bird stock on a regular base.
In another family two persons were also diagnosed with psittacosis. One of the patients unfortunately did not survive. In this case there were also birds at home among which several died and some showed signs of disease. A VWA officer took cloaca swabs of the birds, which were tested for the presence of *Chlamydophila psittaci* at the CIDC. The test confirmed the infection with *Chlamydophila psittaci*.
It had been suspected that imported birds could carry *C. psittaci* after leaving quarantine. As this could possibly be a new source of psittacosis, measuring the prevalence of *Chlamydophila psittaci* in imported birds was strongly advised.
With this in mind, the VWA performed a survey to assess the prevalence of *Chlamydophila psittaci* in imported birds at Dutch quarantine facilities. Five quarantine facilities, which received birds from third countries on a regular basis, were visited. At three of these facilities a questionnaire was answered and fecal samples and cloaca swabs were taken for *C. psittaci* testing. At one facility only samples were taken and at another only a questionnaire was answered. Diagnostic testing of the samples and swabs for *C. psittaci* took place at the CIDC. The PCR method was applied in order to be able to distinguish between *Chlamydophila* genotypes. Of every sampled unit of the quarantine facilities, a fecal sample had also been tested for doxycycline residues with the LCMS method. This was done at the VWA laboratory. None of the birds in quarantine was diagnosed with *C. psittaci*. All quarantine facilities admitted to having used antibiotics. In 3 samples antibiotic residues where shown to be present, doxycycline (1186 µg/kg) and twice oxytetracycline (129 µg/kg and 150 µg/kg). In most cases antibiotic treatment lasted 5 to 10 days, in one instance 3 to 4 weeks.
When birds are treated with antibiotics, shedding of *C. psittaci* ceases and the pathogen cannot be detected in the samples anymore. Effective treatment of infected birds takes about 42 to 45 days, which is in stalk contrast to the duration of the treatment by the quarantine facilities. Most importantly, *C. psittaci* can survive inadequate treatments (too short for example).
It is not entirely clear whether the imported psittacine birds sampled in this survey were in fact infected with *C. psittaci* because of the prophylactic use of antibiotics by the quarantine facilities. By inappropriately shortening the period of treatment, it is possible to create carriers of the pathogen. If imported birds are indeed infected with *C. psittaci* there could be a substantial risk for public health because due to prophylactic use of antibiotics, carriers that temporarily cease shedding, which would cause diagnostic tests to fail, can be created. Control and preventive measures are of great importance and lean on suitable management of imported animals. Usage of antibiotics by the quarantine facilities has to be restricted and a standard psittacosis check of untreated imported birds is recommended.

to assess the risks imposed on humans by psittacosis, that is, if it weren't for frequent prophylactic use of antibiotics in bird trade, hampering chlamydial detection. Nonetheless, measures need to be taken to reduce the likelihood of avian to human transmission. A (compulsory) certification scheme for bird traders and commercial breeders, such as what was in place in the nineties providing safeguards for the absence of *Chlamydophila* in birds, would seem to be the appropriate measure to achieve this.

Psittacosis as a birthday party give-away

Six persons fell seriously ill with high fever, headache and coughing around New Year's Day 2006 after having attended a birthday party in mid-December 2005. Three of the patients were shown to have been infected with *Chlamydophila psittaci* serologically. A budgerigar had been given to the woman whose birthday it was as a present a day before the party. This bird was meant as a replacement for a budgerigar that passed away six weeks prior to the birthday. No further attention was drawn to the fact that the arrival appeared to be unusually calm and lethargic. The birthday party was attended by 30 guests, 6 of which developed severe respiratory disease two weeks later. All patients lived in various parts of the country. Three patients were treated with broad-spectrum antibiotics without improving of the condition. Eventually, a relative of the person whose birthday it was, a physician that herself remained healthy, suspected that psittacosis might be the cause of the trouble within her family. Subsequently, all diseased were given doxycycline, promptly improving the condition.
Apart from the budgerigar that had been given as a birthday present the recipient kept another budgerigar and a canary. These birds had been present in the home for 1.5 years and appeared to be healthy.
All three birds were examined for the presence of *Chlamydophila psittaci* with no result. Two birds had somewhat enlarged spleens and no *Chlamydophila* antigen was detected in spleen smears. Further testing, using more advanced techniques, did not take place.
An investigation held at the pet shop were the incriminated budgerigar had been purchased determined that pet birds were supplied by at least four breeders, of whom only ones full address was known to the shop keeper whereas the others were merely known by name and telephone number. Due to utter lack of record keeping, it could not be ascertained from which breeder the incriminated bird originated and further investigation ceased at this point. All birds present at the shop appeared healthy and none of the psittacine birds tested positively for *Chlamydophila psittaci*.
Even though *Chlamydophila psittaci* was not detected in any of the birds kept by the woman in question, which may well be due to insensitive diagnostics, the budgerigar given as a present has to be regarded as the most likely source of the outbreak as the dates of arrival and the birthday party as well as the incubation periods all coincide nicely.

Prevalence of Chlamydophila psittaci in droppings from feral pigeons in Amsterdam

In the city of Amsterdam, the urban feral pigeon (*Columbida livia*) is an abundant bird species that often lives in close contact with humans. It is known that pigeons, like many other bird species, can harbour *Chlamydophila psittaci*. This bacterium is a pathogen of birds, but can cause zoonotic disease. In a study, the prevalence of *C. psittaci* shedding in faeces from feral pigeons in Amsterdam was determined and all the PCR positive samples were genotyped.
Pigeon samples were obtained at 9 locations in 8 town councils. These locations were geographically widely distributed in Amsterdam. All were situated in the public area and chosen based on previous research of assembling locations of feral pigeons. Pigeons were attracted with food and their fresh faecal droppings were sampled with sterile cotton swabs. The samples were taken on the 3rd of February and the 8th of March 2005, when breeding activity was low and on the 2nd of May 2005 when breeding was frequent.
In total 331 faecal samples were obtained. 160 samples before and 171 in the breeding period (Table). On each location at least 15 samples were collected. In the low-breeding period 5% of all samples were PCR positive. In samples obtained during the breeding period 10% were positive, hence the prevalence of positive samples during the breeding

Table

Town Council	Low-breeding period	Breeding period
Oost Watergraafsmeer	0/15[a]	6/15
Oud Zuid	3/15	0/20
Binnenstad (Dam)	0/20	2/27
Binnenstad (Leidse plein)	2/25	3/27
Zeeburg	0/15	3/15
Zuider Amstel	0/15	3/15
Geuzenveld	0/15	0/15
Bos en Lommer	0/20	0/15
Oud West	3/20	1/22
Total	8/160 (5%; 95% CI 2-10%) [b]	18/171 (10%; 95% CI 6-16%)

a) Number of positive samples/ samples per council tested
b) CI: Confidence interval

period was twice the prevalence in the low-breeding period (Fisher's exact test: p=0.07) It was possible to genotype 10 of the 26 PCR positive samples. The obtained sequences were all 100% similar to a reference genotype B.

This study shows that between 5 and 10% of our sample of the urban feral pigeons in Amsterdam shed *C. psittaci* in their faeces. A previous study indicated that in 2001 the pigeon population size in Amsterdam averaged approximately 30,000. A substantial amount therefore sheds *C. psittaci* in the environment. Our isolates were all identical to genotype B. Currently, at least nine genotypes are known. Each genotype is more or less associated with a specific group of birds from which it is most commonly isolated. Others also found genotype B to be mainly associated with pigeons. Although the majority of human psittacosis cases seems to be related to psittacine birds, pigeons have also been a well known reservoir for zoonotic disease. This study shows significant infection of urban fowl and it alerts clinicians to the potential role of this organism in presentations of pneumonia in urban dwellers with extensive contact with pigeons or their faeces.

Source tracing would greatly benefit from patient sample testing by means of PCR because this allows for genotype comparison with avian sources, thus enabling inferences as to the relatedness of patient and incriminated avian source.

Chlamydophila abortus

Prior to the reclassification of chlamydiacae taxonomy *Chlamydophila abortus* used to be referred to as the mammalian abortion strain of *C. psittaci*. *C. abortus* is endemic among ruminant species and is the aetiological agent of enzootic abortion in sheep and goats. Although, it is of economic importance in small ruminant farming, it is zoonotic and may cause abortion or stillbirth in pregnant women.

Animals

Despite its zoonotic potential and its economic importance *C. abortus* infections are not notifiable.

It seems that the incidence of *C. abortus* related abortion in sheep and goats is increasing, as may be judged by the fact that in 1990 *C. abortus* related abortion was absent in the northern parts of the country whereas it is now seen regularly in all parts of the country. Experts of the Animal Health Service estimated the prevalence in 2003 to be 5 to 10 % in goat herds and 1 % in sheep flocks. The actual prevalence, however, is

Chlamydophila abortus infection in a pregnant woman associated with indirect contact with infected goats.

In March 2003, a 29-year-old pregnant woman in week 25 of gestation was admitted to a hospital when vaginal bleeding started following 3 days of influenza-like illness with headache, abdominal pain, dry cough, and malaise.
With the diagnosis of septic shock, disseminated intravascular coagulation and multi-organ failure, the patient was admitted to the intensive care unit where she gave birth to a stillborn immature son. The patient was intubated, mechanically ventilated and dialysed. Therapy was started with amoxicillin/clavulanate and gentamicin. Blood cultures were negative and low amounts of Escherichia coli were cultured from vaginal swabs. Three days after admission, further discussion with the patient revealed that her husband was a goat farmer. A possible infection with *Chlamydophila abortus* was considered and doxycycline therapy (100 mg b.i.d.) was started. After the serological confirmation of chlamydial infection was obtained, amoxicillin/clavulanate and gentamicin therapy was stopped at day 8 while doxycycline therapy was continued. The patient improved over the next week. Mechanical ventilation and dialysis were discontinued on day 15, and the patient was transferred to the gynaecology ward on day 17. After 38 days of hospitalisation the patient was discharged from the hospital in good condition.
The patient, her husband and two companions were operating a goat farm with approximately 900 goats. The patient and her husband lived at another location, about 5 km from the farm. The patient reported explicitly that she did not help with kidding nor did she have direct contact with kid goats. However, she had visited the stable where goats were kidding several times and she washed the clothes her husband wore during help with kidding. Her husband paid no special attention to personal hygiene before returning home after work.
The history of the herd showed an increased number of abortions (7.5% in 2002; normal range, 3–5%). In July 2003, sera were collected from four groups of goats and analysed in a *C. abortus* antibody-specific ELISA (Institut Pourquier, Montpellier, France). *Chlamydophila abortus*-specific antibodies were detected in six of 14 older goats that had abortions in 2003, in eight of 14 older goats that had normal pregnancies in 2003, in six of 14 yearling goats that had a successful first pregnancy, and in 13 of 15 yearling goats that had a first pregnancy abortion.
In conclusion, this case again demonstrates the importance of advising pregnant women to avoid both direct and indirect contact with (lambing/kidding) sheep/goats, especially when the herd is suspected or has been confirmed to be infected with *C. abortus*. In addition, this report stresses the importance of including questions about contact with animals when taking the anamnesis of pregnant women who present rapidly worsening influenza-like illness.

not known as prevalence studies have not been performed yet. This is due to several factors, most importantly the lack of reliable serologic procedures (another member of the chlamydiae, *Chlamydophila pecorum*, causing arthritis and conjunctivitis, is also found in ruminants and induces cross-reacting antibodies) but difficult culturing of the organism and absence of registration of ruminant *C. abortus* cases contribute as well.
In infected sheep abortion occurs in the last 2- 3 weeks of gestation, irrespective of the time of infection. Shedding of *C. abortus* may take place up to three weeks after abortion. After the primary abortion event ewes usually do not abort in subsequent gestations but remain persistently infected and shed *C. abortus* around the time of ovulation. Infected rams spread the organism venereally. Under favourable conditions *C. abortus* may survive in the environment up to several months.

Humans

Human infection with *C. abortus* is a rare but severe cause of abortion in pregnant women and respiratory disease in non-pregnant persons. Zoonotic transmission is thought to occur via the oral or respiratory route, either directly through contact with the placenta or uterine discharge of aborting animals or indirectly via various fomites.

As of 2003, 3 cases of zoonotic chlamydial infection during human pregnancy have been known to have occurred since 1997, two of which were ascribed to ovine or caprine contacts.

Conclusion
Even though human infections with *C. abortus* seem to be rare events, the devastating effect of the infection on mother and foetus justifies control of enzootic abortion in sheep and goats, thus preventing human infections.

4.5 *Coxiella burnetii*

Coxiella burnetii is a worldwide zoonotic intracellular Gram-negative bacterium, which is the causal agent of Q-fever. The main characteristic of Q-fever in humans is its clinical polymorphism. The incubation time is usually between 2 and 4 weeks. About half of the individuals infected with *C. burnetii* develop symptoms of mainly self-limiting febrile illness lasting a few days to two weeks. This acute disease can include pneumonia, menigoencephalitis or hepatitis. A chronic form of the disease can develop as long as 20 years after initial infection and includes mainly endocarditis.

Animals
Coxiella burnetii can be carried by a wide variety of animals, including sheep, cows, and goats. An inventory in the Netherlands in 1987 demonstrated that *C. burnetii* is often found in dairy cows (21,4% seropositive), less frequently in sheep (3,5% seropositive) and rarely in goats, cats and dogs. Healthy looking animals can excrete large numbers of *C. burnetii* in faeces, urine, milk, placenta and amniotic fluids. At environmental temperatures *C. burnetii* can survive from months to years in dried animal secretions and soil. Inhalation of dust containing *C. burnetii* derived from stables, meadows, wool, hides, clothing etcetera can cause human infection. There have been reports of Q-fever patients with *C. burnetii* originating from sheep up to 18 km up wind from their home or daily occupation.

Food products and drinking water
Infection with *C. burnetii* can occur via consumption of raw milk, cheese, or raw meat from infected animals. Also, drinking infected water is a possible source of Q-fever. Heating for 15 seconds at 73^{o}C or 30 minutes at 63^{o}C is sufficient to kill *C. burnetii*, and render products safe for consumption.

Humans
Specific diagnosis of a *C. burnetii* infection is based on serological tests. IgM and IgG antiphase II antibodies are detected 2 to 3 weeks after infection. IgG antiphase I antibodies indicate a chronical infection. The antibodies against *C. burnetii* are known to persist for months or years after initial infection. PCR can be used to determine the presence of the bacterium. Both methods are available in the Netherlands at the RIVM, but the PCR is still under development. In the Netherlands all Q-fever infections should

be reported to IGZ. On average, 13 cases have been reported annually in the last five years, with 20 cases in 2004, 5 cases in 2005 and 12 cases in 2006. It is important to notice that many cases are not diagnosed at all due to absent or mild symptoms, which leads to an underestimation of the real number of cases. Underreporting makes it difficult to determine the source of the infections and increases the risk of missing a beginning outbreak.
The highest incidence of *C. burnetii* infection is in men between 25 and 60 years of age, most probably due to their occupation in farming, and slaughterhouses. This is confirmed by the relative high incidence in rural areas. However, the number of cases is too low to draw definite conclusions. In addition, Q-fever can be acquired abroad as indicated by a cluster of 21 Dutch Q-fever cases after holidays at the same farm in France in the period that the sheep were lambing.

Conclusions
The presence of *C. burnetii* in cattle and small ruminants in the Netherlands is a risk for persons in contact with those animals. However, the true incidence of this infection in the Netherlands is hard to estimate, due to underdiagnosis and underreporting of cases and limited knowledge of the infection rate of the animals. Detection of the source for human cases would be facilitated by a more accurate overview of the infections. The limited number of reported cases complicates containing an outbreak early.

4.6 *Cryptosporidium* spp.

Cryptosporidium is a world wide distributed protozoan parasite with a broad variety of hosts including both vertebrates (e.g. humans, cattle, birds) and invertebrates (e.g. clams). In most cases, including those in humans, *Cryptosporidium* causes an enteric infection leading to gastrointestinal (GI) problems such as severe diarrhoea.

Animals
The role of *Cryptosporidium* as a cause of diarrhoea in neonatal calves was reported already in 1982. In 2001, a 1-year study performed at a dairy farm in the Netherlands with neonatal problems in young calves showed that the prevalence of cryptosporidiosis was higher (up to 39%) in calves 1-3 weeks old compared to older animals; younger animals were also shedding a higher number of oocysts in their faeces. In addition, Dutch studies in veal calves showed high prevalences on herd bases from 99% herd prevalence at an age of 1-6 weeks to 70% at an age of 35 weeks. In 2006-2007 genotyping of clinical farm animal, mainly calves and cattle, isolates showed that only *C. parvum* was found. Using microsatellite typing (ML1) two variants were present C1 (8%) and C2 (92%). Using GP60 marker, 17 different *C. parvum* IIa variants were recognized and of these 60% were of *C. parvum* subtype IIaA15G2R1. Comparison with human isolates indicated that this strain may be zoonotic.
Recently epidemiological studies on the presence and identification of *Cryptosporidium* in pet animals were carried out. Although *Cryptosporidium* has been found in faecal samples of cats and dogs, no identification to species or subgenotype level is available

yet. Several studies have been carried out to identify *Cryptosporidium* spp. in muskrats, deer, foxes and wild birds. *C. bailly* has been identified in falcons and geese. Moreover, *C. parvum* has been detected in fecal samples of musk rats and roe deer.

Humans

Cryptosporidium is not notifiable in the Netherlands. In most routine laboratories *Cryptosporidium* is only investigated at special request or in specific patients (e.g. HIV positive patients). The parasites will not be detected in the routine diagnostic test.

In several Dutch epidemiological studies the incidence of gastroenteritis caused by *Cryptosporidium* was found in about 2-3 % of the gastroenteritis cases, compared to a prevalence of about 0.2% in the general population. In these studies only some of the *Cryptosporidium* isolates were further genetically analyzed. To study the zoonotic potential of *Cryptosporidium* infections, in 2005-2006 isolates of clinical human cases were used for genotyping and compared with cattle isolates Results in humans showed 70% *C. hominis*, 19% *C. parvum,* 10% a mix of both and 1% *C. felis.* The majority (80%) of the human cases originated from children between 0 and 9 years of age and counted for >70% of *C. hominis*. *C. hominis* peaked in autumn, and subtyping (GP60 marker) revealed that *C. hominis* subgenotype IbA10G2 is predominant. This strain is not found in farm animals, indicating that infection most probably occurs via human to human transmission. In humans, C *parvum* was found relatively constant throughout the year. Subgenotyping both *C. parvum* of human and cattle using the same marker showed that C. *parvum* IIaA15G2R1 was predominant in cattle and this subgenotype was also identified in humans indicating that this strain may be zoonotic.

4.7 *Echinococcus* spp.

Hydatid disease is caused by *Echinococcus granulosus,* the tapeworm of carnivores (dogs, wolves, jackals and foxes). The larval stage (hydatid cyst) occurs in sheep, cattle, horses, pigs, camels, goats and buffaloes. In humans a similar hydatid cyst may develop causing cystic echinococcosis. Alveolar echinococcosis is caused by *E. multilocularis*, one of the most serious parasitic zoonoses. The parasitic cycle is mainly sylvatic (wildlife). In Europe, red foxes are the major definite canid host, but adult worms can also occur in dogs and cats. Humans may get infected by oral uptake of eggs shed by infected definite hosts. Historically, the disease is endemic in central Europe (Germany, Austria, Switzerland, France). But recently, the known geographic range of the parasite in west and central Europe seems extended to include regions in Austria, Switzerland, France, Germany, Liechtenstein, Luxemburg, Belgium, the Netherlands, Poland, Czech Republic, Slovak Republic, Denmark, the Norwegian island of Svalbard and Lithuania. *E. granulosus* and *E. multilocularis* are both notifiable in animals

Echinococcus granulosus

Animals

Surveillance for *E. granulosus* is performed through meat inspection. The previously endemic cattle strain has not been found for decades, not even recently during a pilot study in slaughter animals in 2002. The aim of this pilot study was to detect and to identify *E. granulosus* genotypes using molecular identification methods in suspected liver and lung specimens of slaughter-cattle and sheep. Although it has been the intention to continue this surveillance, no more cattle specimens were investigated after this pilot study.

Humans

Hydatid echinococcosis is not a notifiable disease in the Netherlands. Patients with hydatid echinococcosis, mostly originating from endemic countries (Mediterranean, South America, Africa) are regularly seen. Between approximately 20 to 30 serologically positive patients are being detected at the RIVM annually. This represents about 30-50% of all cases diagnosed by serology annually. Most of these patients originated from Mediterranean countries that are endemic for *E. granulosus*. In 2004, 34 serologically positive patients were identified at RIVM. One of the positive patients, a woman of 43 years old, has not been outside Western Europe and lives in the northern parts of the Netherlands. She was diagnosed at the age of 17 with a pulmonal localisation of *Echinococcus*. At that time (in 1977) the cyst fluid was not typed. In 2004, she presented herself with a liver cyst that was positive for *Echinococcus* protoscolices. By molecular identification of DNA from the cyst fluid, *E. granulosus* was identified and typed as Genotype 5, the *E. granulosus* genotype originating from cattle. Comparing the DNA sequence of this *E. granulosus* G5 with the DNA sequence of the same marker from a previously described patient from the Netherlands (Bowles ea 1992), revealed two identical isolates. This is the second time that *E. granulosus* genotype 5 (*E. ortleppi*) has been isolated in a Dutch patient not traveling abroad. This case indicates that there is still an endemic cattle strain in the Netherlands with the potential for human infections. In 2005, 26 positive patients were identified at RIVM.

Echinococcus multilocularis

Animals (wildlife)

The occurrence of *E. multilocularis*, the etiological agent of human alveolar echinococcosis, in foxes poses a potential infection risk for humans since oral uptake of eggs shed by the definite host is the usual route of infection. This risk may actually exist in all endemic regions in Europe.

In the Netherlands, *E. multilocularis* was detected for the first time in foxes sampled between October 1996 and March 1997 in two distinct areas, in the northern region of Groningen adjacent to Germany and the southern region of Limburg adjacent to Belgium. In Groningen, the base line prevalence in foxes was 9.4% in 2000. In addition, a similar study in the province of Limburg adjacent to the border with Belgium revealed a base line prevalence of 12.6% in 2003. Analysis of positively and negatively

tested foxes from several studies carried out in Belgium and the Netherlands showed corresponding spatial patterns of infection in Belgium and the neighbouring region in the Netherlands, indicating a continuous distribution of *E. multilocularis* in foxes across the national borders.

Surveillance

Surveillance studies were carried out periodically in 2003, 2005 and 2006. In Limburg there is a strong indication that the parasite population is increasing in number and spreading into a wider region into the north at the speed of 2.7 km per year. Distribution of the reproduction number R_0 as estimated from the surveillance data showed that it is likely to be greater than 1 but not exceeding the value 4. Using the estimated R_0 and its expression in terms of the parameters of the parasite transmission processes, the amount of effort to control the parasite by keeping the fox population density low was determined. The fox population density would have to be maintained at 0.3-1 per km^2, which is difficult to achieve by human intervention. This is the first evidence of increased infection pressure for humans living in the Netherlands. However, modeling studies showed that controlling the spread of the parasite in wildlife is extremely difficult to achieve.

Figure 4.7.1. Geographical distribution of E. multilocularis positive foxes in the Netherlands (black spots positive foxes, white spots negative foxes, source RIVM)

Humans

Alveolar echinococcosis is not a notifiable disease in humans. Until now, no autochthonous human cases have been reported in the Netherlands, but because of the long incubation time (5-15 years), the first human case may only be recognized years after the first introduction of the parasite into the wildlife population. In addition, cases may be overlooked or misdiagnosed due to a lack of medical awareness of this disease. For example, in 2006, a Turkish patient living in the Northern region of the Netherlands was diagnosed with *E. multilocularis*, after she had been treated as an *E. granulosus* case for several years. Results from the studies in Limburg in foxes revealed an increasing prevalence and worm burden in foxes, whereby modeling indicated a northward

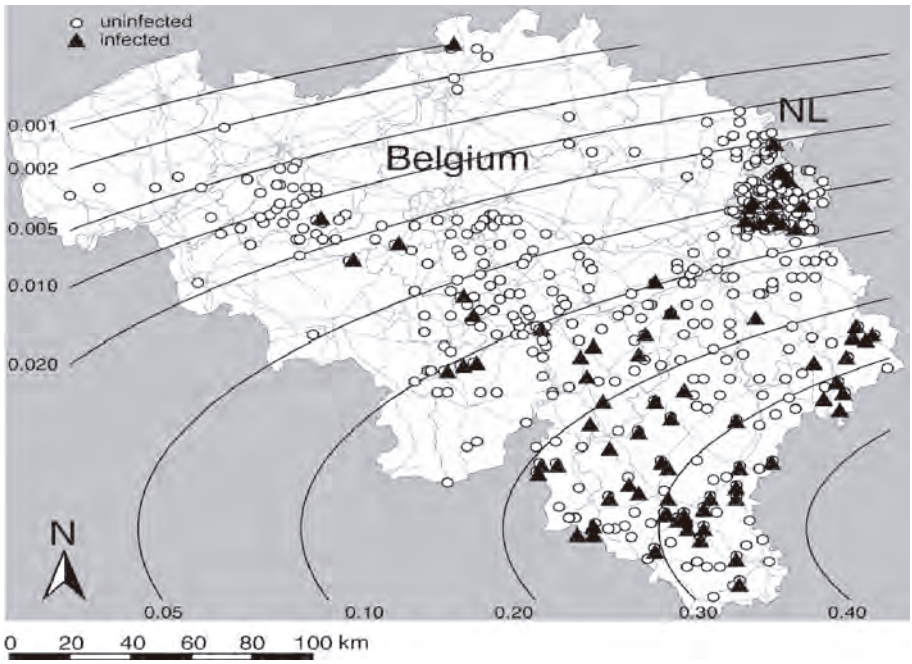


Figure 4.7.2. Spatial distribution of E. multilocularis in foxes across the border of Belgium and the Netherlands

spread of the parasite in combination with a similar prevalence in foxes in the adjacent part of Belgium. In this part of Belgium, close to the border with Limburg, in 2003 the first autochthonous cases of human echinococcosis have been reported, indicating that *E. multilocularis* is an emerging parasite in the Netherlands and the risk of alveolar echinococcosis is increasing.

Conclusion

Results of the studies in foxes in Limburg revealed an increasing prevalence in foxes, whereby modeling indicate a northward spread of the parasite in combination with a similar prevalence in foxes in the adjacent part of Belgium. In this part of Belgium, close to the border with Limburg, in 2003 the first autochthonous cases of human echinococcosis have been reported indicating that *E. multilocularis* is an emerging parasite in the Netherlands and the risk of alveolar echinococcosis is increasing. Moreover, modelling studies showed that controlling the spread of the parasite in wildlife is extremely difficult to achieve.

4.8 *Escherichia coli* Shiga toxin-producing (STEC)

Shiga toxin-producing Escherichia coli O157 (STEC O157), also called enterohaemorrhagic E. coli O157, was recognized as an important human pathogen in 1982. In the first half of the 1990s, attention to this pathogen increased due to several large outbreaks of STEC-related gastro-enteritis and complications like the haemolytic uraemic

syndrome (HUS) in Japan, Scotland and the USA. Since January 1999, an enhanced surveillance of STEC O157 has been implemented in the Netherlands. In 2005, the first national outbreak of STEC O157 was seen with 21 patients involved.

