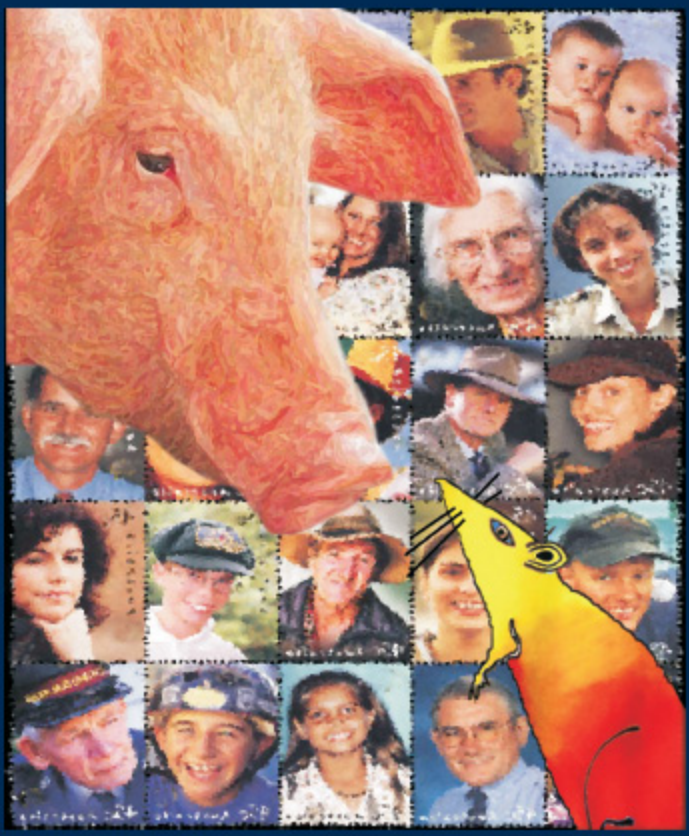
ZOONOTIC RISKS OF RODENTS IN LIVESTOCK PRODUCTION



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Zoonotic Risks of Rodents In Livestock Production

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“You care for nothing but shooting, dogs and rat-catching and you will be a disgrace to yourself and all your family.”

Robert Darwin, to his son Charles (*recounted in Darwin’s autobiography*)

“The trouble with the rat race is that even if you win, you're still a rat.”

Lily Tomlin (1939 -)

“One of the simple but genuine pleasures in life is getting up in the morning and hurrying to a mousetrap you set the night before.”

Kin Hubbard (1868 - 1930)

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This year it will have been 400 years ago that Rembrandt van Rijn, Holland's greatest 17th century painter, was born. Rembrandt (1606-1669) was only 26 years old when he completed an etching called "The Rat Poison Peddler." In terms of size it is a very modest work (it is smaller than a postcard), nothing compared with the famous “Nachtwacht”, but yet it is a marvellous expression of Rembrandt's etching skills; a vivid recreation of what was then a common street vending craft, the selling of rat poisons. The streets of 17th century Amsterdam were literally crawling with rats and mice, breeding mindlessly, destroying food supplies, contaminating kitchens, transferring diseases and some stories even claim that they were biting infants in their cradles. Rembrandt portrays the peddler with a cage containing a live rat upon his head and with the carcasses of many dead rats suspended from the lower rim of the cage. The peddler tries to convince a reluctant houseowner on the merits of his rat poisons. If Rembrandt had lingered a little longer before capturing the scene on his copper plate, he might have witnessed the peddler giving the caged rat some of his poison to demonstrate its lethal effectiveness.

Chapter 1

General Introduction

Introduction

Rodents are often seen as pests because of their gnawing habit, which can cause economic losses, spoilage of food and lead to structural damages. However, rodent presence can also have serious implications for public health. Rodents are hazardous, as they can amplify pathogens from the environment and form reservoirs of zoonotic disease (Gratz, 1994; Webster and MacDonald, 1995). Zoonotic disease (also called zoonosis) refers to those diseases that can be passed from either wild or domesticated animals to humans. A previous analysis has estimated that about 60% of all infectious disease agents affecting humans are zoonotic in origin (Taylor *et al.*, 2001) and most of the zoonotic reservoir species are rodents (Mills and Childs, 1998). Viral, bacterial and protozoan pathogens responsible for zoonotic diseases are excreted by rodent hosts or are transferred via the bite of a bloodsucking arthropod and then enter the human body via inhalation, swallowing or skin punctures (Ostfeld and Holt, 2004). The most famous zoonotic disease associated with rodent presence is probably the infection of rodent fleas with bubonic plague (caused by *Yersinia pestis*), resulting in many millions of casualties during its first (6th and 7th century AD), second (14th to 17th century AD) and third (late 19th and early 20th century AD) pandemics. Natural transmission of plague to humans remains a possibility in many regions of the world, where foci exist in sylvatic rodent populations. Even today, there are an estimated 1000–3000 cases of the bubonic plague each year worldwide, mainly in Africa, the Americas and Asia (Keeling and Gilligan, 2000). Besides bubonic plague, wild rodents can also be vectors of many other disease agents. An overview of the most important disease agents associated with rodent presence is presented in Table 1.1.

In Table 1.2 an overview is given of confirmed cases of pathogens from Dutch laboratories and an estimation of the total number of cases in The Netherlands. Although it is impossible to point out the role of rodents in transmission of pathogens specifically, Table 1.2 demonstrates the overall importance of these disease agents on public health. Of all diseases mentioned in these tables, leptospiroris is among the most well-known. It is an infectious disease of mammals and humans with a worldwide distribution, caused by the family *Leptospiracae* which contains over 200 serovars. According to the World Health Organization (www.who.int), this bacterial infection is probably the world's most widely spread zoonosis with over 100,000 human cases and 1,000 deaths annually. It is endemic to feral and domestic mammals, to amphibians and reptiles. However, *Rodentia* are the most important infection source for humans. The highest prevalence rate of leptospirosis is associated with urban

population growth, urban decay and flooding. As a result, the highest prevalence rates are found in tropical developing countries (Plank and Dean, 2000). About 90% of the *Leptospira* infections are mild (i.e. non-specific fever).

Table 1.1 Overview of the most important pathogens associated with rodents or their ectoparasites

Pathogen	Disease	Type	Reference
Yersinia pestis	Bubonic plague	Bacterial	(Keeling and Gilligan, 2000)
Leptospira spp.	Weil's disease	Bacterial	(Bunnell *et al.*, 2000a; Green *et al.*, 1978)
Yersinia spp.	Yersiniosis non-pestis/ Pseudotuberculosis	Bacterial	(Webster, 1996; Battersby *et al.*, 2002)
Listeria spp.	Listeriosis	Bacterial	(Webster, 1996; Battersby *et al.*, 2002)
Salmonella spp.	Salmonellosis	Bacterial	(Henzler and Opitz, 1992; Battersby *et al.*, 2002; Hilton *et al.*, 2002; Garber *et al.*, 2003)
E. coli 0157	Diarrheal disease etc	Bacterial	(Battersby *et al.*, 2002)
Campylobacter spp.	Campylobacteriosis	Bacterial	(Fernie and Park, 1977)
Borrelia burgdorferi	Lyme disease	Bacterial	(Nakao *et al.*, 1994; Sato *et al.*, 1996; Stafford *et al.*, 1999)
Rickettsia typhi	Murine typhus	Bacterial	(Chaniotis *et al.*, 1994; Richards *et al.*, 2002)
Ehrlichia spp.	Granulocytic ehrlichiosis	Bacterial	(Nicholson *et al.*, 1998; Stafford *et al.*, 1999)
Lassa virus	Lassa fever	Viral	(Mills and Childs, 1998)
Junin virus / Machupo virus	Argentine and Bolivian haemorrhagic fevers	Viral	(Chastel, 1993; Salazar-Bravo *et al.*, 2002)
Hepatitis E virus	Hepatitis E	Viral	(Kabrane-Lazizi *et al.*, 1999)
Cowpox virus	Human cowpox	Viral	(Hazel *et al.*, 2000; Wolfs *et al.*, 2002)
Avian Influenza virus	Avian Influenza	Viral	(Choi *et al.*, 2005)

Table 1.1 continued

Pathogen	Disease	Type	Reference
Hanta viruses	Hantavirus pulmonary syndrome etc	Viral	(Webster, 1996; Bayard *et al.*, 2004; Levis *et al.*, 2004)
Tick-borne encephalitis virus	Tick-borne encephalitis	Viral	(Takeda *et al.*, 1999)
Leishmania spp.	Leishmaniasis	Parasitic	(Chable-Santos *et al.*, 1995; Brandao-Filho *et al.*, 2003)
Babesia spp.	Babesios	Parasitic	(Sebek, 1975; Sebek *et al.*, 1980; Karbowiak and Sinski, 1996)
Entamoeba spp.	Amoebic dysentery	Parasitic	(Abd el-Wahed *et al.*, 1999; Battersby *et al.*, 2002; Bayard *et al.*, 2004)
Toxoplasma gondii	Toxoplasmosis	Parasitic	(Battersby *et al.*, 2002; Hill and Dubey, 2002; Marshall *et al.*, 2004)
Trichinella spiralis	Trichinosis	Parasitic	(Leiby *et al.*, 1990)
Fasciola hepatica	Human fasciolosis	Parasitic	(Menard *et al.*, 2000)

However, about 10% of the patients will experience hepatocellular necrosis, resulting in jaundice. Jaundice appears during days 5-9 of illness and is most intense 4-5 days later, continuing for about 1 month. Other symptoms of infection can include fever, vomiting, abdominal pain, skin rashes, conjunctival hemorrhage (characteristic, particularly with suffusion and scleral yellowing) and uveitis. There is often a severe headache, retro-orbital pain, and photophobia and a severe myalgia (lower back and legs) is common.

A relative new development is the recognition of the role of rodents in the spread of viral pathogens. Hantavirus pulmonary syndrome (HPS) is more and more recognised as an important zoonosis (Schmaljohn and Hjelle, 1997). It is a deadly disease transmitted by infected rodents through urine, droppings or saliva. The disease was first encountered in the United States in 1993. Humans can contract the disease when they inhale aerosolized virus.

Hantaviruses are carried by rodents worldwide and transmission to humans causes an estimated 60,000 to 100,000 hospitalizations annually (McCaughey and Hart, 2000).

The majority of pathogens that are mentioned in Table 1.1 are direct hazards: direct contact of humans with rodent or their ectoparasites (ticks, fleas, sand flies etc.) can result in disease. On the other hand, some pathogens that are spread by rodents are indirect hazards, which mean that they can contaminate food products (sometimes via infection of livestock). This may result in a health hazard for the human population at time of consumption. Examples of these pathogens are *Salmonella*, *Campylobacter*, *Listeria*, *E. coli*, *Trichinella spiralis* and *Toxoplasma gondii*.

Salmonella and *Campylobacter* are generally regarded as the most important cause of gastro-enteritis in the world. Furthermore, these bacteria are linked to other diseases such as reactive arthritis (which can also be caused by e.g. *Yersinia enterocolitica, Yersinia pseudotuberculosis* and *E. coli*), cardiac abnormalities (*S. Typhimurium*) and the Guillain-Barré Syndrome (Lindsay, 1997). Reactive arthritis may cause arthritic symptoms such as joint pain and inflammation, as well as urinary tract symptoms and conjunctivitis. Guillain-Barré Syndrome (or GBS) is a subacute, acquired, inflammatory demyelinating poliradiculoneuropathy that frequently occurs after acute gastrointestinal infection and is linked to previous infection with *C. jejuni* (Lindsay, 1997). The disease is characterised by motor paralysis with mild sensory disturbances and an acellular increase in the total protein content in the cerebrospinal fluid.

Control and elimination of zoonotic bacteria is a priority throughout the food chain and proper rodent management is an important preventive measure. Rodents (especially rats) frequently visit sites where these bacteria thrive (i.e. sewers & garbage disposals). If infected, rodents can transmit *Salmonella* and *Campylobacter* to humans via their droppings. More about these agents and the role of rodents can be found in Chapters 2 and 3 of this thesis.

Toxoplasma gondii is a protozoan parasite with a complex life cycle. Rodents and birds carrying the tissue cysts stage are the main source of infection for cats (Lind and Buxton, 2000). Cats play a key-role as definitive hosts: infected cats shed oocysts with their feces. Livestock animals that take up oocysts from their environment can form tissue-cysts in their meat and organs (Van Knapen, 1989). If their meat is consumed without proper cooking, the parasite may still be viable and is transferred to humans. This process is called acute infection and most cases via this route are asymptomatic (although the immunosuppressed form an exception). Congenital infection is another route of transmission of the disease and may have

Table 1.2 Confirmed cases of pathogen presence and an estimation of the total number of cases in the Netherlands

Pathogen name	**# of laboratory cases in NL in 2004**	**# of estimated cases in NL year $^{-1}$**
Yersinia pestis	0	0
Leptospira spp.	31	<50
Yersinia spp.	38	6,500
Listeria spp.	14	30-60
Salmonella spp.	2,078	50,000
E. coli 0157	29	600
Campylobacter spp.	757	80,000
Borrelia burgdorferi	Unknown	Unknown (~6,000?)
Rickettsia typhi	Unknown	<5*
Ehrlichia spp.	0	<5
Lassa virus	0	<5*
Junin virus / Machupo virus	0	0*
Hepatitis E virus	Unknown	Unknown*
Cowpox virus	Unknown	<5
Avian Influenza virus	89	89*
Hanta viruses	0	Unknown*
Tick-borne encephalitis virus	0	0*
Leishmania spp.	Unknown	100-150*
Babesia spp.	0	0*
Entamoeba spp.	16	<25
Toxoplasma gondii	Unknown	12,000
Trichinella spiralis	0	<10
Fasciola hepatica	Unknown	Unknown

* imported diseases from other countries, e.g. by travelling (source: RIVM Infectieziekten Bulletin, other sources mentioned in the text)

serious and sometimes even fatal consequences. This occurs when a woman is infected for the first time during pregnancy and the parasite crosses the placenta and invades the tissues of the foetus (Gilbert, 2000). The severity of infection depends on the time of infection during the pregnancy: severity is the greatest during the early stage of pregnancy and infection may then

result in hydrocephalus, mental retardation or even spontaneous abortion. Infection at a later stage during gestation may lead to milder symptoms such as e.g. ocular toxoplasmosis.
The risk of transmission from mother to foetus is highest during the third trimester of the gestation, when contact of maternal and foetal circulation is more likely to occur (Rothova and Kijlstra, 1989). Of these infected children, only a part will perceive clinical symptoms at birth. At a later age however, the disease may manifest itself and result in eye lesions.
For the Netherlands, it is estimated that each year 0.5-1.0% of the population (80,000 to 160,000 persons) acquires *T. gondii* infection (Kortbeek *et al.*, 2004). Most of these infections are asymptomatic, but in a minority of cases (about 12,000) the infection is symptomatic (Van Kreijl *et al.*, 2004). In Chapters 4, 5, 6 and 7 more attention will be paid to the life cycle and the prevention of infection with *Toxoplasma gondii*.

As mentioned above, rodents play a substantial role in transmission of diseases to humans and thus can form a serious health threat. Therefore, prevention of rodent presence in farming systems is necessary to reduce the risk of transmission. For this purpose, we need more knowledge about rodents: about their evolutionary development, ecology and characteristics.

Rodent ecology: field rodents vs. commensal rodents

Within *Rodentia*, a division can be made between field rodent species and commensal rodent species (in Europe: *Rattus norvegicus*, *Rattus rattus* and *Mus musculus*). Commensal rodents have the key characteristic that they share resources (feed, water, shelter) with humans, contrary to field rodents who prefer to live outside. Originally, all rodents were field rodents. However, as a result of the development of human sedentism (small villages), systematic cereal harvesting and storage and of increased human pressure on their natural habitat (Tchernov, 1984, 1991), commensal rodents probably evolved during the later Paleolithic. Another advantage of their close contact with humans was the better protection against meteorological variation and climatic changes (Cucchi and Vigne, 2005).
Commensal rodents were introduced to Europe from Asia by the exchange of goods and human migration. From archaeological small mammal collections it was learned that the progression of the house mouse started with a quick but limited diffusion in the Eastern Mediterranean region around the 8th millennium BC (Cucchi and Vigne, 2005). Then the invasive process seems to have stopped or drastically slowed until the first millennium BC, despite the increasing opportunities of passive transport during the Bronze Age. During the

first millennium BC, there was mass colonization by the house mouse of the entire Western Mediterranean Basin and Northern Europe, probably caused by increased human pressure on their habitats (Cucchi and Vigne, 2005).

The black or roof rat (*Rattus rattus*) originates from Asia and is thought to have colonized the Mediterranean basin and spread throughout northern Europe together with Roman trade, mainly between the year 400 BC and 100 AD (Audoin-Rouzeau and Vigne, 1994, 1997). The Norway rat (*Rattus norvegicus*) originates from Siberia and reached Europe after the 16th century (Cheylan, 1984), while spreading massively throughout Western Europe during the 18th century (Vigne and Villié, 1995).

Rodent classification & characteristics

Rodentia is the most abundant and diversified order of living mammals, representing about 43% of the total number of mammalian species (Wilson and Reeder, 1993; Huchon *et al.*, 2002). Its species are distributed on every continent except Antarctica and include many of the most abundant and taxonomically diverse mammals. Paleontological and molecular studies have hypothesized that the origin of rodents should have taken place close to the Cretaceous-Tertiary boundary (Asher *et al.*, 2005), about 65 million years ago. During the early Cenozoic era (Paleocene and Eocene), rodents further evolved through the process of adaptive radiation (Krause, 1984). Adaptive radiation is the process of filling ecological niches through rapid speciation of species. It is driven by both natural selection and mutation.

Because of the diversity of *Rodentia*, the phylogenetic relationships within this order are still controversial. All rodents are feeding on vegetables and this uniformity in their food and in the mode of obtaining it, namely by gnawing, has led to general uniformity, which renders their classification difficult. The traditional view is that the superfamily *Muroidea* includes over 1300 species. This superfamily consists of the *Spalacidae* (bamboo and blind mole rats), *Calomyscidae* (mouse-like hamsters), *Nesomyidae* (gerbil mice, rock mice), *Cricetidae* (voles, lemmings, muskrats, true hamsters, New World rats and mice) and the *Muridae* (Old World rats and mice) families. The *Muridae* form the largest family (comprising over 500 species), including the house mouse (*Mus musculus*) and Norway rat (*Rattus norvegicus*). *Muridae* can then be divided in the subfamilies *Deomyniae* (spiny mice, brush furred mice, link rats), *Gerbillinae* (gerbils and sand rats), *Lophiomyinae* (crested rat) and *Murinae* (Old World rats and mice). The clade *Eumuroida* was especially created in 2004 to describe a group of muroid rodents (Steppan *et al.*, 2004). This clade is not defined in the standard taxonomic hierarchy,

but it lies between superfamily and families (see Figure 1.1). All rodents are known for their single enlarged continuous growing incisors in each half of the upper and lower jaws. If these incisors are not worn down by gnawing they can grow in an outward spiral, resulting in problems with eating and increased chance of disease or mortality. The incisors are always followed by a large diastema.

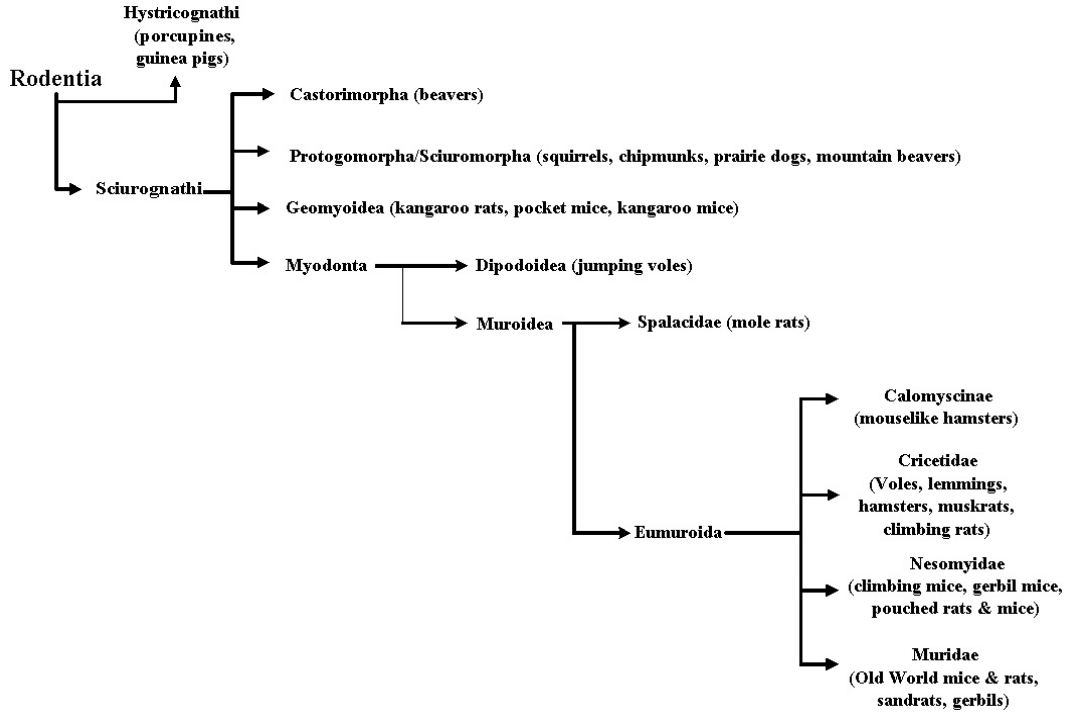

Figure 1.1 The order of *Rodentia* based on nuclear DNA phylogenies, including the Eumuroida clade (as described by Steppan *et al.*, 2004)

Although presence of the majority of rodents species is often not problematic (about 700 hundred species are even listed by the International Union for the Conservation of Nature and Natural Resources (IUCN) as 'at risk', 'threatened' or 'endangered'), some commensal rodent species can cause significant damages, as these animals can foul or consume stored agricultural products, destroy infrastructure and act as disease vectors. Field rodent species can also become pests if they appear in crops and pastures and start to reproduce quickly.

Aim of this thesis

Livestock farming can be prone to rodent infestations as it provides unlimited amounts of shelter, water and food to commensal rodents. Besides economic losses and structural

damages, these rodents may transmit pathogens directly to farmers or via livestock to consumers of livestock products. The aim of this thesis was to obtain a better understanding of the risk of rodent presence on farms for transmission of pathogens to (organic) livestock in the Netherlands and to investigate their potential influence on the safety of food products we consume.

The first objective was to provide insight into the contamination levels of wild rodents (and insectivores) with *Salmonella* and *Campylobacter* in the Netherlands, as these bacteria are the most important causes of human gastroenteritis. The second objective was to obtain more insight into the contamination of rodents with *Toxoplasma gondii*. The third objective was to find a method for sustainable though effective rodent management in (organic) livestock farming.

Outline of this thesis

In Chapter 2, a literature review is presented regarding the role of rodents in transmission of S*almonella* and *Campylobacter*. Chapter 3 describes the results of the field study performed on organic pig and broiler farms, in which the infection levels of rodents and insectivores with *Salmonella* and *Campylobacter* were measured. Chapter 4 provides a risk analysis of infection with *T. gondii* on organic pig-production facilities, while Chapter 5 describes attributable farm management factors. Wild small mammals (both rodents and insectivores) may form a transmission route for *T. gondii* to pigs. Therefore, Chapter 6 describes the results of an analysis on *T. gondii* prevalence in wild small mammals (both rodents and insectivores) present on organic pig farms. In Chapter 7 the infection of organic and regular pig meat with this protozoan parasite is further elaborated. Chapter 8 describes the ethical perspectives of rodent control and explores the humaneness of various control methods. A method for effective and sustainable rodent management for application on organic farms is described in Chapter 9. Finally, the findings in this thesis are discussed and put into a broader perspective in Chapter 10.

Chapter 2

Role of Rodents in Transmission of *Salmonella* and *Campylobacter*

B.G. Meerburg and A. Kijlstra

Submitted

Abstract

Salmonella and Campylobacter are generally regarded as the most important foodborne pathogens in the world. Reduction or elimination of these pathogens in the first part of the food chain (on-farm) is important to prevent disease among consumers of animal products. Rodents are frequently associated with infrastructural damages and eating or spoiling of stored feed and products, but the veterinary risks of rodents are often underestimated. As rodents can amplify the number of pathogens in the environment and transfer them to food animals, farmers should be aware of the need for rodent control from a food safety perspective. Preferably, rodent control should form an integral part of a total package of hygienic measures to prevent transfer of foodborne pathogens. These should also include e.g. control of wild birds and flies and obligatory disinfections of boots/clothes and equipment for farm workers and visitors.

Introduction

Prevention of food hazards in the first part of the food chain is essential to prevent illness of consumers. Control or better elimination of zoonotic bacteria such as *Salmonella* and *Campylobacter* is a priority in today's farming as human campylobacteriosis and salmonellosis are important causes of gastroenteritis in the industrialised world (Jensen *et al.*, 2004). Although rodents are often only associated with infrastructural damages and eating or spoiling of stored feed and products, the veterinary and zoonotic risks of rodents are frequently underestimated. Wild rodents can be reservoirs and vectors of a number of agents that cause disease in food animals and humans (e.g. *Leptospira* spp., *Salmonella* spp., *Campylobacter* spp., *Trichinella* spp.) (Gratz, 1994; Hiett *et al.*, 2002). In this paper we would like to focus on the role of rodents in transmission of *Salmonella* and *Campylobacter* to food animals.

Campylobacter

Campylobacter are mainly spiral-shaped, S-shaped or curved, rod-shaped bacteria. There are 16 species and six subspecies assigned to the genus *Campylobacter* of which the most frequently reported in human disease are *C. jejuni* (subspecies *jejuni*), *C. coli* and *C. fetus.* Other species such as *C. laridis* and *C. upsaliensis* are also regarded as primary pathogens, but are generally reported less frequently in cases of human disease. *Campylobacter* are bacteria that are generally regarded as the most common bacterial cause of gastroenteritis

worldwide (Heuer *et al.*, 2001). In both developing and developed countries, they cause more cases of diarrhea than, for example, foodborne *Salmonella* bacteria. It does not show any seasonal variety in developing countries, as it does in the developed world, where it peaks in summer (Blaser *et al.*, 1982). *Campylobacter* can survive in the environment for several weeks at temperatures around 4°C, but can also be present in surface water at higher temperatures (Skirrow and Blaser, 1992). Although it is unknown why, in almost all developed countries, the incidence of human *Campylobacter* infections has steadily increased the last years, although in 2002 for the first time a 5% decrease was reported in the European Union (Anonymous, 2004). In the Netherlands the rise in *Campylobacter* infections until 2002 could be partially explained by an increase in the amount of Dutch poultry meat consumption, which rose from 17.3 kg per head in 1990 to 22.4 kg in 2002 (Boogaardt *et al.*, 2004). While in the developed world incidence peaks in infants and young adults, in developing countries *Campylobacter* infections in children under the age of two years are especially frequent, sometimes resulting in death (Taylor *et al.*, 1988). Altogether, 149,287 cases of human campylobacteriosis have been reported in the EU and Norway in 2002 (Anonymous, 2004) (39 cases per 100,000 inhabitants). Individuals acquire *Campylobacter* infections mainly through contaminated poultry (chicken and turkey) or consumption of untreated surface water or unpasteurized milk. Further, infection can also be acquired by direct contact with infected animals, mainly in particular situations like for workers in poultry processing plants (Jacobs-Reitsma, 1994).

When ingested, *C. jejuni* moves to the ileum and adheres to the surface of epithelial cells of the mucus membrane. Then, a toxin is released which leads to the over-secretion of electrolytes into the gut. This results in diarrhea, which may be bloody, accompanied by headache, fever, vomiting and abdominal pain. These symptoms last for two to seven days. *Campylobacter fetus* is even more invasive. Infection can lead to spread of the organism from the gut leading to systemic infection. This can result in septicemia, pneumonia, meningitis and in pregnant females infection of the fetus can sometimes lead to spontaneous abortion. Moreover, *Campylobacter* is also linked to Guillain-Barré Syndrome, a rare but serious paralytic autoimmune disease. Serological evidence of *C. jejuni* infection occurs in about 30% of patients with Guillain-Barré Syndrome (Winer, 2001).

Campylobacter is frequently encountered in poultry flocks (Evans and Sayers, 2000). They easily spread between live birds (Shreeve *et al.*, 2000) through feces, shared water sources (Kapperud *et al.*, 1993) or in the slaughterhouse. In 2002, prevalence in broiler flocks in the

Netherlands was 27% and in Denmark 42% (Anonymous, 2004). *C. jejuni* was most often isolated which does not cause disease in chickens, but can result in foodborne illness. In poultry meat, *Campylobacter* prevalence was around 30% in the Netherlands in 2002 (Anonymous, 2004). In France, a contamination rate of 88.7% was shown on poultry meat at retail level (Anonymous, 2004). In pig herds, high infection rates of 50-80% can also be encountered (Anonymous, 2004), but in pig meat low contamination rates (2.1-4.7%) were found. In order to reduce the number of cases of human campylobacteriosis some countries (Denmark, Sweden, The Netherlands and Norway) have started monitoring programmes on *Campylobacter*. In these monitoring schemes, fecal samples or cloacal swabs are taken at farm-level, at the slaughter house or at both locations.

Salmonella

Salmonella is a rod-shaped, motile Gram-negative bacterium of the family *Enterobacteriaceae*. Nonmotile exceptions are *S. gallinarum* and *S. pullorum*. More than 2300 serotypes have been described (Popoff and Le Minor, 1997). Most of the serotypes are non-host specific (Jensen *et al.*, 2004). The seroytpes *S. typhi* and *S. paratyphi* are adapted to humans. Individuals usually obtain *Salmonella* by eating contaminated beef, pork, poultry, eggs, or vegetables contaminated with animal feces. Infection with *Salmonella* often causes gastroenteritis with symptoms similar to those seen in *Campylobacter* infections. Young children, older people and immunosuppressed persons are more susceptible to acquire severe symptoms after infection (Swanenburg, 2000). The onset of a *Salmonella* infection starts with attachment and internalisation of *Salmonella* into the cells of the small intestine. Invasion of enterocytes results in the extrusion of infected epithelial cells into the intestinal lumen with consequent villus blunting and loss of absorptive surfaces. Furthermore, *Salmonella* elicit a polymorphonuclear leukocyte influx into infected mucosa and induce watery diarrhea (Wallis and Galyov, 2000). If the bacteria then are passed out of the mucosa cells into the underlying tissues the more severe type of infection can result as bacteria reach the blood and are widely distributed.

In the European Union (EU-15) and Norway, a total of 145,231 cases of human salmonellosis have been reported (Anonymous, 2004) (38 cases per 100,000 inhabitants). *S. enteritidis* was dominating in human salmonellosis, causing 67% of all notified cases in the European Union and Norway (Anonymous, 2004). *S. typhimurium* caused 17% of all cases. Other important types were *S. infantis*, *S. virchow* and *S. hadar (*Anonymous*,* 2004*)*. Besides health problems,

economic losses of human infection with *Salmonella* and *Campylobacter* are also considerable. In a study on the socio-economic impact of infectious intestinal disease in England, average costs per case were £606 for *Salmonella* and £315 for *Campylobacter* (Roberts *et al.*, 2003).

Results from monitoring programs at slaughterhouses suggest that 20% of the broiler chickens in the United States are contaminated with harmful *Salmonella* strains (Anonymous, 1999). *Salmonella* is also very persistent: in a study on the survival of *S. enteritidis* in poultry units and poultry food, it was found that the organism can persist for at least one year in a trial house in which broilers had been housed (Davies and Wray, 1996a). Moreover, it was shown that *S. enteritidis* can survive at least 26 months in artificially contaminated poultry food (Davies and Wray, 1996a).

Infection of rodents with *Salmonella* and *Campylobacter*

Wild birds and mammals are generally regarded as the main reservoir for *Salmonella* and *Campylobacter* in the environment. These warm-blooded animals can carry both bacteria in their intestinal tracts, mostly without showing any clinical symptoms of disease (Blaser *et al.*, 1983). Infected animals can then cause transmission of pathogens from the farm environment to food animals, as is often mentioned in studies on *Campylobacter* and *Salmonella* epidemiology (Stern, 1992; Van de Giessen *et al.*, 1992; Jacobs-Reitsma, 1994; Davies and Wray, 1995b). Laboratory studies prove that in principle rodents can be infected with *Salmonella* and *Campylobacter*.

Several studies have been undertaken to estimate *Salmonella* and *Campylobacter* prevalence in wild rodents (Table 2.1). In some of these studies this estimation was based on the analysis of fecal pellets. However, as fecal pellets can become infected by deposition in a contaminated environment, the reliability of these studies is probably lower than the reliability of studies based on analysis of swabs or intestinal content.

The degree of contamination and transmission risks may differ substantially between different habitats and a distinction must be made between rodents living in nature such as wood- or grasslands, those living in urban environments and those living on farms (Table 2.1). Studies in wild rodents in woodlands have shown that only limited numbers are infected with *Campylobacter*. During a study in Norway, *Campylobacter* was not detected in any of the 44 bank voles and wood mice investigated (Rosef *et al.*, 1983).

Table 2.1 Studies on *Salmonella* or *Campylobacter* in rodents

Study	Location	Focus	Species trapped	Number	% Infected	Remarks
Rosef *et al*. (1983)	Woodlands	*Campylobacter*	*C. glareolus* (bank vole) *A. sylvaticus* (wood mouse)	24 22	0 0	Swabs
Fernie & Park (1977)	Woodlands/ Grasslands	*Campylobacter*	*C. glareolus* (bank vole) *A. sylvaticus* (wood mouse) *M. agrestis* (field vole)	13 12 17	77 0 0	Fecal pellets
Pacha *et al*. (1987)	Alpine meadows	*Campylobacter*	*M. richardsoni* (water vole) *M. longicaudus* (longtail vole) *Z. princeps* (western jumping mouse) *P. maniculatus* (deer mouse)	In total 551, not specified	<1	Fecal pellets
Hald *et al*. (in prep.)	Livestock farms	*Campylobacter*	Species not specified	44	17	
Henzler & Opitz (1992)	Layer farms	*Salmonella*	*M. musculus* (house mouse) *R. norvegicus* (Norway rat) *P. maniculatus* (deer mouse)	713 2 1	24 (species unspecified)	
Garber *et al*. (2003)	Layer farms	*Salmonella*	*M. musculus* (house mouse)	129	4	Swabs
Singh *et al*. (1980)	Urban area	*Salmonella*	*Rattus* (species not specified) *M. musculus* (house mouse)	254 109	6 10	
Davies & Wray (1995a)	Broiler and layer breeder farms	*Salmonella*	Species not specified	83 dead mice; 152 droppings	47 (intestinal analysis) 35 (liver analysis) 9 (droppings)	
Guard-Petter *et al*. (1997)	Layer houses	*Salmonella*	*M. musculus* (house mouse)	621 (year 1) 526 (year 2)	25 (year 1) 18 (year 2)	Spleens
Pocock *et al*. (2001)	Mixed farms	*Salmonella*	*M. musculus* (house mouse)	341	0	Fecal pellets
Hilton *et al*. (2002)	Urban	*Salmonella*	*R. norvegicus* (Norway rat)	50 carcasses 100 fecal swabs	10 (carcasses) 8 (swabs)	
Davies & Breslin (2003)	Free-range layer	*Salmonella*	*M. musculus* (house mouse) *R. norvegicus* (Norway rat)	25 4	44 0	Fecal pellets
Shimi *et al*. (1979)	Not specified	*Salmonella*	*M. musculus* (house mouse)	170	10	
Jensen *et al*. (2004)	Organic pig farms	*Salmonella*	*M. musculus* (house mouse) *R. norvegicus* (Norway rat) *A. sylvaticus* (wood mouse) *M. agrestis* (field vole)	2 2 9 8	0	Intestinal content
Meerburg *et al*. (2006)	Organic pig & poultry farms	*Salmonella* & *Campylobacter*	*M. musculus* *R. norvegicus* Other (*A. sylvaticus, M. arvalis, M. minutus, M. agrestis, C. glareolus*)	83 8 62	1 (*Salmonella*) 10 (*Campylobacter*) 0 (*Salmonella*) 12.5 (*Campylobacter*) 0 (*Salmonella*) 0 (*Campylobacter*)	Intestinal content

In the United Kingdom, fecal pellets voided by 13 bank voles (*Clethrionomys glareolus*), 17 field voles (*Microtus agrestis*) and 12 wood mice (*Apodemus sylvaticus*) trapped in woodlands and grasslands were investigated for *Campylobacter* presence (Fernie and Park, 1977). These authors were able to detect *Campylobacter* in 10 out of 13 bank voles tested; the other bank voles were not infected. Isolates from the bank voles resembled a type of *C. fetus* associated with infectious infertility in cattle. In a study in small rodents, in which water voles (*M. richardsoni*), longtail voles (*M. longicaudus*), Western jumping mice (*Z. princeps*) and deer mice (*P. maniculata*) were trapped on alpine meadows in the United States of America, *C. coli* was recovered in less than 1% of the isolates (Pacha *et al.*, 1987). However, these authors prove that after artificial inoculation with *C. jejuni*, water voles (*M. richardsoni*) can shed the bacterium for several weeks and have the potential to act as a reservoir in high mountainous areas. Although these studies prove that rodents living in nature can be infected, the risk that they may cause food-borne infections is generally low. For wild rodents living on or nearby agricultural premises, this risk is expected to be higher.