Farm animals

E. coli O157 in dairy cows and fattening calves

In 2005, the estimated prevalence of *E. coli* O157 in herds of dairy cows and fattening calves was 4% and 8.5%, respectively. Possibly, these estimates have been affected by the changes in the execution of the monitoring programme and the detection method applied from July 2005 on. Besides, the increase in the fraction of white veal calf herds in the sample is likely to have influenced the crude prevalence estimates since the prevalence of *E. coli* O157 in herds of pink veal calves (about 40%) is significantly higher than in herds of white veal calves (about 1%). In 2006 146 veal calves herds were sampled, 20 (about 13 %) were tested positive for *E. coli* O157. Furthermore 131 dairy cows herds were sampled. Of those herds 7 (about 5%) were tested positive.

Food

Surveys of STEC O157 in beef, pork, veal and mutton were carried out by the VWA in the period of 2003-2006 (table 4.8.1). STEC O157 was found in meat 6 times out of about 5000 samples. No STEC O157 was detected in mutton samples at retail. In 2004

Table 4.8.1. STEC O157 in cattle on randomly selected farms (Monitoring VWA – RIVM).

	Veal calves			Dairy cows		
Year	# herds	% Positive	90% CI	# Herds	% Positive	90% CI
2000	133	17.3	11.9 – 22.7	158	8.9	5.2 – 12.6
2001	89	12.4	6.7 – 18.1	103	10.7	5.7 – 15.7
2002	159	23.9	18.4 – 29.4	148	14.2	9.5 – 18.9
2004	170	13.5	9.2 – 17.9	153	8.5	4.8 – 12.2
2005	142	8.5	4.7 – 12.3	126	4.0	1.8 – 8.9
2006	146	13		131	5	

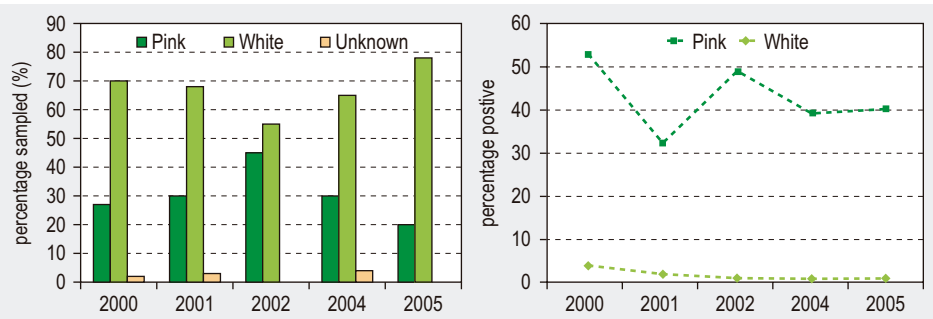


Figure 4.8.1. Distribution of herds of pink veal calves and white veal calves in the sample per year (A) and the percentage of positive herds per type of veal calves per year (B).

Table 4.8.2. STEC O157 in meat and ready to eat products (Monitoring programme VWA)

	2003		2004		2005		2006	
	N	%+	N	%+	N	%+	N	%+
Meat								
Beef	634	*0*	655	*0.2*	763	*0*	793	*0.3*
Pork	208	*0*	306	*0*	401	*0.5*	435	*0*
Veal			233	*0.4*	201	*0*	84	*0*
Mutton	36	*0*	153	*0*	129	*0*	44	*0*
Ready to eat products								
Steak tartare			453	*0.2*			954	*0.1*
Fermented sausage			662	*0*				
Tartare			332	*0*				

and 2006 projects were carried out in ready to eat products by the VWA. In steak tartare STEC O157 was detected in both years.

Humans

Results of enhanced surveillance

With the enhanced surveillance of STEC O157 in 2006, 40 cases (38% male) were identified, corresponding to 0.25 cases per 100,000 inhabitants. This was comparable with the number in previous years (annual number of cases ranging from 36 to 57). In 2005 and 2006, a shift in age distribution was seen from young children (0-4 years of age) to older children. The majority (60%) of the STEC O157 isolates belongs to serotype O157: H7 and are stx_2- and eae-positive. In 2005 and 2006, no sorbitol-fermenting STEC O157 was found. In both years, about one-third of the patients were hospitalised and 8% developed the haemolytic-uraemic syndrome of which one 1 year-old boy died in 2005. In 2006 a larger number of patients (67%, previous years 50-56%) reported a known risk factor, such as contact with farm animals or manure, consumption of raw or undercooked beef (33%, 11-30% in previous years), raw milk (28% in 2006, 29% in 2005 and 7-20% in 1999-2004) or contact with a symptomatic individual. Especially, a relation with the consumption of raw milk or raw milk product and undercooked beef seems to have increased over the years.

In 2005, cluster analyses of the fingerprints of bacterial DNA from the STEC O157 isolates (by pulsed-field gel electrophoresis) suggested nine times a relationship between several patients. For three clusters this was supported by additional epidemiological information. Two clusters were closely related and make up the national outbreak caused by filet américain, except for two patients who fell ill (one two months and the other one month) before this outbreak. Furthermore, one household cluster was identified from which an indistinguishable PFGE pattern was found in an isolate in the manure of cattle from their farm. In addition, an isolate from one individual case could be matched with an isolate from cattle of the neighbours. In 2006, cluster analyses suggested ten times a relationship between several patients. For one cluster this was

supported by additional epidemiological information. An isolate from one individual case could be matched with isolates from goats of a children's farm.

Seasonality

The relationship of contaminated cattle with infections in humans is illustrated by the parallel seasonality of STEC O157 in humans and cattle (Figure 4.8.2). Each year the spring rise of the contamination level of veal calves occurs a few weeks earlier than that in dairy cattle and infections in humans. This suggests that veal calves are the primary reservoir for both dairy cattle and humans.

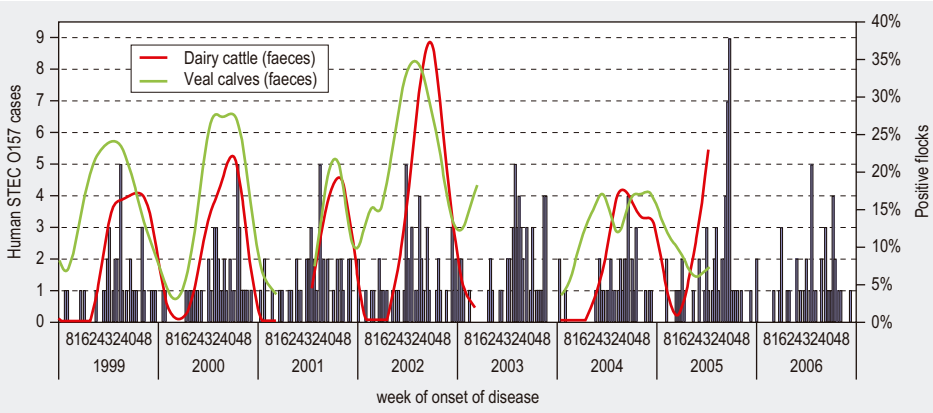


Figure 4.8.2. Reported cases of STEC O157 (Enhanced surveillance RIVM) and the monthly fraction of positive dairy cattle and veal calf flocks on farms (monitoring farm animals).

Antibiotic resistance of STEC *E.coli*O157 and *E. coli* as indicator organism.

Farm animals

Two isolates from cattle were multi-drug resistant. In *E. coli* strains isolated from faeces (indicator organisms for the commensal gut flora) from intensively reared broilers and veal calves, and to a slightly lesser extent from slaughter pigs, resistance levels are very high and show a tendency to increase over time. In dairy cows resistance is only rarely present. Multi drug resistance shows a similar increasing trend, with alarmingly high levels of multi drug resistant isolates in broilers and veal calves.

The occurrence of extended spectrum beta-lactamases (ESBLs) resistance increased substantially in broilers from 9.7% in 2004 to 14.1% in 2005, this in spite of the fact that cephalosporins are not used in these animals. This means that the linkage of resistance genes and co-selection by usage of other antibiotics is most likely the main cause for the observed increase.

Resistance levels in isolates from imported poultry products from Southern America were higher while resistance in isolates from organically reared animals was lower compared to those from Dutch meat products or conventional Dutch animals.

Humans

In 2004, 78 strains of *E. coli* O157 were sent to RIVM for typing purposes, 37 were isolated from specimens taken from human faeces and 41 from veal calves and dairy cattle in an attempt to trace a human clinical infection. In 2005 an outbreak occurred with a cluster of 25 human cases. This outbreak clone was resistant to trimethoprim and sulphamethoxazole.

Conclusion

STEC O157 is regularly found among dairy cattle and veal calves and sporadically found at retail. In humans it is concluded that the incidence of STEC O157 is relatively low in the Netherlands. In 2005, a small increase was seen due to a national outbreak. This shows the epidemic potential of STEC O157. Over the years, the resistance levels for *E. coli* O157 were low and limited to a small number of individual isolates. In the past years, other serogroups than O157 have caused outbreaks in several European countries, demonstrating similar epidemic potential and serious illness. Methods that are able to detect all STEC were therefore developed in the Netherlands and should be implemented. In eight medical microbiological laboratories it was assessed that in 2005-2006 non-O157 serogroups STEC were four times more common in the Netherlands than O157.

First nationwide outbreak of *E. coli* O157

Shiga toxin-producing *Escherichia coli* (STEC) O157 is the most important cause of haemorrhagic colitis and the haemolytic-uraemic syndrome in infants. In adults, STEC infections are mostly limited to mild diarrhoea. Cattle are the major reservoir of STEC O157 and transmission to humans can occur both directly through contact with the animals or the farm environment and indirectly through consumption of raw or undercooked products of bovine origin. Since January 1999, the Netherlands has implemented an enhanced surveillance of STEC O157 and in December 1999, notification of STEC O157 infections became mandatory as part of the Infectious Diseases Act. From 1999 to 2004, the number of patients varied between 36 and 57, corresponding to an annual incidence of 0.22 to 0.35 laboratory-confirmed cases per 100.000 inhabitants. Most of the infections were individual cases, although sometimes, small clusters of fewer than 5 cases were reported.

Within the first week of October 2005, an unusual high number of 18 cases was reported and therefore an outbreak investigation was started. In total, 21 laboratory-confirmed cases of infection were identified and an additional 11 probable cases (two primary and nine secondary cases), for whom no specimens were available for microbiological testing. Based on interviews with patients using a trawling questionnaire, filet americain or steak tartare (raw minced beef mixed with a mayonnaise-based dressing) was suggested as a possible source of the outbreak. A subsequent case-control study supported this hypothesis. Furthermore, it turned out that the majority of the cases who consumed filet americain had bought the product at supermarkets belonging to one specific chain. Samples of filet americain collected at different branches from this chain of supermarkets across the Netherlands, all tested negative for STEC O157. However, sampling was done three days after the onset of symptoms of the last reported case. A trace back investigation led to the identification of the plant that most likely had delivered the incriminated batch of filet americain. Unfortunately, no remnants of the product were available for sampling. The plant processed beef from numerous abattoirs, and therefore a further trace back was not feasible.

Since 88% of the cases became ill within a period of 2 weeks and STEC O157 could not be detected in the samples of filet americain tested, a point source contamination of filet americain has to be regarded as the most likely cause of the first national food-related outbreak of STEC O157 in the Netherlands. Partly because of this outbreak and the recent outbreak of multi-resistant Salmonella Typhimurium DT104, the VWA intends to investigate the production chain of steak tartar in 2008, to be able to act faster and more efficiently in similar situations in the future.

Shiga toxin producing *E. coli* O157 on farms

Infection with Shiga toxin producing *Escherichia coli* O157 in humans can lead to mild or bloody diarrhoea, with haemolytic uraemic syndrome (HUS) as a possible complication. Cattle appear to be important reservoirs of O157 STEC. Prevalence, risk factors, and transmission of O157 STEC were investigated to understand the dynamics of O157 STEC in Dutch cattle. Data from a monitoring programme in Dutch animal herds indicated that O157 STEC is endemic in the Netherlands, with higher prevalence during summer and early fall. Risk factors for infection were identified. Within-herd prevalence, potential environmental reservoirs, intermediate hosts and DNA types of O157 STEC isolates were determined in a longitudinal study of a positive dairy farm. DNA clusters indicated persistence on the farm during winter and spring. Quantification of transmission using data from this diary herd and from an experiment with calves showed that transmission was higher in calves. Transmission of O157 STEC during summer might differ from transmission during winter. Two other experiments indicated that both previously infected heifers and previously contaminated pastures did not function as reservoir of O157 STEC between shedding seasons. A retrospective cohort study established that positive farms from the Dutch monitoring programme were only slightly more likely to be found positive in a next shedding season than previously negative farms, so between-herd transmission seems to occur. Factors associated with a positive test after a second sampling implied (re-)introduction rather than long-term persistence of infection. Results suggest that several types of O157 STEC might persist on a farm for some time, with possibly different strains being the most prevailing types in subsequent shedding seasons. Ultimately, the within-herd infection might become extinct due to limiting numbers of susceptible cattle. Between-herd transmission of O157 STEC can lead to persistence in a larger region, e.g. the Netherlands, maintaining the endemic status in Dutch cattle populations. Because of this, exposure of humans to O157 STEC cannot be ruled out in the Netherlands. Humans might become infected through food- or waterborne transmission and by transmission directly from animals to humans. When aiming at reducing risks for humans by interventions at farm-level, it is of importance to reduce the number of positive animals and farms. For this, more specific research of the effect of intervention measures on introduction, transmission and survival of O157 STEC on farms, and economic (cost-benefit) analyses, should be performed.

4.9 *Giardia* spp.

The flagellated protozoan *Giardia* is an intestinal parasite that can infect many species including mammalian, avian and reptilian wildlife, domesticated animals, and humans. Of the morphologically defined *Giardia* species, *Giardia muris*, *Giardia microti*, *Giardia agilis*, *Giardia psicatti* and *Giardia duodenalis* (syn. *Giardia lamblia*, *Giardia intestinalis*), only the latter is recovered from humans and a wide variety of other mammals. In humans, *Giardia* can cause gastrointestinal infections ranging from mild to severe as well as chronic disease. In domestic animals, *G. duodenalis* is of considerable clinical importance and could have economic significance in the beef and cattle industries. Infection occurs by faecal oral route of transmission, either by direct contact or by ingestion of contaminated food or water. Zoonotic transmission of *G. duodenalis* is still under debate and despite the increasing knowledge on the molecular identification of *Giardia* from different host species, the zoonotic potential of *G. duodenalis* is not clear.

Animals

A study estimating the number of *Giardia* cysts produced by farm animals in the Netherlands found *Giardia* in pooled faecal samples in 77 of 93 veal calf herds and 1 of 55 dairy calf herds that were studied. In faeces from chicken (both broilers and egg layers) no *Giardia* was found. For the veal calf herds almost 100% of the herds between 1 – 6

weeks of age were *Giardia* positive and this decreased from 72% to 57% for herds of 21 – 35 weeks of age. Prevalence of *Giardia* in relation to age and season were investigated on a dairy farm over the course of 1 year. The prevalence varied from 0.8% in June to 15.5% in February. Shedding of *Giardia* cysts was found in all age categories and peaked in animals 4-5 months old (54.5%). Molecular analysis of the *Giardia* isolates from this dairy farm showed that in most cases the livestock specific assemblage E is found and in rare instances assemblage A, indicating a possible zoonotic type. In addition, phylogenetic studies comparing DNA of both human and animal Giardia strains, using multilocus sequence analysis showed species specific assemblage D for dog, assemblage E for ruminants, assemblage A for roe deer and *G. muris* in musk rats. In dogs and cats, preliminary results show that besides the species specific assemblages C, D and F also, but to lesser extent, assemblage A is also present.

Humans

From 1996 to 1999, the incidence of gastroenteritis in general practices and the role of a broad range of pathogens in the Netherlands were studied. All patients visiting a general practitioner for gastroenteritis (cases) and an equal number of patients visiting for non-gastrointestinal symptoms (controls) were invited to participate in a case-control study. *Giardia* was found to be responsible for 5.4% of gastroenteritis cases, and in 3.3% of the control patients. In a study conducted in the region of Haarlem 14.8 % of the cases of gastroenteritis with diarrhoea lasting more than 1 week were positive for *Giardia*. In 1999, a prospective population-based cohort study with a nested case-control study was conducted to estimate the incidence of gastroenteritis and the associated pathogens in the general Dutch population. *Giardia* was reported in 5.0 % of the cases and in 4.9% of the controls and was found more in patients with persistent diarrhoea (>1 week) in the summer and autumn. Molecular analysis of *Giardia* isolates from the stool from human patients showed that only two *Giardia* Assemblages are present. In 35% of the cases an assemblages A genotype and in 65% of the cases an assemblages B genotype was found. In humans, *Giardia* Assemblage B was correlated with more severe complaints in both studies.

Conclusion.

Giardia is the most prevalent protozoal pathogen causing gastro-enteritis in humans. In addition, *Giardia* spp. are also present in many animal populations. Despite molecular epidemiological studies in human and animal populations, the role and transmission of *Giardia* as a zoonotic pathogen is still unclear and needs further investigation.

4.10 *Hantavirus*

Hantaviruses belong to the family of the Bunyaviridae a family of enveloped, negative strand RNA viruses. The family is grouped into 4 genera of animal viruses, 4 of which contain viruses that are zoonotic. A 5th genus consists of plant viruses (Table 4.10.1).
The hantaviruses that are endemic in the Netherlands (Puumala virus) cause an illness typified by acute renal fever named “nephropathica epidemica” (NE), which is a milder

Table 4.10.1. Family of Bunyaviridae

Genus	Most common vector	Examples of zoonotic pathogens	Genus present in NL
Orthobunyavirus	Mosquitoes	California encephalitis virus	No
Hantavirus	Rodents	Puumala virus, Sin Nombre virus	Yes
Nairovirus	Ticks, biting midges	Crimean-Congo haemorrhagic fever virus	No
Phlebovirus	Sandflies	Rift valley-fever virus, Sandfly fever Naples virus	No
Tospovirus	Various insects	None	No

form of the haemorrhagic fevers with renal syndrome (HFRS) caused by other viruses in the hantavirus genus in Central and Eastern Europe and Asia. Nephropathia epidemica occurs in most countries of North-Western Europe. The epidemiology of hantavirus infections is tightly linked with that of their reservoir host, in the sense that the likelihood of exposure to hantavirus increases with increasing population density. In the Netherlands, the main animal reservoir of the infection was shown to be the bank vole (*Myodes glareolus*). Virus is transmitted to humans through inhalation of aerosolized infected animal excreta, i.e. urine, faeces and saliva. In Western Europe, periodic outbreaks of hantavirus infections in humans have been observed. The last year with relatively high activity in the Netherlands was 2001. Persons with regular exposure to rodents such as animal trappers, forestry workers, and farmers have the highest risk of infection.

Animals

There is no active surveillance for the presence of hantaviruses in rodents in the Netherlands. Surveys more than 10 years ago showed that the virus was present in rodents in specific regions in the east and south of the country.

Humans

In 2005, a total of 28 cases were detected. One person had become ill while on vacation in Finland, and was considered to have acquired the infection abroad. Seventy eight percent of all the cases lived in a region of the country that is known to be endemic for Puumala virus. This number of diagnosed cases was not significantly higher than in previous years, in contrast to the situation in France, Germany and Luxemburg, where the incidence of hantavirus infections was greatly increased in 2005. Physicians and nephrologists in The Netherlands were specifically informed of the reported increase in neighbouring countries in June, but despite this increased awareness, no significant rise in the number of cases was observed.

Conclusion

Hantavirus infections are diagnosed occasionally. The current level of surveillance is minimal, with limited data available on human cases, and no information on the presence of hantavirus in reservoir species. Given the observed epidemic of hantavirus infection in three neighbouring countries, the need for enhanced surveillance is presently being discussed and studies in wild life reservoirs have been initiated.

4.11 *Hepatitis E virus*

Hepatitis E virus (HEV) is a non-enveloped, positive-sense, single-stranded RNA virus with icosahedral symmetry. HEV is classified as a member of the family *Hepeviridae*, genus *hepevirus*, species hepatitis E virus. Infection in humans occurs in sporadic and epidemic forms and can cause an acute, self-limited, icteric hepatitis. HEV is endemic in much of the developing world, especially in conflict situations. Large common source outbreaks of hepatitis E have primarily occurred in developing countries through contaminated water mainly due to deficient drinking water systems or poor sanitation. HEV may also be transmitted through other sources, such as consumption of contaminated food, contact with infected animals or animal products or blood transfusion. Still intrafamilial spread seems rare.

Animals and environment

Worldwide evidence is accumulating on HEV infections in a wide range of animals such as pigs, wild boar, deer, *etc*. In the Netherlands evidence for omniprevalence of HEV is also accumulating. Pigs have been suggested to be a potential reservoir for locally acquired human hepatitis E virus (HEV) infections in the Netherlands.

The prevalence of HEV in 2005 was estimated at 55% (HEV in faecal samples at 53/97 pig farms) indicating a significant increase as compared to the prevalence rate of 22% (25/115) as was reported in 1999. This increase is due to the inclusion of appropriate quality assurance controls such as internal amplification controls for RT-PCR.

Four of 62 (6.5%) porcine livers bought in Dutch stores were HEV RNA-positive by RT-PCR and HEV-specific Southern blot hybridization. Each positive liver was estimated to contain ~65 PCR-detectable units per gram. Sequences were obtained for three of the four positive livers, and were classified as HEV genotype 3. At most, 93% similarity to Dutch human sequences was observed and a 97% similarity to Dutch swine sequences.

Other potential reservoirs, such as wild boar and musk rats and surface waters were analysed for the presence of HEV RNA. In 4% of wild boar faeces (n = 26) and 17% of the studied surface water samples (n = 12) HEV genotype 3 RNA was detected but no HEV RNA was detected in musk rat faeces (n = 150). Phylogenetic analysis of all detected sequences showed grouping of sequences mainly within four suggested genotype 3 clusters, 3a, 3c, 3e and 3f, of which cluster 3c is unique to the Netherlands. Moreover, one sequence of an unrelated genotype 3 variant was identified in pigs. Most of the pig HEV sequences and one environmental sequence grouped within cluster 3f. Remarkably, most of the human isolates obtained from Dutch hepatitis E patients clustered with genotype 3c. Of the Dutch HEV genotype 3c isolates obtained between 2004 and 2006, 43% was of human origin. One of the porcine HEV genotype 3c sequences isolated in 2005 appears to be completely homologous to a HEV sequence identified in a hepatitis E patient in 2005 in a fragment of 148 nt of ORF2, suggestive of possible zoonotic transmission.

In another study intravenously (IV) inoculated pigs of approximately 3-4 weeks of age were found to become serologically positive for anti-HEV antibodies an average of 13 (±2) days after detection of viral RNA in faeces. In pigs housed with HEV infected pigs,

seroconversion took 11 (±5) days after detection of viral RNA in their faecal samples. In 90% of the pig farms with pigs of approximately 6 months of age sold to slaughterhouses anti-HEV antibodies were detected.
To determine whether HEV can spread among pigs, the basic reproduction ratio R_0 was estimated from one-to-one infection experiments starting with IV inoculated pigs followed by infection of susceptible pigs by natural contact. The average incubation period was 6 days as determined by RT-PCR on RNA from faecal samples. The R_0 for contact-exposure was estimated to be 8.8, showing that HEV may cause an epidemic amongst pigs.

Humans
To generate hypotheses about possible risk factors for non-travel-related HEV infections in the Netherlands, IgM positive cases confirmed by immunoblot and/or RT-PCR were interviewed about clinical and medical history, food consumption and contacts. Out of 19 cases, 11 had pre-existing disease, 17 were male and the median age was 50 years. Seventeen cases were PCR positive (genotype 3 HEV), two had identical HEV sequences but no identified relation. One patient had an identical sequence to a ditch water sample, in this case probably indicating sewage contamination of surface water consumed by this patient.
A Bayesian stochastical model was used to combine results of seroassays (one IgM and two IgG ELISAs, and subsets with IgG and/or IgM Western blots) on blood samples from three subpopulations (49 swine veterinarians, 153 non-swine veterinarians and 644 randomly selected individuals from the general population). The model accounted for imperfection of each assay by estimating sensitivity and specificity, and it accounted for dependence between serological assays. As expected, discordance among assay results occurred. Applying the Bayesian stochastical model yielded seroprevalence estimates of ~11% for swine veterinarians, ~6% for non-swine veterinarians and ~2% for the general population confirming that exposure to swine or their environment was associated with elevated HEV seroprevalence. However, no differences in prevalences between these three groups would have been found if the currently operating diagnostic scheme based on confirmation of ELISA results by immunoblot would have been used.

Conclusions
The abundant presence of pigs excreting HEV raises concerns on potential zoonotic transmission of the virus, either by exposure through the environment (or to pigs) or by consumption of contaminated pork products. Indeed, phylogenetically related strains were detected in pigs, porcine livers, river water and hepatitis E patients. Specifically some variants of HEV genotype 3 circulate in Dutch reservoirs which more often than other strains cluster with strains originating from hepatitis E cases. These strains may be more pathogenic to humans than viruses in other genotype 3 clusters. Hepatitis E in the Netherlands appears to be acquired from diverse sources, as may be judged from risk factors studies. Host susceptibility may play an important role in development of the disease.

Because multiple reservoirs were found to be positive for HEV, a reduction in exposure might be reached by a range of general preventive measures such as; effective sewage treatment to reduce contamination of surface waters, sufficient heating of porcine livers prior to consumption and strict hygiene measures designed to ensure food safety.

4.12 *Influenza virus*

Influenza virus types A, B and C belong to the Orthomyxoviridae, a family of negative stranded RNA viruses with a segmented genome. A key difference between them is their in vivo host-range; whereas influenza viruses of types B and C are predominantly human pathogens that have also been isolated from seals and pigs respectively, influenza A viruses have been isolated from many species including humans, pigs, horses, marine mammals and a wide range of domestic and wild birds. It is generally accepted that in the human influenza pandemics from the last centuries and numerous outbreaks in domestic and wild animals, interspecies transmission of avian influenza A viruses have played a crucial role. Migratory birds and waterfowl are thought to serve as the reservoir for influenza A viruses in nature. Influenza A viruses representing 16 haemagglutinin (HA) and 9 neuraminidase (NA) subtypes have been described in wild birds and poultry throughout the world. Viruses belonging to antigenic subtypes H5 and H7 may become highly pathogenic upon transmission from wild birds to poultry and thus cause fowl plague, in contrast to viruses possessing other HA subtypes.

Animals

Wild birds

After the influenza A virus zoonoses in Hong Kong in 1997, caused by highly pathogenic avian influenza (HPAI) viruses of subtype H5N1, the department of Virology at the Erasmus Medical Centre (EMC) in Rotterdam initiated a surveillance study in wild birds. This study is performed to gain information on the prevalence of influenza A viruses in birds in Northern Europe and to generate a panel of reference reagents (influenza A virus isolates and antisera). In addition, after the occurrence of HPAI H5N1 virus in wild birds in Southeast Asia, and the spread of the H5N1 virus from Southeast Asia to the west and north, surveillance activities were intensified and have been performed in real-time since September 2005 in order to potentially provide early warning signals for arrival of the virus in new areas.