The environment around livestock farms varies considerably but is usually rural. Wildlife, including rodents, is attracted to spilled feedstuffs, the availability of water and presence of shelter. Generally, the species diversity around farm buildings corresponds to what can be encountered in the surrounding natural or semi-natural environment (Leirs *et al.*, 2004).

Within poultry farms, infected rodents are often reported. Some authors (Henzler and Opitz, 1992) found a high prevalence (24%) of *Salmonella enteritidis* in commensal rodents present on contaminated chicken layer farms. On the other hand, in this study *S. enteritidis* was not detected in mice on clean farms (Henzler and Opitz, 1992). This is logical, as another study revealed that prevalence of *S. enteritidis* in mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses (Garber *et al.*, 2003). In another study, mice (*Mus musculus*) captured in hen houses were assessed for the presence of *Salmonella* in spleens during two consecutive years (Guard-Petter *et al.*, 1997). It was found that during the first and second year, 25% and 18% of the spleens respectively were positive for *S. enteritidis*. Furthermore, passage of *S. enteritidis* in mice may also selectively amplify more egg invasive and virulent strains (Humphrey *et al.*, 1996). The risks of rodents regarding *Salmonella* persistance in poultry houses have only been evaluated in a few studies: in broiler-breeder and layer-breeder houses in the UK (Davies and Wray, 1995b; Evans and Sayers, 2000) and in layer houses in the USA (Henzler and Opitz, 1992; Henzler *et al.*, 1994; Guard-Petter *et al.*, 1997; Garber *et al.*, 2003).

Chances for *Salmonella* persistence on farms are about two times higher when rodents are encountered by farmers (Rose *et al.*, 2000). Rodents can be long-term sources of the infection: it was found that three-week-old chicks can acquire infection via mice artificially infected with *S. enteritidis* two and five months previously (Davies and Wray, 1995b). Artificially and naturally infected rodents (commensal *M. musculus*) were found to excrete 104 – 106 cfu/g in some individual droppings (Blaser *et al.*, 1983; Davies and Wray, 1995b), while their droppings can be contaminated for 3 months post-infection (Davies and Wray, 1995a). Only a few bacteria are necessary to infect a mouse: in case of *Salmonella* only fifteen are sufficient (Welch *et al.*, 1941; Henzler and Opitz, 1992). Mice are also easily colonized by *Campylobacter* (Berndtson *et al.*, 1994). Rodents can further amplify the number of pathogens present in the environment: isolates from mice contained three times more *Salmonella* than isolates from the environment of contaminated houses (Henzler and Opitz, 1992).

It has therefore been suggested that rodents constantly reintroduce unstable orally invasive phenotypes back into the environment of poultry (Parker *et al.*, 2001; Liebana *et al.*, 2003). Presence of a resident infected mouse population is therefore an important risk factor for egg contamination (Anonymous, 1998). Rodents can be a source of oral infection for laying hens with *Salmonella*. In this case, high density cell growth will take place in the intestine of layer hens and the bacteria will have easy access to the eggs. If human-pathogenic variants of *Salmonella* survive within the eggs, this could lead to human disease after consumption of contaminated eggs. Molecular fingerprinting (Parker *et al.*, 2001) demonstrated a close relationship between orally invasive phenotypes in laying hens that resulted in egg contamination and isolates obtained from naturally infected mice. A source for oral infection of poultry are rodent droppings, which are actively sought out by the broilers or layers when mixed in their food or bedding (Davies and Wray, 1995b), thus increasing chances of further colonization. Dead mice can also be a problem, especially if their carcasses are found in poultry houses which have been cleaned and disinfected: *Salmonella* in it may be a hazard for the new flock, as they contain higher levels of organisms than droppings and dead mice may be pecked and consumed by mature chickens (Davies and Wray, 1995b; Davies and Breslin, 2003).

Rodents can acquire their infection from various sources: they can get into contact with feces of infected livestock on the farm (a known *Salmonella* infection route; Oosterom, 1991), acquire it from other wild animals (e.g. wild birds) or get it from their own family members.

Rodents tend to live close to each other, thus enabling the infections to remain resident in the population.

On swine farms, infected rodents can also be encountered. In a recent study, 5% of the mice caught on swine farms (out of 180) were *Salmonella*-positive (Barber *et al.*, 2002). However, in other ecological compartments in this study *Salmonella* was even more abundant: in cats (12% of samples positive), boots (11%), bird feces (8%) and flies (6%). It needs to be said that the pigs on 9 of the 12 investigated farms were shedding *Salmonella* in their feces. In a recent study from our group on organic pig farms we have found that about 10% of the house mice (*M. musculus*) were *Campylobacter* positive and 1% *Salmonella* positive (Meerburg *et al.*, 2006). Moreover, 1 out of 8 Norway rats was *Campylobacter* positive. Other rodent species and all insectivores (shrews) were negative for *Campylobacter* and *Salmonella* (Meerburg *et al.*, 2006). *Salmonella* in rodents is not always encountered on farms, as is shown by two studies (Healing and Greenwood, 1991; Pocock *et al.*, 2001). These authors did not isolate any *Salmonella* in any of the house mice caught on-farm. On the other hand, true *Salmonella* prevalence could be underestimated in these studies as fecal pellets were analysed.

The use of antimicrobials for prophylaxis, therapy and growth promotion in animal production has led to an increase in antimicrobial resistance in animal pathogens and in commensal bacteria, among others, *Salmonella* and *Campylobacter*. For human health, this can have important implications as antibiotics used to cure infected humans are working less effectively. Resistance of food animals to some antimicrobials is high. In a recent study in pigs (Payot *et al.*, 2004), susceptibilities of *Campylobacter* strains were determined for five antimicrobial drugs. Resistance to tetracycline and erythromycin was high (79 and 55%, respectively; Payot *et al.*, 2004). Susceptibility testing of *Campylobacter* isolated from poultry shows similar results concerning antimicrobial resistance (Ledergerber *et al.*, 2003). Unfortunately, no information is currently available on antimicrobial drug resistance of *Salmonella*/*Campylobacter* isolates in rodents.

The presence of rats on farms has been associated with an increased risk of *Campylobacter* introduction into broiler houses (Kapperud *et al.*, 1993). Another study (Kasrazadeh and Genigeorgis, 1987) demonstrated that 87% of rat feces samples tested were positive for *Campylobacter jejuni*. Within poultry operations *Campylobacter* can also be found in mice intestines (Hiett *et al.*, 2002). A study from New Zealand revealed that 7 out of 65 house mice (*M. musculus*) caught in snap traps on a dairy farm were infected with *Campylobacter jejuni* (Adhikari *et al.*, 2002; Adhikari *et al.*, 2004). These infected rodents may contaminate feed and water, which can then become a source for *Campylobacter* colonization of food animals.

Potential transmission risks of rodents are probably even larger in organic farming (Engvall, 2001; Heuer *et al.*, 2001) as rodents live in closer contact with food animals within these farming systems because of the following reasons: 1. food animals have the possibility to go outdoors, 2. food animals are offered roughage and straw in which rodents often hide themselves, and 3. organic farmers are less willing to use rodenticides for rodent elimination as it does not fit into their farming philosophy. Some studies in pigs (Wingstrand *et al.*, 1999; Van der Wolf *et al.*, 2001) have for example shown that risks of meat juice samples being *Salmonella*-positive are higher for free-range and organic than for conventional pig herds (based on cut-off OD%>10, OD%=Optical Density of the sample, relative to the Optical Density of positive reference samples).

Importance of rodent control

Decontamination of farms is an important step in reduction of *Salmonella* and *Campylobacter* infection throughout the food chain. Although the disinfection procedure is the main risk factor of pathogen persistence after cleansing and disinfection (Rose *et al.*, 2000), the efficacy of a proper disinfection procedure is often reduced by the presence of *Salmonella*-infected mice remaining or returning to the farm after cleansing and disinfection (Davies and Wray, 1995a). Mice can acquire infections from inaccessible parts of the livestock houses and then deposit contaminated droppings on places where food animals reside. Because of this food safety risk, even the smallest infestation with rats or mice on farms needs to be addressed (Meerburg *et al.*, 2004). It has been shown that rodent control measures can effectively decrease *S. enteritidis* in the hen house (Davies and Wray, 1996b; Henzler *et al.*, 1998; Anonymous, 1999). In one study (Evans and Sayers, 2000) one hundred flocks were monitored for one production cycle to investigate risk factors for *Campylobacter* infection of broiler flocks. These authors did not encounter evidence of environmental survival of *Campylobacter* in broiler houses after adequate cleansing and disinfection. Furthermore, they did not find that rodents were a source of infection, although they state that most sites operated effective vermin-control programmes (Evans and Sayers, 2000). Farmers generally apply rodent control to prevent economical losses as rodents can cause considerable feed losses or structural damages on their farms (e.g. gnawing on insulations). However, they only do so when rodent densities exceed a certain subjective threshold (Meerburg *et al.*, 2004). This threshold apparently varies between different countries. In Denmark it was shown that mice were regularly observed on 69% of the farms, but that their presence was rarely

considered a problem by farmers (Leirs *et al.*, 2004); only 9% of the farmers considered the presence of mice problematic. Rats were more often seen as a problem (by 26% of the farmers), while they were only seen regularly on 39% of the farms. Of the farmers, 53% performed rat control on a regular basis, while for mice this was 25% (Leirs *et al.*, 2004). On the other hand, a survey in the USA among 526 farmers showed that of 28% of the farmers considered their farms to have a moderate or severe problem with mice and 9% with rats (Anonymous, 1999). However, nearly all (99%) of the farmers used some method of rodent control. Chemicals or baits were by far the most common methods of rodent control. Traps or sticky tape were used by almost one-half (46%) of farm sites, but were the primary method of rodent control for only 7% of farm sites. A professional exterminator was used on 14% of farm sites that used at least one method of rodent control (Anonymous, 1999).

A survey (Rodenburg *et al.*, 2004) has demonstrated that there is also a difference in rodent control methods between conventional and organic farmers. Conventional farmers mainly use rodenticides, whereas organic farmers also use traps and cats. Sometimes organic farmers also make use of natural predators by stimulating presence of barn owls, buzzards and kestrels on their farm by placing perches or nest boxes (Meerburg *et al.*, 2004).

Conclusion

In conclusion, both *Salmonella* and *Campylobacter* can cause serious health problems in humans. Therefore, elimination of these pathogens in the first part of the food chain should have a priority. Wild rodents are generally not much of a problem as they do not get into close contact with food animals, but rodents in agro-ecological surroundings can be infected with *Salmonella* and *Campylobacter* and transfer these pathogens to food animals or amplify the number of bacteria in the farm environment. A resident infected rodent population could lead to continuously returning infections in the farm environment. Rodent control should therefore not only be applied by farmers to prevent economic losses, but also from a veterinary perspective. Although many farmers already use various methods of rodent control, most of them only apply these after a certain subjective threshold is passed and mainly to prevent economic losses or structural damages. Therefore, there is a clear need to stress the importance of rodent control for food safety purposes. Preferably, rodent control should form an integral part of a total package of hygienic measures. These should also include e.g. control of wild birds and flies, obligatory disinfections of boots/clothes and equipment for farm workers and visitors.

Chapter 3

Presence of *Salmonella* and *Campylobacter* spp. in Wild Small Mammals on Organic Farms

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Abstract

The presence of Salmonella and Campylobacter presence in rodents and insectivores (n=282) was investigated. Infections were encountered in house mice (8 of 83 Campylobacter-positive and 1 of 83 S. Livingstone-positive) and Norway rats (1 of 8 Campylobacter-positive), not in other species. No shared Campylobacter genotypes were found between rodent and pig manure isolates. On-farm rodent management is essential.

Salmonella and *Campylobacter* spp. are the most important causes of bacterial gastroenteritis in humans and are responsible for 24% of foodborne diseases caused by known pathogens in the United States of America (Mead *et al.*, 1999; Tauxe, 2002). Food from animal origin is one of the main sources of infection (Tauxe, 2002). Prevention of the introduction of zoonotic agents in the primary production is strongly dependent on the level of biosecurity. Wild rodents may can spread zoonotic bacteria between farms (Stern *et al.*, 1985; Gratz, 1994; Hiett *et al.*, 2002; Leirs *et al.*, 2004). This risk may even be greater in organic production, where contact with livestock is more likely and rodenticides are used less often.

Rodents and insectivores were trapped on ten organic farms (nine pig farms, one broiler farm) by using live-traps between August-October 2004. Farms were sub-divided into five areas: feeding passage (near the feed trough), storage (inside the stable), outdoor area (next to the stable, solid/slatted concrete floor, sometimes roofed), pasture, and "nature area" (further from stable, wild vegetation). After CO_2 euthanasia, trapped animals were taken to the laboratory within hours. Upon arrival, their ceca and colons were processed.

Pig manure samples were collected on six out of 10 farms in October 2004. Four farms were omitted because the livestock batch had meanwhile changed. On each farm, five fresh manure samples (10g) were taken throughout the stables. These samples were pooled and analyzed for the presence of *Salmonella* and *Campylobacter*.

Rodent intestinal contents or pig manure were directly streaked on CCDA *Campylobacter*-selective agar plates (Oxoid CM739 + SR155) by using cotton swabs. These swabs were subsequently transferred to 5 ml of Preston enrichment broth (Oxoid CM67 + SR84 + SR204) without blood (Jacobs-Reitsma *et al.*, 2003). Enrichment broth was incubated for 24 h at 41.5°C under microaerobic conditions and plated onto CCDA plates. All CCDA plates were incubated for 48 hours at 41.5°C under microaerobic conditions and examined for presence of characteristic colonies of *Campylobacter*. Confirmation was performed by checking

morphology and motility microscopically. The Amplified Fragment Length Polymorphism (AFLP) method was used for species identification and genotyping (Duim *et al.*, 1999; Duim *et al.*, 2001).

For *Salmonella* detection, a swab containing 0.1 to 1 g of rodent intestinal content or pig manure was added to 9 ml of nonselective preenrichment medium, buffered peptone water (Biotrading K168B009). After preenrichment for 18 h at 37°C, 3 drops were transferred to Modified Semi-Solid Rappaport Vassiliadis agar plates (MSRV; Lab M, Lab150). MSRV plates were incubated at 41.5°C for 24 h. *Salmonella*-suspected growth on MSRV plates was streaked on Brilliant Green Agar (BGA, Oxoid CM329) and examined for presence of suspected colonies after incubation for 18 to 24 h at 37°C. A suspected colony was then agglutinated with polyvalent anti-specific O-antisera (Oxoid) and specific flagellar H antisera. Each isolate was biochemically confirmed (triple sugar iron [Oxoid] agar, urease and lysine decarboxylase).

In total, 282 animals were examined and 9 were positive for *Campylobacter* (8 of 83 house mice, 1 of 8 brown rats). One *Salmonella* sp. strain Livingstone-positive house mouse was detected. Positive animals were only found in the storage, feeding passage and outdoor areas (Table 3.1). On three farms (A, B, and C), three single rodent isolates were obtained. On farm A, 1 of 6 (16.7%) house mice was positive (*Campylobacter coli*). On farm B, 1 of 8 (12.5%) brown rats caught was positive (*C. coli*). On farm C, 1 of 30 (3.3%) house mice was positive (*Campylobacter* spp.). This isolate was lost in follow-up and could not be speciated. On farm D, 1 *C. hyointestinalis*, 2 *C. coli* and 3 *C. jejuni* strains were isolated from 6 of 15 (40%) house mice that were caught. The *Salmonella* spp. strain Livingstone-positive house mouse (also positive for *C. jejuni*) was caught here. *Salmonella* and *Campylobacter* spp. were not isolated from the pig manure at this farm. Overall, *Campylobacter* was isolated from pig manure on three of six farms (farms A, B and E), but *Salmonella* strains were not encountered.

AFLP analysis revealed no direct shared genotypes of *Campylobacter* isolates from rodents and those from pigs at farms A and B (Fig. 3.1; a cut-off point of 95% similarity to define identical isolates was chosen). C*ampylobacter* strains in two house mice caught at the same time at exactly the same trapping point on farm D were indistinguishable (Fig 3.1, numbers 244 and 245). Further examination of this particular farm showed that almost all (five of six)

Campylobacter-positive animals were found in one pig stable. The sixth infected animal was trapped in the feeding passage of another stable, only 25 metres away. No infections were encountered elsewhere.

Although we realize the limitations concerning the number of herds and their microbiological status, our study shows that on Dutch organic farms two rodent species were found to be carriers of *Salmonella* and C*ampylobacter*. *Salmonella* prevalence was limited, a finding similar to a previous study (Pocock *et al.*, 2001) in which fecal pellets from house mice caught on-farm were analyzed (0 of 222 animals infected). However, *Salmonella* prevalence may increase when rodents are caught near a *Salmonella* sp.-positive herd or flock. The *Campylobacter* species found in rodents in the present study were *C. jejuni*, *C. coli* and *C. hyointestinalis* subsp. *hyointestinalis*.

Figure 3.1 AFLP analysis of *Campylobacter*-strains identified in the study, linked to farms of origin. M, house mouse; R, rat; P, pig.

Although no shared *Campylobacter* genotypes in rodents and pigs were found, horizontal transmission by infected rodents cannot be excluded. Pigs can carry multiple *Campylobacter* strains (mixed infections), a phenomenon also observed in poultry (Jacobs-Reitsma *et al.*, 1995; Bang *et al.*, 2003). In turn, this phenomenon may facilitate genetic exchange between different *Campylobacter* strains in the pig gastrointestinal tract under normal farm conditions. Such genetic instability under natural conditions could undermine the application of genetic subtyping (De Boer *et al.*, 2002). Hypothetically, infected rodents and pigs can acquire their infection from the same source or by infecting each other after having acquired the infection from some other source. For example, this could result from the consumption of an infected rodent by a pig or by a mouse foraging through infected pig manure.

Understanding of pathogens is important to ensure livestock health and food safety. Here, house mice and brown rats were found to carry *Salmonella* and *Campylobacter*, but rodents can also transmit other pathogens, such as *Toxoplasma gondii* (Kijlstra *et al.*, 2004b). Transmission risks might be limited, but a reliable assessment is necessary to quantify the risks posed by rodents. Organic farmers should apply effective rodent management that is in line with organic principles to protect livestock and human health (Meerburg *et al.*, 2004).

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Table 3.1 Cross-tabulation of animal species (n = 283), their numbers and the area in which they were encountered[a]

Species	Latin name	Total no.	*No. found in (area of farm)[a]:*				
			Feed passage	Storage	Outdoor area	Pasture	Nature area
White-toothed shrew	*Crocidura russula*	*119*	4	20	29	23	43
Common shrew	*Sorex araneus*	10	0	0	0	2	8
House mouse	*Mus musculus*	83	37(2*)	21 (1*)	14 (5*,1†)	7	4
Common vole	*Microtus arvalis*	31	1	2	2	12	14
Wood mouse	*Apodemus sylvaticus*	19	0	1	0	3	15
Brown rat	*Rattus norvegicus*	8	8 (1*)	0	0	0	0
Harvest mouse	*Micromys minutus*	6	0	0	0	0	6
Field vole	*Microtus agrestis*	*3*	0	0	0	2	1
Bank vole	*Clethrionomys glareolus*	3	0	0	0	2	1
Northern vole[b]	*Microtus oeconomus*	1	0	0	0	0	1

[a] Numbers indicate number of animals captured, positives between brackets (* *Campylobacter* positive; † *Salmonella* positive).
[b] This animal was released again because of its endangered status in the Netherlands.

Chapter 4

Animal-Friendly Production Systems may cause Re-emergence of *Toxoplasma gondii*

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Abstract

Toxoplasmosis is still one of the most common parasitic infections in the world, although in Europe improvements in hygiene and introduction of 'total' indoor farming in livestock production have rapidly diminished the problem during the past decades. As result of public dislike however, introduction of alternative and more acceptable animal-friendly livestock production systems including outdoor access are gaining ground. Potentially these systems can lead to increased prevalence of certain zoonotic diseases, including toxoplasmosis. To retain prevalence of this disease in humans at current levels, emphasis should be on disease control at farm-level. This article provides an analysis of various risk factors for farm animals to get infected with Toxoplasma gondii. Access of cats on farm premises, use of compost, goat whey and rodent control were identified as possible risk factors that should be addressed on these farms. Consumers should be aware of the fact that Toxoplasma gondii infection, besides through meat, can also be caused by the uptake of contaminated water, soil, fruit and vegetables.

Introduction

Toxoplasmosis, caused by *Toxoplasma gondii* is currently the most prevalent parasitic zoonotic disease throughout the world (Tenter *et al*., 2000). It is an important cause of abortion in humans and livestock (sheep) and was recently shown to be the third cause of death following foodborne illnesses (Mead *et al*., 1999). In humans it is further known to cause mental retardation, encephalitis and blindness. Although not fatal, ocular toxoplasmosis is probably the most frequently occurring complication of this disease (Holland, 2003) whereby eventually 24% of the affected persons becomes blind (Bosch-Driessen *et al*., 2002). Over the years a large number of anti-parasitic drugs have been developed to treat patients with toxoplasmosis, with good results under certain conditions. However, the drugs developed so far were not always effective in the treatment of ocular toxoplasmosis (Stanford *et al*., 2003). So at present, prevention of *Toxoplasma* infection is the only strategy to combat the blinding complications of toxoplasmosis. Since meat consumption is one of the main risk factors, prevention should also be aimed at the stage of livestock production.

Over the past decades animal farming in Western Europe has changed drastically. Some of these changes were not in favour of animal welfare and have led to the development of alternative production systems such as organic animal husbandry. At this moment, the impact of these animal-friendly production systems on certain zoonotic diseases such as

toxoplasmosis is not clear. In this short review we shall discuss risk factors for an animal to become infected with *Toxoplasma gondii* and present a risk analysis with a number of control points to limit the on-farm risk.

Parasitology

Toxoplasma gondii is a ubiquitous protozoan parasite capable of infecting virtually all warm-blooded vertebrates throughout the world. It is an obligate intracellular organism belonging to the Coccidian family. Three strains of *Toxoplasma gondii* have been defined, (type I, type II and type III), of which type I is extremely virulent for mice and type II has been associated with the majority of toxoplasmosis cases in AIDS patients (Boothroyd and Grigg, 2002; Klaren and Kijlstra, 2002). Type III is present in animals and has been detected in AIDS patients, but does not seem to be associated with ocular toxoplasmosis (Boothroyd and Grigg, 2002).

The parasite has a complex life cycle whereby *Felidae* (cats) function as the definitive host, i.e., the sexual part of the life cycle takes place in these animals. Fusion of gametocytes and zygotes takes place in the gut of catlike animals leading to the formation of eggs (oocysts). Cats have been shown to shed millions of oocysts via their feces into the environment during a period of a few weeks (Dubey, 2001). Oocysts must mature (sporulate) for 1–5 days to become infective for other hosts. Sporulated oocysts can remain in a moist environment for a number of years (Dubey and Beattie, 1988), since they are resistant to a large number of threats such as heat and cold. Further prolongation of the oocysts' lifetime may be due to the uptake by other organisms in the soil or water. A number of Western European countries (e.g. The Netherlands, Belgium, United Kingdom, Germany and Denmark) have optimal climatic conditions for a parasite like *Toxoplasma gondii* to thrive, due to their moist summers and generally temperate winters. As there is also a large population of pet cats in these countries, it is not remarkable that toxoplasmosis is now recognized as a serious health problem in these countries.

When an intermediate host ingests a sporulated *Toxoplasma* oocyst the parasite transforms into a stage called the tachyzoite. Tachyzoites can virtually infect any nucleated cell type, although a tropism for certain cell types (for instance retinal vascular endothelial cells) has been reported (Smith *et al.*, 2004). After invading a cell, the tachyzoites can rapidly divide and after death of the host cell they will invade adjacent cells or – after traveling through the blood stream – attach to cells elsewhere in the body. The tachyzoite stage can transform into a

slowly dividing bradyzoite. This stage of the parasite is able to form a cyst wall around a large family of dividing parasites, thereby rendering the parasite protection against the mounting immune response of the host. The stage differentiation from tachyzoite to bradyzoite is thought to be triggered by certain cytokines of the cellular host immune response (Klaren and Kijlstra, 2002). The parasite can remain dormant in its encysted stage whereby release of parasites from the cyst is associated with a weaning cellular immune response (Nath and Sinai, 2001). The cysts have a predilection for certain sites in the host such as muscle, brain or retinal tissues. Ingesting tissues of an intermediate host containing cysts infects carnivorous or omnivorous animals. This is also the route whereby cats (and other *Felidae*) become infected thereby closing the life cycle of the parasite.

Congenital transmission is a unique method that this parasite has developed to maintain an infectious reservoir in certain intermediate hosts. During pregnancy the tachyzoites can transfer the placenta and infect the developing offspring. In some species this can occur during successive pregnancies (Owen and Trees, 1998; Webster, 2001; Marshall *et al.*, 2004). In humans, congenital transmission is thought to be a one-time event occurring when a pregnant woman becomes infected with the parasite for the first time in her life. For sheep and rodents, evidence has been reported indicating that this may occur during successive pregnancies. Although congenital transmission has been reported in pigs, it is not known whether transmission can occur during multiple pregnancies. In rodents, the congenitally infected offspring may transfer the disease to their offspring leading to a long-lasting reservoir of *Toxoplasma gondii* even in the absence of *Felidae* as definitive host (Webster, 1994). In immunocompetent humans a primary infection is followed by a lifelong immunity causing the parasite to remain in its encysted stage during lifetime, preventing transfer of infection to the fetus in women who have encountered the infection prior to pregnancy.

Epidemiology

Due to the ubiquitous presence of the parasite, infection in humans is quite common. In some populations up to 100% of the individuals have been shown to be seropositive for *Toxoplasma* (Tenter *et al.*, 2000). Prevalence rates differ, depending amongst other things on the environmental conditions of oocyst survival. Prevalence of *Toxoplasma* is high in humid tropical areas and low in hot and dry areas. Prevalence in cold areas is also low. The prevalence of infection in a number of European countries (e.g. United Kingdom, Sweden and The Netherlands) shows a rapid decline over the past years (Walker *et al.*, 1992; Nokes *et al.*,

1993). A study performed in the Netherlands in 1987 showed that 50% of the population aged 30-34 years had experienced a previous *Toxoplasma* infection, whereas a study performed in 1996 showed that 37% of this age group was seropositive (Figure 4.1; L.M. Kortbeek: unpublished results). It is not clear why prevalence has dropped over the past years. It may be due to a change in consumption patterns, food handling and in outdoor activities. On the other hand, the decreased prevalence in the human population parallels a decrease in *Toxoplasma* infection rates observed in pigs (Figure 4.2). The change to intensive farm management practices whereby animals are confined within buildings may have contributed to a decrease in *Toxoplasma* seroprevalence. This in turn may have led to fewer consumers becoming infected.

Risk factors

Risk factors for both humans and livestock include the ingestion of fruit, vegetables, soil or water contaminated with sporulated oocysts shed into the environment by cats during a few weeks following infection (Tenter *et al.*, 2000). It is assumed that the latter only occurs once in the lifetime of a cat. Prevalence studies in European cats have shown that approximately 50% of the cats have experienced a *Toxoplasma* infection, indicating that these pets are the cause of a serious environmental burden of parasites (Webster, 2001). Disposal of cat litter boxes into compost garbage collection systems or via the toilet may pose as yet unknown environmental problems. Infection of marine mammals along the west coast of the United States of America is thought to be due to the outlet into the Pacific Ocean of sewage systems containing cat box litter disposed via home toilets (Dubey *et al.*, 2003b). Compost production systems may not reach temperatures high enough to kill sporulated oocysts. Compost, which is used by a few pig farms to improve iron uptake, may be involved in the transfer of toxoplasmosis to pigs.

The main risk factor for humans to contract toxoplasmosis is the consumption of raw or undercooked meat from animals that have been previously infected with the parasite (Cook *et al.*, 2000). It is not known how many cysts result in the infection of human beings, but ingestion of one cyst (containing hundreds of bradyzoites) is sufficient for a cat to become infected. Pigs, goats, sheep and poultry are the major meat sources of infection for humans (Tenter *et al.*, 2000; Aspinall *et al.*, 2002). One pig may be consumed by 200–400 different individuals so that there is a tremendous amplification of the risk to become infected (Fehlhaber, 2001).

During the production of various meat products, meat of many animals is mixed, which also amplifies the risk in cases where only a few animals would be infected (Aspinall *et al.*, 2002). Of interest is the fact that beef is not an important source of infection for humans (Dubey and Beattie, 1988). Although cattle can become infected with *Toxoplasma gondii*, this does not result in the appearance of infectious cysts in their meat.

Epidemiological studies in Europe have indicated that meat consumption could account for almost 60% of the *Toxoplasma* infections, whereas contact with soil (gardening) may be held responsible for approximately 20% of the cases (Cook *et al.*, 2000). Kitchen hygiene with respect to handling of meat may also be a risk factor for humans to contract toxoplasmosis. Examples include the use of the same knife to cut raw meat and subsequently cut fresh salad or the tasting of raw minced meat during flavouring with salt and pepper (Kapperud *et al.*, 1996; Tenter *et al.*, 2000).

Toxoplasma gondii can also be transmitted via the milk of infected goats (Riemann *et al.*, 1975; Tenter *et al.*, 2000). It is not known whether goat cheese prepared from animals shedding the parasite is still infectious. Byproducts of goat cheese processing (whey) are sometimes fed to pigs, which may potentially lead to the transfer of *Toxoplasma* to these animals. *Toxoplasma gondii* is effectively killed by heating at temperatures above 67°C for a few minutes (Dubey, 2000). Overnight freezing at –12°C also kills the majority of cysts (Kotula *et al.*, 1991), whereas curing of meat with salt does not seem to affect the parasite immediately (Dubey, 2000). Irradiation with low dosages of Cesium 137 can also be a suitable method to destroy the parasite (Dubey *et al.*, 1998).

Animal-friendly production systems

Indoor housing of farm animals is not regarded as being in favour of animal welfare and due to social pressure the bioindustry in Western-Europe is urged to reintroduce outdoor housing. In the Netherlands different animal-friendly pig-production systems have therefore been introduced. On modern intensive farms, pigs are housed indoors following a high hygiene protocol, mostly kept on concrete-slatted floors and are fed regular pelleted pig feed.

So-called free-range pigs are allowed outdoor access, are given straw bedding and are also fed regular pelleted pig feed. Pigs from organic farms are held according to regulations set up by the European Union (EU regulation nr 2092/91), which includes outdoor access, straw bedding and ‘organic’ pig feed. Organic pig feed often contains the same (plant) ingredients as regular pig feed, but is produced on farms that do not use inorganic fertilizers or pesticides. Since the BSE crisis, in the European Union it is not allowed to feed pigs with products of

'animal' origin. So pigs can be considered as vegetarians, although they are in fact omnivorous animals.

Whether animal-friendly production systems lead to a re-emergence of *Toxoplasma* infections is not yet known. This question was therefore the subject of research performed in the past few years by the Animal Sciences Group of Wageningen University and Research Centre. Results show that indoor housed pigs are free from *Toxoplasma* infection whereas almost 3% of the animals raised in animal-friendly production systems had previously been infected with *Toxoplasma* (Kijlstra *et al.*, 2004a). Analysis of sows showed that 70% of the tested organic farms had seropositive animals whereby on average 15% of the sows per farm showed evidence of a previous *Toxoplasma* infection (I.A.J.M. Eijck; personal communication).

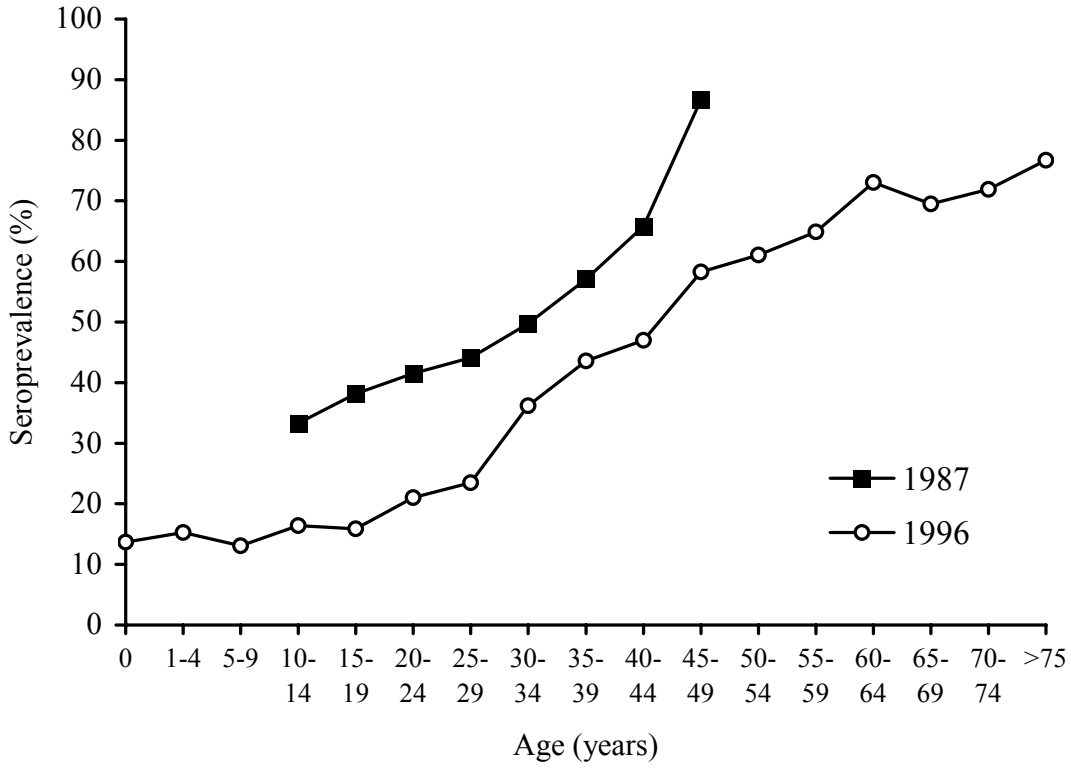

Figure 4.1 *Toxoplasma gondii* seroprevalence in the Dutch population by age groups in the years 1987 (n=28,000) and 1996 (n=7,521).

The source of infection is not yet known exactly, but may include ingestion of sporulated oocysts deposited in the environment via cats. Pigs are known to catch rats and mice and earlier research concerning risk factors for *Toxoplasma* infection on pig farms has already shown that poor rodent control is involved (Weigel *et al.*, 1995). Our own field studies confirm these observations. All conventional pig farmers use chemical rodenticides, whereas up to 30% of farmers on animal-friendly farms do not use rodenticides but rather rely on the

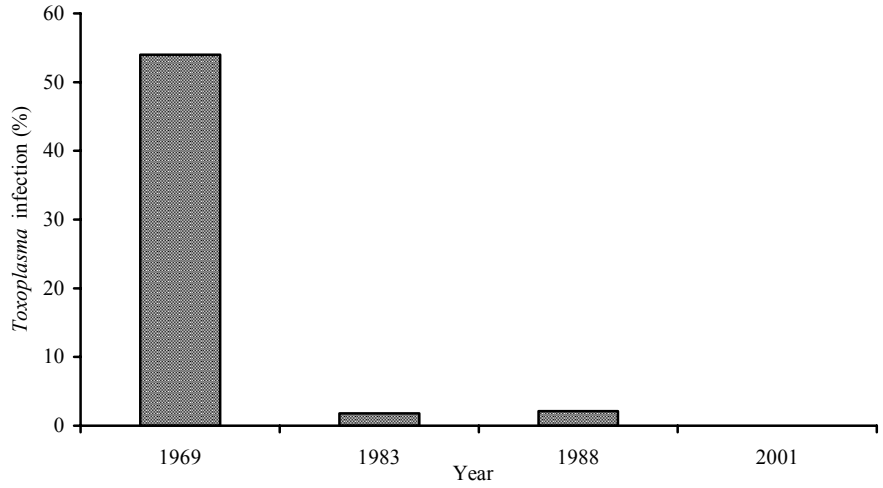

Figure 4.2 *Toxoplasma* infection in Dutch slaughter pigs in the period 1969-2001.

use of cats for rodent control on their premises. An analysis of risk factors and possible means of controlling *Toxoplasma* infection on organic pig farms, based on HACCP methodology is presented in the Appendix of this Chapter.

Since cats have been recognized as an important risk factor (Dubey *et al.*, 1995a), the effect was studied of *Toxoplasma* vaccination of farm cats on *Toxoplasma* seroprevalence in pigs. A number of years following the introduction of the cat vaccine, the seroprevalence showed a small but statistically significant decrease in seroprevalence of pigs where cats had been vaccinated (Mateus-Pinilla *et al.*, 1999). The small change could point to other more important risk factors, such as transfer via the ingestion of rodents. As will be mentioned later, rodents can be an important reservoir of the parasite even in the absence of cats for a long period of time (Webster, 2001).