Cloacal swabs or fresh dropping samples are collected primarily from bird species known to harbour influenza A virus such as ducks, geese and gulls, but a wide variety of other birds species are tested as well. Samples are collected by a large number of ornithologists and sent to the laboratory of the EMC virology department where they are tested using highly specific and sensitive RT-PCR procedures for the matrix gene and the H5 gene. From RT-PCR positive samples, viruses are subsequently isolated in embryonated chicken eggs and characterised using serological tests and genomic sequencing. The results of the surveillance in the year 2005 is summarised in Table 4.12.1.

Table 4.12.1. Results of influenza A virus surveillance in wild birds in 2005

Type of bird	Species	Positive/total	%
Ducks	Mallard	148/1540	9.6
	Eurasian wigeon	49/586	8.4
	Common Teal	45/253	17.8
	Gadwall	2/60	3.3
	Pintail	4/43	9.3
	Northern Shoveler	4/34	11.8
	Tufted duck	0/6	
	Common pochard	0/1	
Geese	White-fronted goose	51/1877	2.7
	Barnacle goose	7/316	2.2
	Pinkfooted goose	6/206	2.9
	Canada goose	0/343	
	Greylag goose	1/121	0.8
	Bean goose	2/115	1.7
	Egyptian goose	0/48	
	Brent goose	1/41	2.4
Swans	Tundra swan	2/25	8.0
	Mute swan	1/12	8.3
Gulls	Black-headed gull	2/708	0.3
	Common gull	2/239	0.8
	Herring gull	0/8	

Up until December 2005, virus isolates containing HA subtypes 1, 3, 4, 5, 6, 7, 8, 9, 11, 12 and 16 and NA subtypes 1, 2, 6, 7 and 8 were detected. A number of virus isolates have not yet been fully characterised. Sequence analysis of the HA gene of the H5 and H7 isolates revealed the absence of a basic cleavage site, indicating that these were not HPAI viruses. The accumulated collection of influenza A viruses from Northern Europe now includes viruses containing all known HA subtypes, with the exception of subtypes 14 and 15, and all 9 known NA subtypes. This collection of virus isolates was obtained from more than 50.000 samples collected from more than 330 bird species.
The influenza A virus isolates collected within this surveillance network and the antisera raised against them are used as reference reagents in a number of laboratories. In addition, studies on the zoonotic potential of these viruses, pathogenesis, and virus evolution are currently in progress.

Poultry
All breeding flocks of *Gallus gallus*, which are in production, are clinically inspected monthly for List A disease in accordance to Directive 90/539/EC by the authorised practitioner. For the export of birds or hatching eggs some third countries demand serological investigation of the flocks of origin for avian Influenza. Mostly only the agar gel precipitation test (AGPT) is requested, but sometimes also the HI test for H5 and/or H7 have to be included. When the results of the AGPT were positive the HI test for H5

and H7 were used for confirmation. All 4380 samples received in 2002 from 152 flocks were negative.

Pigs
Investigations for influenza infections in pigs are requested in cases of clinical respiratory problems. Primarily blood samples for serological investigation are sent to the laboratory of the GD. In a small number of occasions material is forwarded for virus isolation. For the serological investigation the Haemagglutination Inhibition (HI) tests for H1N1, H3N2 and since the last months of 2002 also for H1N2 were used. Material from 5 pig herds was sent in for virus isolation. All samples were negative for influenza A virus. From 724 herds, a total of 9619 blood samples were received for serology. Of these, 3046 samples were positive for H1N1 and 3501 for H3N2. For H1N2, only 643 samples were tested from 43 herds and 64 were positive.

Humans
Influenza B virus and influenza A viruses of subtype H3N2 and H1N1, descendants of the 1968 Hong Kong pandemic and 1918 Spanish influenza pandemic respectively, are the main causes of influenza-like illnesses in humans. The World Health Organization coordinates a global influenza surveillance network, currently consisting of 112 national influenza centres (NIC) and 4 collaborating centres for reference and research. This network routinely characterizes the prevalence, antigenic properties and genetic properties of circulating influenza viruses. The combined antigenic, epidemiological, and genetic data are used to select strains for use in influenza vaccines. The NIC of the Netherlands is operated by the department of Virology at the EMC and the RIVM. Samples, collected within a network of general practices (NIVEL) or sent in by hospitals, nursing homes, and individual general practitioners, are analysed. In 2005, there were no influenza viruses from avian or swine origin detected in humans in the Netherlands.

Pandemic preparedness
The rapid spread of H5N1 HPAI from China throughout Asia, into Europe, The Middle-East, and Africa has raised the level of alert at the World Health organisation to level 3 on the pandemic preparedness scale (incidental transmission to humans, no sustained human to human transmission). Public health authorities worldwide have worked on pandemic preparedness protocols. In the Netherlands, the experience of the H7N7 outbreak in 2003, which resulted in a high rate of transmission of HPAI to humans involved in the culling, was used to improve preparedness protocols for avian influenza as well. These protocols were made available to the European CDC for use in other countries.

Conclusions
It is not clear whether the current surveillance systems are sufficiently sensitive to detect incidental zoonotic transmissions. According to a publication of the CDC, a considerable number of pig farmers show serological evidence of exposure to pig viruses. If further transmissions do not occur and clinical symptoms remain mild, it is almost

Zoonotic threat of avian influenza in the Netherlands in 2003.

In 2003, the Netherlands experienced a real-life drill for emerging disease preparedness, when an increasing number of persons were involved in control of an epizootic of highly-pathogenic avian influenza A virus (HPAI), subtype H7N7. At the end of February 2003, the ministry of Agriculture announced an outbreak of HPAI in a poultry-dense, central region of the country. On May 23rd , 2003 the last farm was found to be infected, which heralded the end of the outbreak after the culling of flocks from 255 infected farms, 890 other farms and poultry of 16.490 recreational farmers. Initially, this virus was considered of low risk to humans and control measures recommended by public health authorities were debated. Active case-finding was started through a health post stationed near the crisis centre where poultry workers were assigned to specific culling teams. A total of 453 persons reported health complaints, of which the majority was conjunctivitis. H7N7 infection was diagnosed by detection of the virus in 85 of these. In addition, infection was confirmed in three household contacts of poultry workers with conjunctivitis due to HPAI. Late in the course of the outbreak, one person was hospitalised with influenza-like illness and pneumonia, but initially tested negative for H7N7. After his death, the diagnosis HPAI infection was made. The virus isolated from this case had a significant number of mutations compared with the viruses detected in poultry and in humans with mild illness. Based on further characterisation of the virus, it was shown that the virus indeed had mutated to a more virulent form, causing severe illness. Fortunately, this did not coincide with increased transmissibility of the virus between humans. Since then, the full extent of the outbreak has been studied with striking conclusions. Based on the combined results from serological studies and risk factor analyses, we estimate that at least 1000 people and possibly more were infected during this outbreak. Our results also suggest person-to-person spread, with detection of H7-specific antibodies in almost 60% of household contacts with H7-PCR positive poultry workers. After the initial diagnoses of 19 cases of conjunctivitis, two weeks after the start of the outbreak, all poultry workers and otherwise exposed persons were given prophylactic treatment with neuraminidase inhibitors, in addition to the protective measures that had been instructed throughout (glasses, masks, protective clothing). Risk factor analyses showed that the use of antivirals had a protective effect, in contrast to other protective measures. Based on these findings, avian influenza control protocols have been thoroughly revised to include more stringent health monitoring and health protection measures.

certain that current surveillance would be unable to detect this. Clearly, a more targeted surveillance approach needs to be developed.

Serological monitoring of Avian Influenza in the Netherlands

After the avian influenza outbreak in the Netherlands an obligatory serological monitoring of AI started in 2004. The monitoring is based on legislation of the Production Boards for Poultry and Eggs (PVE). GD organises the sampling and the testing of the samples. Flocks are sampled based on the production type, housing method and age. Flock information is gathered from the central data base of the PVE. Until October 2005 haemagglutinin inhibition tests and agar gel precipitation tests were performed. From October 2005 on these tests were replaced by ELISA tests. The AGP test was continued only for broilers. The specificity of the AI ELISA is 99.5 %. Samples, which are not negative in the ELISA test, are sent to the reference laboratory in Lelystad (CIDC) and tested in the HI test with different H5 and H7 antigens. On average, 0.5% of the samples are sent to the reference laboratory. A negative result of the H5 and H7 test by the reference laboratory leads to a negative monitoring result.

Table 4.12.2. Total number of flocks and samples tested for the presence of antibodies against Avian Influenza by GD Deventer.

	2004 Flocks	2004 Samples	2005 Flocks	2005 Samples	2006 Flocks	2006 Samples
OLF			1	10	13	330
LF	35	3500	26	260	15	180
LO	36	354	5	50	29	445
LV	17	1210	67	670	45	560
OL	1251	12292	778	7490	597	11032
LL	2660	26747	1452	14688	1070	19220
SF	228	2415	166	2220	63	680
SO	265	2650	149	2670	86	1451
SV	710	7098	377	3770	303	3923
• SS	839	22040	752	20867	263	3520
KO	0	0	1	10	565	15508
KV	6	60	5	50	5	100
KS	154	1737	134	1360	6	90
EV	5	90	0	0	97	1819
ES	81	2361	44	1292		
EO			1	30	97	30
Unknown	70	699	59	630	29	579

• Flocks tested with AGP test.

OLF= rearing layer type grandparents	OSF= rearing meat type grandparents	KV=parents turkey
LF=grandparents layer type	SF=grandparents meat type	KS=meat turkey
LO=rearing layer type parents	SO=rearing parents meat type	ES=meat ducks
LV=layer type parents	SV=parents meat type	EO=rearing parents ducks
OL=pullets	SS=broilers	EV= parents ducks
LL=layers	KO=rearing parents turkey	

When antibodies against H5 or H7 are found by the reference laboratory a team of specialists is sent to the farm to inspect the flock and to take samples to check for the presence of AI virus. These samples are again tested by the reference laboratory in Lelystad.

Results 2004, 2005 and 2006.

In 2004, in 4 flocks antibodies against Avian Influenza were detected. As a result 2 flocks were destroyed, although there were no AI virus detected with PCR and viral culturing. In 2005, the serological monitoring did not necessitate the destruction of flocks. In 2006 the serological monitoring detected the presence of a low pathogenic Avian Influenza virus in the centre of the Netherlands. Positive serology showed a H7 infection and surveillance within a 1 kilometre zone around that farm detected the H7 virus on at least one other farm. Destruction followed.

General Monitoring system rules

Obligatory monitoring for Avian Influenza antibodies in poultry.

- Broilers: from all farms blood samples from a flock older than 28 days have to be tested once a year. (30 samples/year)
- Broiler ducks: from all farms blood samples from flocks older than 28 days have to be tested once a year. (40 samples/year)
- Meat turkeys: blood samples from every flock have to be tested at the end of the fattening period. (30 samples/every flock)
- Reproduction: every house has to be tested once a year. (30 samples/house/year).
- Layer (not free range): every house has to be tested once a year. (30 samples/house/year).
- Layer birds (free range): on every farm with free range layers blood samples from every house have to be tested every 3 months. (30 samples/house/3 month).

4.13 *Leptospira* spp.

Leptospirosis is an important, worldwide occurring bacterial zoonosis caused by spirochetes of the genus *Leptospira*. This genus *Leptospira* comprises pathogenic and saprophytic species, the number of which depends on the classification system applied. According to the conventional system 2 species, saprophytic *L. biflexa* and pathogenic *L. interrogans*, are known. Based on serological criteria, numerous serovars, with related serovars clustering in serogroups, can be distinguished. Currently, almost 300 pathogenic serovars, clustering in 25 serogroups, are recognized. Generally, each serovar is adapted to a certain host, e.g. serovars Icterohaemorrhagiae and Copenhageni are associated with rats, Grippotyphosa with field voles, Hardjo with cattle and Canicola with dogs. Certain serovars have the propensity to cause serious forms of leptospirosis, while others are associated with milder forms of the disease. Often, infection with serovars Icterohaemorrhagiae and Copenhageni give rise to a serious form of leptospirosis, Weil's disease, whereas infections with serovars Hardjo or Grippotyphosa frequently result in less serious disease.
The highest incidences of leptospirosis are found in humid tropical and subtropical areas. It has been estimated that globally at least 350,000 persons suffer from a serious form of leptospirosis annually. As such, leptospirosis is the most widespread zoonosis worldwide, but its importance is largely underrated.

Animals

Clinical symptoms are comparable in all animals, although some species seem to be more resistant to acute infections. Infections may be asymptomatic or cause various signs, including fever, icterus, hemoglobinuria, renal failure, infertility, abortion, and death. After acute infection, leptospires frequently localize in the kidneys or reproductive organs and are shed in the urine, sometimes in large numbers for months or years, especially with host-adapted serovars. Because leptospires survive in surface waters, such as swamps, streams, and rivers, for extended periods, the disease is often water-

borne and floods frequently result in an increase of disease outbreaks. The organism survives well in mud and moist, alkaline soil, such as riverbanks as well.
In Dutch animal husbandry, mainly the serovar Hardjo that has adapted to cattle is of importance. Infected dairy cattle rarely show signs of clinical disease but abortion, stillbirth or decrease of milk production can occur. In calves acute infections tend to be more serious. Because of its economical relevance and its zoonotic potential, infections of cattle with Hardjo are notifiable. For the same reasons, absence of Hardjo in dairy herds has been made compulsory since 2005. In 2006, 99.2% of the dairy herds where shown to comply with this provision. The Hardjo free status is monitored by bulk milk or blood sampling and only 0.5% of herds lose their status annually, mostly due to buying stock from non-free herds.
Of cow-calf herds, 33% are certified free of Hardjo. In 2006, the Animal Health Service investigated the prevalence of Hardjo in non-free cow-calf herds. In 7% of these herds infected animals were shown to be present. These animals pose a risk for the cattle industry and for their owners.

Humans
In humans, leptospirosis is a protean disease, manifesting itself from mild to very severe, and potentially fatal. Clinical symptoms vary widely and are not specific to leptospirosis. Combinations of fever, myalgia, headache, chills, diarrhoea, oligo- or anuria and icterus are frequently seen, implying the occurrence of clinical pictures resembling those of a large number of other disorders. In about 10 % of the cases the infection takes a very serious course with renal failure, icterus and haemorrhagic diathesis (Weil's syndrome). Of all reported cases in the Netherlands, 5 % have a fatal outcome. Infections are usually contracted directly through contact with infected hosts or indirectly through contact with urine contaminated environment.
Leptospirosis is a notifiable disease in the Netherlands. The annual number of patients with severe leptospirosis is about 30. Currently, leptospirosis is a recreational disease for the most part, with half of the cases having acquired the infection during holidays abroad. Leptospirosis is a seasonal disease that peaks in late summer and autumn. Most cases acquired in the Netherlands are due to infection with serovars Icterohaemorrhagiae, Copenhageni and Grippotyphosa. Infections with Hardjo have not been seen for several years due to successful eradication of this serovar in cattle stock.
The variety of signs and symptoms of leptospirosis often make clinical diagnosis very difficult. Support of the laboratory is therefore indispensable. Several diagnostic procedures, of which the microscopic agglutination test (MAT) is the gold standard, are available, but all tests suffer from a varying degree of drawbacks. A number of rapid and easy-to-use tests have, however, become commercially available recently but require confirmation by MAT.

Conclusion
Conventional leptospirosis tests are laborious, unreliable or require expertise. The MAT is very complicated and hence restricted to only a few expert centres. In the Netherlands, these are the WHO/FAO/OIE and National Leptospirosis Reference Centre, KIT Biomedical Research, KIT in Amsterdam. The difficult diagnosis of leptospirosis, both

in the clinic and laboratory, probably forms the main reason for its under/ misdiagnosis and consequently its underestimation and the unawareness of practitioners. The new commercial leptospirosis tests will hopefully contribute to an improved case detection and an increased awareness worldwide.

Leptospira ballum infection in 16 year old girl

In early 2004, a 16-year-old girl fell ill with symptoms of fever, myalgia and neck pain. The symptoms being indicative for Weil's disease, blood was drawn for leptospirosis testing. A discriminative ELISA showed a positive serum reaction to *Leptospira* serovar Ballum. Upon request of the Municipal Health Service of the girl's hometown, VWA initiated a source finding investigation.
The patient followed training outside her hometown and stayed at a host family during courses. A month prior to the onset of her symptoms she had bought four mice at a small animal fair. One of the animals died within a day of purchase whereas the family cat had consumed a second animal at some time. The remaining mice were kept alternately at her host family's house and her own place. Two of the three children of her host family fell ill with similar symptoms around the same time but tested negatively for leptospirosis. The two remaining mice were tested for *Leptospira* at the CIDC. Both animals were shown to have a *Leptospira* serovar Ballum titre in a micro-agglutination test. However, an attempt to culture *Leptospira* failed due to overgrowth of the culture medium with unspecific bacteria. The mice originated from a pet wholesaler who said to receive weekly consignments of up two 2000 mice from the Czech Republic. Most of the animals are sold out-of-the-box to a large number of customers. No costumer records are kept whatsoever. Thus, other recipients potentially exposed to infected mice could not be tracked.
The outcome of the serological laboratory investigation is strongly suggestive of a human *Leptospira* Ballum infection through contact with infected mice imported from the Czech Republic. This case clearly highlights risks to public through unregulated pet trade and underpins the necessity of proper record-keeping by animal traders.

4.14 *Listeria monocytogenes*

In the genus Listeria, human illness is predominantly caused by *Listeria monocytogenes*, especially by serotypes 1/2a, 1/2b and 4b. The bacterium is found in humans and a variety of animal species, but is also widespread in the environment. Most human infections are caused by food (of animal origin).

Food
The VWA monitors the occurrence of *L. monocytogenes* in foods at retail level. According to the Dutch Commodity Act, investigations are to be performed, regularly, on food products prepared for direct consumption. The maximum level of contamination of food products with *L. monocytogenes* is set at 100 bacteria per gram. The results of this monitoring as carried out between 2001 and 2006 are shown in Table 4.14.1.

Humans
In 2005, the existing surveillance of *L. monocytogenes* in the Netherlands was intensified. In 2005, 91 cases were reported, including six pregnancy-related cases. In 2006 this number decreased to 69 cases. Of the patients, 37% were in the age-group 65-79 years. Seventeen patients died (18%) of which three were neonates. Serotypes 4b (46%) and 1/2a (43%) were mostly found. For 69 patients (76%), a questionnaire was answered. Of these patients, 86% had predisposing conditions for listeriosis, most often use of

Table 4.14.1. Ready to eat meat (-products) at retail with >100 cfu/g L. monocytogenes (Monitoring VWA)

	2001		2002		2003		2005		2006	
	N	%+	N	%+	N	%+	N	%+	N	%+
Beef and veal	716	*0.6*	1,012	*0.7*	774	*0.4*	729	*0.3*	920	*2*
Pork										
Lamb									180	*0,6*
Poultry	39	*3*			19	*5*	340	*0*	477	*1*
Fermented sausages	260	*0.4*								
Sliced meat products	1,070	*1*	951	*0.1*						
Meat, other			1,864	*0.6*	2,384	*8*				
Cheese	330	*0*	234	*0.4*	148	*7*			805	*0*
Ice-cream	175	*0*	96	*0*						
Custard	50	*0*								
Milk products			40	*0*						
Milk, raw			50	*4*	18	*0*				
Fish products			301	*0.3*	180	*1*				
Smoked eel	113	*0*								
Smoked salmon	183	*0*	113	*0.9*						
Smoked mackerel	80	*4*	24	*0*						
Smoked fish									568	*4*
Herring	101	*23*								
Shrimps	14	*7*	15	*0*						

n.i. no investigation

immunosuppressive therapy, malignancies and use of antacids. Other non-predisposing diseases, such as heart diseases and vascular disorders, were present in 72% of all patients. The most common diagnoses were septicaemia (28%), pneumonia (25%), meningitis (23%) and gastroenteritis (20%). Relatively high percentages of patients reported consumption of cooked or smoked ham (76%), sausages (62%), sliced cold chicken or turkey meats (61%), raw vegetables or salad (77%), smoked salmon (49%), or eating in a restaurant (55%). In 2005, the number of reported cases in the enhanced surveillance system was much higher than expected (incidence of 5.6 per million inhabitants per year). This can at least partially be explained by improved submission of *Listeria* isolates by the laboratories, as a result of which, also, less severe *Listeria* infections are notified. The lower number in 2006 remains in accordance with the increasing number of reported cases since 2003.

Antibiotic resistance

All strains isolated from 2001 to 2004 and sent to the RIVM for confirmation and typing (N = 146) were tested for susceptibility using broth micro dilution. The origin of the strains was predominantly human; 55% were isolated from blood samples and 25% from liquor. The remaining 20% was isolated from environmental specimens and various food products. The purpose of this study was to determine the susceptibility level of *Listeria* spp. to a wide variety of antimicrobial agents used in human and veterinary medicine. The strains were tested for susceptibility to amoxicillin, neomy-

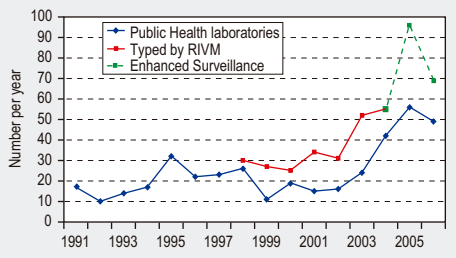


Figure 4.14.1. L. monocytogenes infections in humans reported by 15 PHLs and typed by the RIVM for all Dutch laboratories.

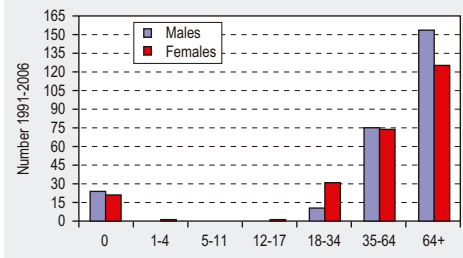


Figure 4.14.2. Age and gender distribution of cases with a L. monocytogenes infection, 1991-2006

cin, gentamicin, tetracycline, doxycycline, erythromycin, ciprofloxacin, chloramphenicol, florfenicol, imipenem, sulphamethoxazole, trimethoprim, linezolid, salinomycin, quinu/dalfopristin and vancomycin.
Most strains were susceptible to all antibiotics listed, except for 6 that were resistant to sulphamethoxazole (MIC >1024 µg/ml).

Conclusions
Listeriosis rarely occurs in generally healthy persons. In almost 90% of the laboratory confirmed cases, predisposing conditions for listeriosis played a role, most often the use of immunosuppressive therapy, malignancies and the use of antacids. The incidence of listeriosis is increasing steadily since 2003. In the next few years, the enhanced surveillance system should show whether the increasing incidence of listeriosis in the Netherlands can be explained by improved surveillance, or whether the increase is genuine. With the start of enhanced surveillance, the VWA has intensified their testing of food products for presence of *L. monocytogenes* and also types the isolates with serotyping and PFGE. This should improve the identification of important sources for human infection. Because the clinical consequences of listeriosis are severe and the case-fatality rate is high, the current recommendations for pregnant women to avoid high-risk foods, such as soft cheeses and smoked fish products, should be continued. These dietary recommendations should also be given to individuals with predisposing conditions, since they, too, are at risk of *Listeria* infection.

4.15 *Mycobacterium* spp.

The Dutch cattle population has been officially declared free of tuberculosis since 1992, a situation that has been ratified by decision of the European Commission 95/138/EC. However, *Mycobacterium bovis* infections still occasionally occur in man, cows and other animals. *Mycobacterium bovis* is the causative agent of tuberculosis in cattle, but a wide range of other animals is also susceptible to infections with these bacteria. In a large number of countries reservoirs of *M. bovis* have been found in wildlife species such as badgers, brush-tailed possum, elk and deer. Fortunately *M. bovis* infections have never been diagnosed in wildlife in this country. Occasionally *M. bovis* infections have been diagnosed in zoo or hobby animals.

Animals

The surveillance on bovine tuberculosis currently depends mainly on the official post mortem examination of all animals slaughtered. Due to the high speed of the slaughtering process, the meat inspectors do not observe small tubercles and outbreaks sometimes go undiscovered for a long period. This explains why when positive animals are discovered, usually a high percentage of the animals at the farm of origin seem to be affected. In addition, tuberculin testing of cattle takes place as a part of the accreditation procedure for cattle sperm centres (Directive 88/407/EC), export to non-member states and with the tracing and screening of cases of bovine tuberculosis. Slaughter pigs are checked for caseous lymphadenitis (mostly caused by *M. avium*) by slicing the mandibular lymph nodes. Zoo animals are checked for tuberculosis when entering or leaving a zoo, or when the animals have been immobilized. The animals are tuberculinated and blood samples are taken for experimental tests for tuberculosis. The control of outbreaks of tuberculosis in animals is carried out by the VWA.

In 2005, 5 cases of tuberculosis suspected animals were reported to the VWA. Two cases of suspicion in slaughterhouses proved to be negative after further investigation. A case of suspicion after import of a calf that originated from an infected farm in another member state was also negative. In two zoo's, infections of animals with *M. tuberculosis* were confirmed positive. One was in an onager and one in a hyrax. Both animals were euthanised and rendered. Further tracing and tuberculination revealed no further infections in these zoos. The personnel of these zoos were checked for infections of tuberculosis with a negative result. In 2006, there was a similar pattern to what occurred in 2005. All investigations resulting from suspicions in slaughterhouses (5), at import (1) or in sperm centres (3) proved to be negative. Two suspicions in two zoos were investigated. One in sea lions was identified as *M. pinnipedii*. As a result of this outbreak 28 sea lions were euthanised. The personnel of the zoo were checked for tuberculosis and 5 of them were found positive. They showed no symptoms but were treated according to protocol.
The second suspicion rose in a tapir. This animal was not euthanised but treated. The causative mycobacterium species is unknown.

Due to all-in all-out procedures and strict hygienic measures, *M. avium* infections are very rarely seen in commercial poultry. Studies for the presence of *M. avium* complex bacteria in slaughter pigs, in slaughterhouses, in the mid nineties made clear that those animals can be infected. From the examined animals, 0.5-1% seemed to have caseous lesions in the mandibular or mesenteric lymph nodes. Genotyping analyses of a few hundred strains of birds, pigs and humans during that same period suggested that pigs and humans share common sources of infection, or that pigs are a vehicle for *M. avium* infections in humans. The studies make clear that the monitoring of caseous lesions in slaughterhouses is still of great importance. Examining pig farms to identify other unknown risk factors for the infection of pigs should be taken into consideration, in order to get a clearer insight of the infection risks for humans. Based on slaughterhouse data, farms with a high incidence of *M. avium* infections would be selected for that purpose.

A dead sea lion at the zoo - A hazard for man and animal?

A large-scale investigation was instigated among congeners, other animals, staff and visitors when a South American sea lion was diagnosed with open lung tuberculosis.