Chickens are also a potential source of *Toxoplasma* infection in humans (Tenter *et al.*, 2000). Most chickens used for consumption are raised indoors and probably do not have access to a source of *Toxoplasma* infection during their short lifetime. Organic chickens raised for meat production are allowed outdoor access and are slaughtered at an older age. Due to their longer life and due to the fact that they have access to various *Toxoplasma* sources, organic chickens could potentially become infected with *Toxoplasma*. So far this has not been studied but recent data shows that a large percentage of free ranging chickens has been infected with the parasite (Dubey *et al.*, 2003a). At present there is no epidemiological data supporting the hypothesis that 'animal-friendly' farming will have impact on the incidence of human toxoplasmosis. But this is also because at present the market share of organic meat is only a few percent.

Toxoplasma infection in rodents: prevalences and routes of transmission

Many studies have focused on the prevalence of *Toxoplasma* infection in wildlife animals. In this section we shall confine ourselves to rodents that may play a role in the transmission of infection to farm animals like pigs and poultry. From field studies conducted on pig farms in Illinois (USA) during 1992 and 1993 it was learned that 2.1% of the house mice (*Mus musculus*) were seropositive for *Toxoplasma* (Dubey *et al.*, 1995b). Infectious parasites were recovered from heart or brain tissue in 0.5% of the mice investigated. Sera from Missouri and Kansas (USA) collected in the period December 1974 - December 1987 and analysed for the presence of antibodies to *Toxoplasma gondii* showed a low prevalence (3%) of antibodies in mice (*Mus musculus* and *Peromyscus* spp.) and rats (*Rattus norvegicus* and *Sigmodon hispidus*), while medium-sized herbivores, like squirrels (*Sciurus* spp.), rabbits (*Sylvilagus floridanus*) and muskrats (*Ondatra zibethicus*) had prevalences of about 18% (Smith and Frenkel, 1995). Webster (1994) studied the prevalence of *Toxoplasma gondii* within 6 UK farmstead wild rat populations (*Rattus norvegicus*) and reported a mean prevalence of 35%. No statistically significant age, sex or site differences were observed in prevalence between or within populations irrespective of habitat type or presence of cats.

The prevalence of *Toxoplasma gondii* in the Czech Republic was < 1% in insectivores (n = 578), 12% in carnivores (n = 112), 1% in rodents except muskrats (*Ondatra zibethicus*) (n = 5163), 24% in muskrats (n = 437), 5% in lagomorphs (n = 293), 0% in ruminants (n = 456) and 2% in wild boars (*Sus scrofa*) (n = 136) (Hejlicek *et al.*, 1997). Another study from the Czech Republic showed *Toxoplasma* infection in 47% of the muskrats from a site with water heavily polluted with municipal wastes and 9% in muskrats from 3 sites with water slightly polluted with wastes, stressing the role of waste water as a source of *Toxoplasma* infection (Nezval and Literak, 1994). A study from Ontario (Canada) showed that 11% of the mice (*Mus musculus*), 5% of the deer mice (*Peromyscus*), 3% of the rats (*Rattus norvegicus*) and less than 2% of the sparrows (*Passer domesticus*) investigated were seropositive. All samples from short-tailed field mice (*Microtus pennsylvanicus*), squirrels (*Sciurus carolinensis*), chipmunks (*Tamias striatus*), meadow jumping mice (*Zapus hudsonius*) and starlings (*Sturnus vulgaris*) were seronegative (Tizard *et al.*, 1978). How wild rodent populations become infected with *Toxoplasma gondii* is not known exactly, but congenital transmission may perpetuate the infection over successive generations. Webster (2001) concluded that this mode of transmission is the predominant route for *Rattus norvegicus*. Marshall and colleagues (Marshall *et al.*, 2004) presented evidence that 75% of the transmission in the house mouse

(*Mus musculus*) also occurs via the congenital route (Owen and Trees, 1998). Also studies in sheep favour this mode of transmission (Duncanson *et al.*, 2001).

Toxoplasma gondii has been shown to affect the behaviour of its intermediate rat host: its chance of being predated by cats is increased. Rats, which normally avoid areas with cat scent, become attracted to cat urine odour following infection with *Toxoplasma gondii* (Berdoy *et al.*, 2000).

Conclusions

Toxoplasmosis is an important parasitic disease worldwide causing substantial health problems in humans as well as in farm animals. Currently no vaccine is available and anti-parasitic drugs are either not effective (ocular toxoplasmosis) or are associated with serious adverse effects. This means that prevention is currently the best method to manage the disease. Since the majority of human infections are due to the consumption of meat, emphasis should be on control of the disease at farm level or on implementing measures to test animals before the meat reaches the consumer. Indoor housing of farm animals as practised in intensive animal husbandry has resulted in the abrogation of the *Toxoplasma* problem in the pig industry. However, animal-friendly livestock production systems are associated with a potentially higher prevalence of *Toxoplasma* infections and extra attention is needed to control transfer of infection. The access of cats on the farm premises, use of compost, goat whey and rodent control have been identified as risk factors that should be addressed on these farms.

Acknowledgements

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Appendix Risk analysis of *Toxoplasma gondii* infection on organic pig-production facilities

Part	Hazard	Chance[1]	Severity[2]	Motivation	Control measure
Environment	Introduction of *Toxoplasma* due to pigs rooting in the earth	1	2	Oocysts can survive in soil for prolonged periods of time, after cat has shed infected feces on the pasture. Not all pigs will become infected.	- Limit the number of cats on the farm - No kittens - Vaccination of the cats - Sterilization of the cats - Keep cats away from pasture - Take male cat to defend farm area against cats from neighbours
	Introduction of *Toxoplasma* by pig due to uptake of dead or live worms or flies	1	1	Uptake by a pig of worms and flies infected with *Toxoplasma* is a seldom event. Only an individual pig may become infected.	- Fly control
	The pig may become infected with *Toxoplasma* by eating an infected dead mouse	2	1	The chance of a pig eating a dead mouse can occur a few times per year. It is not known how many mice are infected with *Toxoplasma* (estimated a few percent). Only an individual may become infected.	- Rodent control - Use of live traps to prevent dead animals in the outdoor area or in the pigsty.
	Introduction of *Toxoplasma* on the farm via a live mouse or a dead or live bird.	1	1	The chance of a pig eating a live mouse or a dead or live bird is small. If it does occur only an individual animal may become infected.	- Rodent control - Improvement of farm hygiene
	Introduction of *Toxoplasma* by pig via cat feces	2	2	In the Netherlands there are always cats in the local farm environment. The defecation of cats on the outdoor area may be limited. On the other hand, at least 50% of the cats will have shed oocysts in their lifetime. Shed oocysts can persist for years. Many pigs can take up these oocysts, indicating that several pigs on the farm may become infected.	- Limit the number of cats on the farm - No kittens - Vaccination of cats - Sterilization of cats - Keep cats away from pasture - Take male cat to defend farm area against cats from neighbours
Management	Infection of pigs with *Toxoplasma* through application of compost (iron supplementation)	2	2	Compost may contain litter or feces from cat-boxes, possibly containing oocysts. Not all parts of compost reach temperatures high enough to inactivate oocysts. Since piglets are sometimes given compost, they are at risk of becoming infected with *Toxoplasma* via this route.	- According to Dutch farming regulations, pig farmers are forbidden to use compost during pig production

Appendix cont'd.

Part	Hazard	Chance	Severity	Motivation	Control measure
Water	Pigs can become infected with *Toxoplasma* by drinking water contaminated with oocysts	2	3	If pigs drink water from ponds, ditches or canals there is a fair chance of them becoming infected with *Toxoplasma.* Cats can drop feces in the neighbourhood of ponds canals and ditches, which may drain into the water following rainfall. Whether this can occur in the Netherlands is not known. All pigs having access to this water source may become infected.	- Do not allow access to water from ponds, canals or ditches
	Pigs can become infected by drinking water from an infected well	1	3	The chance of infection of a well is small if the well is deep. If the well does become infected then nearly all pigs on the farm will become infected.	- Test well water
	Spreading of *Toxoplasma* due to infected insects or rodents getting into a local intermediate water reservoir	1	3	The chance of an infected mouse drowning in a water reservoir is low. The chance of insects drowning in a water reservoir is higher. The number of oocysts in insects is not exactly known and probably very small. Due to dilution in the water the overall chance of a pig becoming infected via this route is small. The severity may be large because the water is supplied to various pens and many animals may become infected.	- Close the water reservoir
	Pigs become infected due to a dead bird in a local intermediate water reservoir	1	1	The chance of an infected bird drowning in a water reservoir is low. The cysts will probably not leave the dead bird and thus will not infect the pigs.	- Close the water reservoir
Feed	Pigs become infected with *Toxoplasma* because the feed is contaminated with cat feces or dead infected rodents	1	3	Pelleted pig feed is made at high temperatures and under high pressure. This limits the chance of oocysts present in the original ingredients to survive. If temperatures are too low and feed ingredients are contaminated, infectious oocysts may remain in the feed leading to several pigs becoming infected.	- Feed producers should guarantee temperatures during feed processing of at least 65°C during 5 minutes
	Pigs become infected with *Toxoplasma* because cat feces or dead rodents enter the feeding system	2	3	If the feces are from an infected cat or if the rodent is infected, several pigs may become infected.	- No access of cats to feeding system - No access of rodents to feeding system - Farm hygiene

Appendix cont'd.

Part	Hazard	Chance	Severity	Motivation	Control measure
Feed	Pigs become infected with *Toxoplasma* because non-processed feed (by-products and individual ingredients, including hay and straw) are contaminated with cat feces or dead rodents	1	3	If infected cats can drop feces onto stored feed products or if infected rodents have access to these products (and can die there), this can lead to contamination of the feed products with both oocysts and (rodent tissue) cysts. Several animals can become infected if fed with these products.	- No access of cats or rodents to farm storage sites of by-products or other feed ingredients - Feed producers should guarantee a cat- and rodent-free storage system (including control system)
	Pigs become infected with *Toxoplasma* because they are fed (infected) goat-whey	1	3	Some pig farmers feed organic goat-whey to their pigs. If whey is obtained from *Toxoplasma*-infected goats, the whey can contain *Toxoplasma* tachyzoiytes. This may lead to infection of all animals on the farm.	- Forbid feeding goat whey
Piglets	External supply of piglets may introduce *Toxoplasma* on the farm	2	2	Piglets can become congenitally infected during pregnancy or during the lactation period. Transport of such pigs to a finishing farm will lead to the presence of infected slaughter pigs. Not all pigs will become infected.	- The supply of piglets should come from *Toxoplasma*-free farms
	Piglets become infected due to cannibalism	2	1	There is a chance of *Toxoplasma* infection being transmitted through cannibalism. Cannibalism of ears or tails containing infectious *Toxoplasma* cysts may lead to the infection of other piglets. A few piglets will become infected via this route.	- Prevent cannibalism by introducing distracting elements (playing material)
Instruments and tools	Pigs can become infected with *Toxoplasma* due to the presence of oocysts or cysts on instruments and tools	2	1	The chance of instruments or tools on the farm being infected with oocysts (cat feces or dead rodents) is very small. Carry-over of cysts via instruments (castration or vaccination) is low too. Only an individual animal may become infected.	- The use of clean instruments and tools
Visitor and animal care-taker	Pigs can become infected with *T. gondii* through visitors and animal caretakers via oocysts on their shoes or clothing	1	1	The chance of shoes or clothes from visitors becoming infected with cat feces is low.	- The use by visitors of clean shoes and clothes before entering the farm area

Appendix cont'd.

Part	Hazard	Chance	Severity	Motivation	Control measure
Dung	Pig can become infected with *Toxoplasma* via dung transport	1	1	Removal of dung from the farm may lead to introduction of pathogens on the farm if the trucks or other machines used for transport are not clean prior to entering the farm. Presence of *Toxoplasma* oocysts on these trucks via cat feces is hypothetical. If this is the case only an individual pig may become infected.	- Only clean wagons and trucks have access to the farm
Finishing pigs	Finishing pigs can become infected with *Toxoplasma* due to the presence of cat feces or dead rodents in the trucks used for transport	1	2	Regulations oblige transporters to clean their trucks before loading new animals. The chance of trucks containing cat feces or dead rodents is small. If trucks are not clean this may lead to several animals becoming infected. The time between loading and slaughter is such that this will not lead to gross infection of the animals.	- Always use clean transport
Insemination and boars	Pigs become infected with *Toxoplasma* via sperm	1	1	Transfer of *Toxoplasma* via sperm has not yet been reported.	
Cadavers	Pigs become infected with *Toxoplasma* through cannibalism of cadavers or due to uptake of cadaver material by cats or rodents	1	2	The chance of transferring *Toxoplasma* infection via the pig cadavers is small. Pigs are not infectious on the outside and transfer only occurs through cannibalism or uptake by rodents or cats, which in turn can serve as a risk factor. Several pigs may become infected via this route.	- Remove cadavers immediately

[1] Chance estimates whether the described risk occurs on the farm. It is ranked from 1 to 3; with 1 = chance of occasion is low, it occurs rarely or is theoretically; 2 = chance of occasion is mediocre, it can occur or occurs several times a year; 3 = chance of occasion is high, it occurs frequently.

[2] Severity gives an estimate of the number of animals possibly affected by *Salmonella* when the risk becomes manifest. It is also ranked from 1 to 3; with 1 = the occasion has an influence on a single pig to all pigs in the pen; 2 = the occasion has an influence on a part of all the pigs on-farm (pig unit); 3 = the occasion has influence on (almost) all the pigs on-farm.

Chapter 5

Cats and Goat Whey associated with *Toxoplasma gondii* Infection in Pigs

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Submitted

Abstract

In organic livestock production systems, farm-management factors are thought to play an important role in the on-farm prevalence of Toxoplasma gondii. Serological results and the results of an HACCP analysis were combined to determine important risk factors for the prevalence of this protozoan parasite. Mathematical analysis demonstrated that feeding goat whey to pigs and the presence of a high number of cats were positively correlated to T. gondii seroprevalence in pigs. Not covering roughage and the farmers' assumption that pigs can come into contact with cat feces also showed a positive relationship. In order to decrease the risk of T. gondii infecting their pigs, farmers should limit the access and number of cats on their farms and refrain from feeding goat whey to their pigs.

Introduction

Preventing contact between farm animals and zoonotic pathogens is important in both conventional agriculture and organic animal husbandry. This is difficult in organic production systems since the animals are allowed outdoors and thus have easy access to potential sources of hazardous bacteria and/or parasites. The protozoan parasite *Toxoplasma gondii* is a good example of such a microbial food safety hazard. It causes toxoplasmosis, the most prevalent parasitic zoonotic disease in the world (Tenter *et al.*, 2000), which can result in substantial health disorders in humans like mental retardation, encephalitis and blindness.

Consumption of raw or undercooked meat (pig, goat, sheep, or poultry) is known to be an important risk factor for humans in contracting toxoplasmosis (Cook *et al.*, 2000). *T. gondii* parasites remain viable in cysts if the meat is not well prepared and can thus cause infections in humans. Unfortunately, treatment of toxoplasmosis is difficult because available drugs are not always effective (Gilbert and Gras, 2003; Stanford *et al.*, 2003). Prevention of the parasite's presence at the farm level, therefore, is one of the strategies in the battle against toxoplasmosis (Kijlstra *et al.*, 2004a).

T. gondii has a complex life cycle. Cats function as definitive hosts during one stage of *T. gondii*'s complex life cycle and transmit the parasite to the environment through defecation (Dubey *et al.*, 1995a). In fact, an infected cat can shed millions of *T. gondii* oocysts via its feces that, after sporulation and upon intake by intermediate hosts such as rodents, can infect other species. Farm animals, for example, can become infected by ingesting the tissues of intermediate hosts or by consuming soil, water, or feed that is contaminated with oocysts. Currently, the Netherlands has a population of 3.3 million cats in a total area of 35,054 km^2

(94 cats per km^2). This large feline population and the Dutch climate (moist summers and mild winters, similar to that of other West European countries) offer conditions conducive to *T. gondii* growth.

The number of Dutch slaughter pigs infected with *T. gondii* decreased rapidly from over 50% seroprevalence in 1969 to 0% seroprevalence in 2001 (Kijlstra *et al.*, 2004a), because of intensive farm-management practices whereby the animals were confined indoors (Van Knapen *et al.*, 1995). During the last decade, however, consumer demands for farming practices that offer better animal welfare have led to an increase in organic animal production where pigs have outdoor access and straw bedding and are fed roughage. As suggested by the results of a theoretical HACCP analysis (Kijlstra *et al.*, 2004b), these circumstances could lead to an increased risk of pigs contracting the parasite (Kijlstra *et al.*, 2004 a,b). In order to test the results of that analysis, we conducted a questionnaire-based survey and serological testing in 2004 to determine the prevalence of *T. gondii* in pigs on 36 organic pig farms in the Netherlands.

Material & Methods

In total, 2796 pigs from 41 organic pig farms were tested for *T. gondii* infection at the slaughter line. Between one and seven batches of pigs (each batch consisting of 15-28 animals) per farm were tested from June to September 2004. The total number of animals tested varied from 15 to 140 pigs per farm. A competition enzyme-linked immunosorbent assay (ELISA) with a peroxidase-labeled monoclonal IgM anti-SAG1 antibody (Lind *et al.*, 1997) was used for the serological detection of *T. gondii* infection.

During the same period, a questionnaire-based survey was conducted among 36 of the participating farmers (on-site). These farmers already knew about the study's background and the potential consequences *T. gondii* infection has on food safety. The goal of the questionnaire was not primarily to analyze the risk of infection, but to function as a tool for the farmers to limit the *T. gondii* infection rate. The questionnaire consisted of nine questions (including sub-questions, see Appendix) about cats, feed, farm management and piglet supply, because these aspects had been identified as possible risk factors during the earlier HACCP methodology study (Kijlstra *et al.*, 2004b).

The goal of this study was to combine the serological results with the results of the questionnaire in a mathematical model in order to verify the important risk factors for *T. gondii* infection. First, a bi-plot analysis with Genstat 6.0 software (Rothemsted Research,

Harpenden, UK) was used to determine the coherence between the different variables in the questionnaire. A bi-plot (Gabriel, 1971) is a graphical representation of the relationships between n individuals and p variates. If these variates are arranged as a matrix X(n´p), the singular value decomposition of X (X=USV¢) is used to express the least-squares approximation to X in two dimensions in the form X2=AB¢, where X2 is (n´2); A (n´2) and B (p´2) are given in the first two columns of (USr) and (VS(1-r)), respectively. When strongly correlated variables were found, we preferred those with a low non-response (unfortunately not all questions were answered by all farmers) and an objective nature (some questions were subjective, e.g., "are only older cats present?", where "older" is of course a subjective term).
The preliminary base model was then expanded using step-by-step regression with significant variables that had a lower non-response or were less objective. Variables that had a low non-response and were objective, yet not significant were eliminated from the model. Thus, a number of significant explanatory variables remained: x1 (pigs not fed goat whey vs. pigs fed goat whey), x2 (low vs. high number of cats present), and x3 (not covering vs. covering roughage). Explanatory variables are those variables that can be used in a relationship to explain or predict changes in the values of another variable (in our case *T. gondii* infection).
Finally, the number of *T. gondii*-seropositive pigs was analyzed for the selected explanatory variables. A logistic model (Generalized Linear Mixed Model) was used both during the selection phase of explanatory variables and in the final model. A dispersion parameter was also estimated because of overdispersion in the data (McCullagh and Nelder, 1989).
The model describes the relationship between the risk of infection (p, where $0<p<1$) of pigs tested in the slaughterhouse and the explanatory variables. We used the logit-link function:

$$\text{Logit}(p) = \ln(p/(1-p)) = b0 + b1x1 + b2x2 + b3x3 + b4x4$$

Where:
b0 = mean (on logit-scale) of the combination of all factors in the lowest class
x1 = pigs not fed goat whey (x1=0) vs. pigs fed goat whey (x1=1)
x2 = having less than 3 cats (x2=0) vs. more than 3 cats (x2=1)
x3 = not covering roughage (x3=0) vs. covering roughage (x3=1)
x4 = assuming contact between pigs and cat feces as impossible (x4=0) vs. assuming it possible (x4=1)
b1 = effect (on logit-scale) of not feeding goat whey vs. feeding goat whey
b2 = effect (on logit-scale) of having less than 3 cats vs. having more than 3 cats

b3 = effect (on logit-scale) of not covering roughage vs. covering roughage
b4 = effect (on logit-scale) of assuming contact with cat feces as impossible vs. assuming it possible

A model assumption is that the variance of the observed number of infected pigs of a hypothetical farm Y can be described by the variance (YIp) = φ*n*p(1-p). In this formula *n* is the total number of pigs provided by farm Y. Because of missing answers with regard to variables x2 and x3, the final model was based on data from 26 of 36 farms.

Results

Of the 2796 samples tested, 85 (3%) were positive for *T. gondii*. On the farm level, 19 of the 41 (46%) farms were *T. gondii*-negative, while 22 (54%) were positive. There was only one seropositive pig, however, on 6 of these farms (14%). Forty-one of the 148 batches that arrived at the slaughterhouse contained at least one seropositive pig (27.7%, Table 5.1).
Table 5.2 presents a selection of replies to the questions that offer insight into the current farm-management practices of organic pig farmers. These questions were selected because of their low level of non-response and their simple interpretation. For mathematical (bi-plot) analysis, all questions that were answered with 'yes' were given a value of 0, while all questions answered with 'no' were given a value of 1.
Figure 5.1 shows a graphic display of the coherence (bi-plot) between the answers to questions concerning cats. This bi-plot analysis was performed to determine the correlations between all variables and to optimize the model by selecting variables that had a low non-response and were objective. The variables in Figure 5.1 are represented by vectors in the bi-plots and the direction and length of the vectors indicate how each variable contributed to the two principal components in the plot. The vectors are situated close together if the variables were positively correlated, point in opposite directions if they were negatively correlated and lie perpendicular to each other if they were not correlated. The observations in this plot are represented by dots and their locations indicate the score of each observation for the two principal components in the plot.
As noted above, only 36 of the 41 farmers received the questionnaire (Table 5.1). The results of 26 questionnaires were selected for the final model, because of missing answers with regard to variables x2 and x3.

Table 5.1 Serological data of the farms

Farm	# of batches	# of batches	Total # of	# of pigs	% of pigs
A	1	1	20	1	5
B	4	1	66	1	1.5
C	4	1	80	1	1.3
D	4	1	51	1	2
E	5	1	95	1	1.1
F	4	1	75	1	1.3
G	5	5	95	26	27.4
H	1	1	15	14	93.3
I	2	0	35	0	0
J	6	0	110	0	0
K	3	0	50	0	0
L	4	2	75	2	2.7
M	4	0	75	0	0
N	4	2	75	2	2.7
O	4	0	70	0	0
P	6	2	115	3	2.6
Q	5	0	78	0	0
R	5	3	95	3	3.2
S	2	2	44	3	6.8
T	4	0	70	0	0
U	4	1	75	2	2.7
V	3	0	55	0	0
W	5	0	95	0	0
X	5	0	98	0	0
Y	4	0	75	0	0
Z	3	3	50	4	8.0
AA	6	2	115	5	4.3
AB	3	2	60	3	5.0
AC	7	2	140	2	1.4
AD	1	0	20	0	0
AE	2	0	40	0	0
AF	4	2	80	3	3.8
AG	4	2	80	2	2.5
AH	3	0	60	0	0
AI	4	0	76	0	0
AJ	3	1	60	1	1.7
AK	3	2	60	2	3.3
AL	4	0	80	0	0
AM	1	0	20	0	0
AN	1	1	20	2	10
AO	2	0	48	0	0
Total	**148**	**41**	**2796**	**85**	**3.0**

Table 5.2 Summary of replies to some questions on the questionnaire

Factor	Question no.	% Yes	% No	# replies
Contact with cat feces is assumed possible	1	61.1	39.9	36
>3 cats present on the farm	1.2	33.3	66.7	31
Goat whey is fed to pigs	5	8.3	91.6	36
Rodent control is practiced	6.5	100	0	36
Roughage is covered	6.7	87.5	12.5	32
Feed manufacturer guarantees heating >70°C	8.1	17.1	82.9	35

Because not all questions concerning cats were answered by all farmers, the main data set had to be divided into subsets. In Figure 5.1 the subset (17 farms) is presented in which all cat questions were answered.

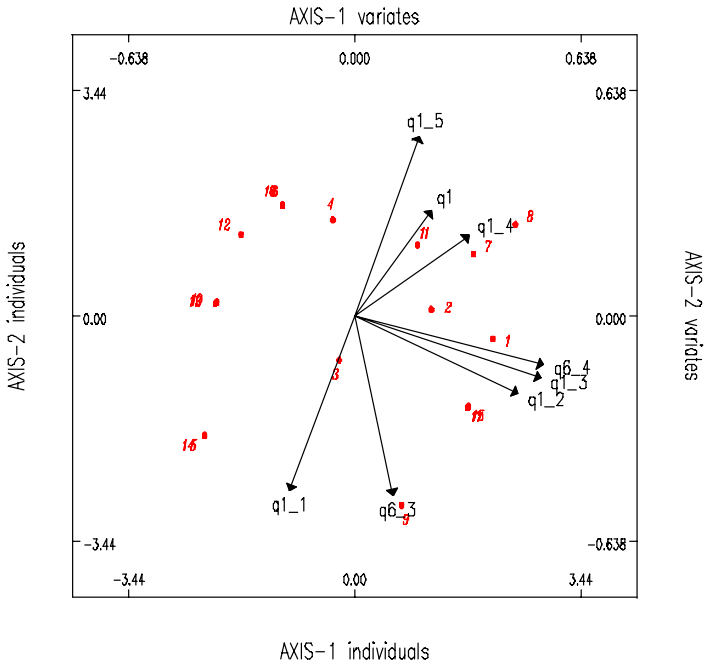

Figure 5.1 Coherence of questions concerning cats (bi-plot, question numbers are preceded by the letter q, e.g., q1_5 refers to question 1.5) from the subset of 17 farms.

Observations (farm results) are represented by points in this plot. Some have exactly the same location in the plot and overlap each other. Answers to questions 1.2, 1.3, and 6.4 (see Appendix for questions) showed a strong positive correlation to each other. Answers to questions 1, 1.4, and 1.5 were positively correlated to each other, but negatively to that of

question 1.1. Questions 1 (is contact between pigs and cat feces possible?) and 1.2 (are there <3 cats present?) were selected as independent cat variables for the final stage of analysis.

Figure 5.2 provides a graphic display of coherence (bi-plot) between the answers to questions on feeding aspects and the selected variables concerning cats. The answer to question 1.2 was negatively correlated to that of question 5 and positively to those of questions 3, 6.6, 6.7, and 6.8. Answers to questions 6.7 and 6.8 were positively correlated to each other and negatively to those of questions 8.1 and 1. The answer to question 7 was positively correlated to questions 4 and 5 and negatively to those of questions 6.1, 9, and 1. Interpretation of the bi-plot models is complicated because of the manner in which the questions were formulated in the questionnaire. The horizontal axis in Figure 5.2 represents the effects of goat whey, drinking basins and cats. The farms on the left side of the plot did not feed goat whey to their pigs (q5), did not use floating drinking basins (q4), and did not have more than three cats (q1.2), while the farms on the right did.

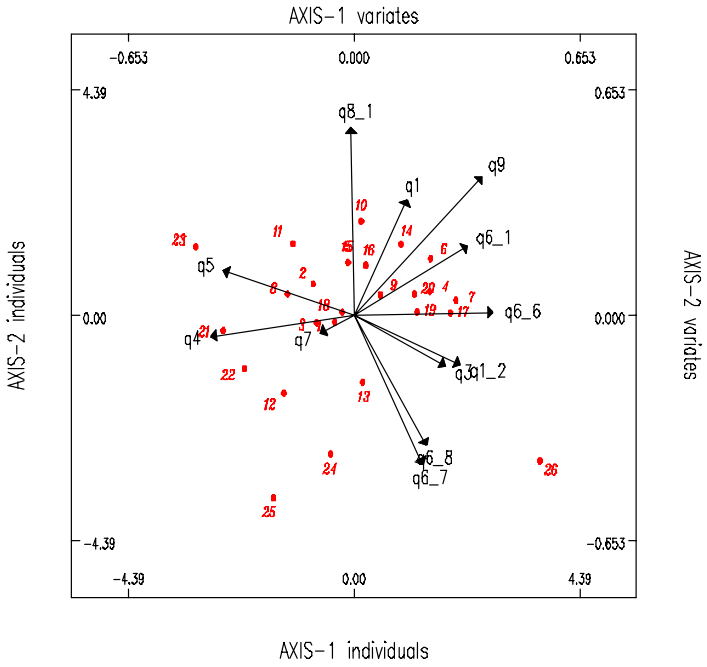

Figure 5.2 Coherence of questions concerning feeding and selected cat variables (bi-plot, question numbers are preceded by letter q) from the main data set (26 farms).

Thus, it can be deduced from Figure 5.2 that 'feeding goat whey' and 'having more than three cats' are positively correlated to each other. All these variables were selected for the final model. Odds ratio estimates of the selected explanatory variables are displayed in Table 5.3.

Toxoplasma gondii-infection levels varied between 0% and 10% on all but two farms: farms G and H had infection levels of 27% and 93%, respectively. Animals from farm G were tested

in five consecutive batches consisting of 15-20 animals each. Of these, 2-8 animals per batch were seropositive. Farm H (number 26 in Fig. 5.2) was a smaller farm and only delivered one batch of 15 pigs, 14 of which were infected. The estimated effects of the factors 'not covering roughage' (parameter b3) and 'assessing contact between pigs and cat manure as impossible' (b4) can be highly attributed to these farms. As a result, we also analyzed our data excluding these two farms (see Table 5.4).

Table 5.3 Odds ratio estimates of the selected explanatory variables

Parameter	Factor	Question no.	Odds ratio	Significance
b1	Goat whey is fed to pigs	5	6.67	<0.01
b2	>3 cats present on the farm	1.2	2.07	0.15
b3	Roughage not covered	6.7	13.45	<0.001
b4	Contact with cat feces assumed possible	1	4.55	<0.01

Table 5.4 Odds ratio estimates excluding data from the two extreme farms

Parameter	Factor	Question no.	Odds ratio	Significance
b1	Goat whey is fed to pigs	5	3.57	<0.01
b2	>3 cats present on the farm	1.2	3.24	0.04

Discussion

The use of odds ratios is advantageous because one can then speak about the increase in risks of a certain situation compared to a reference situation. Using observational data, the results of the present study show a relationship between *Toxoplasma gondii* in pigs at the slaughter line and several farm-management aspects. We could conclude that the number of cats present on-farm and feeding pigs goat whey were both positively related to the seroprevalence of *T. gondii* in pigs. The first relationship did not come as a surprise since earlier studies recognized cats as an important risk factor (Dubey *et al.*, 1995a; Meerburg *et al.*, 2004). To our knowledge, however, the second relationship (transfer of *T. gondii* to pigs through goat whey consumption) has not been previously reported, even though drinking milk has been implicated in the transfer of toxoplasmosis. A study by Sacks *et al.* (1982, cited in Tenter *et*

al., 2000) did relate acute toxoplasmosis in humans to the consumption of unpasteurized goat's milk and *T. gondii* tachyzoites have been found in the milk of sheep, goats, and cows (Tenter *et al*., 2000). Further, a theoretical HACCP analysis of *T. gondii* infection at organic pig-production facilities (Kijlstra *et al.,* 2004b) revealed that the chance of contamination (whether the described risk occurs on-farm) may be relatively low, but the severity (number of pigs affected by *Toxoplasma* when the risk becomes manifest) is high.

Not covering roughage and farmers assuming a possible contact between pigs and cat feces also seem to have a certain influence on the seroprevalence of *T. gondii* in pigs, although this can mostly be attributed to the two 'extreme' farms G and H where *T. gondii* seroprevalence reached 27% and 93%, respectively. Even though farm H was a small farm, the seroprevalence was high because the farmer assumed possible contact between pigs and cat feces, more than three cats were present, roughage was not covered, and pigs were fed goat whey. Interestingly, farm G produced a continuous level of infection. This continuity may be related to poor rodent control since another study by our group (Meerburg *et al.*, 2006) reported numerous rodents at this particular farm. Because other farm-management factors may be involved, more research is necessary to find the source(s) of infection at this particular farm.

Feeding goat whey (a byproduct of cheese-making) to pigs had a strong coherence with the keeping of many (3 or more) cats (see Figure 5.2). Not covering roughage was strongly correlated to failure to repel cats and pest animals from hay, straw, roughage and feedstuff. Although the latter (failure to avoid cats etc.) may not be a very suitable question (6.8) because of its diverse nature, it does provide good insight into a farm's hygiene status. Further, the effect of assuming no contact between pigs and cat feces was strongly correlated to the fattening of a farm's own piglets and with neglecting to question feed manufacturers about guarantees concerning feed-heating temperatures.

An earlier study pointed out that poor rodent control is a risk factor for *T. gondii* on pig farms (Weigel *et al.*, 1995). The effect of pest control (including rodents), however, could not be directly estimated in this study, because all 36 farmers answered question 6.5 positively, i.e., they use some form of pest control. Since it remains uncertain to what extent this control is indeed performed, one cannot claim that this factor is unimportant for the occurrence of *T. gondii* in pigs. Moreover, the farmers were aware of this risk factor so their answer could be biased.

In order to guarantee safe organic pork, more emphasis should be placed on the importance of certain farm-management factors. For example, organic farmers can easily switch from

feeding goat whey (or sheep whey) to providing other products that have the same nutritional values, but are heated during the production process. In our opinion, feeding unpasteurized goat or sheep whey to pigs should be prohibited. Further, although it is impossible to prevent all contact between pigs and cats/rodents in an organic setting, it is possible to restrict the frequency. Pest-proofing farm buildings and removing access to feed, water and shelter will help limit not only the presence of cats, but also the number of pest animals (Meerburg *et al.*, 2004). It will also lower the number of birds, thus further minimizing the amount of prey for cats. Although it is not recommendable from a food-safety point of view, organic farmers frequently use cats as rodent exterminators instead of applying rodenticides (Kijlstra *et al.*, 2004a). These agents are relatively easy to use, but may eliminate non-target species, be cruel in their action and weaken rodents, thus facilitating their consumption by, e.g., pigs and increasing the risk of *T. gondii* infection. Better options include the use of traps or installing perches or nest boxes to stimulate the presence of birds of prey (Meerburg *et al.*, 2004).

Another option to limit the risk caused by the presence of cats is the administration of a feline *Toxoplasma gondii* vaccine. The use of this vaccine was found to reduce *T. gondii* incidence in pigs (Mateus-Pinilla *et al.*, 1999). Vaccination, however, is only possible with the farmers' own cats, not with all other cats in the neighborhood, as they have other owners. In order to overcome this problem, we recommend the nationwide integration of *Toxoplasma* vaccination into the standard kitten vaccination schedules. Until this is realized, farmers should reduce the number of cats on their farms and limit their access to the farm premises in order to decrease the risk of *T. gondii* infection. Moreover, farmers should be aware of the risks of feeding their pigs "animal products" such as whey.

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Appendix Questions concerning farm management and *Toxoplasma gondii*

1. Do you assume that pigs can come into contact with cat feces (e.g. in the pens or in the outdoor area)?
1.1. Do you bar cats from the outdoor area?
1.2. Are less than three cats present on-farm?
1.3. Are only older cats present?
1.4. Are the female cats sterilized?
1.5. Do you have a male cat that defends the outdoor area?

2. Are the piglets or pigs fed compost?
If so:
2.1. Do you plan not to feed compost anymore because of possible transmission of various pathogens? (yes=no more compost will be fed)

3. Do the pigs have access to water from ditches?
If so:
3.1. Do you prevent in the future that pigs have access to ditch water because of potential contamination risk? (yes = it will be prevented in the future)

4. Do you use floating drinking basins?
If so:
4.1. Are your floating drinking basins closed to prevent contamination?

5. Do you feed goat whey to your pigs?
If so:
5.1. Do you plan to stop feeding goat whey to the pigs?

6. Do you feed cut products, roughage, straw or hay to your pigs?
If so:
6.1. Is it right that cats do not have access to feed or feeding equipment (yes=yes, that is right)?
6.2. Do you repel pest animal access to feed or feeding equipment?
6.3. Do you acquire your roughage from farms with only a few cats?
6.4. Do you only have older cats (can be repeating if question 1 is answered as “yes”)
6.5. Do you apply pest control?
6.6. Do you apply pest control by trapping?
6.7. Are you covering your roughage?
6.8. Do you always prevent contact between cats or pest animals and the cut products, roughage, hay or straw on your farm?

7. Do cats, birds or pest animals have access to the pelleted feed trajectory (silo, feed pipes, feed cart or trough)?
If so:
7.1. Is it right that cats do not have access to feed or feeding equipment (yes=yes, that is right)?
7.2. Do you apply pest control by trapping (can be repeating if question 6 is answered as “yes”)?
7.3. Do you control the feed and feeding equipment on its cleanness (free from manure etc.)?

8. Do you provide pelleted feed to your pigs?
If so:
8.1. Do you ask feed manufacturers any guarantees concerning feed heating temperatures above 70° Celsius?

9. Do you supply piglets of farms that guarantee these to be free of *Toxoplasma gondii*?
If so:
9.1. Do you obtain piglets of farms that guarantee they are free of *Toxoplasma gondii*?