In September 2006, VWA was informed by CIDC about a South American sea lion that had died of generalized tuberculosis caused by *Mycobacterium pinnipedii* with rupture of lesions into bronchi. *Mycobacterium pinnipedii* has been isolated from pinnipeds (sea lions, seals and walruses) in the wild and in capture from 1986 on. Since 2003, *M. pinnipedii* has been added to the *M. tuberculosis* complex, a group of organisms with the capacity to cause disease in humans. Other members of the *M. tuberculosis* complex are *M. tuberculosis* (humans and non human primates), *M. bovis* (cattle, mammals, humans), *M. africanum* (humans), *M. microti* (voles, humans), *M. canetti* (humans) and *M. caprae* (goats, humans). The *M. pinnipedii* spoligotype resembles that of some *M. bovis* strains. This, together with the known pathogenicity of *M. pinnipedii* for other mammals and humans, raised concerns that its host range might be broad and possibly comparable to that of *M. bovis*.

With these alarming facts in mind, a large-scale investigation into the possible spread of *M. pinnipedii* from the sea lion enclosure commenced. The sea lions were housed in outside basins with access to inside dwellings. Direct contact with other animals was not possible. The water in the basins and the feeding yard were strictly separated from the rest of the zoo. All caretakers involved in the care of the sea lions were called in for a tuberculin skin test. Furthermore, all sea lions and a large number of other mammals were tuberculin skin tested as well. Some of the caretakers that had been in close contact with the sea lions showed a positive tuberculin reaction. Some of them were offered medical treatment. A number of sea lions reacted positively as well, whereas no reaction was seen in any of the other mammals tested. Sea lions that reacted positively were euthanised. During post mortem, several of these animals where shown to have tuberculous lymph nodes. Up to this point, the possibility of infection of visitors could not be excluded. In order to address this issue, it was decided to repeat the tuberculin skin testing in staff that did not come into contact with the sea lions as these where thought to be representative for visitors, as far as exposure to mycobacteria was concerned. No positive skin reactions where seen in this group. Hereupon, it had been assumed that visitors have not been at risk of contracting tuberculosis. After a third round of tuberculin skin testing in sea lions, positive reactions where seen again. So, it was finally decided to euthanize the entire sea lion group, thoroughly clean and disinfect the animal housing and rebuild the group.

4.16 *Poxviridae*

Two members belonging to the family of Poxviridae that cause zoonotic disease, Orf virus (genus Parapoxvirus) and cowpox virus (genus: Orthopoxvirus), are present in the Netherlands.

Orf virus

Orf virus is a common cause of vesicular type lesions in small ruminants, particularly goats and sheep, and is occasionally diagnosed in humans. Orf is highly endemic in animals in the Netherlands, but the incidence of infection of humans is likely to be underreported. Besides direct contact, resulting in Orf lesions on hands and fingers, recent reports suggest the possibility of ulcerative lesions in the oral cavity of humans, following consumption of undercooked meat. It is highly unlikely that in persons with this clinical presentation, the differential diagnosis of Orf would be made.

Cowpox virus

Cowpox is a zoonotic skin condition caused by the cowpox virus (CPXV). CPXV is a member of the genus orthopox virus and is related to variola, vaccinia and monkeypox

Cowpox outbreak at a sanctuary for exotic animals

In the summer of 2003, a Barbary macaque kept at a sanctuary for exotic animals acquired papular gingival lesions followed by facial swellings and total malaise. A couple of weeks later, two more Barbary macaques developed a similar clinical picture. In a biopsy sample of one of the gingival lesions of the first diseased monkey, characteristic inclusion bodies were shown to be present, giving rise to suspicions of a poxvirus infection. Initial concerns that the animals might suffer from a monkeypox virus infection, one of the more serious zoonoses, could quickly be dismissed when additional biopsy specimens where examined virologically, showing that the animals were in fact infected with CPXV. Nonetheless, tight measures were taken in order to prevent zoonotic infection of the staff. The premises of the sanctuary were faced with a considerable plague of brown rats at the same time that the problems occurred. One Barbary macaque was even observed to take a rat as a pet, demonstrating close contact between rats and monkeys. In order to keep the rats at bay, cats were introduced in the three of the premises of the sanctuary. In October 2003 one of the caretakers developed an oral lesion that was reminiscent of CPXV infection in monkeys. Fortuitously, CPXV could not be shown to be involved. In the course of autumn 2003, five more Barbary macaques developed oral lesions all of which, as well as four apparently healthy Barbary macaques, where shown to have neutralizing CPXV antibodies. Altogether, another seven individuals belonging to four different monkey species were shown to have neutralizing CPXV antibodies as well. The three cats that were meant to keep the brown rats at bay were also shown to carry CPXV antibodies, indicating that they had been exposed to CPXV. More than 50 % of the rats that were killed during pest control were shown to be CPXV PCR positive. Sequence comparison of rat and Barbary macaque CPXV isolates revealed identical sequences, strongly hinting at rats as being the primary cause of the outbreak.

virus. Rodents are considered to be the reservoir of the agent, whereas cats, cows and humans act as incidental hosts. All orthopox viruses infections induce cross immunity against other members of the genus, thus with the cessation of routine vaccination with vaccinia virus, cohort immunity of the human population is decreasing. Zoonotic CPXV infections are, however, rare events. Infection in immunocompetent persons mostly manifests itself as localized lesions on fingers, hands or face, whereas in immunocompromized patients fatal, generalized infections have been known to occur.

4.17 Prions

Bovine Spongiform Encephalopathy (BSE) is a prion disease (transmissible spongiform encephalopathy (TSE)) of cattle. After the diagnosis of the first BSE case in the United Kingdom (UK) in 1986, a huge epidemic developed in the UK where since 1986 more than 181,000 cases in cattle were diagnosed. The BSE epidemic in the UK reached its peak in 1992 and has since steadily declined as a consequence of the control measures taken. From the UK, the BSE agent was spread through export of infected animals and feed to other countries, where it was again recycled and propagated via the feed chain. The first BSE case in a Dutch cow was diagnosed in 1997.

BSE is transmissible to sheep and goats as demonstrated by experimental infection; small ruminants develop a disease that is indistinguishable from scrapie, the TSE specific to small ruminants. So far, naturally occurring BSE has been diagnosed in two goats (France, UK) and no cases were found in sheep. The control of BSE in Europe was enforced in 1996 when a new disease in humans was noticed in the UK, which

was designated as the variant Creutzfeldt-Jakob disease (vCJD). The current view on vCJD is that it resulted from transmission of infection from BSE in cattle to humans via infectivity in food. Human to human transmission of vCJD (via blood transfusions) also occurred.

Animals

Passive surveillance

The passive surveillance for BSE started on 29 July 1990 when BSE became a notifiable disease in the Netherlands. In addition to cattle notified as BSE suspects by veterinary practitioners and farmers, cattle showing clinical signs resembling those of BSE noticed by the official veterinarians at the ante-mortem inspection at slaughterhouses were tested with histopathology and immunohistochemistry, and from 2001 onwards also with EU approved rapid BSE tests.

Active surveillance

In 1999-2000 so-called "BSE rapid tests" were developed, which became available at the end of 2000. With these tests it became possible to test large amounts of brainstem samples and to have results within 24 hours. The passive surveillance system was extended with active surveillance from 1 January 2001. The active surveillance system consists of the testing of brainstem samples with EU approved rapid tests of:

- All healthy slaughtered bovine animals above 30 months; from February 2001 till 1 July 2006, slaughter animals imported from Germany were tested above 24 months of age.
- All fallen bovine animals above 24 months; from 1 January 2001 till 31 March 2001 not all fallen animals were tested, but approximately 25% of all fallen animals older than 30 months; from 1 April 2001 onwards, all fallen stock above 24 months was sampled and tested.
- All casualty slaughtered bovine animals above 24 months; cattle slaughtered in emergency and bovines showing symptoms of any disease or of a disorder of their general condition.

BSE is suspected in bovine animals, which have produced a positive result from a rapid BSE test. The final diagnosis is made at the national reference laboratory (NRL) CIDC-Lelystad with histopathology an/or immunohistochemistry.

All rapid tests till April-May 2002 were performed by the NRL. Private laboratories became involved in rapid testing of slaughter cattle since April 2002. The five approved private laboratories for rapid tests are controlled by the NRL.

Control measures

Legislation has been introduced and implemented in the Netherlands to reduce the exposure of people to the agent. The most prominent measures from 1989 onwards were:

- Ban on the use of meat-and-bone meal (MBM) from ruminants in feed for ruminants (1989).
- Ban on the import of MBM from ruminants originating from the UK (1990)
- Ban on production of feed for ruminants in machines (mixers) after the production of feeds for other species, containing more than 6% MBM (1993)
- Ban on the presence of MBM originating from the UK, Ireland and Switzerland in a ruminant feed production unit (1993)
- Ban on the import of MBM from mammals in feed for ruminants (1994)
- Trade in mammalian proteins allowed if feed has been treated to 133°C, 3 bars, >20 minutes, particles size < 50mm (1997)
- The removal of specified risk materials at slaughter and its exclusion from the food and feed chain by a pre-treatment and by rendering and subsequent incineration (1997)
- Production of ruminant feed completely separated from production of feeds for other species, which may contain animal proteins (1999)
- Ban on the use of fish meal and feather meal in ruminant feed (2000)
- Ban on the feeding of animal proteins to all farm animals; ban on trading, supplying, transporting and processing animal proteins for farm animal feed (end 2000)
- Ban on the production of mechanically recovered meat from ruminants (2001)

Results

The results of the Dutch surveillance system are listed in Tables 4.17.1 (passive surveillance), 4.17.2 (active surveillance since the end of 2000) and Figure 4.17.1. With only the passive surveillance system in place for 1990-2000, 8 cases were diagnosed, two cases each year from 1997 until 2000. In the period from 2001-2006, more than 3,1 million bovines were tested: 74 Dutch BSE cases were diagnosed and confirmed; 2 cases were found in Belgian cows. From 1997 till 2000, 7,186 bovines from BSE positive herds were culled; all culled animals tested negative for BSE

Analysis of the Dutch BSE situation

BSE has only been diagnosed in cattle within the Netherlands. Up until 2006, 82 cases have been diagnosed in Dutch cows in the Netherlands. The first case was diagnosed in March 1997 and the last one in March 2006. Eighty cases were found in dairy cows and two cases in suckler cows. In 69 of the cases, the BSE cow was born and raised on the infected farm. In 13 cases, the positive cow had stayed on two or more farms during her lifetime. In almost all these 13 cases, the farm of birth was determined as the farm of the origin of infection. The youngest case was 50 months old (Figure 4.17.1); the oldest 164 months. The average age of the Dutch BSE cases is 6.47 years.

In addition to the 82 BSE cases in Dutch cows diagnosed in the Netherlands, BSE was diagnosed outside the Netherlands between 1995 and 2002 in 7 cows that were born in the Netherlands (UK: 4 cases, Ireland: 2 cases, Germany: 1 case). Considering the date of exportation, the date of diagnosis and the average incubation period of BSE, at least one case was infected in the Netherlands; it concerns a cow born on 24 August 1996 on a farm in Ysselsteyn (province of Limburg), exported to Germany in April 2002,

where in November 2002 BSE was diagnosed. The location of infection of the second case, an animal born on 22 February 1994 on a farm in Boekel (province of Noord-Brabant), exported to the UK on 11 May 1996, where BSE was diagnosed in October 1998, remains questionable. With regard to the 5 remaining cases, Dutch authorities consider the cases as UK and Irish cases.

Two BSE cases per year were diagnosed from 1997 till 2000 (passive surveillance). In 2001, an apparent increase of the number of BSE cases (20 cases) was noticed, similar to findings in other European countries as a consequence of the implementation of the active surveillance. After 2001, the number of BSE cases per year in the Netherlands remained low and stable: 24 in 2002 and 19 in 2003. As of 2004, the number of BSE cases per year dropped to very low numbers (6 in 2004, 3 in 2005 and 2 in 2006). With the same surveillance system in place from 2001 till 2007, these results indicate that the Dutch (and European) control measures to eradicate BSE are very effective.

Year of birth distribution

It is difficult to interpret the year of birth distribution of all 82 Dutch BSE cases because the surveillance systems before and after 2001 were different. Considering the average age of detected cases (6.5 years), the results of the birth cohorts of 1995-2000 can be compared in a reliable way. These results show that the birth cohort of 1996 (with 32 cases and one case detected in Germany) especially was exposed to more infectivity than others. Control measures, such as the different feed bans for ruminants (1989, 1994), and especially the removal of specified risk materials in slaughterhouses and subsequent incineration (1997), the total separation of the production of feeds for the different animal species (1999), and the total feed ban for all domestic farm animals (2001) seemed to be very effective in reducing the exposure to infectivity of the birth cohorts from 1997 onwards to negligible levels.

Geographical distribution

The geographical distribution of the BSE cases is not fully in accordance with the dairy cattle distribution in the Netherlands. Cattle born in the provinces of Overijssel, Gelderland, Zuid-Holland and Noord-Brabant had the highest chance to develop BSE. Most of the BSE cases originated from the middle part of the country. Two significant clusters were found. The first one was located in the eastern part of the country of cattle of the birth cohort 1996, followed by a cluster in the western part of the country of the birth cohort 1997. These clusters were associated with feed producers.

Cause of the Dutch BSE cases

Two studies on the origin of infection of the Dutch BSE cases have been performed. The first one was conducted by the General Inspection Service of the Ministry of Agriculture, nature and Food Quality and was based on all data obtained from the official inspections of the first 43 BSE cases. The second study of the National Reference Institute for TSEs (CIDC-Lelystad) involved the first 76 BSE cases, and included a case-control study to determine animal and farm related BSE risk factors. The last 6 cases were also evaluated after they were diagnosed. Both studies concluded that all Dutch BSE

cases could be explained by the feeding of contaminated meat-and-bone meal that was present in the cattle feed as a consequence of cross-contamination with feed for non-ruminants. The only significant risk factor found by multivariate analysis in the case-control study was the feed producer

BSE types (strains)

TSE strains can be differentiated by their behaviour in bioassays (strain-typing in conventional mice or in transgenic mice expressing the PrP gene of different species and by molecular analyses of the disease associated protein (PrPSc)). Until recently, isolates from BSE cases of cattle in different countries appeared to be very homogenous. However, a limited number of atypical BSE isolates have recently been identified. Based on molecular mass, glycoprofile and the reaction pattern with several anti PrP antibodies, at least two different types (H-type and L-type) have been identified in addition to the C-type (classical BSE). The first results from strain-typing in mice indicate that the H-type and the L-type are indeed different strains compared to the classical BSE strain of the big epidemic in the UK and other European countries. All Dutch BSE isolates have been examined in Western blots; 79 isolates were defined as C-type. One isolate from a 13 year old cow was defined as H-type, and two isolates were defined as L-types, from two cows 10 and 12 years old. Currently, these isolates are strain-typed in mice bioassays. It is speculated that these H- and L-type isolates are sporadic strains of BSE, which may occur in older cattle.

Table 4.17.1. Number of clinical suspects for laboratory examination in the Netherlands (1991-2006).

Year	Number of clinical suspects	Number of positives
1991	2	0
1992	0	0
1993	2	0
1994	0	0
1995	1	0
1996	22	0
1997	35	2
1998	25	2
1999	32	2
2000	40	2
2001	97	6
2002	39	2
2003	25	2
2004	19	0
2005	7	0
2006	12	1
Total	358	19

Other TSEs

Other TSEs that have been diagnosed in the Netherlands are scrapie in small ruminants and variant Creutzfeldt-Jacob disease (vCJD) in man.

Table 4.17.2. Results of the Dutch active BSE surveillance per year of detection (October 2000-2006): number of tested animals and positives per category

	Healthy slaughtered cattle		Casualty slaughtered cattle		Fallen animals	
Year	Number tested	Positive	Number tested	Positive	Number tested	Positive
2000	0	0	289	0	416	1
2001	454.649	9	13,281	2	31,056	2
2002	491,069	10	17,710	4	46,611	8
2003	439,403	11	15,418	1	50,525	5
2004	467,448	5	15,705	0	50,425	1
2005	451,507	1	17,936	2	47,715	0
2006	432,042	1	10,739	0	49,084	0
Total	2,736,118	37	91,078	9	275,832	17

Scrapie in small ruminants

Scrapie in sheep is endemic in the Netherlands. Scrapie in small ruminants has been a notifiable disease since 1993. Since 1 January 2002, an active surveillance system is also in place in compliance with EU regulations (testing of samples of slaughter animals and fallen animals, both in sheep and goats). Similar to BSE an apparent increase of the number of positives was noticed after the introduction of the active surveillance in 2002.

Control measures: in 1998, a national breeding programme for resistance to TSEs in sheep was started on a voluntary basis, and was made compulsory afterwards. Eradication measures (culling of positive flocks) are in compliance with EU legislation. Scrapie in goats has been diagnosed only once; it was an isolated case diagnosed in December 2000 in goats from a children's farm. All sheep and goat TSE isolates (including those of culled animals) since 2000 were tested with EU approved discriminatory Western blotting methods and immunohistochemistry to differentiate between BSE and scrapie. All isolates were defined as classical or atypical scrapie. BSE was not detected.

Humans

CJD in human has been a notifiable disease in the Netherlands since 1 September 2002. The annual incidence of sporadic CJD is approximately 1 case per million, similar to other countries. So far, two cases of vCJD have been diagnosed in the Netherlands: one case in 2005 (female, 26 years), and one case in 2006 (male, 16 years). Both patients died, and had no history of a stay in foreign countries with a high incidence of vCJD, such as the UK or France.

Conclusions

The results of the Dutch BSE surveillance system clearly demonstrate that the control measures are very effective in reducing the number of BSE cases in cattle in the Netherlands. The Dutch authorities are confident that the (external) challenge of the national herd with respect to infectivity has decreased to a negligible level, and that the very stable, optimised national system will assure that the Dutch national herd is no longer exposed to BSE infectivity, which will lead to an absence of BSE cases in the near future.

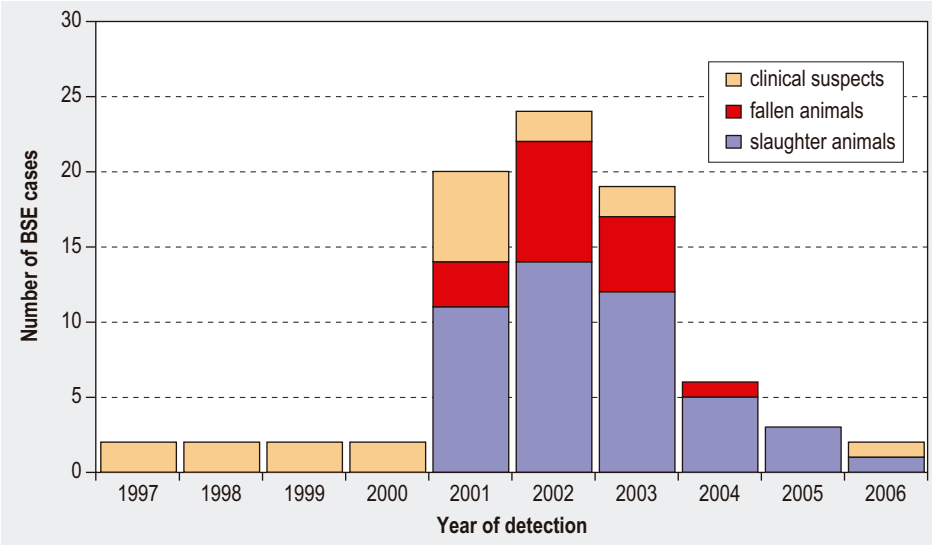


Figure 4.17.1. Year of detection of the 82 Dutch BSE cases and distribution in different target groups.

It is expected that in 2007 or 2008 no cases or the last cases of BSE will be diagnosed. This prediction does not reckon with speculations that "spontaneous" or sporadic BSE cases in cattle may exist.

4.18 *Rabies virus*

The rabies virus belongs to the genus Lyssavirus of the Rhabdoviridae family. The rabies virus is divided into 7 genotypes. For the Netherlands, two types are important: the classical virus (genotype 1) and the European Bat Lyssa viruses (EBLV). The EBLVs can be subdivided in several subtypes. EBLV-1 and EBLV 2 are classified as genotypes 5 and 6, respectively. EBLVs are endemic in wild insectivorous bats in Europe. In the Netherlands the viruses can be isolated from two bat species, the serotine bat (*Eptesicus serotinus*) and the pond bat (*Myotis dasycneme*). EBLV-1 is endemic in serotine bats, whereas EBLV-2 is found in pond bats, although very rarely.

Bats are the only reservoir for rabies in the Netherlands at this moment. When bats are infected with the virus, their saliva becomes infective. The infection is spread by bites from infected bats or contact with infected saliva. EBLV infections in humans have been reported and have resulted in fatal rabies. Therefore, bats involved in direct-contact incidents with humans or pets are examined for the presence of the EBLV at the CIDC in Lelystad. As most of the human rabies cases worldwide are caused by the classical rabies virus, all other animals with an abnormal behaviour are examined for the presence of rabies (classical virus) as well. Terrestrial mammals with symptoms of classical rabies have not been reported in the Netherlands since 1988.

Animals

In the Netherlands there is an ongoing surveillance of lyssavirus in bats. Analysis has been performed on all data collected in the period, between 1984-2003. In total 3,873 bats were investigated of which 1,219 were serotine bats. Of the serotine bats 251 (21%) were tested positive for lyssavirus antigen by specific immune fluorescence staining of brain tissue. Five (4%) of 129 specimens from the pond bat were positive. All viral sequences from serotine bats clustered with genotype 5 lyssavirus (EBL-1) sequences. In the period of 2003-2006 435 bats were investigated for rabies virus and 34 (7.8%) were found positive. The incidences of lyssavirus in the examined bats ranged between 4.3% and 15.4%. All suspected animals investigated for classical rabies virus were found negative. The number of animals investigated during the period of 2003-2006 is summarized in Table 4.18.1.

Table 4.18.1. Animals examined for rabies in the period 2003-2006.

Animal species	Positive/total (%)			
	2003	2004	2005	2006
Bats (wildlife)	7/130 (5.4)	14/91 (15.4)	4/94 (4.3)	9/120 (7.5)
Foxes	0/3 (0.0)	0/12 (0.0)	0/2 (0.0)	0/3 (0.0)
Dogs	0/5 (0.0)	0/4 (0.0)	0/4 (0.0)	0/1 (0.0)
Cats	0/5 (0.0)	0/2 (0.0)	0/5 (0.0)	0/4 (0.0)
Muskrats	0/0 (0.0)	0/0 (0.0)	0/1 (0.0)	0/0 (0.0)
Squirrels	0/0 (0.0)	0/1 (0.0)	0/1 (0.0)	0/0 (0.0)
Polecats	0/0 (0.0)	0/0 (0.0)	0/1 (0.0)	0/0 (0.0)
Ferrets	0/0 (0.0)	0/0 (0.0)	0/1 (0.0)	0/0 (0.0)

Rabies (bats)

A risk assessment was performed with the aim to study the frequency and the nature of contact incidents of the serotine bat, *Eptesicus serotinus,* with humans and with companion animals (specifically cats and dogs), in the Netherlands between 2000 and 2005. Out of 17 bats involved in bite incidents with humans, 5 were tested positive for European bat lyssavirus (EBLV) type 1a. Cats had the most numerous contacts with bats (49 times) but a relatively low number of these bats were EBLV-positive (6 times). It has been estimated that the average incidence of human bat rabies infection might be between once per year and once per 700 years, depending mainly on the number of infectious viral particles in bat saliva. The risk of bat rabies is higher between April and October, and in the northern half of the country. This is the first study in Europe describing the risk of human bat rabies after bat contact incidents

Humans

Persons who have been involved in direct contact incidents with animals diagnosed rabid receive post-exposure vaccinations. If possible, these were combined with the application of anti-rabies immunoglobulin. The treatment is also applied when a suspected animal is not available for rabies testing. The treatment can be carried out by general practitioners, who can obtain information about (the necessity) of the treatment at the National Poisons Control Centre (NVIC) of the RIVM, but also at the LCI.
No human cases have been reported for many years in the Netherlands.

Conclusion

Rabies caused by the classis virus has not been reported for many years in the Netherlands, whereas EBL virus is endemic in bats. The results of the identification of the bats show that the Serotine bats are still the main reservoir of lyssavirus in the Netherlands. As the risk of introduction of classic virus is still present and both the classic and the EBL virus may cause fatal rabies in humans, it is important that investigation of rabies-suspected animals is continued in the Netherlands.

4.19 *Salmonella* spp.

Salmonella has been recognised as an important pathogen for many years. In the Netherlands between 1984 and 2006 about 1,000 serotypes and phage types have been found. The pathogen has drawn a lot of attention and much progress has been made in the control of the pathogen in feeds, animal husbandry and at slaughter, in the improvement of the HACCP norms for the production of foods and the ongoing education of the public on improving food handling and kitchen hygiene. Combining recent epidemiological studies performed in the Netherlands in the general population and in general practices with data from laboratory surveillance, produces to the estimate of about 35,000 cases of salmonellosis occurring in the general population in 2004, of whom about 5400 consulted a general practitioner, 510 were hospitalised and an estimated 39 died. The burden of disease was estimated to be 600 (450-900) Disability Adjusted Life Years (DALYs) per year. Cost-of-illness is estimated at € 7 million (3-17) per year.

Feed

Results of the monitoring programme in feed ingredients and end products (compound feed).

Table 4.19.1 shows that the percentage of positive *Salmonella* samples from (imported) by-products of rape seed was 3.4% in 2006 and 6.8% in 2005. Imported by-products of sunflower-seed and soybeans, toasted soy beans and untreated fishmeal were less contaminated with *Salmonella* in 2006 than in 2003-2005 (1.4%, 1.5%, 2.0% and 0.8% respectively). It may be concluded that the suppliers of feed materials have steadily improved their performance. Of the end products (i.e. the compound feed), only 0.3% of poultry feed was *Salmonella* positive. This is better than in the period 1999-2005. The percentage of *Salmonella* positive samples of pig feed and cattle feed end products was 0.3% in 2006, which was better than in previous years.

The main causes for contamination of the end products are, inadequate processing of the (positive) feed components and/or post-process contamination (cross- and recontamination). Corrective actions undertaken by the feed industry include: repelletizing of the feed (if positive) at higher process temperatures; acidification of the feed with (for example) formic acid; intensified cleaning and disinfection of the plant and processing equipment, feedback of results regarding the *Salmonella* monitoring to sup-

Table 4.19.1 Percentage of Salmonella positive samples of feed ingredients

	2003		2004		2005		2006	
	samples	%+	samples	%+	samples	%+	samples	%+
Feed ingredients								
Barley and by-products	151	*0.7*	252	*0*	208	*0*	185	*1.6*
Wheat and by-products	1218	*0.7*	1,802	*0.2*	1,608	*0.1*	1,608	*0*
Maize and by-products	625	*0.6*	5/920	*0.5*	716	*0.7*	607	*0*
Extracted rape seed/ -expeller	3,395	*7.0*	3,932	*11.8*	4,360	*6.8*	4,337	*3.3*
Palm kernel and by-products	173	*1.2*	169	*0*	154	*0.6*	89	*1.1*
Toasted soy beans	2,020	*3.2*	2,805	*4.9*	2,370	*4.3*	1,729	*2.0*
Extracted soybeans/ -expeller	2,712	*4.7*	2,552	*5.6*	2,806	*4.1*	4,514	*1.5*
Extracted Sunflower seed	527	*3.0*	401	*4.7*	1,132	*2.8*	1,137	*1.4*
Linseed flakes	54	*1.9*	77	*6.5*	81	*6.2*	73	*0*
Fishmeal	306	*1.7*	821	*0.9*	508	*0.8*	386	*0.8*
Compound feed								
Poultry Feed (total)	6,126	*0.4*	7,851	*0.6*	9,768	*0.4*	1,267	*0*
• Top breeding	413	*0*	298	*0*	693	*0.1*	486	*0*
• Raising parent stock	165	*0*	151	*0*	274	*0*	239	*0*
• Parent stock	916	*0.5*	789	*0.5*	993	*0.1*	952	*0.1*
• Laying hens	2,262	*0.4*	3,037	*1*	3,357	*0.8*	3,001	*0.7*
• Consumption turkeys	324	*0*	145	*0.7*	98	*0*	105	*0*
• Broilers	1,818	*0.4*	2,753	*0.3*	2,939	*0.2*	2,158	*0.1*
Pig Feed	2,857	*0.6*	3,048	*0.6*	3,301	*0.4*	2,917	*0.3*
Cattle Feed	1,375	*0.7*	2,188	*0.4*	2,467	*0.5*	2,438	*0,3*

pliers of the feed ingredients and a more intensive monitoring of the Salmonella status of the (critical) feed materials delivered at the compound feed plant.

Classification of Salmonella types in feed

After the determination of *Salmonella* in feed materials and compound feeds, *Salmonella* is serotyped. The purpose of this classification is to establish any relationship between *Salmonella* types in feed materials, the compound feeds produced from them, as well as animals and animal products more accurately. It is an aid for the investigation of the possible cause of *Salmonella* contamination in subsequent links in the production chain. The classification of *Salmonella* types for 2005 and 2006 is shown in Table 4.19.2 and 4.19.3.

Farm animals

***PVE** monitoring of the poultry meat production chain*

The 2000 objective of the plan of approach for *Salmonella* control in poultry meat was to achieve a lower level of infection with *Salmonella* spp. of less than 10% in broiler flocks. Although contamination of fluff at the hatcheries was consistently decreasing between 1999 and 2002 and practically absent in the past three years, contamination increases just before and after arrival of the chickens at the farm (Table 4.19.4)

Table 4.19.2. Salmonella serotypes found in critical feed ingredients in 2005 and 2006

2005	N	Salmonella serotypes
Extracted rape seed and expeller	301	3x Infantis, 7x Cubana, 16x Mbandaka, 2x Orion, 16x Tennessee, 2x Livingstone, 103x Senftenberg, 23x Lexington, 14x Agona, 8x C1-groep, 4x Java, 4x Montevideo, 5x Havanna, 3x Cubana, 6x Oranienburg, 85x unknown.
Extracted sunflower seed	32	1x Havanna, 19x Agona, 3x Lexington, 2x Senftenberg, 1x Infantis, 1x Derby, 1x Albany, 1x Kentucky, 1x Java, 2x unknown.
Toasted soy beans	103	50x Rissen, 18x Minnesota, 1x Anatum, 2x Livingstone, 2x Virchow, 1x Montevideo, 24x Agona, 5x unknown.
South-American Extracted soybeans and expeller	114	17x Lexington, 11x Kentucky, 11x Mbandaka, 9x Livingstone, 7x Tennessee, 9x Senftenberg, 2x Montevideo, 3x Swarzengrund, 3x Agona, 1x Grumpensis, 1x Infantis, 1x Anatum, 1x Wortington, 1x Pisa, 1x Orion, 3x Carrau, 3x Yoruba, 2x C1-groep, 2x Virchow, 2x Havanna, 1x Cubana, 1x Rissen, 1x Ouakam, 1x Adelaide, 1x Salamae, 1x Java, 1x Enteritidus, 17x unknown.
2006		
Extracted rape seed and expeller	144	2x Cubana, 1x Lexington, 2x Anatum, 28x Lexington, 18x Senftenberg, 10x Parath. B. Java, 9x Tennessee, 12x Agona, 5x Serogr E1, 5x Mbandaka, 5x Infantis, 2x Kentucky, 1x Livingstone, 1x Rissen, 1x C1-groep, 1x E4-groep, 1x B-groep, 1x Virchow, 1x Enteritidus, 1x Stanley, 1x Duisburg, 1x Brandenburg, 35x unknown
Extracted sunflower seed	15	2x Senftenberg, 3x Havana, 2x Tennessee, 1x Kentucky, 1x Rissen, 1x Infantis, 3x Agona, 1x Livingstone, 1x B-groep
Toasted soy beans	35	1x Lexington, 1x Infantis, 1x Rissen, 1x Virchow, 6x Anatum, 2x Agona, 19x Rissen, 4x unknown
South-American Extracted soybeans and expeller	40	7x Livingstone, 7x Senftenberg, 4x Agona, 4x Infantis, 2x Orion, 3x Rissen, 1x Lexington, 2x Havana, 2x Mbandaka, 1x Give, 1x Tennessee, 1x Salmonella C1-groep, 5x unknown

Much progress has been made between 1998 and 2006 in the reduction of *Salmonella* contamination in all links of the poultry meat production chain. In 2000 the target of 10% was not attained for the tested caeca or neck skins at the slaughterhouse nor for breast skins tested in 2001. In the 2000+ control programme the new objective was set at a maximum *Salmonella* contamination level for produced meat of 10% at the end of 2002, which has succeeded and halved again between 2002 and 2006. Since 2001, breast skins have been tested as a more realistic proxy for the “end product”. The large difference between the results for caeca and neck skins suggests a strong influence of cross-contamination at slaughter which is not representative for the real contamination of the meat. This notion is supported by the findings at retail (Table 4.19.5) as the

Table 4.19.3. Salmonella serotypes found in the end product (compound feed) in 2005 and 2006

2005	N	Salmonella serotypes
Poultry feed	32	1x Havanna; 1x Newport, 5x Mbandaka, 6x Senftenberg, 5x Livingstone, 2x Lexington, 1x Cerro, 1x Montevideo, 1x Infantis, 1x Enteritidis, 1x Tennessee, 1xAnatum, 7x unknown.
Pig feed	14	1x Indiana, 3x Lexington, 5x Senftenberg, 2x Wortington, 1x Agona, 1x Mbandaka, 1x Havanna
Cattle feed	12	3x Senftenberg, 2x Lexington, 1x Mbandaka, 1x Havanna, 1x Anatum, 1x Stourbridge, 3x unknown.
2006		
Poultry feed	23	1x Heidelberg, 5x Havana, 2x Mbandaka, 3x Senftenberg, 2x Parath. B Java, 1x Lexington, 1x Bredeney, 1x Anatum, 1x Livingstone, 6x unknown
Pig feed	10	2x Livingstone, 2x Senftenberg, 1x Agona, 1x Tennessee, 1x Rissen, 1x Anatum, 1x Corvallis, 1x unknown
Cattle feed	8	2x Infantis, 1x Lexington, 5x unknown

Table 4.19.4. Percentage of Salmonella-positive broiler flocks/batches, from different links in the production chain. At slaughter about 550 flocks are tested each week (Control programme PVE).

Matrix	link	1998	1999	2000	2001	2002	2003	2004	2005	2006
Fluff	hatchery	4.3	2.0	2.3	1.3	0.5	0.8	0.0	0.0	0.0
Leaflets	transport	9.0	5.5	4.3	4.0	1.8	3.0	0.8	1.3	0.8
Faeces	farm (overshoes)	16.5	14.0	12.0	9.5	7.3	7.5	4.0	3.8	3.5
Caeca	slaughterhouse	22.0	20.8	14.5	12.5	10.0	9.0	6.0	5.3	5.0
Neck skin	slaughterhouse	44.5	41.3	32.0	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Breast skin	slaughterhouse	n.i.	n.i.	n.i.	16.8	12.0	11.3	6.5	7.0	6.3

n.i. no investigation

Table 4.19.5. Salmonella in farm animals on randomly selected farms (Monitoring VWA – RIVM - GD)

	Dairy cattle		Veal calves		Finishing pigs				
	2001	2002	2001	2002	2000	2001	2002	2004	2005
Farms	103	148	89	155	194	153	157	221	163
% positive	9	5	10	7	36	27	30	29	21

contamination of breast skins between 2001 and 2006, although consistently lower, shows a close resemblance to the level of contamination found in poultry meat at retail (Table 4.19.5). The end target in 2010 is a contamination degree of 0^{+}%.

Figure 4.19.1 shows that the change of contamination levels in the different links of the poultry meat production chain observed over the years (Table 4.19.4) is generally in agreement with the changes observed in the different quarters of a year. A minor set back is seen in the 3rd quarter of 2003, which is probably related to repopulation of broiler flocks after the end of the avian influenza outbreak in poultry.

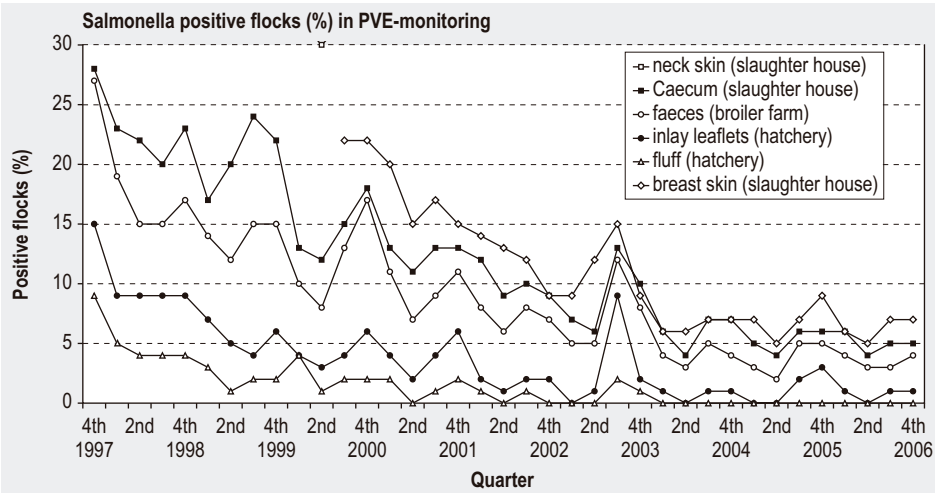


Figure 4.19.1. Percentage of Salmonella positive flocks from hatchery to the end of the slaughter line.

Table 4.19.6. Salmonella in poultry meat (+/-12%organic) at retail (Monitoring programme VWA)

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
sample size	1,314	1,077	859	1,454	1,578	1,600	1,510	1,482	1,474	1,539
% *Salmonella* spp. (organic)	29.1	20.2	17.6	21	16.3	13.4	11.3 (3.4)	7.4 (2.1)	9.4 (1.9)	8.4 (4)
Main serovars as a fraction of all isolates. Regular production (%)										
Paratyphi B Java	15.0	11.4	13.9	33.1	43.2	53.5	45.6	58.2	46.8	38.5
Enteritidis	20.2	12.8	26.4	6.6	8.2	2.3	8.8	5.5	7.2	6.6
Hadar	10.1	6.1	4.5	3.3	4.2	0.9	1.8	-	1.4	5.7
Indiana	6.1	8.3	9.3	10.2	11.6	6.5	6.4	1.8	2.2	4.1
Infantis	9.2	5.0	3.6	6.6	7.0	7.9	11.7	-	11.5	13.9
Virchow	4.6	2.8	2.6	10.2	3.5	5.6	5.8	4.5	8.6	11.5
Typhimurium (DT104)	7.8 (..)	3.6 (1.8)	1.3 (0.7)	0.1 (0.1)	7.4 (7)	7.4 (2.8)	5.8 (5.3)	3.6 (..)	5 (2.2)	1.6 (0)
Corvallis									4.3	1.6
Other types	27.0	53.6	39.7	30.0	22.3	23.3	19.9	26.4	13.0	16.5

National serological monitoring of fattening pigs at farms and at slaughterhouses

Since 1 February 2005, monitoring for *Salmonella* in fattening pigs at farms and slaughterhouses has been obligatory, which prescribed in a regulation of the Product Board for Livestock and Meat (PVV). The results of the first monitoring year are presented in section 3.1.4.

Monitoring of farm animals by VWA, the RIVM and the GD

Since 1997, a voluntary surveillance programme runs in farm animals on a varying sample of farms with the main objective to monitor trends in the occurrence of zoonotic bacteria (see section 3.1.4). Monitoring of *Salmonella* in cattle was discontin-

ued after 2002 but it showed infected herds in up to 10% of the farms (Table 4.19.5). Infected herds of fattening pigs are found much more frequently, *i.e.* in about 30% of the farms.

Food

In 2006, the VWA monitoring programme for poultry meat at retail showed that the gradual decrease of contamination of poultry meat with *Salmonella* spp. since 1996 has stabilised between 2004 and 2006 (Table 4.19.6). In 2006, 8.4% was contaminated with *Salmonella* spp.. Still about 40% of these consisted of *S.* Paratyphi B var. Java, which now clearly seems to be decreasing. The *S.* Enteritidis and *S.* Typhimurium contamination reached a level of approximately 0.5% in 2006. *S.* Corvallis, appeared in 2005, and was also found more frequently in humans, but decreased again in 2006 (Addendum 4.19.4).

The results of the investigations of meat derived from cattle, pigs and sheep indicates that contamination occurs at retail (Table 4.19.7). A large survey in 2006 of meat intended to be eaten raw was performed after several incidents with *Salmonella* and STEC in 2005. It is shown that even in beef intended to be eaten raw, contamination can be found.

The serotype distribution in farm animals (Addendum 4.19.1)

Isolates received and typed at the *Salmonella* reference laboratory at the RIVM generally agree with the predominant serotypes found in pigs, cattle and poultry found in the more specific surveillance of healthy animals at farms (VWA - RIVM), slaughterhouses (PVE), at retail (VWA) and in animals for clinical diagnostic purposes (GD).
In 2006, *S.* Typhimurium is still the predominant serotype in pigs (61%), *S.* Livingstone is slightly emerging as is SI 1,4,5,12:i:2ef nat in cattle. The latter type is strongly emerging in other EU-countries, both in humans and in farm animals and is probably a monophasic variant of *S.* Typhimurium ft507. No other shift of serotypes has occurred in cattle examined for diagnostic purposes, wherein *S.* Dublin still predominates, followed by *S.* Typhimurium.

Since 1998, the RIVM database allows for the distinction between broilers (including products) and layers (including animals from reproduction and eggs). It clearly shows the continuing increase of *S.* Paratyphi B var. Java in broilers that is found in layers as well (Addendum 4.19.1). Java is a persistent infection in broilers. In Germany and Belgium as well, it is multiple resistant, but hardly causes clinical infections in humans

Table 4.19.7. Salmonella in 25g of raw meat at retail (Monitoring programme VWA)

	1999		2002		2003		2004		2005		2006	
	N	%+	N	%+	N	%+	N	%+	N	%+	N	%+
Beef and veal	746	0.9	532	3	678	0.6	956	1	484	0.2	1,159	2
Consumed raw											983	0.7
Pork	533	6	105	11	227	5	333	1	356	2	469	3
Mutton									120	0	49	0

(Figure 4.19.2). As shown at retail (Table 4.19.6), *S.* Infantis is still high in broilers whilst *S.* Virchow is clearly (re) emerging. The (relative) importance of *S.* Enteritidis continues to decrease in broilers and layers but it is still the predominant serotype among layers. *S.* Senftenberg has remarkably emerged in 2006 in layers but is of minor public health concern, as it does not transmit to humans by eggs as *S.* Enteritidis does. Several other more minor changes in poultry are also indicated in the table.

The phage type distribution of S. Typhimurium in farm animals (Addendum 4.19.2)

In pigs and cattle *S.* Typhimurium was isolated relatively more often in 2006 than in the five previous years (addendum 4.19.2). DT104 became relatively less important in cattle (and poultry) but remained on the same level in pigs (figure 4.19.4). Ft-507 has strongly emerged in the past six years and that increase is probably related to an increasing number of small outbreaks in humans. Phagetype ft-507 is in fact a monophasic variant of *S.* Typhimurium that has currently been strongly emerging in both the Netherlands and other European countries. This type now ranks third after *S.* Typhimurium and *S.* Enteritidis in human salmonellosis.

In 2006, DT7 appeared (frame). The majority of these isolates were related to an outbreak involving large amounts of contaminated hard cheese. So far it is not indicated whether this new type is really emerging in the Netherlands.

The phagetype distribution of S. Enteritidis in farm animals (Addendum 4.19.3)

The serotype *S.* Enteritidis was hardly ever found in pigs but does occur sporadically in cattle. It was strongly reduced in poultry and rarely found among broilers, or at retail (Table 4.19.6). In layers *S.* Enteritidis is still the predominant serotype although the *Salmonella* contamination level itself is low and among the lowest in Europe as shown in the EU-baseline study. As in humans, the predominant Pt-4 has strongly decreased in

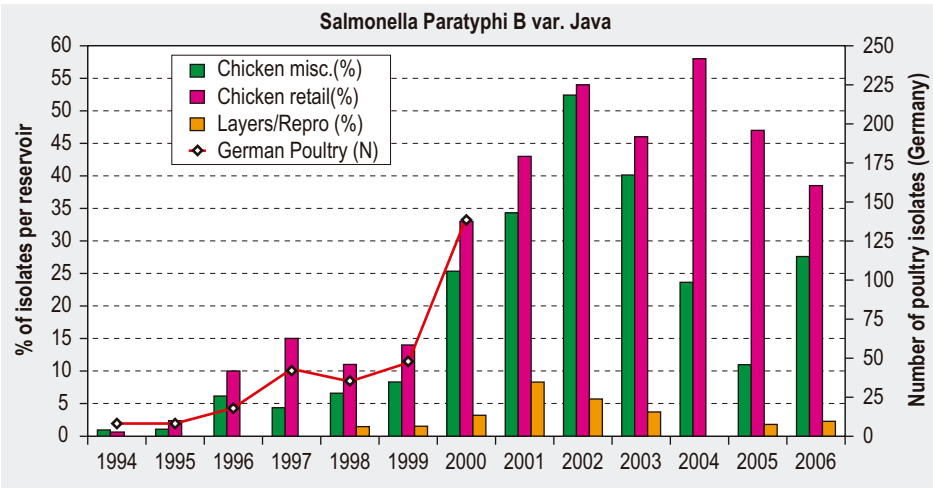


Figure 4.19.2. The emergence of S. Paratyphi B var. Java in broilers the Netherlands and Germany. The recent large decrease in broilers is an artefact due to selective typing requests of the broiler industry; the retail monitoring (statistical sampling) indicates a much smaller decrease. The number of isolates found per year in humans is indicated at the top of the figure.

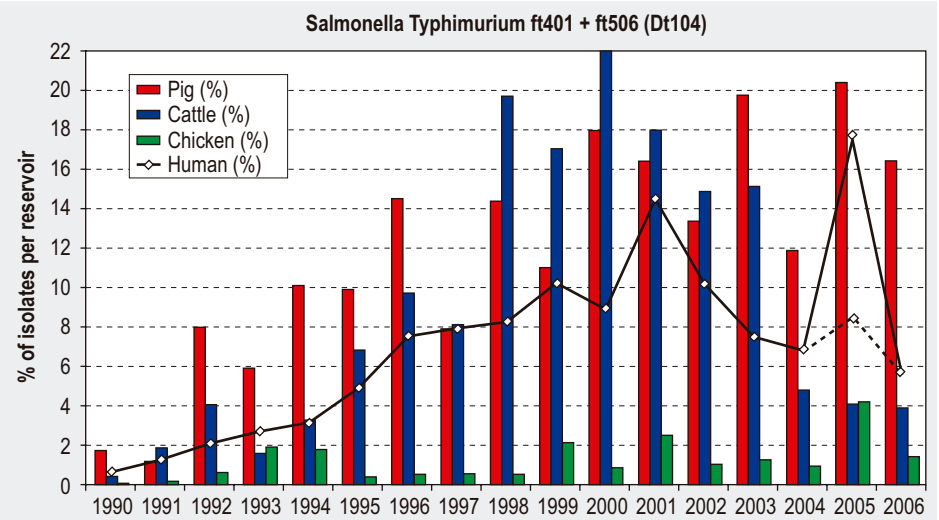


Figure 4.19.4. The emergence of S. Typhimurium Dt104 in the Netherlands. In recent years a clear decrease is indicated in cattle but not in pigs. Contaminated imported beef caused the large increase in humans in 2006; the dotted line indicates the endemic course of DT104 cases.

the past decade to be replaced by Pt-1, Pt-6, Pt-8 and Pt-21 but the 2006 data indicate that this trend is reversing, except for Pt-1. Clear differences exist in types circulating among broilers and layers and between the years examined. From the detailed typing data it is estimated that about 33% of all human *Salmonella* infections in 2006 were still related to the consumption of contaminated eggs. In 2003, this amounted to 47% but about 15% were due to imported eggs replenishing the shortage of eggs that arose due to the avian influenza outbreak (figure 4.19.5).

Humans

In 2005, a considerable reduction of the number of *Salmonella* isolates from relevant patient material was observed by the 16 PHLs (coverage of 64% of the Dutch population) but numbers returned to the level of 2003 in 2006. A total 1,667 isolates were sent in for typing in 2006, *i.e.* a drop of more than 40% in 10 years (addendum 4.19.4). This decrease holds true for both the predominant serotypes *S.* Typhimurium and *S.* Enteritidis that have completely different food origins. In the entire 10-year period, but also in the 10 years before 1995, the decrease primarily concerned a reduction of infections among young children, and in recent years mainly those aged between 1-5 years. The decreasing trend was interrupted several times by different incidents. First by a temporary epidemic rise of multiresistant *S.* Typhimurium DT104 in 2001, probably reflecting increasing problems with this type in cattle and pigs, and again in 2005 (addendum 4.19.5) caused by DT104 in imported beef. In 2006 more than 200 isolates of *S*. Typhimurium DT7 (provisionally typed as the Dutch phagetype ft560) were related to contaminated hard cheese in a relatively small area in the east of the Netherlands. In 2003, an explosion of cases with *S*. Enteritidis took place that was related to highly contaminated table egg imports. The imports were meant to replenish shortages due to the avian influenza outbreak in poultry in the same year. Albeit on a lower level, the

increased egg imports caused an excess of *S.* Enteritidis cases in the previous year as well (figure 4.19.5). The percentage of additional *Salmonella* isolates received due to the import of contaminated eggs in 2003 is estimated to be 17%. As in Southern Europe, travel related *S.* Enteritidis infections often involve naladixic acid resistant strains. *Salmonella* infection related hospitalizations confirm the trend in the laboratory data.

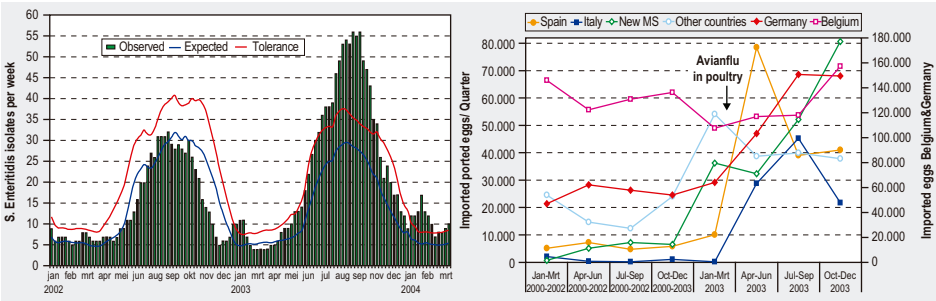


Figure 4.19.5. Seasonal course of human cases showing excess in cases in 2003 during the avian influenza outbreak far above expected (left figure: based on trends in time series five years before); concurrently table egg shortages were replenished by extra egg imports in that period (right figure).

The 1,667 laboratory-confirmed cases in 2006 amount to an incidence of 16.0 cases per 100,000 inhabitants. Dutch epidemiological studies show that in order to arrive at the true incidence of patients with salmonellosis visiting a GP or in the general population, this figure should be multiplied by about 2 and 14, i.e. 5,200 and 37,000 cases respectively.

The serotype distribution in humans (Addendum 4.19.4)

The two main serotypes in 2006 were still *S.* Enteritidis and *S.* Typhimurium comprising about 75% of all isolates. A good third that emerged in 2005 and again in 2006 is SI 1,4,5,12:i:2ef nat. The latter type is strongly emerging in other EU-countries as well, both in humans as well as in all farm animals and is probably a monophasic variant of *S.* Typhimurium ft507 and is also often multiresistant. The percentage of serotypes next in rank is about 2% or less. Strong differences exist between serotypes being travel-related or not. An average of 9% of the *Salmonella* infections between 2004-2006 were travel-related. This figure, however, is an underestimation due to underreporting. After interviewing cases in the CASA study, it proved to amount to about 25%, an underreporting of a factor of almost 3. Obvious extremes in the underreporting are the typically non-endemic infections by *S.* Typhi and *S.* Paratyphi B that in reality should have been close to 100%. *S.* Paratyphi B var. Java in humans did not enter the list of the top serotypes found in humans. The fraction in humans (0.1 - 0.9%) is negligible compared to the almost 50% of all isolates found in poultry at the end of the production chain and at retail (figure 4.19.2 and addenda 4.19.1 and 4.19.4).

The phagetype distribution of S. Typhimurium in humans (Addendum 4.19.5)

Phagetyping shows multiresistant DT-104 (the Dutch phagetypes ft-506 and ft-401) to have become predominant in the last decade (addendum 4.19.5, figure 4.19.4). In 2001 the temporary epidemic rise and the explosion of cases in 2005 of DT-104 can also be seen in *S.* Typhimurium overall, *Salmonella* spp. overall and in hospital discharges for salmonellosis in those years (addendum 4.19 4).

As in pigs, ft-507 is emerging in humans as well and is related to numerous small explosions in the north-eastern provinces of the Netherlands. Ft-507 is often multiresistant, but to a lesser extent than DT-104. This may even be an underestimation as the strongly emerging SI 1,4,5,12:i:2ef nat might be a monophasic variant of ft507 (addendum 4.19.4). *S*. Typhimurium infections are rarely reported to be travel-related and are typically domestic.

The phagetype distribution of S. Enteritidis in humans (Addendum 4.19.6)

In 2005 *S.* Enteritidis Pt-4 is still the predominant phagetype of *S.* Enteritidis but it is considerably reduced as compared to the end of the 1990's (addendum 4.19.6). Since that time Pt-21, Pt-1, Pt-6, Pt-6a, Pt-8, Pt-14b and in 2005 Pt-3, Pt-11, Pt-34 and Pt 13 became more important to some extent, reflecting their emergence in layers. In 2002 the emergence of Pt-21, Pt-8 and Pt-1 became noteworthy. To some extent this is reflected by the emergence of these phagetypes in Dutch poultry. However, some of the emergence of these phagetypes is related to travel, most noteworthy for Pt-1 and Pt-6a. This is reflected by their fluoroquinolone resistance which did not occur in domestically derived Enteritidis isolates at all until 2005. In 2006 a turning point seemed to be reached, when the relative importance of Pt-4 was comparable to 2002 again. During the explosion of 2003 the Enteritidis isolates related to contaminated egg imports could be characterized by their typical phagetypes and high levels of nalidixic acid resistance (figure 4.19.4). The most important phagetypes Pt-4 and the emerging Pt-8 seem more strongly domestically derived.