Chapter 6

Wild Small Mammals: a Pathway to Livestock Infection with *Toxoplasma gondii*?

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Submitted

Abstract

Toxoplasma gondii can infect most warm-blooded vertebrates, including humans, and can cause congenital toxoplasmosis with severe problems in unborn children and babies, including fatal premature birth, hydrocephalus, retinochoroiditis and neurological disorders. Acquired infections may cause eye problems, fever, enlarged lymph nodes and muscle aches, while reactivation of latent cysts can have fatal consequences in immunosuppressed persons. Most human infections are considered to be the result of the ingestion of infected meat, the main sources of which are pig, chicken, sheep and goat. Thus, preventing T. gondii from infected food animals on farms is important from a food safety perspective. Previous studies showed that poor rodent management is a risk factor for T. gondii transmission. The present study investigated wild small mammals as possible transmission route to food animals (pigs and poultry) on organic farms. Both serological and PCR testing were performed because there are indications that there are differences between serological and PCR detection concerning T. gondii prevalence. The results showed that T. gondii antibodies could be detected in 6.4% of the blood samples taken from 235 wild small mammals. The PCR test demonstrated that 71.1% (n=182) of the tissue samples taken from 256 wild small mammals were positive. Moreover, a significant difference in the T. gondii contamination level was observed between house mice and white-toothed shrews (p=0.004). We conclude that if small wild mammals can come into contact with food animals, proper pest management is necessary to prevent T. gondii transmission.

Introduction

Infected pork has traditionally been considered as one of the main sources of toxoplasmosis in the Western world (Mead *et al.*, 1999; Cook *et al.*, 2000; Montoya and Liesenfeld, 2004). Pigs may acquire their infection by ingesting *T. gondii* oocysts directly from the environment, e.g. by drinking water contaminated with sporulated oocysts or by ingestion of contaminated soil. They may also become infected via vertical transmission, i.e. from sow to piglets (Dubey and Urban, 1990), a phenomenon that has been previously reported in sheep (Williams *et al.*, 2005), rodents and humans (Tenter *et al*., 2000). A third transmission route is infection via scavenging, i.e. pigs eat the infected carcasses of wild small mammals (rodents and insectivores).

Because of several of its management features, organic pig farming may offer an increased risk of exposure to *T. gondii* through the third route. For instance, organic pigs have outdoor access and can thus come into contact with wildlife more easily than pigs in conventional

production systems that house the animals indoors. In addition, organic pigs are provided with roughage and straw beddings that provide an excellent habitat for some small mammal species. Finally, although allowed in the European Union, some organic farmers do not apply rodenticides or other types of rodent management because their use does not follow organic farming philosophy. Earlier studies (Assadi-Rad *et al.*, 1995; Dubey *et al.*, 1995b) have shown, however, that poor pest management is a risk factor for the transmission of *T. gondii* to pigs. Because of the lack of field experiments to date, the present study was set up to investigate the infection rate of rodents and insectivores on organic farms in the Netherlands.

Materials & Methods

Wild small mammals (rodents and insectivores) were captured on ten organic livestock premises (9 pig, 1 poultry) in the Netherlands. The traps were set between early September and October 2004, which is the best season for trapping wild small mammals. Maximum temperatures during this period varied between 9.3°C and 32.5°C and minimum temperatures between 2.4°C and 20.8°C. Daily precipitation varied between 0 mm and 28.7 mm throughout the country (Royal Netherlands Meteorological Institute KNMI, De Bilt, The Netherlands). The trapping time per farm was three consecutive 24-hour periods. Longworth life traps (Longworth Scientific Instruments, Abingdon, UK) and Tomahawk rat traps (Tomahawk Live Trap Co, Tomahawk, WI, USA) were used to capture the small wild mammals. After CO_2 euthanasia was performed, the trapped animals were identified according to species and if possible, blood was collected from their hearts. Samples of heart tissue were also taken on eight of the farms incorporated in this study.

The prevalence of *T. gondii* in these small mammals was assessed using two methods: one was based on serology of the blood samples, the other was a nested B1 PCR procedure performed on the heart tissue (Burg *et al.*, 1989) (samples originating from 8 organic pig farms). A competition enzyme-linked immunosorbent assay (ELISA) with a peroxidase-labeled monoclonal IgM anti-SAG1 antibody was used for the serological detection of *T. gondii* infection in blood (Lind *et al.*, 1997). DNA was extracted using the QIAamp Tissue Kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany) and its concentration determined spectrophotometrically. All PCR reactions were performed using 20-µl samples containing 50-200 ng DNA. Positive and negative controls were added to each microtiter plate. B1 PCR primers, made according to published sequences (Burg *et al.*, 1989), were used in this study. Amplification of the B1 locus is widely used in human clinical specimens because it is a tandemly arrayed 35-fold repetitive gene (Burg *et al.*, 1989;

Hohlfeld *et al.*, 1994; Grigg and Boothroyd, 2001). Invitrogen E-gels-48 4% agarose were used in the analyses and configuration of the gel images accomplished using E-Editor Software (Invitrogen, Carlsbad, CA, USA). Non-parametric chi-square tests were then carried out to discover possible differences in *T. gondii* infection rates between the two main species (*Mus musculus* and *Crocidura russula*) observed by the B1 PCR and between male and female specimens of all species studied with the B1 PCR. For both serology and PCR, *T. gondii* prevalences were only recognized as representative if the total number of a species trapped exceeded fifty specimens.

Results

In total, 283 wild small mammals were trapped on ten organic livestock farms (9 pig, 1 poultry). Blood samples were taken from 235 of them. The difference in the number of animals in each group was caused by the hyperthermic deaths of some animals despite the use of live traps, deaths which rendered blood sampling impossible due to clotting. On eight farms, heart tissue was collected (256 animals in total). Unfortunately, we were unable to test hearts of all animals trapped, as some hearts were used in order to collect blood for serology.

The serological tests showed that *T. gondii* was present in 6.4% of the 235 blood samples (see Table 6.1 for a specification per small mammal species). In contrast, the nested B1 PCR tests indicated that 71.1% of the 256 heart tissue samples were positive for the pathogen (Table 6.2). Table 6.2 also presents an overview of the number of infected specimens per farm and per species. The prevalence reliability is limited for a number of species due to small capture numbers. There was a significant difference in contamination levels between house mice and white-toothed shrews ($p=0.004$), but not between the sexes of all the infected small mammals ($p=0.164$).

Discussion

Wild small mammals and especially rodents, are frequently associated with structural damage and the consumption and/or spoiling of stored feed and food products. The zoonotic risks of these animals, however, are often overlooked.

Our serological results agree with those of previous serology studies assessing *T. gondii* prevalence in rodents (Tizard *et al.*, 1978; Dubey *et al.*, 1995a; Smith and Frenkel, 1995; Hejlicek *et al.*, 1997). Those studies reported that 2-11.0% of the house mice tested were positive (Tizard *et al.*, 1978; Dubey *et al.*, 1995a; Smith and Frenkel, 1995). Based on this

Table 6.1 *T. gondii* prevalence per wild mammal species based on serology (n=235)

English name	Latin name	# tested	# positive	% infected
Field vole	*Microtus agrestis*	3	0	n.r.*
Wood mouse	*Apodemus sylvaticus*	18	1	n.r.
Common shrew	*Sorex araneus*	2	0	n.r.
Harvest mouse	*Micromys minutus*	6	0	n.r.
House mouse	*Mus musculus*	74	1	1.3
White-toothed shrew	*Crocidura russula*	98	9	9.1
Norway rat	*Rattus norvegicus*	6	0	n.r.
Bank vole	*Clethrionomys glareolus*	2	0	n.r.
Common vole	*Microtus arvalis*	26	4	n.r.
Total		235	15	6.4

* n.r. = % infected is not representative because of the small numbers caught.

Table 6.2 Different mammal species per farm incorporated in the B1 PCR test and their infection rate (infected specimens indicated in parenthesis)

Farm	Period	White-toothed shrew	Common shrew	House mouse	Common vole	Wood mouse	Norway rat	Harvest mouse	Field vole	Bank vole	Total # of animals in B1 PCR
A	Sept	46 (37)	2 (1)	6 (4)	2 (1)	4 (3)	0	6 (6)	1 (1)	0	67 (53)
B	Oct	8 (2)	0	6 (6)	1 (1)	9 (3)	0	0	0	0	24 (12)
C	Oct	17 (12)	0	6 (3)	0	0	8 (3)	0	0	0	31 (18)
D	Oct	15 (7)	0	15 (14)	1 (1)	2 (1)	0	0	0	0	33 (23)
E	Sept	6 (6)	0	8 (7)	0	0	0	0	0	0	14 (13)
F	Sept	0	0	9 (8)	1 (1)	0	0	0	0	0	10 (9)
G	Sept	5 (4)	3 (3)	2 (2)	0	1 (1)	0	0	0	0	11 (10)
H	Sept/ Oct	19 (8)	0	30 (24)	12 (9)	3 (2)	0	0	1 (1)	1 (0)	66 (44)
Totals		116 (76)	5 (4)	82 (68)	17 (13)	19(10)	8 (3)	6 (6)	2 (2)	1 (0)	256 (182)

low seroprevalence, some investigators concluded that rodents are not a major factor in the transmission of the pathogen (Smith *et al.*, 1992).

The present study found a marked difference between serological and nested B1 PCR test results. Earlier studies on *T. gondii* in house and field mice showed a similar pattern of PCR-based assays compared to serology (Owen and Trees, 1998; Hafid *et al.*, 2001).

The difference between PCR and serological results can be explained in several ways. Bioassays already revealed that congenitally infected rodents can harbor viable *T. gondii* in their brains even though their antibody titers are negative (Jacobs, 1964; Dubey, 1997). The study by Lee and colleagues, for example, reported that antibody production patterns can vary after infection with different *T. gondii* strains (Lee *et al.*, 1995). In addition, the dosage of the pathogen, the route of transmission, the life-cycle stage of the parasite, the age of the small mammal at infection and the gestational stage can influence serological responses. Moreover, in the case of vertical transmission, the immune response of the offspring vanishes after a few months even though the parasite remains present in the host tissue (Dubey, 1997; Dubey and Frenkel, 1998). A study by Marshall and his colleagues (Marshall *et al.*, 2004) using PCR detection based on the amplification of the SAG1 gene, reported a 59% prevalence in house mice in an urban environment. The overall *T. gondii* prevalence in small mammals on organic farms as shown by our PCR test (71.1%), therefore, was not entirely surprising. On the other hand, some authors question the specifity of the B1 PCR test due to co-amplification of genomic host sequences (Chabbert *et al.*, 2004; Kompalic-Cristo *et al.*, 2004). Whether this is also the case in rodent species has not been thoroughly investigated until now. Further optimization of molecular diagnosis of toxoplasmosis is therefore needed (sequencing PCR products, confirmation tests using hybridisation with gene specific probes).

Our results demonstrate that, similar to farms in the USA (Dubey *et al.*, 1995b), a substantial percentage of small mammals on European (Dutch) farms can carry *T. gondii* and thus could play a role in the transmission of the pathogen to pigs. Furthermore, we are the first to show a substantial *T. gondii* prevalence in the white-toothed shrew (*C. russula*). It is possible that this insectivore is involved in parasite transmission to pigs.

Our results support an earlier report (Lehmann *et al.*, 2003) based on (serological) research conducted on one farm and incorporating only a limited number of rodents. That paper suggested that the transmission of *T. gondii* near pig sties is considerably higher than in the surrounding environment and farms should be considered as hyperendemic foci of parasite infection. Small mammals are known to amplify a number of pathogens (e.g. *Salmonella* sp.

and *Campylobacter* spp.) and transfer them to food animals and through them to humans. Although our serological and B1 PCR results showed that *T. gondii* is prevalent among small mammals, it remains uncertain whether these animals are able to transmit the parasite to pigs. A bioassay of pigs fed *T. gondii*-infected small mammals should provide some insight into the matter. For the time being, farmers must first become more aware of the veterinary risks of the presence of small mammals. They then need to apply efficient rodent management to decrease their numbers and thus the risk of *T. gondii* transmission by infected small mammals. Limiting the number of wild small mammals on farms will also contribute to a disruption of the parasitic life cycle of *T. gondii*, because the definitive hosts in which the sexual reproduction of the parasite takes place (cats) often acquire their infection from eating infected rodents.

Chapter 7

Toxoplasma gondii Detection in Pork: Discrepancy between Serology and Molecular Detection provides further Food for Thought

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Submitted

Abstract

Consumption of not thoroughly heated pork is considered an important risk factor for acquiring toxoplasmosis in humans, which is estimated to be the third cause of death by foodborne pathogens and the most important cause of infectious posterior uveitis worldwide. Although Toxoplasma gondii seroprevalence estimates in finishing pigs have declined to below 5% in Northern Europe and the USA, recent studies based on molecular detection show a higher prevalence. Estimation of true parasite prevalence in pork is relevant for the development of T. gondii free meat and was therefore the purpose of our study. Diaphragm samples were obtained from finishing pigs following slaughter. In these fresh pork samples T. gondii prevalence was assessed by amplification of the B1 gene using a nested PCR. Samples originated from 33 conventional and 15 organic farms.

A positive PCR reaction was encountered in 55 (29.6%) out of 186 slaughter pigs raised on conventional farms (n=33 farms; 16 [48.5%] farms positive), whereas 106 (55.2%) out of 192 samples from organic pigs tested positive (n=15 farms; 15 [100%] farms positive). Toxoplasma seroprevalence (n=11 organic farms) was demonstrated in 34 (4.3%) out of 794 pigs. This discrepancy could be the result of: 1. higher sensitivity of PCR in combination with a low humoral response, 2. cross-reaction of the parasite with other closely related protozoa, 3. different serological responses after infection with different strains or 4. the possibility of amplification of a genomic DNA product instead of parasite DNA. Further optimization and standardization of detection techniques are required in order to determine true T. gondii prevalence in pork.

Introduction

Toxoplasmosis caused by the *Toxoplasma gondii* parasite affects most vertebrate species including humans (Montoya and Liesenfeld, 2004). It is estimated in the USA as the third cause of death by foodborne pathogens (Mead *et al.*, 1999) and is currently considered to be the main cause of infectious posterior uveitis. Congenital toxoplasmosis leads to severe problems in unborn children and babies, including abortion, hydrocephalus, neurological disorders and retinochoroiditis. Reactivation of undiagnosed congenital toxoplasmosis can lead to ocular toxoplasmosis later in life. Next to congenital disease, acquired infections may cause intraocular inflammation, fever, enlarged lymph nodes and muscle aches. Recent studies have shown that despite treatment, 25% of eyes affected by toxoplasmosis eventually end up as being blind (Bosch-Driessen *et al.*, 2002). Reactivation of latent *T. gondii* cysts can

occur in immunosuppressed individuals (e.g. transplant patients) and is an important cause of lethal encephalitis in AIDS patients (Montoya and Liesenfeld, 2004). Each year, considerable resources are invested in health programmes to prevent toxoplasmosis in pregnant women. However, recent European studies have only demonstrated small effects of both pre- and neonatal screening programmes, and led to uncertainty about the cost-effectiveness of screening (Wallon *et al.*, 1999; Montoya and Liesenfeld, 2004). Furthermore, treatment is disappointing. Although anti-*Toxoplasma* drugs are available, it is not yet clear how effective they are in the treatment of ocular toxoplasmosis. Stanford *et al*.(2003) performed a meta-analysis on this subject in immunocompetent patients and came to the conclusion that to date none of the properly conducted clinical trials have shown a beneficial effect of treatment. Moreover, Gilbert *et al*. (Gilbert *et al.*, 2001; Gilbert and Gras, 2003) did not find a marked difference in transmission risks or neonatal clinical manifestations between seroconverted women that were prenatally treated and those who were not. The combination of these aspects consequently emphasizes the need for prevention of acquiring infection earlier in the food chain, for instance by the introduction of *Toxoplasma*-free food.

T. gondii infections in humans are often caused by consumption of undercooked infected meat or meat products (Cook *et al.*, 2000), whereby pig meat has traditionally been considered as one of the main sources (Tenter *et al.*, 2000). Within Western Europe, *T. gondii* seroprevalence in pigs has decreased markedly over the past decades, which has been attributed to improvements in farm hygiene, increasing farm sizes and introduction of indoor pig production (Tenter *et al.*, 2000; Kijlstra *et al.*, 2004b). A previous study using serological methods showed a reduction of *T. gondii* seroprevalence in finishing pigs from 54% in 1969 to 1% in 1985 (Van Knapen, 1989). More recently, *T. gondii* infection could not be detected in regular pork in the Netherlands, while “animal- friendly” produced pork (with outdoor access of pigs) showed a seroprevalence of 1 to 2.9% (Kijlstra *et al.*, 2004a). Despite these low seroprevalence figures, a recent study from the UK, using the polymerase chain reaction (PCR) method has demonstrated that a very large proportion (38%) of pig meat products available in retail stores, contained *T. gondii* DNA (Aspinall *et al.*, 2002). As pork consumption in the EU has risen from 23 Kg per person per year in 1961 to 45 Kg per person per year in 2002 (FAOSTAT, http://faostat.fao.org), these findings prompted us to investigate the presence of *T. gondii* DNA in fresh pork samples from finishing pigs in the Netherlands.

Methods

Conventional vs. organic pig production systems

In the Netherlands finishing pigs in conventional systems are housed indoors on mostly concrete or semi-concrete beddings and are fed regular pig feed. In contrast, pigs in organic livestock production systems have outdoor access, straw beddings and are fed organic pig feed that is produced without the use of pesticides or fertilizer. In organic pig farming, use of antibiotics and drugs is strictly limited, according to regulations set up by the European Union (EU2092/91).

Sample collection and B1 PCR-test procedure

Diaphragm meat samples of finishing pigs (approximately 6 months old at slaughter) were routinely collected for various research purposes following slaughter and stored at -20^0C. Blood samples from these animals were not available. The meat samples originated from 33 conventional pig farms (n=186) and from 15 organic pig farms (n=192). *T. gondii* prevalence in these pork samples was assessed using the nested B1 PCR procedure. Amplification of the B1 locus is widely used in human clinical specimens, as it is a tandemly arrayed 35-fold repetitive gene (Burg *et al.*, 1989; Hohlfeld *et al.*, 1994; Grigg and Boothroyd, 2001). DNA was extracted using the QIAamp Tissue Kit according to the manufacturers' recommendations (Qiagen, Hilden, Germany). The concentration of extracted DNA was determined spectrophotometrically. All PCR reactions were done in 20µl reactions containing 50 - 200 ng of DNA. Positive (*T. gondii* DNA) and negative (water) controls were added to each microtiter plate. The B1 PCR primers (Burg *et al.*, 1989) were according to the published sequences. For analysis, we used Invitrogen E-gels 48 (4% agarose). To configure gel images, E-Editor Software (Invitrogen, Carlsbad, USA) was used.