The seasonal evolution of salmonellosis

Similar to the data of the occurrence of campylobacteriosis (section 4.3), the incidence of salmonellosis in humans shows a seasonal variation. The number of *Salmonella* isolates from humans strongly increases at the end of the second quarter of each year and peaks in early September. In contrast with *Campylobacter* however, the number of *Salmonella*-positive poultry flocks does not show an explicit seasonal fluctuation. On average, 550 flocks are tested weekly and on a compulsory basis in accordance with to the PVE-monitoring programme for poultry meat. The secular trend in humans is loosely related to the consistent gradual decrease of the level of contamination of poultry flocks. Obviously, explosion incidents as mentioned above, strongly influence the number of cases but these are not related to salmonella derived from broilers. However, the higher incidence in the 3rd quarter of 2003 in both trends probably reflects the effects of repopulation of the broiler flocks after the avian influenza explosion in poultry in the spring of 2003.

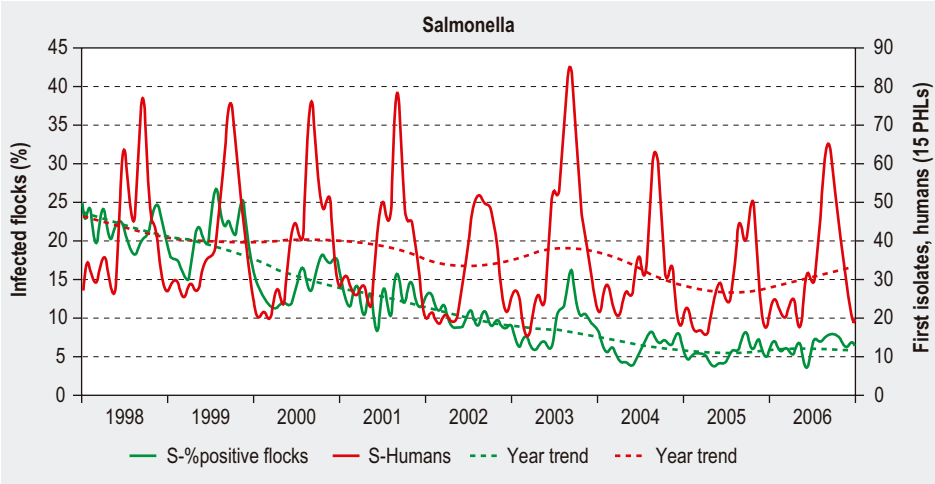


Figure 4.19.6. Seasonal and secular trend of the weekly occurrence of human cases of salmonellosis (Laboratory surveillance RIVM) and the percentage of positive flocks in the slaughterhouses (caeca). Compare with Addendum 4.19 4 and Table 4.19.4.

Contribution of travel, farm animals and their products to human salmonellosis

Using typing data of *Salmonella* spp. isolates, it was estimated which fraction of cases of human salmonellosis could be attributed to which category of farm animal and their products, or which fraction is of unknown origin, including travel (figures 4.19.7 and 4.19.8). Retrospective data of isolates derived from humans and farm animals that were routinely sent in to the NRL at the RIVM for serotyping and phagetyping were used. The estimate exploits the relative host-specificity of *Salmonella* serotypes and phagetypes. In addition to typing data from human isolates, data from isolates sent in from broilers (droppings from farms, caeca and meat products), layers (including raw materials for egg products, consumption eggs and materials from farms and hatcheries such as inlay leaflets, fluff, etc.), pigs (both adults, piglets, healthy and sick animals) and cattle (mainly dairy cattle and veal calves, healthy and sick animals) were used.

Apart from the general decrease of human salmonellosis, within poultry a gradual shift is visible from the contribution of types circulating in broilers to those found in layers. The latter is most probably related to the consumption of contaminated eggs. The slight increase in 2001 can be attributed to the epidemic increase of *S.* Typhimurium DT-104, explaining the increase of the estimated contribution of pigs and cattle in that year. In 2003, the contribution of eggs was considerably larger due to the explosion of S. Enteritidis cases related to highly contaminated imported eggs, particularly from Spain. The 17% increase related to this event is singled out in figures 4.19.7 and 4.19.8. The imported beef related explosion of DT104 in 2005 and the hard cheese related explosion with Dt-7 in 2006 have been singled out as well. This gives a more comprehensive picture of the contribution of cattle and pigs to human salmonellosis.

Figure 4.19.7 shows the estimated contributions to human salmonellosis in 2006 as compared to the average contribution in 2003-2005. The human cases were estimated

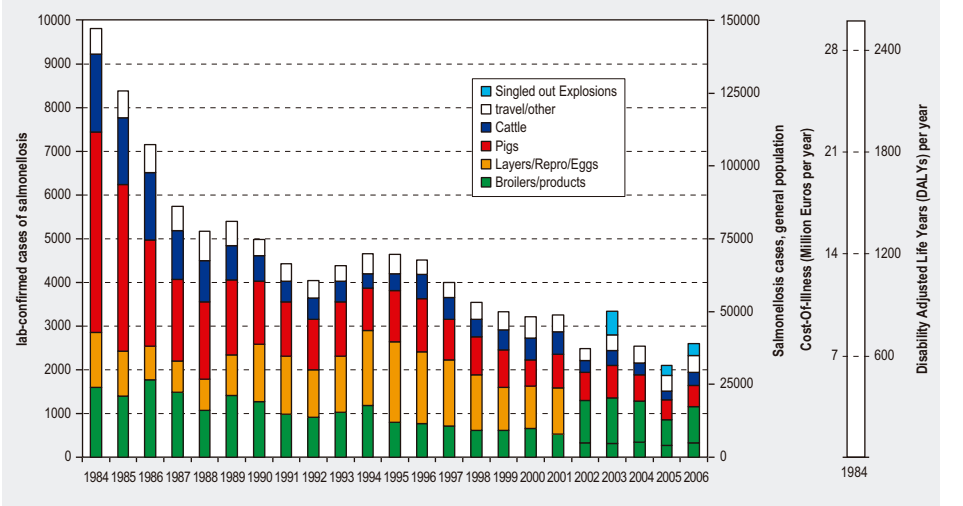


Figure 4.19.7. Estimated contribution of travel, farm animals and their products to laboratory-confirmed human salmonellosis and estimated Salmonella infection cases in the general population (Laboratory surveillance RIVM). Related estimates of Disability Adjusted Life Years and Cost-of-Illness are calibrated for 2004.

to be related to types circulating in broilers (12%), eggs (33%), pigs (18%) and cattle (12%). About 14% of the human cases were either travel-related (probably an underestimation) or from an unknown source. The hard cheese DT-7 explosion (in principal cattle derived) contributed 11%.

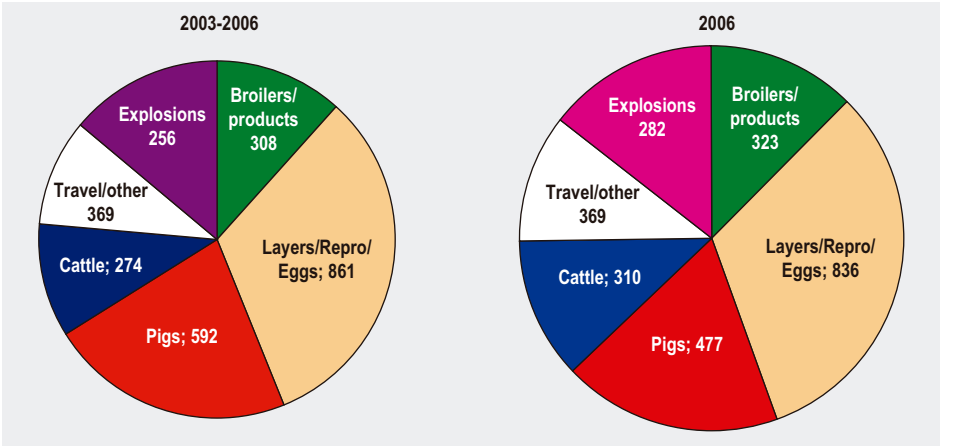


Figure 4.19.8. Estimated contribution of travel, farm animals and their products to laboratory-confirmed human salmonellosis in 2006 as compared to 2003-2005 (Laboratory surveillance RIVM).

Antibiotic resistance in Salmonella

Humans

High level resistance to ciprofloxacin was incidentally detected in *S.* Kentucky strains isolated from human patients (also detected in 2002, 2003, 2004, 2005, 2006 and 2007). These strains were related to travel to North African countries and not to Dutch food-animals. Quinolone resistance in Enteritidis from human patients was predominantly related to travel related Pt-1 and to a lesser extent to Pt-4. This indicates that quinolone resistant strains of *S.* Enteritidis isolated from humans predominantly originate from imported animal products or from travel related infections.

Farm animals

Resistance levels in *S.* Enteritidis showed a dramatic change in Pt-4 from Dutch layers in 2005. For the first year a high percentage of quinolone resistance was observed in Pt-4 from Dutch layers. This was most probably related to import of resistant organisms and not by selection through usage of (fluoro) quinolones in these animals. Striking is the increase in resistance to modern third-generation cephalosporins in *Salmonella* from poultry. This is most likely caused by transfer of resistance plasmids from commensal *E. coli* in these animals.

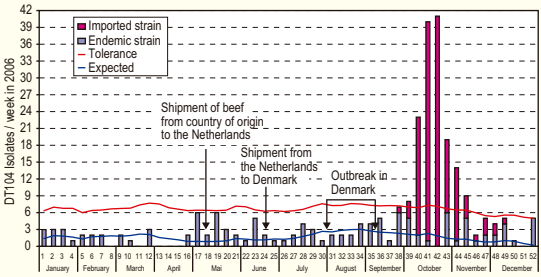
Conclusions

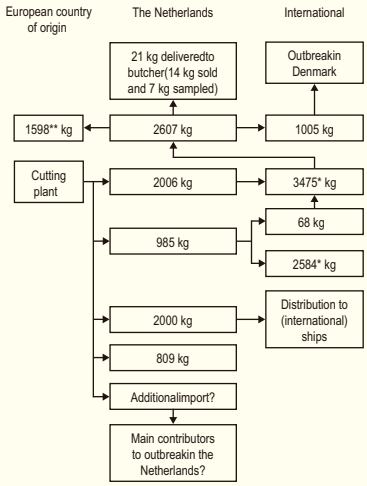
Human salmonellosis has been decreasing steadily during the past 25 years from about an estimated 150,000 cases in the general population at the beginning of the eighties up to about 37,000 in 2006. However, large outbreaks in 2003, 2005 and 2006 have shown that incidental introductions of the pathogen into the food chain are difficult to be ruled out and may have large public health consequences. Part of the reduction in recent years can probably be attributed to control programmes put into effect since the end of 1997 in the poultry production chain. In all the links of the poultry meat production chain, including retail, a gradual decrease of contamination levels is seen, especially with respect to *S.* Enteritidis. However, only about 12% of human salmonellosis can be attributed to *Salmonella* types predominating in poultry meat. Not much progress has been made in the reduction of the levels of contamination of cattle and especially of pigs in the past four years. This is of concern because pigs and cattle are the main reservoirs for multiresistant *S.* Typhimurium DT-104, which is one of the more important causes of human salmonellosis in the Netherlands. In 2001, an estimated 250 extra laboratory-confirmed cases were due to DT-104 infections, many of whom were hospitalised. The implementation of the control programme for the pig sector in February 2005 may give more control of the DT-104 problem.

A beef-associated outbreak of Salmonella Typhimurium DT104 in the Netherlands in 2005 with implications for national and international policy

Salmonella enterica serovar Typhimurium definitive phage type (DT) 104 has emerged as an important pathogen in the last two decades. The increase of DT104 has primarily been observed in Western Europe and North America and the lineage is strongly associated with multidrug resistance. The Dutch laboratory-based *Salmonella* surveillance detected an about ten-fold increase of DT104 cases in the Netherlands in September–November 2005 (Figure 1). This prompted an outbreak investigation to identify the infection source and prevent further cases. A population-based matched case-control study included 56 cases and 100 controls. Risk factors for infection were consumption of a pre-processed raw beef product (odds ratio 4.2, 95% confidence interval 1.5–12.0) and of food from mobile caterers (odds ratio 4.9, 95% confidence interval 1.1–22.1). Bacterial molecular typing established a link with another DT104 outbreak in Denmark caused by beef from a third European country. The contribution of the outbreak strain above the endemic ones is indicated in figure 1. The molecular typing included MLVA (multiple-locus variable-number tandem repeats analysis), which has been proposed as a more suitable alternative to typing DT104 and other bacteria. Product tracing of the incriminated beef was hampered by the complexity of the distribution chain and missing information (Figure 2). The beef shipments were distributed through several EU Member States. A part of the shipments was traced in the Netherlands and sampling of this beef yielded DT104 of the outbreak-associated molecular type. The investigation concluded that this outbreak was caused by imported contaminated beef. Consumers should be informed about presence of raw meat in pre-processed food products. This outbreak investigation also underlines that optimal utilization of international networks and testing and traceability of foodstuffs has the potential to prevent foodborne infections.

Right. Distribution routes uncovered by product tracing, showing the shipment of incriminated beef from the European country of origin to the Netherlands and from there further internationally, May–November 2005.
Bottom. Distributions of all *S.* Typhimurium DT104 cases in the Dutch laboratory-based surveillance by week of registration. The timings of key events that preceded the outbreak in the Netherlands are indicated.





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CASA-study. Risk factors for Salmonella Enteritidis and Typhimurium (DT104 and non-DT104) infections in the Netherlands: predominant roles for raw eggs in Enteritidis and sand boxes in Typhimurium infections

Since 1996 *Salmonella* Typhimurium DT104 salmonellosis has increased in the Netherlands. This prompted a case-control study of risk factors for salmonellosis to inform transmission routes for this phage type. Cases were laboratory-confirmed patients with a *Salmonella* infection and controls were selected from population registries by frequency matching for age, sex, degree of urbanization and season. Cases and controls received a questionnaire on risk factors. Of the 1171 cases, 573 (49%) responded: 245 *S.* Enteritidis and 232 *S.* Typhimurium cases (both DT104 and non-DT104), of which 58 were DT104. Of the 10,250 controls, 3409 (33%) responded. Use of H2-antagonists and proton pump inhibitors, consumption of raw eggs and products containing raw eggs were associated with endemic *S.* Enteritidis infection. Risk factors for endemic *S.* Typhimurium infection were use of proton pump inhibitors, occupational exposure to raw meat, playing in a sandbox (for children 4-12

years), consumption of undercooked meat and use of antibiotics. Use of proton pump inhibitors and playing in a sandbox were the only risk factors for *S.* Typhimurium DT104 salmonellosis. This study confirms known risk factors for salmonellosis. However, playing in a sandbox was a predominant new risk factor for *S.* Typhimurium salmonellosis in children (population attributable risk, PAR = 14%), and especially for S. Typhimurium DT104 (PAR = 32%).

Table Risk factors for endemic infections with Salmonella Enteritidis and S. Typhimurium in their final multivariate models.

	Controls	S. Enteritidis cases		S. Typhimurium cases	
Total N (response %)	3106 (33)	167 (47)		243 (53)	
Risk factors for endemic infections	N(%)	N(%)	PAR(%) (95%CI)	N(%)	PAR(%) (95%CI)
Undercooked meat	316 (10)	—	ns	26 (13)	7 (2-10)
Playing in sandbox (4-12 yr old)	183 (6)	—	ns	46 (24)	14 (8-17) DT104 32 (19-35)
Raw egg consumption	50 (2)	9 (5)	4 (1-5)	—	ns
Product with raw egg	237 (8)	20 (12)	5 (1-8)	—	ns
Use of antibiotics last 4 wks	105 (3)	—	ns	16 (8)	4 (0-6)
Use of proton pump inhibitors	69 (2)	15 (9)	7 (5-8)	15 (9)	8 (7-8)
Use of H2-antagonists	23 (1)	5 (3)	2 (1-3)	—	ns
Total risk factors			18		33

ns. non significant

A prolonged regional outbreak of salmonellosis by contaminated hard cheese from raw milk in the Netherlands.

A regional cluster of an unusual high number of cases with *Salmonella* Typhimurium phagetype 560 (provisionally, ft560: Dutch phagetyping system; DT7 in the Colindale system) was detected in early February 2006. At the end of 2006 more than 200 laboratory-confirmed cases of ST560 were found. This corresponds to an estimated 3.400 cases of ST560 gastroenteritis in the community. In the first 6 months of the outbreak 75% of the cases came from the same region, expanding to other parts of the counry afterwards.

Trawling questionnaires incriminated a dairy farm with a production of 5.000 liters of raw milk per day for the local production of cheese predominantely for regional retailers. Environmental samples, manure and dairy cattle of this farm were positive for ST560. However, no *Salmonella* could be detected in any product of the farm up to the end of October and no shortcomings could be shown in the hygiene control measures. Molecular typing (MLVA) largely, but not consistently, pointed to the isolates found at the dairy farm. On this evidence no formal action was taken. In August-September a case-control study was performed enrolling 51 cases and 105 regional matched controls. This strongly implicated hard cheeses from raw milk, as well as the suspected dairy farm. Closer scrutiny of the cheeses demonstrated ST560, however in very low concentrations (4 bacteria per kg); legally, Salmonella should be absent in 25gr. The number of cases dropped considerably after it was decided that all new, recent and old batches that could be traced from the farm were to be screened and destroyed if found positive. However, in 2007 up to April, another 13 cases were found.

Due to long shelf life, contaminated hard cheese may cause prolonged outbreaks. As shown for several other vehicles (soft cheese, chocolate) levels of contamination below legally accepted sampling levels may effectively cause a massive outbreak. This implies either a necessary adaptation of the Food Act for specific foodproducts or additional HACCP regulations for specific settings. MLVA has been shown to be a useful additional tool combined with sero- and phage typing to exclude non-outbreak related cases, farms and farm animals. Finally, for preventive action to be effective for public health, epidemiological evidence alone should be enough, even when microbiological proof is lacking.

Salmonella infections on Dutch dairy farms

Voluntary certification programme on Salmonella status for dairy farms in the Netherlands

A certification programme for *Salmonella* Dublin on Dutch dairy farms was started in 2000 by the Animal Health Service. This is a voluntary programme for dairy farms without signs of salmonellosis and no detectable antibody titre against *Salmonella* Dublin in bulk-milk samples. From the start of the programme about 10% of the interested farms could not be certified because of a positive bulk-milk sample. Surveillance of certified herds is performed by testing of bulk-milk samples using an LPS-ELISA three times per year. If bulk-milk samples test positive, an additional sample is tested immediately after the result of the first one is known. Herds lose their certificate if bulk-milk samples test positive on both samples. Approximately 3% of the certified herds lose their certificate annually, and about 25% of these herds will show clinical signs of salmonellosis at the time the bulk-milk samples tested positive. Farms that lose their certificate are advised to contact their veterinary practitioner to draw up a plan to control the disease.

The voluntary programme was extended in 2003 to include both *Salmonella* Dublin and *Salmonella* Typhimurium by using a combination (bulk-milk ELISA) for both Salmonella serovars. At the start of 2006 more than 3100 herds were certified, representing approximately 14% of the Dutch dairy herds.

Risk factors for Salmonella infections on Dutch dairy farms

To reduce the number of positive herds percentage knowledge about risk factors for salmonellosis is important. Therefore, a case-control study was set-up. Seventeen dairy farms with a sudden rise in antibodies in bulk tank milk (case farms) were compared with 65 herds with no rise in antibodies of bulk tank milk (control farms). A questionnaire with closed questions was used to obtain information on the clinical signs of the *Salmonella* infection (only on case farms) and on potential risk factors (on both case and control farms). The outcome of the study confirmed insights on risk factors identified earlier in the Netherlands and in Denmark enhancing the importance of factors such as purchase of manure and location of farm. Earlier studies on risk factors in the Netherlands have shown that using a closed-herd policy can decrease the introduction of the disease on a farm. The role of manure as risk factor for the introduction of *Salmonella* seems underestimated and relates to regional differences in the prevalence of *Salmonella* Dublin. The frequency of change to a positive serological status of bulk-milk samples was related to the serological status of neighbouring farms that, presumably by raising the infection level of the environment, increased the risk of introduction of *Salmonella* Dublin. A long known association exists between *Salmonella* Dublin and liver fluke (*Fasciola hepatica*).

Addendum 4.19.1. The serotype distribution of Salmonella in animals (NRL, RIVM)

Serotypes	2006					2001-2005				
	Pigs	Cattle	Poultry	Broilers	Layers	Pigs	Cattle	Poultry	Broilers	Layers
Total number	140	205	657	384	93	1863	816	7159	3130	1319
Serotype%Total	%	%	%	%	%	%	%	%	%	%
Typhimurium	60.7	27.8	4.7	3.9	8.6	54.0	19.1	3.9	4.4	4.3
Enteritidis	0.7	2.4	7.0	2.9	23.7	0.1	3.6	10.5	3.7	31.9
Paratyphi B, var, Java	0.7	—	27.5	31.0	2.2	0.3	0.4	37.2	51.7	3.2
Dublin	—	52.7	—	—	—	0.2	43.9	—	—	—
Senftenberg	0.7	—	6.8	0.5	43.0	0.3	0.2	2.8	0.9	10.3
Infantis	2.9	1.5	13.4	16.4	3.2	2.7	1.2	16.0	13.0	13.3
Virchow	—	—	8.5	8.9	2.2	0.1	2.3	3.0	2.8	4.9
Livingstone	5.7	—	2.3	2.6	2.2	2.7	1.3	3.1	3.1	4.8
Mbandaka	2.1	0.5	2.6	3.4	2.2	0.1	1.6	5.0	6.0	3.8
SI 1,4,5,12:i:2ef nat	2.9	8.3	0.9	1.3	—	0.3	0.5	—	0.1	—
Derby	9.3	—	0.3	0.5	—	10.8	0.9	0.4	0.6	0.6
Heidelberg	0.7	—	1.8	0.5	6.5	0.1	1.6	0.7	0.7	1.0
Saintpaul	—	0.5	3.2	3.4	1.1	—	—	0.2	0.1	0.5
Hadar	—	—	3.0	3.1	—	0.2	1.6	0.7	0.8	0.6
Agona	0.7	—	1.8	2.3	—	0.4	0.7	2.8	0.9	3.4
Indiana	—	—	2.0	2.9	—	—	0.1	2.2	2.4	1.2
Anatum	0.7	—	1.7	2.3	—	1.9	2.0	0.5	0.5	0.5
Goldcoast	1.4	2.4	0.2	0.3	—	2.0	0.6	0.1	0.1	0.2
Brandenburg	2.9	—	0.2	0.3	—	4.7	1.3	0.3	0.2	0.4
Kentucky	—	—	0.6	0.3	3.2	—	0.1	0.5	0.4	1.1
SI 4,5,12:d:2ef nat	1.4	—	1.1	1.3	—	1.0	1.0	0.1	—	0.5
London	0.7	—	1.1	1.6	—	2.1	0.2	0.1	0.2	—
Panama	2.1	1.0	—	—	—	2.2	0.6	0.1	0.1	0.2
Stanley	—	—	1.4	1.0	—	—	—	—	—	0.1
Lexington	—	0.5	0.5	0.8	—	0.1	1.7	0.3	0.1	0.4
SI 9,12:l,v:2ef nat	—	0.5	0.3	0.3	1.1	0.4	—	—	—	—
Cerro	0.7	—	0.2	—	1.1	0.1	—	0.2	0.1	0.3
Braenderup	—	—	0.6	0.8	—	—	—	0.5	0.3	1.8
SI 3,10:1,6:2ef nat	—	—	0.8	1.0	—	—	—	0.1	0.2	0.1
Kedougou	1.4	—	—	—	—	0.5	—	—	—	0.1
Bovismorbificans	—	0.5	—	—	—	4.7	0.1	0.1	0.1	0.2
Bredeney	—	—	0.9	0.3	—	0.1	0.7	0.1	0.1	0.4
Yoruba	—	—	0.5	0.8	—	0.1	—	0.2	0.1	0.6
Thompson	—	—	0.3	0.5	—	—	0.5	0.8	0.6	0.8
Jerusalem	—	—	0.5	0.8	—	—	—	—	—	—
Manhattan	—	—	0.2	0.3	—	1.4	0.9	1.0	0.6	—
Minnesota	—	—	0.5	0.5	—	—	—	0.1	0.2	—
Montevideo	—	—	0.3	—	—	—	2.1	0.4	0.2	0.8
Kottbus	—	—	0.3	0.5	—	—	—	0.1	0.1	0.2
Rissen	—	—	0.2	0.3	—	1.3	0.2	0.2	—	0.6
Bareilly	—	—	0.2	0.3	—	—	0.2	1.0	0.3	0.3
Corvallis	—	—	0.5	—	—	—	—	0.3	0.3	0.2
Other serotypes	1.4	1.5	1.5	2.3	—	5.2	8.6	4.3	4.0	6.7

Poultry: all chicken categories together; Broilers: including chicken products; Layers: including repro and eggs

Addendum 4.19.2. The S. Typhimurium phagetype distribution in animals (NRL, RIVM)

Phagetypes (Dutch)	2006					2001-2005				
bzw Colindale DT	Pigs	Cattle	Poultry	Broilers	Layers	Pigs	Cattle	Poultry	Broilers	Layers
Total number	85	57	31	15	8	1006	156	276	137	57
Typhimurium%Total	60.7	27.8	4.7	3.9	8.6	54.0	19.1	3.9	4.4	4.3
Ft%Typhimurium	%	%	%	%	%	%	%	%	%	%
Ft-506+/-DT-104	25.9	14.0	32.3	26.7	25.0	22.9	30.8	40.9	49.6	35.1
Ft-507, some DT	25.9	31.6	19.4	26.7	—	18.2	13.5	11.6	14.6	7.0
Ft-401+/-DT-104	1.2	—	3.2	—	12.5	6.2	—	3.6	5.1	3.5
Ft-296+/-DT-12, some DT	1.2	1.8	—	—	—	4.2	1.9	0.4	—	1.8
Ft-655, some DT	1.2	—	—	—	—	3.5	1.3	1.1	—	1.8
Ft-510, some DT	4.7	1.8	—	—	—	3.0	3.2	0.4	—	—
Ft-353	—	—	—	—	—	3.7	—	0.4	—	—
Ft-508, some DT	3.5	—	—	—	—	1.4	—	2.5	3.6	1.8
Ft-2+/-DT-2	1.2	1.8	—	—	—	0.8	1.3	4.3	—	5.3
Ft-90	—	—	—	—	—	2.1	1.3	0.4	—	1.8
Ft-350+/-DT-193, some DT	1.2	—	—	—	—	1.9	—	0.7	1.5	—
Ft-60+/-DT-12, some DT	2.4	1.8	—	—	—	1.7	0.6	—	—	—
FT560 DT-7	4.7	12.3	—	—	—	0.5	3.2	—	—	—
Ft-301, some DT	—	1.8	—	—	—	1.1	—	0.7	—	3.5
Ft-61+/-DT-12	1.2	—	—	—	—	1.4	—	0.0	—	—
Ft-80	—	3.5	—	—	—	1.0	—	0.4	—	—
Ft-20 +/-DT-124	—	—	—	—	—	1.1	—	0.4	—	—
Ft-504	4.7	1.8	—	—	—	0.1	1.3	—	—	—
Ft-656+/-DT-17	1.2	—	—	—	—	0.5	0.6	—	—	—
Other Ft	20.0	28.1	45.2	46.7	62.5	25.0	41.0	32.2	25.5	38.6

Poultry: all chicken categories together; Broilers: including chicken products; Layers: including repro and eggs. S. Typhimurium phagetypes are based on the Dutch phagetyping system. If correspondence is known with the Colindale scheme (Definitive Type: DT), this is indicated

Addendum 4.19.3. The S. Enteritidis phagetype distribution in animals (NRL, RIVM)

Phagetypes			2006					2001-2005		
(Colindale)	Pigs	Cattle	Poultry	Broilers	Layers	Pigs	Cattle	Poultry	Broilers	Layers
Total number	1	5	46	11	22	2	29	750	117	421
Enteritidis%Total	0.7	2.4	7.0	2.9	23.7	0.1	3.6	10.5	3.7	31.9
Pt%Enteritidis	%	%	%	%	%	%	%	%	%	%
Pt 4	—	60	47.8	36.4	40.9	—	24.1	37.9	36.8	39.7
Pt 21	—	—	15.2	—	31.8	—	—	16.9	25.6	7.4
Pt 6	—	—	2.2	—	4.5	—	—	8.9	7.7	11.4
Pt 7	—	—	2.2	9.1	—	50	—	8.1	3.4	10.7
Pt 1	—	—	13.0	45.5	—	50	—	5.9	9.4	5.7
Pt 8	—	—	2.2	—	—	—	3.4	5.5	5.1	5.9
Pt 6a	—	—	4.3	—	4.5	—	—	2.3	1.7	2.6
Pt 14b	—	—	—	—	—	—	—	1.9	0.9	2.4
Pt 3	—	—	2.2	—	4.5	—	6.9	0.4	—	—
Pt 11	100	20	4.3	—	9.1	—	—	—	—	—
Pt 33	—	—	2.2	9.1	—	—	—	—	—	—
Other Pt	—	20	4.3	—	4.5	—	65.5	12.3	9.4	14.3

Poultry: all chicken categories together; Broilers: including chicken products; Layers: including repro and eggs. S. Enteritidis phagetypes correspond to those in the Colindale scheme (Pt) used since 1997

Addendum 4.19.4. The evolution of the main Salmonella serotypes in humans, reported by 16 PHL's (NRL, RIVM)

Human												Travel
	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	>2003
Total number	2889	2556	2266	2127	2059	2086	1591	2142	1626	1340	1667	10%
Serotype%total	%	%	%	%	%	%	%	%	%	%	%	
Enteritidis	44.0	45.5	43.2	40.5	46.5	43.2	44.4	55.2	47.3	35.7	37.7	11%
Typhimurium	34.7	30.8	30.3	31.9	29.4	34.0	31.9	24.0	28.5	39.9	37.1	3%
SI 1,4,5,12:i:2ef nat	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	1.9	4.9	3%
Virchow	1.2	1.5	1.3	1.3	1.3	1.4	1.9	1.0	0.8	1.3	1.5	32%
Infantis	2.3	2.3	2.2	1.3	1.2	1.2	2.0	1.3	1.4	1.4	1.1	15%
Hadar	2.3	2.0	2.2	1.7	1.2	1.0	1.1	1.0	1.0	0.9	1.0	27%
Brandenburg	1.3	1.4	1.4	2.7	2.0	1.3	2.1	0.9	1.2	1.8	0.5	3%
Bovismorbificans	1.8	1.5	1.6	1.6	1.5	1.3	0.9	0.5	0.6	0.2	0.3	10%
Goldcoast	1.1	1.1	0.7	1.4	0.3	0.4	0.8	2.1	0.8	0.1	0.4	1%
Newport	0.2	0.5	0.7	0.6	0.4	0.8	0.5	0.8	0.8	0.5	0.7	24%
Derby	0.6	0.5	0.7	0.8	0.8	0.5	0.7	0.8	0.6	1.0	0.4	8%
Panama	0.6	1.0	0.7	0.8	0.4	1.8	0.3	0.3	0.5	0.4	0.3	10%
Typhi	0.8	0.9	0.7	0.9	0.8	0.6	0.6	1.0	1.1	0.7	0.5	31%
Kentucky	0.0	0.2	0.3	0.2	0.1	0.2	0.7	0.3	1.3	0.7	0.7	47%
Dublin	0.4	0.1	0.4	0.4	1.4	0.6	0.3	0.2	0.4	0.1	0.5	0%
Muenchen	0.0	0.3	0.3	0.0	0.3	0.2	0.2	0.1	0.1	0.4	0.7	20%
Heidelberg	0.1	0.3	0.8	0.7	0.5	0.2	0.1	0.5	0.8	0.4	0.4	12%
Blockley	0.2	0.2	0.3	0.4	0.2	0.2	0.3	0.2	0.1	0.4	0.5	12%
Paratyphi B	0.2	0.7	0.4	0.9	0.2	0.7	0.2	0.5	0.4	0.1	0.4	26%
Braenderup	0.2	0.4	0.6	0.8	0.6	0.2	0.5	0.3	0.1	0.4	0.4	24%
Agona	0.4	0.5	0.8	0.3	0.5	0.2	0.5	0.2	0.5	0.1	0.3	17%
Paratyphi A	0.1	0.1	0.4	0.3	0.3	0.3	0.7	0.3	0.4	0.1	0.4	51%
Stanley	0.1	0.2	0.1	0.4	0.4	0.2	0.3	0.5	0.4	0.7	0.4	33%
SI 9,12:l,v:2ef nat	0.0	0.1	0.3	0.8	0.1	0.2	0.4	0.3	0.2	0.5	0.5	0%
Corvallis	0.1	0.4	0.2	0.0	0.1	0.1	0.1	0.1	0.4	1.3	0.5	30%
Senftenberg	0.3	0.3	0.4	0.1	0.2	0.1	0.0	0.1	0.2	0.4	0.5	17%
Saintpaul	0.1	0.1	0.5	0.2	0.0	0.2	0.2	0.1	0.4	1.3	0.4	29%
Montevideo	0.2	0.4	0.2	0.4	0.5	0.1	0.2	0.2	0.4	0.4	0.4	13%
Oranienburg	0.2	0.2	0.1	0.1	0.3	0.7	0.3	0.2	0.2	—	0.4	29%
Mikawasima	—	—	0.1	—	—	—	—	—	0.2	—	0.5	0%
Bredeney	0.2	0.2	0.1	0.2	0.0	0.3	0.3	0.0	0.1	0.1	0.3	17%
Javiana	—	0.0	0.2	0.1	0.0	0.1	0.1	0.0	0.3	0.1	0.3	20%
Paratyphi B, var, Java	0.2	0.2	0.2	0.3	0.2	0.4	0.4	0.1	0.2	0.1	0.2	8%
Other serotypes	5.8	6.2	8.0	7.8	7.8	7.0	7.2	6.6	8.3	6.6	5.2	

* Changes in a year as compared to other years of any significance are indicated

Addendum 4.19.5. The S. Typhimurium phagetype distribution in humans (NRL, RIVM)

Humaan												Travel
Ft (Dutch), DT (Colindale)	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	>2003
Typhimurium%Total	34.7	30.8	30.3	31.9	29.4	34.0	31.9	24.0	28.5	39.9	37.1	3%
Total number	1002	786	686	679	605	710	508	514	463	534	618	
Ft%Typhimurium	%	%	%	%	%	%	%	%	%	%	%	
Ft-506+/-DT-104	18.2	19.6	20.7	25.9	28.6	42.4	30.7	27.8	19.0	40.3	12.8	2%
Ft-507, oth DT	5.7	3.1	1.5	2.1	1.7	7.6	12.8	25.3	22.5	18.9	18.8	2%
Ft-510, oth DT	15.4	9.5	8.3	8.7	6.4	2.3	7.7	3.9	5.0	3.6	4.4	7%
Ft-296+/-DT-12, oth DT	5.0	4.7	7.9	6.5	10.6	3.7	3.1	2.9	0.0	0.0	3.4	2%
Ft-560+/-DT-7	1.4	0.3	0.9	0.9	2.3	2.0	1.4	0.6	0.4	0.2	29.9	0%
Ft-401+/-DT-104	3.6	6.1	6.6	6.0	1.7	0.6	1.4	2.9	3.9	2.1	1.3	2%
Ft-20 +/-DT-124	2.1	2.9	9.3	6.2	3.8	0.7	1.0	0.8	2.4	0.2	0.2	6%
Ft-508, oth DT	1.9	2.4	2.5	3.1	1.0	0.6	3.1	4.7	6.0	1.7	1.1	3%
Ft-60+/-DT-12, oth DT	1.7	4.2	1.9	2.4	2.8	1.5	0.8	0.6	0.9	0.4	1.1	6%
Ft-80	3.9	2.2	3.2	3.8	2.3	1.1	1.0	0.8	1.7	0.4	0.6	5%
Ft-655, oth DT	0.8	1.3	1.3	1.2	1.0	2.3	2.2	2.3	3.7	0.2	0.2	6%
Ft-61+/-DT-12	2.2	1.4	1.7	1.6	1.8	1.8	1.4	2.1	0.4	0.6	0.6	0%
Ft-350+/-DT-193, oth DT	2.8	2.9	0.4	2.5	1.8	1.4	1.2	1.4	0.4	0.2	0.2	0%
Ft-295, oth DT	0.8	1.1	0.3	0.4	0.8	0.8	1.8	0.4	0.6	0.4	0.2	11%
Ft-651, oth DT	0.5	1.1	0.9	0.7	0.5	0.4	0.8	0.2	0.2	0.0	0.3	0%
Ft-3, oth DT	0.6	0.6	0.6	0.3	0.2	0.0	0.2	0.6	1.1	0.2	1.0	12%
Ft-10, +/-DT-3	0.8	0.5	0.6	0.3	0.5	0.6	0.2	0.8	–	0.6	0.3	11%
Ft-460	–	–	–	0.6	0.0	0.1	–	–	0.9	0.2	2.3	7%
Ft-2+/-DT-2	0.6	0.4	0.1	0.1	0.7	0.6	0.2	0.2	0.4	0.7	0.3	25%
Ft-292	0.1	0.5	0.0	0.4	0.2	0.8	–	0.2	0.4	0.2	0.6	0%
Ft-1	–	–	0.6	–	0.5	0.8	0.2	0.2	0.2	0.2	0.3	29%
Ft-290	0.2	0.1	0.1	0.1	0.2	0.1	0.6	–	0.9	0.7	0.5	23%
Other Ft	31.8	35.0	30.6	26.1	30.7	27.7	28.3	21.4	28.9	28.3	19.6	

Typhimurium phagetypes are based on the Dutch phagetyping system. If correspondence is known with the Colindale scheme (Definitive Type: DT), this is indicated. Changes in a year compared to other years of any significance are marked

Addendum 4.19.6. The S. Enteritidis phagetype distribution in humans (NRL, RIVM)

Addendum 4.19.6. The S. Enteritidis phagetype distribution in humans (NRL, RIVM)

Humaan Phagetypes (Colindale)	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Travel >2003
Enteritidis%Total	44.0	45.5	43.2	40.5	46.5	43.2	44.4	55.2	47.3	35.7	37.7	11%
Total number	1270	1163	979	862	958	902	707	1183	768	478	629	
Pt%Enteritidis	%	%	%	%	%	%	%	%	%	%	%	
Pt 4	–	80.5	70.8	62.8	54.3	49.7	51.2	34.4	29.2	30.8	50.1	7%
Pt 21	–	2.8	3.0	6.0	9.6	8.9	17.0	22.1	18.5	15.9	8.7	11%
Pt 1	–	2.3	4.3	4.1	7.1	8.1	9.2	14.3	11.1	9.8	7.5	18%
Pt 6	–	2.5	3.6	3.2	5.2	8.4	5.0	5.1	8.2	11.5	11.0	10%
Pt 8	–	0.5	0.7	0.6	0.6	1.3	4.1	8.3	13.2	7.7	6.5	8%
Pt 28	–	0.3	2.3	9.0	7.6	11.8	2.4	0.3	–	–	0.3	0%
Pt 14b	–	1.2	4.0	2.2	1.8	2.7	2.3	4.3	2.0	4.4	1.4	15%
Pt 6a	–	1.9	2.9	2.8	2.2	1.8	1.0	2.6	3.8	2.9	2.7	22%
Pt 3	–		0.4	0.2	0.4	0.3	0.7	0.6	0.5	1.5	1.6	39%
Pt 11	–	0.1	0.3	0.5	0.4	0.4	0.4	0.3	0.3	1.9	1.3	0%
Pt 4b	–	–	–	–	2.8	0.4	–	–	0.3	0.6	0.3	0%
Pt 34	–	0.2	0.4	0.7	–	0.1	–	0.3	0.8	1.5	0.8	4%
Pt 7	–	0.4	0.2	0.3	0.2	0.1	0.1	0.8	0.5	0.4	0.3	16%
Pt 13a	–	–	–	–	0.6	0.3	0.3	0.2	0.1	0.8	0.2	0%
Pt 13	–	–	–	–	0.3	0.2	–	0.1	–	1.3	0.2	0%
Pt 19	–	–	–	–	0.2	0.4	–	0.1	–	–	0.5	75%
Other Pt	100	7.3	7.2	7.5	6.6	5.0	6.4	6.4	11.7	9.0	6.7	

Enteritidis phagetypes correspond to those in the Colindale scheme (Pt) used since 1997.
Changes in a year compared to other years of any significance are indicated

4.20 *Staphylococcus aureus*, methicillin resistant

Worldwide, methicillin-resistant *Staphylococcus aureus* (MRSA) causes hospital- and community-acquired infections in humans. The prevalence of MRSA has historically been very low in the Netherlands due to the stringent 'search-and-destroy' policy and the restrictive usage of antibiotics. In 2006, a total of 2012 MRSA isolates (one per patient) were sent to the RIVM, which is an increase of 16% compared to 2005 (Figure 4.20.1). A detailed questionnaire was received for 1426 (71%) MRSA isolates. Of these MRSA isolates, 21% were isolated from wounds/abscesses/furuncles. The proportion of persons who acquired MRSA abroad (through admission or work in a hospital abroad) was 11% (Figure 4.20.1), mainly from Belgium and Germany. About 75% of the MRSA isolates were found in hospitals and 15% in nursing homes. The remaining isolates (10%) came from patients who acquired MRSA at home (possibly community-acquired MRSA). A significant proportion (14%) of all MRSA isolates in 2006 was non-typeable by Pulsed Field Gel Electrophoresis (NT). The NT isolates showed ST398 and were related to animal husbandry.

Occasionally MRSA has been cultured from dogs, cats and diseased horses, but in a survey of 200 healthy horses no MRSA was found. Recently, MRSA has been isolated from three patients who had contact with pigs. Twenty-six pig farmers were also tested and

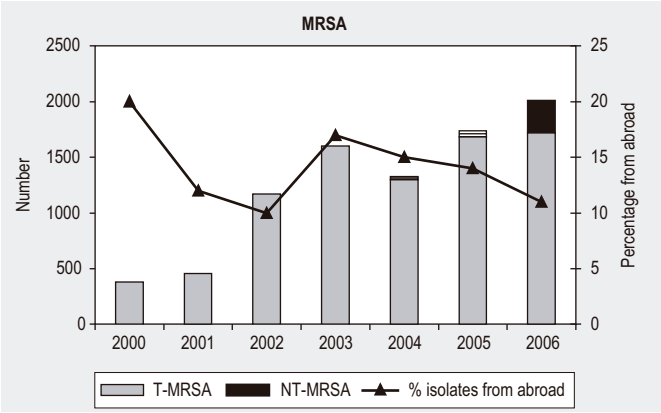


Figure 4.20.1. Numbers and the origin of MRSA in the Netherlands

six of them (23%) carried MRSA. Subsequently, MRSA was isolated from several members of a family living on a pig farm and 8 out of 10 pigs at that same farm appeared to carry MRSA. All MRSA-isolates, from human and porcine origin in these investigations, were non-typeable. Prompted by these observations, a survey on the prevalence of MRSA in slaughter pigs was conducted by RIVM and VWA.

MRSA in farm-animals

At the end of 2006, a two-year research programme on MRSA in farm animals and food was launched and was sponsored by the Dutch Ministry of Agriculture. The aim of the programme is to estimate the prevalence of NT-MRSA in pig production, in the veal calf sector, in poultry and in dairy cattle, and to determine the MRSA-contamination of food of animal origin. On pig and veal calf farms, risk factors for farms to be MRSA-positive will be analysed. Flanking projects are focussing on harmonisation of detection techniques, on typing, susceptibility testing, molecular characterization of MRSA ST398, transmission of MRSA within the pig production chain, and on the role of dust in transmission of MRSA. On pig farms and veal calf farms, farmers and their families are included in the studies to estimate the MRSA-carrier rate of the people working closely with the animals. In the beginning of 2008 slaughterhouse workers will be included in the studies to extend the study on the transmission of MRSA from farm animals to humans. As a positive MRSA-status of farms and the people living on farms, have a large impact from a social point of view, a specific project on the communication of the results towards farmers, agricultural sector and general public is part of the research programme.

MRSI. In the second half of 2006, a sharp increase in isolation rate of MRSI/MRSA (Methicillin Resistant *Staphylococcus intermedius*/Methicillin Resistant *Staphylococcus aureus*) from samples from companion animals and horses was noticed at the Veterinary Microbiological Diagnostic Centre of the Faculty of Veterinary Medicine. Not the total number but the increase is alarming (see Table 4.20.1):

Table 4.20.1. Number of isolates MRSA/MRSI from companion animals and horses

Year	Number
2005	4
2006	14
2007 (till August 22nd)	62

The increase is not the result of an increased screening or another sampling strategy. Comparable results are reported from Belgium and Sweden. The reason for the increase remains unknown. In the second half of 2007 a research project on MRSI/MRSA in companion animals and horses started. The aims of the project are to identify risk factors for these animals to become MRSI/MRSA positive, to study the dynamics of MRSI/MRSA positivity over time, and to study the transmission of MRSI/MRSA towards other animals and humans.

Survey on MRSA in slaughter pigs

From November 2005 to January 2006, a survey on MRSA in slaughter pigs was conducted by the RIVM in collaboration with the VWA. Screening of 540 pigs from 9 slaughterhouses all over the Netherlands showed 209 (39%) of the pigs to carry MRSA in their nares. Forty-four of the 54 investigated batches from different farms were affected. The percentage of MRSA positive pigs was significantly different among slaughterhouses and among batches within slaughterhouses, indicating presence of MRSA in pigs delivered to the slaughterhouse as well as cross contamination during lairage. All MRSA isolates showed Multi-Locus Sequence Type 398 and closely related *spa* types (mainly t011, t108 and t1254). Three types of the Staphylococcal Chromosome Cassette (SCC*mec*) were found: III (3%), IVa (39%) and V (57%). All isolates were resistant to tetracycline, reflecting the high use of tetracycline in pig husbandry, but were susceptible to a range of other antibiotics. Patients who had contact with pigs are now being screened and isolated to avoid MRSA from entering hospitals.

4.21. Tick-borne zoonoses

Blood sucking ticks that parasitize animals and humans are found worldwide and many of them are involved in zoonotic disease transmission (transmission of micro organisms i.e. viruses, bacteria and parasites from animal reservoirs to humans). Probably the best-known tick transmitted disease is Lyme disease, which is caused by the spirochete, *Borrelia burgdorferi,* after the bacterium has travelled from the tick salivary gland to the host's blood stream. Other well-known tick transmitted pathogenic microorganisms are: the intracellular bacteria *Anaplasma, Ehrlichia*, and *Rickettsia*; the intracellular eukaryotic protozoan parasites *Babesia* and *Theileria;* and the tick born encephalitis virus (TBEV). Different subspecies of these organisms have been associated with different diseases in humans. For instance, *B. garinii* has been associated with neuroborreliosis, *B. burgdorferi senso stricto* (s.s.) with arthritis and *B. afzelii* with acrodermatis chronica atropicans (ACA). *Ehrlichia* and *Anaplasma* species may also cause disease in canines. Worldwide there are over 800 different tick species. The most prevalent species in the Netherlands is *Ixodes ricinus* also known as the sheep tick, which is a 3-host tick. This tick has a lifecycle of two years depending on the environmental conditions

during egg, larva, nymph, and male and female adult stages. This tick species will bite many different hosts ranging from reptiles to birds and mammals including man and can transmit a variety of pathogens. Previous studies have shown that about 5 to 20% of the Dutch *I. ricinus* ticks are infected with various closely related *B. burgdorferi* species, which is also designated as *B. burgdorferi* sensu lato. In countries like Germany, Austria and Russia the tick-borne encephalitis virus (TBEV), which is also transmitted by *I. ricinus*, cause many cases of serious human disease each year. Until now TBEV has not been found in ticks in the Netherlands. In most Mediterranean countries in Europe infections with *Rickettsia* species, in particular *Rickettsia conorii*, frequently occur. The protozoan pathogen *Babesia divergens and Babesia microti* are pathogens that rarely causes disease in humans and only 25 European cases of disease caused by infection with *B. divergens* have been described so far. Mainly patients without a spleen are at risk, but in these patients the disease is often lethal. *B. divergens* is the cause of occasional outbreaks of babesiosis among cattle in the Netherlands.

Ticks

Since 2000, the tick densities in different habitats and the presence of different pathogenic *Borrelia*, *Anaplasma* and *Ehrlichia* species found in these ticks are being studied by PCR and subsequent reverse line blot (RLB) analysis by a collaboration between RIVM/ASG/Alterra. Results from 2000-2004 showed that the lowest tick density was observed in the heather area (1 - 8/100m2). In the oak forest and the city park densities ranged from 26 - 45/100m^2. The highest density was found in the dune area (139 - 551/100m^2). The infection rates of these ticks varied strongly between the four regions and years, ranging between 0.8 – 11. 5 % for *Borrelia* spp. and between 1 - 16% for *Ehrlichia/Anaplasma* spp.. *Borrelia* infection rates were highest for the dune area, followed by the oak forest and the city park and the lowest in the heather region. In contrast, the prevalence of *Ehrlichia/Anaplasma* was highest in the oak forest and lowest in the city park. RLB analyses showed the following *Borrelia* species: unspeciated *B. burgdorferi* sensu lato (2.5%); *B. afzelii* (2.5%)*; B. valaisiana* (0.9%); *B. burgdorferi* sensu stricto (0.13%); and *B. garinii* (0.13%). For *Ehrlichia/Anaplasma* these were: unspeciated *Ehrlichia/Anaplasma* spp. (2.5%); *A. schotti* variant (3.5%); *A. phagocytophilum* variant (0.3%); and *E. canis* (0.19%) is reported here for the first time in ticks in the Netherlands. About 1.6% of the ticks were double infected with *Borrelia s.l.* and *Ehrlichia/Anaplasma* spp., which was approximately 3 times more than the predicted one based on the individual infection rates. Results in 2005 and 2006 showed that the infection rates varied considerably by year. In addition, these ticks were also analysed for *Rickettsiae spp.*. The infection rate varied per region and in the dune area infection rates of 60% were found. Genotyping revealed one predominant species *R. helvetica*. The high infection rates indicate that the exposure risk to humans is high. In addition, in 2003 and 2004 in the dune area ticks were also screened for *Babesia.* This protozoan parasite was detected in 0.69% of the ticks by PCR and DNA sequence. Analysis of the partial18S rRNA showed that four ticks carried *Babesia* EU1, one *B. microti* and one *B. divergens.* This was the first finding of *Babesia* EU1 (*B. venatorum*). This *Babesia* species has been detected several times in Europe and also in a patient in Italy. Surveillance studies on ticks in the vegetation and on different animal species to elucidate the reservoirs of

tick borne human pathogens need to be further continued. In 2006, a national tick working group including RIVM, ASG, Alterra, Entomology of WUR-Wageningen and the Tick Centre of the Faculty of Veterinary Medicine was founded with the aim to collaborate more intensively in future.

Humans

Lyme disease, a multi-system infectious disease, which is transmitted by ticks and caused by the spirochete *Borrelia burgdorferi*, is the most common vector-borne infection in temperate areas of the Northern Hemisphere. A characteristic clinical sign of Lyme disease is erythema migrans, a slowly expanding skin lesion, which is recognized in about 75 - 90% of patients with objective evidence of *B. burgdorferi* infection. At this relatively harmless early stage of Lyme disease, the infection can be treated with antibiotics. Nevertheless when *B. burgdorferi* infection remains undiscovered and untreated, the infection can progress into a severe debilitating disease (neuroborreliosis) with clinical manifestations like meningoencephalitis, carditis, arthritis and dermatitis. Since Lyme disease is not notifiable in the Netherlands, retrospective studies were performed to determine the occurrence of tick bites, erythema migrans (EM) and Lyme disease in the Netherlands. In 1995, 2002 and 2006, all general practitioners in the Netherlands (approximately 8,000 general practitioners, population coverage 88, 68 and 71%) were asked to complete a postal questionnaire on tick bites and EM case-patients seen in the previous year. Annual counts of hospital admissions for Lyme disease were obtained from the Dutch National Medical Register using International Classification of Diseases-code 1048 (other spirochaetoses).

The incidence of EM consultations was estimated at 39 per 100,000 persons in 1994, and it then increased by twofold to 74 in 2001, and tripled to 103 in 2005. The incidence of tick bite consultations increased from 191 per 100,000 persons in 1994 to 372 in 2001, and continued to increase to 446 in 2005. Risk areas were found in the north and east of the country and a strip along the coast. The strongest increase in tick bites and EM was seen in the south-east of the country, the northeast and several locations along the coast (figure 1). The occurrence of hospital admissions displayed the same geographical distribution as was seen for tick bites and EM. The estimated number of hospital admissions for Lyme disease increased from 170 patients in 1994 to 229 patients in 2001, and 435 patients in 2005, increasing mainly between 2002 and 2004. It is not certain whether the increase of hospital admissions in the last years represents a true doubling in the occurrence of Lyme disease, since a new guideline for diagnosis and treatment of Lyme disease was published halfway 2003. This guideline encouraged treatment of severe Lyme disease with intravenous antibiotics, which are usually administered in the hospital. Analyses of the role of and changes in ecological risk factors and outdoor recreation, between regions and years, are forthcoming.

Conclusions

Lyme disease appears to be an increasingly important health care problem in the Netherlands. According to the afore mentioned study in which ticks were collected from 2000 till 2004 in the Netherlands, the prevalence of contamination of ticks with Bor-

relia burgdorferi (sensu lato) was 0.8 to 11%, and other pathogenic micro-organisms were also demonstrated in these ticks. In 2007 a prospective study in sentinel general practices to determine the regional differences in the level of infection of different tick-borne pathogens of ticks removed from patients is planned. Also serological tests in EM-case patients from these general practices are planned and their clinical outcome will be observed.

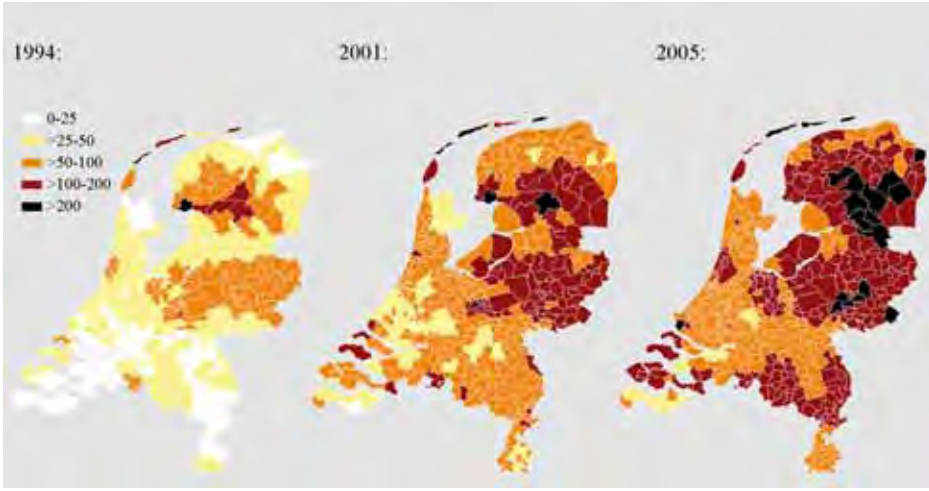


Figure 1: Geographical distribution of erythema migrans (cases / 100.000 inhabitants) in the Netherlands in 1994 (a), 2001 (b), and 2005 (c).

4.22 *Toxoplasma* spp.

Infection by the protozoan parasite *Toxoplasma gondii* is widespread in humans and many other species of warm-blooded animals. There is a widespread distribution of *Toxoplasma* infection in a variety of livestock, wild animals and pets. *Toxoplasma* may be transmitted to humans either by ingesting environmentally robust transmissive stages (oocysts with sporozoites) or by eating raw or undercooked meat containing infective tissue stages (cysts with bradyzoites).

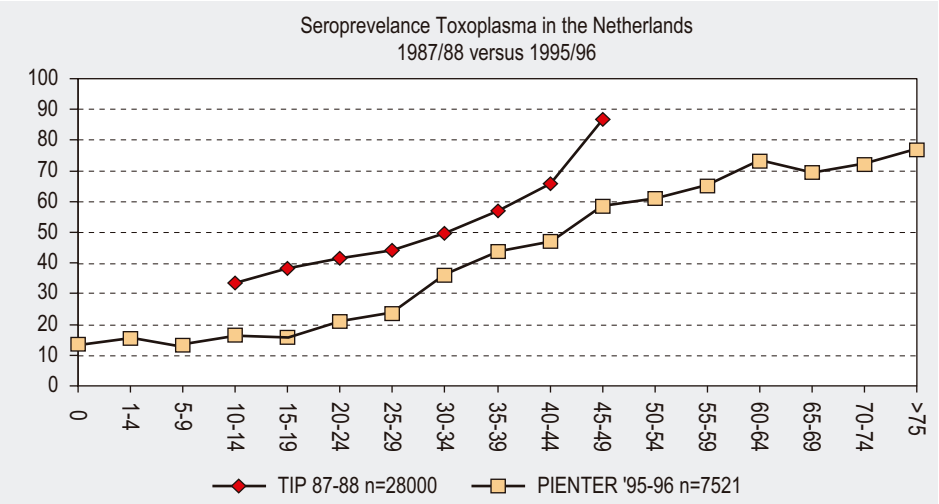
Animals

Although toxoplasmosis a notifiable disease in animals, it is almost never reported. Probably, this might only happen if the epidemiological situation gives reasons for this. However, it is important to know the status of *Toxoplasma* infections in different food producing animals in order to estimate the infection risk for humans. It is possible to significantly reduce the risk of *T. gondii* infection in livestock using intensive farm management with adequate measures of hygiene, confinement, and prevention. Previous studies in the Netherlands in pigs show a decreasing seroprevalence from 50% in 1985 to almost 0.5% in 1995. The change from extensive to industrialised indoor kept farming systems in that decade might have influenced the seroprevalence in pigs. Recent change towards more animal friendly farming systems might have an impact again on the infec-

tion risk these animals pose for humans. Indeed, studies comparing organic, free ranging and intensive farming systems in the Netherlands show that there is an increasing risk related to the animal friendly systems (see frames). Recent studies in cattle show that the seroprevalence in cattle is almost the same as 20 years ago, however the survival of tissue cysts in cattle and thus the infection risk of raw beef are still under debate. This must be clarified because eating raw or undercooked beef is an increasing food habit.

Humans

Human toxoplasmosis in not a notifiable disease in the Netherlands and there is no formal screening programme during pregnancy or in neonates to identify congenitally infected cases. During 1995-1996 a population-based seroprevalence study was conducted in the Netherlands. Risk factors were established for postnatally acquired toxoplasmosis. The results were compared with a study conducted during 1987-1988 in pregnant women in the Southwest of the Netherlands in order to estimate the change in seroprevalence. The overall seroprevalence was 40.5 %. The seroprevalence among women of 15-49 years was 10 % lower (35.2%) in the study of 1995-1996, compared to the *Toxoplasma* study in 1987-1988 (45,8%). Living in the Northwest, having professional contact with animals, living in a moderately urbanized area, being divorced or widowed, being born outside the Netherlands, frequent gardening and owning a cat were independently associated with *Toxoplasma* seropositivity. Risk factors like eating undercooked meat could not be studied. Therefore, main transmission routes in the Netherlands are not known. A study to estimate the disease burden of several gastrointestinal pathogens showed that toxoplasmosis is one of the most important diseases. However the uncertainty of the estimates was high, due to lack of data on incidence of both congenital and acquired toxoplasmosis and the lack of data on the occurrence of clinical symptoms in both groups of patients.



Conclusion

Although studies on the disease burden of toxoplasmosis indicate that it is an important disease, the system for its routine monitoring or reporting is considered to be inadequate. As such, the incidence of human disease and parasite occurrence in animals and food is undoubtedly underestimated.

Role of farming systems in the occurrence of zoonotic parasitic infections

Prevalence of parasitic infections in pigs from different housing systems may vary, due to their contact with the environment. This might pose consequences for food safety. In a collaborating study between RIVM and VWA, 40 organic, 9 free-range and 24 intensive farms were selected and a total of 845 serum samples were tested for antibodies specific for *Toxoplasma* and *Trichinella* using ELISA assays. The overall seroprevalence of *Toxoplasma* in the total number of 845 serum samples tested was 2.6%, ranging from 0.38% in intensively raised pigs to 5.62% in free-range pigs. Of the housing systems tested, 4% (intensive farms) to 33% (free-range farms) were infected with *Toxoplasma gondii*. The chance of detecting *Toxoplasma* antibodies in a free-range farm are statistically higher (almost 16 times) than in an intensive farm. We observed that the chance of detecting specific antibodies is twice as high in free range compared with organic farms. Seropositivity of *T. spiralis* antibodies was 0.12 to 0.35% (depending on the cut-off value at the 99.5% or 97.5% level). There was a tendency for *Trichinella* seropositivity to be higher in organic pig farming (0.24%), but this amount was not significant. This serological study in pigs from different farming systems shows that the seroprevalence of antibodies specific for *Toxoplasma gondii* is higher (and for *Trichinella* equivalents) in pigs raised in systems where there is contact with the environment than in pigs raised in intensive, indoor farming systems. This indicates that the prevalence of parasitic infections is higher in outdoor farming systems than in indoor farming systems.

***Toxoplasma gondii* surveys in pigs**

Introduction

Because of changes in pig production systems, the incidence of *Toxoplasma gondii* infection has declined rapidly over the past decades. In the late 1960s, pigs were often kept outdoors, and up to 75% of animals were shown to be infected with Toxoplasma gondii. Because of the indoor housing systems used today, the infection rate has dropped below 1%. The effect of the introduction of animal-friendly production systems on the incidence of Toxoplasma infection in slaughter pigs was, however, not known. Therefore a series of studies was started by the ASG of WUR in 2001

Approach

The first study in 2001/2002 was designed to investigate *Toxoplasma* seroprevalence amongst various pig production systems. Blood samples were obtained at slaughter from conventional, free range and organic pigs. Serum was tested for *Toxoplasma* antibodies by using latex agglutination and indirect immunofluorescence testing, with confirmation by immunoblotting.

In 2004 a larger number (n=2796) of organic pigs were retested from 41 different farms for *Toxoplasma* antibodies using a competitive ELISA system. At the same time the participating farmers were also interviewed to get an insight in current risk factors. All famers were associated with a slaughthouse/ distribution company and represented a selected population of organic producers.

In 2006 a questionnaire concerning *Toxoplasma* risk factors was sent to all certified organic pig holders.

Table 1. Effect of farm type on Toxoplasma seroprevalence of slaughter pigs (2001/2002 study)

Farm type	% Toxoplasma positive pigs	% Toxoplasma positive farms
organic	1.2	18
free-range	4.7	59
regular	0	0

Fig 1. Distribution of Toxoplasme positive organic pigs per farm in 2004

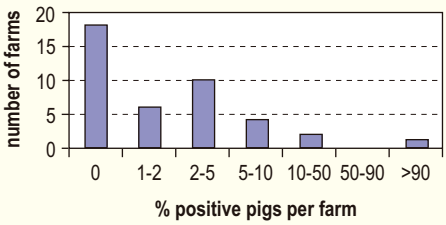


Table 2. Odds ratio estimates for Toxoplasma seroprevalence on organic pig farms.

Factor	Odds ratio	Significance
feeding goat whey	6.67	<0.01
> 3 cats on the farm	2.07	0.15
roughage not covered	13.45	<0.001
contact cat feces possible	4.55	<0.01

Results

In 2001/2002 660, 635 and 621 pigs from 16 organic, 17 free range and 30 regular farms were tested.

From June to September 2004 batches of organic pigs were consecutively tested at slaughter. The number of batches per farm ranged from 1 to 7. The total number of animals tested per farm ranged between 15 to 140 pigs per farm. Of the 2796 samples tested, 85 (3%) were positive. The number of positive animals per farm ranged from 1 to 93 % (figure 1).

During the same time period that blood samples were collected an on site survey was performed among 36 of the participating farms concerning various possible risk factors for *Toxoplasma* infection. Odds ratio estimates are shown in table 2.

As shown in figure 1 the *Toxoplasma* seroprevalence distribution is skewed to the right. When repeating the odds ratio estimate analysis excluding two extreme farms (one with 93% and the other with 27% seropositive pigs), two factors, feeding goat whey and the presence of more than 3 cats, were significantly associated with *Toxoplasma* infection.
In 2006 all certified organic pig farmers in the Netherlands were interviewed concerning *Toxoplasma* risk factors. The study also included a large number of small farms, that were not associated with the slaughterhouse/distribution company and who slaughtered their animals at a local butcher. *Toxoplasma* risk factors were each assigned a risk score which was calculated by multiplying the chance whether the described risk occurs on the farm with the severity. The severity gives an estimate of the number of animals possibly affected when the risk becomes manifest.
Of the 81 eligible organic pig farms, 52 sent in their questionnaire (64% response). Of the *Toxoplasma* risk factors, the highest number of points (9 points) were attributed to the use of an unpaved outdoor run. Only a few small farms use this type of outdoor run, whereas the larger farms all use a concrete paved run, which can easily be cleaned. Feeding animals goat or sheep whey was also given a large number of points (6 points). The presence of more than 3 cats was given 3 points. Various other factors concerning the presence of cats on various locations were given between 1 and 2 points each. In total a farm could obtain 32 points. Most of the farms had less than 10 points (33 farms) in total whereby five farms even had 0 points. Nineteen farms had a score higher than 10. The highest score was 24 points. Figure 2 shows that a relation exists between *Toxoplasma* risk score and the size of the farm.

Discussion and Conclusions

Animal friendly pig production systems are associated with a small but significant increase in the infection rate with *Toxoplasma gondii*. Sources of infection for pigs include soil, food, water or straw bedding contaminated with oocysts shed with the faeces of an infected cat. With a temperate climate and high density of cats (3.4 million), the Netherlands is an ideal country for a parasite like

Toxoplasma gondii. Other possible source of infection of pigs include tachyzoites in milk products or by the consumption of raw meat containing tissue cysts (for instance a rodent). As yet it is not clear what the main source of infection is.
Although the presence of an outdoor run has often been implicated to play an important role in *Toxoplasma* infection in pigs, a recent survey has shown that this aspect deserves further analysis. Most outdoor runs from professional organic pig farms consist of a concrete paved run which can easily be cleaned. It is unlikely that cats defecate on this run. Of interest is the observation that small "hobby like" farms who only keep a small number of pigs often have a pasture or "barren" soil type of outdoor space for their animals. This pasture may be a site where cats defecate. As yet no data are available concerning *Toxoplasma* oocyst density in soil from the outdoor runs.
Cats often have unrestricted access to all areas of the farm except the food supplies for the pigs. Roughage, which is an obligatory food source according to organic ruling, on the other hand is not always covered and can be a site were cats defecate. Straw is also accessible for cats and can be a site where young kittens defecate. A feasibility study is needed to investigate the effect of *Toxoplasma* vaccination of cats.
Water as a source of infection is unlikely since most pigs do not drink surface water, but are given tap water or water obtained from deep wells. Analysis of water samples for the presence of oocysts is needed to definitely rule out this source of infection on the farms.
Whey as a source of infection of pigs is possible although definitive proof is still lacking. Many organic pig farms also have goats, sheep or cows and local production of organic cheese is quite common. Whey from cheese making is still given to pigs. As long as transfer of infection via milk has not been excluded it is advised not to feed non pasteurised whey.
It can not be excluded that mice in the stables or in the roughage, transfer *Toxoplasma* infection to pigs. It is advised to cover roughage to prevent mouse access. Rodent control should be a continuous focus of attention on the farm. The latest survey indicates that pigs from small scale pig producing farms have a higher chance to be infected with *Toxoplasma*. Most of these farms sell their (frozen) meat products via farm stores. Since freezing is known to kill the parasite this does not pose a risk to the consumers. Some larger farms however also incidentally deliver *Toxoplasma* infected pigs for slaughter. Since one pig may be consumed by 200-400 different individuals the chance of transfer of infection is potentially present. The exact risk for the consumer has not yet been calculated since data about the actual number of *Toxoplasma* cysts in organic pig meat are not yet available. Furthermore little is known about consumer handling of organic meat (barbecues, thoroughness of cooking procedure, kitchen hygiene). Further issues include the question whether a *Toxoplasma* monitoring should be set up at slaughter, the type of monitoring (serology versus parasite detection) that might be useful, or whether risk meat should be preferentially decontaminated post slaughter.

4.23 *Trichinella* spp.

Trichinellosis is a parasitic zoonosis with a worldwide distribution and is caused by nematodes of the genus *Trichinella*, which is divided into several species (*Trichinella (T.) spiralis (T1), T. nativa (T2), T. britovi (T3), T. pseudospiralis (T4), T. murrelli (T5), T. nelsoni (T7), T. papuae (T10)*), *T. zimbabiensis (T11)* and 3 additional genotypes *T6, T8 and T9*. In Europe, *T. spiralis, T. britovi, T. nativa* and *T. pseudospiralis* have been described. Eating raw or insufficiently cooked infected meat causes human trichinellosis. Pork and horsemeat infected with *T. spiralis* (domestic life cycle) is of special importance. Infected wild or game meat can also be a source of infection.

Animals

Trichinella investigation in accordance with the Meat Inspection Law
Pork, horsemeat and game may be infected with muscle larvae of the zoonotic nematode *Trichinella*, which can cause severe disease in humans In the legislation (Directive 64/433/EEC, in force until December 31st, 2005), meat from all susceptible animals must

be examined for the presence of *Trichinella* using specific detection methods (Directive 77/96/EEC). From 2000 until 2006, a total of 104.923.305 slaughtering pigs, 18.449 horses and 5,658 wild boars were tested in the Netherlands (Table 4.23.1). Since 2006, for certain types of animals kept under specific management conditions; an alternative system for *Trichinella* control is possible (EU legislation 2075/2005). Meat inspection results from 2000-2006 indicate that the Netherlands is practically free of *Trichinella* in industrialised indoor-kept pigs. During meat inspection, only one positive pooled sample of slaughter pigs in 2002 was identified morphologically as *Trichinella*, however, it could not be confirmed by PCR. In addition, no positive pigs could be identified during follow up testing. Since 2002, all carcasses slaughtered were negative.

Table 4.23.1. Investigation in pigs with the digestion method (routine sampling, VWA)

Year	Trichinella species	Positive/ tested	%
2000		0/18,426,093	0.0
2001		0/15,378,046	0.0
2002	Not known	1/15,413,023	< 0.1
2003		0/13,983,838	0.0
2004		0/14,340,981	0.0
2005		0/13,421,601	0.0
2006		0/13,959,723	0.0

Table 4.23.2. Investigation in horses with the digestion method (routine sampling, VWA)

Year	Trichinella species	Positive/ tested	%
2000		0/3,654	0.0
2001		0/3,207	0.0
2002		0/2,638	0.0
2003		0/2,395	0.0
2004		0/2,187	0.0
2005		0/2,345	0.0
2006		0/2,023	0.0

Wild boar carcasses were investigated using 5 g of diaphragm. The results are listed in Table 4.23.3. So far no positive wild boars are being detected, indicating the low level of infections per animal (see Table 4.23.3).

Surveillance

From 1979-1995, a serological surveillance programme in an annual sample size of 100.000 fattening pigs showed that no serologically positive animals were found, indicating that indoor bred industrialised pig production is practically free of *Trichinella* infections. Since 1999, seroprevalence in wild boars varied between 3.1 and 9% but there was no significant increase (Table 4.23.5). Despite the positive finding during this serosurveillance, no positive wild boar was identified during the individual carcass control in accordance with Directive 77/96/EEC. The discrepancy between serosurveillance results and digestion results was analysed in a field study. Since 2005, the serological assay was adapted and this resulted in lower seroprevalence results.

Table 4.23.3. Investigation of wild boars with the digestion method (routine sampling, VWA)

Year	Trichinella species	Positive/tested	%
2000		0/882	0.0
2001		0/784	0.0
2002		0/1,013	0.0
2003		0/900	0.0
2004		0/945	0.0
2005		0/652	0.0
2006		0/482	0.0

Study to compare digestion and serology in wild boars (2003-2005)

A study comparing serological results and the results of a large portion of digested meat (diaphragm) of wild boar was conducted between September 2003 and March 2005. Only one animal was positive by digestion, but this animal was negative in the serology. Molecular identification of this isolate revealed that this was the first isolation of *T. pseudospiralis* (Figure 4.23.4) (*Trichinella pseudospiralis* infected animals will also be positive in the serological assay performed). All serologically positive animals were digestion negative, indicating that there was bad correlation between serological results and digestion. Moreover, the infection level in wild boar in the Netherlands was low. Since 2005, the assays performed in the serosurveillance are more correlated to the digestion results resulting in lower seroprevalence results

Table 4.23.4 Study to compare digestion and serology in wild boars (2003-2005) (source RIVM)

	Year	Positive/tested in digestion	% Positive/tested serology
Veluwe	2003-2005	0/108	20
Limburg	2004	1/47	15

Figure 4.23.1. (A). Two populations of wild boar are tested annually. In the central part of the Netherlands (Veluwe) and the southern part (Meinweg). T. pseudospiralis was first isolated in wild boar in the Netherlands in 2004 in the Meinweg (black dot) source RIVM. (B) Picture of the T. pseudospiralis isolate from wild boar, morphologically all trichinella species are indistinguishable.

Table 4.23.5. Serological investigation for Trichinella antibodies in sera of wild boars 1999-2006 (Source RIVM).

	2003	2004	2005#	2006 #
Wild boar tested	234	277	366	311
Trichinella positive	23	17	1	0
Prevalence %	9.8	6.1	0.3	0.0
95% CI	6.7-14.4	3.8-9.6	0.067-0.14	
Cumulative tested	1471	1748	2114	2425

ELISA test adapted

Humans

Trichinellosis is a notifiable disease category C. Endemic trichinellosis in humans is absent in the Netherlands since decades, but annually a few imported cases are diagnosed. In 2002, 4 human cases were reported based on serological examination and confirmation by western blotting. In 2003 there were 5 positive cases, including a family that was infected in Montenegro eating sausages at a village barbeque. In 2004, only one patient was detected, most probably with an old infection. *Trichinella* antibodies can last for a very long time, even IgM antibodies (> 15 years) and therefore it is difficult to establish the precise moment of infection.

Conclusions

In the Netherlands, all slaughter pigs are tested by the digestion method according to the Directive 77/96/EEC. Routine meat inspection showed that the parasite is practically absent in indoor raised fattening pig population. Annual serosurveillance in wild boar showed that *Trichinella* does occur, however, only at very low infection levels. In 2004, a study to compare serological results with digestion of 50 gr. diaphragm of the same animal revealed only a very low infection level. In addition, *T. pseudospiralis* was identified for the first time in wild boar Despite the occurrence of *Trichinella spp.* in the wildlife population, this has no consequences for indoor raised fattening pigs and thus for public health.

4.24 *West Nile virus*

West Nile virus (WNV) is an arthropod-borne (arbo) flavivirus that is endemic in Africa, Europe, East Asia and has quite recently also been reported in North America. The introduction of WNV into North America in 1999 was followed by a fast dissemination across the country, into Canada, Middle- and possibly South America. WNV is transmitted in natural cycles between birds and mosquitoes. Humans, horses and other susceptible mammals are incidental hosts. They do not produce a significant viremia and therefore do not contribute to the transmission of the virus. WNV has been detected in over 65 mosquito species, particularly *Culex* species, and more than 130 species of birds. Migratory birds have been implicated as long-distance transport agents for WNV in North America and recently birds in the UK were shown to be infected with WNV. Susceptible birds will be viremic for 4-7 days after exposure, after which the host

develops lifelong immunity. In the US, the highest rate of infection has been detected in birds belonging to the family Corvidae in which mortality is also high. There are two lineages of West Nile virus. Lineage 1 has emerged in the past two decades, and appears to be more virulent.
Until 1996, WNV was considered a minor public health concern in Europe. However, several outbreaks in horses and/or humans with lineage 1 viruses have occurred since then, increasing the threat of WNV infections in Europe. In 2003, WNV was found to have circulated in August and September in the South-East of France. WNV was also diagnosed in two British tourists, who developed symptoms in Portugal. Until the end of 2005, no new human cases were detected. Detection of WNV strains in Central Europe, with considerable genetic differences to the known lineage 1 and 2 viruses, demonstrates that there is reason to be alert for the introduction of new and possibly more virulent WNV strains into Europe in general, but particularly in the Netherlands.
At present, it is unclear which factors determine the dissemination of WNV, and therefore, it is difficult to estimate a risk for the potential of such an epidemic in Europe. Therefore, surveillance of WNV in humans, horses and birds was initiated in 2000. Recently, monitoring for WNV in birds has been started.

Animals

Horses
An awareness campaign was started for veterinary practitioners through a letter in the Dutch Journal of Veterinary Medicine. Practitioners were asked to contact the Equine Clinics at the Veterinary Faculty of the Utrecht University for cases of sudden neurological disease in horses. Cases were reviewed and selected for inclusion in the follow-up when no other causes for the disease were found. Horses with fever and neurological symptoms were routinely tested only for Equine Herpesvirus type 1 (EHV1) infection. From 2002-2005, a total of 109 horses with neurological symptoms were referred for further evaluation. No WNV cases were detected.

Birds
To investigate whether WNV circulates in resident birds, the WNV seroprevalence was studied in two members of the family Corvidae, the *Corvus monedula* and the *Corvus corone*. To this end, 120 serum samples of *Corvus monedula* and 80 samples of *Corvus corone* were tested by means of an inhibition ELISA followed by confirmation of positive results in a neutralisation test. Only one crow (*C. monedula*) tested positive in both tests. Furthermore, heart and kidney tissue was analysed with a polymerase chain reaction test suitable for detection of lineage 1 and 2 viruses. Preliminary results suggest that WNV may be present at a very low incidence in crows in the Rotterdam area.

Humans
In humans, most WNV infections are mild and often clinically unapparent after an incubation period of 2-14 days. However in less than 1% of WNV infections, severe neurological disease, including meningitis and (meningo) encephalitis, with some fatalities

may occur. From 2002 until November 2004, a total of 666 CSFs were collected from 6 clinical virological laboratories in the Netherlands and subsequently tested for the presence of IgM and IgG against West Nile virus. 150 samples were collected in 2002, 294 in 2003, 222 in 2004, and 187 samples were collected in 2005. None of the CSF contained detectable levels of WNV specific IgM or IgG. In addition, all CSF samples were tested negative for the presence of antibodies to Venezuelan Equine Encephalitis, Japanese Encephalitis, Yellow fever or Dengue virus.
In order to understand the level of underdiagnosis of neurological illness of humans, a syndromic surveillance approach was developed to study trends in unexplained neurological illness. The aim of the study is to assay sensitivity and specificity of this approach for use as an early warning system for unusual clusters of cases. A full report was published in 2006.

Conclusions
No cases of WNV were detected in humans or horses. The possible presence of WNV in crows, however, needs further evaluation to determine whether infections are acquired locally. At present, there is no screening of mosquito pools for WNV.

5 CONCLUSIONS

In our neatly arranged world it remains difficult to accept that animal pathogens continuously threaten public health. Effortlessly crisscrossing between species, those opportunistic critters could not care less about barriers that seem to exist between the human and the animal world. This has, however, been proven to be a misconception as human and animal health are, in fact closely intermingled.

It comes as no surprise that successful zoonoses control is about staying ahead of the game. Therefore many monitoring and surveillance programmes have been put in place in the Netherlands. Some are compulsory, some are on a voluntary basis, some are carried out on a short-term basis, and some have been implied for many years already.

This report describes the different monitoring and surveillance systems, the way these are organized and the results gathered per pathogen. Data obtained from those systems provide us with important tools to gain insight in the epidemiology of diseases and to indicate where we should intervene to minimize the risks for human health. Fortunately, control programmes such as executed for poultry have shown that indeed cases of human salmonellosis originating from poultry production have decreased over the years, demonstrating that monitoring systems can lead to successful interventions. Meanwhile, systems are being developed that not only follow up diseases but that should help us to be prepared for emerging or re-emerging pathogens.

The animal world, and the ways by which pathogens can infect us, is far from being a simple and uniform realm. We tend to incriminate animal husbandry as the most important source of trouble. Of course, animal density in many housing systems is impressive and many diseases come to us via food of animal origin. Related problems such as antibiotic resistance are a serious problem, too. However, the increase in incidence of Lyme disease that is transmitted via ticks, the spread of *Echinococcus multilocularis* via foxes, the transmission of avian influenza via wild birds, or rodents as a reservoir for hantavirus, remind us that the wildlife population, possibly via an arthropod vector or contaminating the environment, as a source of zoonoses is certainly not a negligible factor. Not to forget the many pet animals living in close contact with people, spreading diseases such as cat-scratch disease and psittacosis but also campylobacteriosis and salmonellosis.

Many people, from many different disciplines, contributed to this report. This resulted in a broad overview of the occurrence of zoonoses in the Netherlands over the period 2003-2006. Hopefully this will not only have given the reader insight in what has happened in recent years, but also inspire the many specialists from different institutions to an even closer collaboration in the future to fight zoonotic infectious diseases.

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