Comparison of nested B1-PCR outcome with serological data

Although we did not have the opportunity to test paired blood and diaphragm samples from individual pigs, serological data were obtained for another study from 41 organic pig farms (n=2796) two months earlier. A competition enzyme-linked immunosorbent assay (ELISA) with a peroxidase labeled monoclonal IgM anti-SAG1 antibody was used for the detection of *T. gondii* infections in these blood samples as described earlier by Lind and his colleagues (Lind *et al.*, 1997). Samples of 11 out of the 15 organic farms incorporated in the present study were also analysed as part of that study. We compared the outcome of our nested B1

PCR of those 11 organic farms with the serological results from the study that was performed two months earlier.

Results

The presence of *T. gondii* DNA in pork was tested by collecting diaphragm samples in the slaughterhouse and analysis of these samples using a PCR method whereby the B1 gene was amplified. A PCR product was obtained in 55 (29.6%) samples (n=186) from slaughter pigs raised on conventional farms (n=33 farms; 25 [75.8%] farms positive), whereas 106 (55.2%) of 192 samples of slaughter pigs from organic farms tested positive (n=15 farms; 15 [100%] farms positive).

A comparison of the outcome of the nested B1 PCR results per organic farm with the serological results obtained from the same farms two months earlier that year is shown in Table 7.1. Farms A to K were included in both the serological and the PCR study, while farms L, M, N and O were only tested in the PCR-study (Table 7.1). Overall, the serological study (n=41 organic farms, 2796 animals) showed that the seroprevalence for *T. gondii* was 3.0%. The serological results of farms A to K (n=11 organic farms, 794 animals) showed an overall seroprevalence of 4.3%. Positive *Toxoplasma gondii* serology in the pigs from these 11 farms included in the PCR study ranged between 0% and 27% and seropositive animals were encountered in 5 farms. The nested B1 PCR, on the other hand, showed that positive pigs were found in all 11 farms and that PCR positive frequency per farm ranged between 20% and 100%. Serological results and PCR results of the same farm sometimes differed markedly (farm K), while in other occasions this difference was less substantial (farm C).

Discussion

Our results show a marked discrepancy between the serological and PCR detection of *T. gondii* in slaughter pigs. It was demonstrated that, depending on the production system, between 29-55% of the animals were positive when tested in the *Toxoplasma* B1 PCR test. However, serology on the same farms two months earlier indicated that only a few percent of the animals present at that moment were infected with *T. gondii*. These serological results are in agreement with earlier serological observations from our group (Kijlstra *et al.*, 2004a). On the other hand, our PCR results are in line with previous observations: an earlier study from the UK (Aspinall *et al.*, 2002) showed that a high percentage of meat products (38%) containing pork ingredients harboured *T. gondii* DNA. Moreover, a recent study from Brazil

(Dias *et al.*, 2005) has shown that fresh pork sausages can indeed contain viable *T. gondii* parasites. The difference between these earlier molecular studies and ours is that we tested individual pigs whereas pig meat products/sausages are usually made from a pool of meat obtained from many pigs. In this situation a few (heavily) infected pigs can contaminate a whole batch of meat products.

Table 7.1 Comparison of detection of *T. gondii* contamination of pigs using serology and B1 PCR

Farm	No. positive pigs in serological study	No. positive pigs in nested B1 PCR
Farm A	0/46 (0 %)	6/20 (48 %)
Farm B	0/98 (0 %)	8/20 (40 %)
Farm C	26/95 (27%)	13/20 (65 %)
Farm D	0/75 (0%)	4/20 (20 %)
Farm E	3/95 (3 %)	4/10 (20 %)
Farm F	0/35 (0 %)	8/12 (67 %)
Farm G	1/80 (1%)	8/10 (80 %)
Farm H	0/20 (0%)	6/10 (60 %)
Farm I	1/95 (1%)	5/10 (50 %)
Farm J	3/80 (4%)	5/10 (50 %)
Farm K	0/75 (0%)	10/10 (100 %)
Farms L, M, N & O	Not tested	29/40 (73 %)
Totals	**34/794 (4.3%)**	**106/192 (55.2%)**

Various explanations for the discrepancy between PCR and serology in the current results can be envisaged. A lab contamination is unlikely since controls were always negative and strict separation of lab rooms for the various procedures during the PCR analysis was enforced. The obtained results could also be explained by assuming a cross reaction of our B1 PCR assay with other parasites in pigs. The only other Apicomplexan parasite that has been found in muscle tissue of pigs is *Sarcocystis*, most likely *S. miescheriana* or *S. suihominis.* The *Sarcocystis* sequence for the gene resembling the Toxoplasma B1 gene is unfortunately not yet available in the EMBL library. Others have shown however that *S. neurona* DNA did not cross-react with the B1 primers also used in our study (Vashist *et al.*, 2005). Therefore, a

cross-reaction with a closely related protozoan seems unlikely. Another possibility is that molecular approaches for *T. gondii* detection still have some pitfalls; recent studies in humans for instance reported that amplification of the protozoan B1-gene may co-amplify human genomic sequences (Chabbert *et al.*, 2004; Kompalic-Cristo *et al.*, 2004), a phenomenon that could also occur in other species such as the pig. Further characterisation of the B1 PCR products (sequencing, hybridisation with specific probes) found in our study should be performed to resolve this issue.

Another possible explanation is a combination of factors such as a high sensitivity of a nested PCR for *T. gondii* DNA in combination with a low humoral immune response against the parasite. A low humoral immune response in pigs may be related to the source of infection (oocysts in soil, feed or water; tissue cysts in rodents, tachyzoites in milk), whether the infection was congenital or acquired, the time of infection during the six month finishing period or the strain of *T. gondii* involved. Little is known about the role these factors play in the degree of the serological response in pigs. Recently the group of Fehlhaber performed a longitudinal study on a number of pig farms in Germany and made some interesting observations (Schulzig and Fehlhaber, 2005). Testing of individual animals took place during the period from birth to slaughter. At slaughter seroprevalences varied between 0 and 15.2 %. Curiously enough, all slaughtered seropositive pigs (6 months old) were tested negative at an age of 9 weeks, even while high titres of *T. gondii* antibodies were detected shortly after birth (3 to 4 days). Furthermore, many newborn piglets were seropositive, but became seronegative at the age of six months. Further studies are needed to explain these anti-toxoplasma responses during the six month lifetime of a slaughter pig.

Strain differences may also account for different reactions after *T. gondii* infection, as was found in a previous study in pregnant minipigs (Jungersen *et al.*, 2001). Some strains led to abortions and mummified fetuses, whereas others led to minimal transplacental transfer. One identified *T. gondii* strain was apathogenic to both minipigs and mice, but parasites were transferred to all fetuses. Unfortunately, immune responses in surviving fetuses were not analysed post partum (Jungersen *et al.*, 2001). Studies in mice have also shown that antibody production patterns can vary after infection with different *T. gondii* strains (Lee *et al.*, 1995; Lee and Kasper, 2004). Another study in rats has shown that congenitally infected rodents harboured viable *T. gondii* in their brains, while their antibody titers were negative (Dubey, 1997). A longitudinal study in congenitally infected children has also shown that *Toxoplasma*

antibodies can decline to very low levels during lifetime, despite a reactivation of ocular lesions (Rothova, 1986). Taken together, the above mentioned studies show that *Toxoplasma* infection can lead to the presence of parasites in the tissues in combination with a low serological response.

The presence of viable *T. gondii* in pigs is generally shown by the administration of tissue samples to mice or cats followed by the determination of infection in these latter animals. In general a good agreement was observed between serological tests in pigs and these bioassay results (Dubey *et al.*, 1995a; Dubey *et al.*, 1996), although occasionally seronegative pig meat samples have been shown to be infective after administration to cats or mice (Dubey *et al.*, 1995a). Also others have found discrepancies between serological and bioassay results. Using molecular methods, a discrepancy was observed in pigs where *T. gondii* ribosomal RNA was demonstrated, whereas the mouse bioassay of the same samples was negative (Gajadhar *et al.*, 1998). Omata *et al*. isolated *T. gondii* in 14 out of 109 pig diaphragm samples, but only 8 out of these 14 samples were virulent when fed to mice (Omata *et al.*, 1994). These findings could be explained by assuming that certain *T. gondii* strains residing in pigs do not induce a vigorous humoral immune response and may also not be infectious for "indicator" mice. This is in agreement with the results by the group of Lind, showing that some strains of *T. gondii* can seem apathogenic for both mice and pigs (Jungersen *et al.*, 2001). Whether these strains are infectious for humans is also not clear. Detailed analysis of strains from infected humans and comparison with strains residing in pigs may provide an answer to this question. Until the time that these virulence and typing studies are performed, freezing of all pig meat products might be a solution to reduce infectivity of any potential virulent *T. gondii* cysts for humans. This will especially be important with meat that is used in those meat products that are not heated before consumption such as certain fresh pork sausages. Moreover, as our PCR-results once again demonstrate that a substantial discrepancy exists between serological and molecular detection methods of *T. gondii*, more efforts should be put in standardization of these techniques and improvement of their technical sensitivities and specificities in order to facilitate detection.

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Chapter 8

The Ethics of Rodent Control

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Submitted

Abstract

Because Western societies generally see animals as objects of moral concern, demands have been made on the way they are maintained and treated, e.g, during food production and animal experimentation. In the case of rodent pests, however, inhumane control methods are often applied. This inconsistency in the human-animal relationship demands clarification. The present paper analyses the criteria that must be fulfilled when judging the use of animals during experiments and investigates whether they can be applied in the rodent-control situation. This is important, because until now animal welfare has not been an issue in pest control: effectiveness, hygiene and cost-efficiency have been the leading principles underpinning rodent control methods. Two options are available to solve the inconsistency: the first is to abandon the criteria used in animal experimentation; the second is to apply the criteria to both animal experimentation and rodent control. This latter, more preferable option implies that ethical rodent control methods should not lead to intense pain or discomfort; this discomfort should have a short duration and should allow escaped rodents to lead a natural life. Adherence to this option will, however, require a shift in the design of rodent control methods: effectiveness will no longer be the main leading principle. It will have to share its position with animal welfare and humaneness.

"When mice do little more than nibble our food, we justify using some of the most abominable painful mechanical and chemical means to exterminate them. However, when kept as laboratory animals, we accord them rights".
Andrew Moore, EMBO reports, 2001, vol 2, no. 7, 554-558.

Introduction

Animals have a moral value and are considered by many as appropriate objects of moral concern. Because of this, society has drawn up laws about the way animals should be treated. In fact, there is serious concern by the general public whether animal experiments are useful for human welfare and whether animals need to be harmed at all. The number of animals that are harmed is also important (Morris, 2000).

In contrast, less attention is paid to the harm done to animals during rodent control. Moreover, the criteria used to judge animal experiments do not seem to apply to rodent pests in a persons' own home or professional environment. This noteworthy inconsistency in human-

animal relationships becomes even more evident when the amount of scientific literature focusing on animal welfare issues in animal experimentation is compared to that on animal welfare in rodent control. The present paper examines this problem. It first describes the criteria used in the debate on animal experimentation and the core moral values of the human-animal relationship in this field. Then, it describes the development and characteristics of rodent control and shows that ethical considerations, like animal welfare, play no role in this area. Finally, it investigates the differences between the two different contexts and proposes recommendations that might provide more consistency.

Criteria used in animal experimentation

A multiformity of positions can be observed throughout the society with regard to the use of animals in research. Some individuals believe it imperative to protect animal welfare no matter what, while others believe that moral arguments favour the continuation of research using animals. A common framework has, nevertheless, been derived from these opposite positions. It forms the foundation of many laws that apply to the use of animals in research, such as the Animal (Scientific Procedures) Act 1986 (A(SP)A) in the United Kingdom and the Council Directive 86/609/EEC of the European Union.

The authoritative report of the Nuffield Council of Bioethics (NCB, 2005) contains an analysis of ethical issues that are important in animal experimentation and further investigates the framework used in this type of research. The debate on research involving animals is often reduced to the question of defining the moral status (or moral importance) of humans and animals (NCB, 2005). In the report, the Council asks itself which features of humans and animals qualify them as moral subjects, thus imposing constraints or limits on how they may be treated. It presents five relevant features: sentience, higher cognitive capacities, the capacity to flourish, sociability and possession of a life.

According to the Nuffield Council (NCB, 2005), all research licensed in the UK under the Animal (Scientific Procedures) Act 1986 (A(SP)A) has the potential to cause pain, suffering, distress, or lasting harm to the animals used. Because of this, the nature of any pain, suffering, or distress first needs to be determined in order to assess the ethical implications of animal experimentation. The Council does agree that, philosophically, it is extremely difficult to determine exactly the subjective experiences of animals. Practically, however, it is often

possible to make meaningful approximations. Evaluating clinical signs or determining animal choices, familiarity with ethology and ecology, and consideration of physiological and neurological features are all important in this respect. In practice, welfare consequences of animal use in research vary greatly, which makes it impossible for the Council to generalise about the way animals are affected by their use in research.

The Council has, however, proposed criteria that can be used to judge animal experimentation. These criteria are extremely useful for policy-making since there is a need for a single policy on animal experimentation. The criteria are mainly based on the 'Three Rs' (Refinement, Reduction and Replacement). According the Council, the concept of the 'Three Rs' and its legal implementation should be acceptable, or at least tolerable, to all individuals holding reasonable views. Moreover, it should prevent instability, protest, and, in extreme cases, civil unrest. The criteria that the Council mentions have been cited in earlier studies (Degrazia, 1999; Heeger and Brom, 2000). They are:

1. Care for research animals. The benefits of research for humans should be obtained with a minimum of pain, suffering, distress, lasting harm, or death to animals involved in research.
2. The search for alternatives. If possible, alternatives should be used instead of animals. This would significantly reduce the number of animals used in research.
3. Offer research animals the possibility to lead natural lives. When the animals are not (yet) used for research, their accommodation should enable them to lead natural lives.
4. Animal experiments involving animal suffering should result in an equivalent or better alleviation of suffering in equal or greater numbers of humans.

With these criteria in mind, let us now look at the rodent control situation. Is it feasible to apply these criteria there? Before discussing this matter, the paper will present an overview of the development of rodent control and the characteristics of the most important rodent control methods.

Development and characteristics of rodent control

Rodents are among the most significant pests worldwide (Prakash, 1988; Singleton *et al.*, 1999). They are known for their impressive reproductive rates and gnawing capabilities (Meehan, 1984) and their ability to spoil food and destroy infrastructure. Fear of rodents has been embedded in European culture for centuries and has gradually developed into a general

antipathy to the presence of commensal rodents in and around our premises. Public attitude towards rodents reflects a connection with filthy environments and ill health. Indeed, rodents are known to carry a variety of organisms that may cause diseases in human and domestic animal populations (Gratz, 1994). The black rat (*Rattus rattus*), for example, played a key role in spreading the bubonic plague during the Middle Ages (Scott and Duncan, 2001). Rodents can also carry other pathogens and diseases, including *Leptospira* (Bunnell *et al.*, 2000b), hantaviruses (Webster, 1996), porcine parvovirus (Joo *et al.*, 1976), Aujeszky disease virus (Maes *et al.*, 1979), Foot and Mouth Disease virus (Capel-Edwards, 1970), avian influenza (Le Moine *et al.*, 1987), *Toxoplasma gondii* (Marshall *et al.*, 2004), and *Salmonella* and *Campylobacter* (Fernie and Park, 1977; Davies and Breslin, 2003). The need for effective rodent control has led to the development of many control methods that cause animal suffering. A few of the methods used to kill rodents in homes include poisoning, trapping, hunting, shooting, fumigants and deliberately introducing diseases (Oogjes, 1997; Meerburg *et al.*, 2004). In general, these methods can be classified into three different types: bait poisons, fumigant poisons and non-poisonous measures (Mason and Littin, 2003).

Until 1950, bait poisons, acute rodenticides in particular, were the mainstay of the pest-control profession. These poisons were known for their quick-acting, high toxicity to all life forms and for the absence of antidotes. Their palatability had to be good to ensure that a lethal dose was consumed in one feeding. Rodents were, however, often able to link bait consumption with poisoning of their congeners, resulting in bait shyness of the remaining animals. The British Government forbade the use of acute rodenticides in the Animal Cruel Poisons Act 1963 because the effects of this type of poison were extremely cruel. Another important disadvantage of acute rodenticides was the poisoning of non-targets, such as birds, domestic animals and sometimes even children, with lethal consequences.

New series of ‘chronic’ rodenticides were developed to overcome the limitations and hazardous nature of acute rodenticides. They were sold under the name anticoagulants. ‘Chronic’ means that rodents must consume the bait over a number of days before a lethal dose is reached. These anticoagulants inhibit the production of clotting agents in the rodent’s liver (Bell and Caldwell, 1973; Zimmermann and Matschiner, 1974; Thijssen *et al.*, 1986), resulting in death due to internal haemorrhages. Death usually occurs five to fifteen days after bait consumption. If the uptake of poisonous bait is insufficient, the animal will recover due to its own production of vitamin K which restores blood-clotting ability. This delayed action

has a decided safety advantage because it leaves time to administer the antidote (vitamin K) and thus save pets, livestock, and, of course, people who may have accidentally ingested the bait.

First-generation anticoagulants (e.g. warfarin, fumarin, pindone) were developed in the late 1940s and 1950s. The major disadvantage of these first-generation anticoagulants was that rodents became genetically resistant (Greaves and Ayres, 1969, 1982), which resulted in a gradually decreased drug efficacy (Hildebrandt and Suttie, 1982). This resistance was the result of sustained applications of this first-generation toxicant. Resistance to warfarin has been widely documented in United Kingdom rodent populations; in fact, in some cases rodents fed freely on toxic bait with no harmful effects (MacNicoll, 1985; Quy *et al.*, 1995). In response to emerging resistance (Gratz, 1973), more potent second-generation anticoagulants, such as brodifacoum and flocoumafen, started being developed in the 1970s.

Studies have shown that continued use of second-generation anticoagulants, e.g. brodifacoum, may have a lower risk of inducing resistance. This advantage, however, is offset by the persistent nature of these compounds (second-generation anticoagulants appear to be more persistent in animal tissues) and thus an increased potential for adverse effects on non-target species, especially sublethal or lethal poisoning through secondary exposure (Eason *et al.*, 2002). Secondary exposure in this case means that a poisoned rodent is consumed by another species, such as birds of prey (e.g. barn owl). Removal of dead poisoned rodents is, therefore, always necessary.

Fumigant poisons (e.g. carbon disulphide, carbon dioxide, sulphur dioxide, aluminium phosphide and cyanide gas) are gases used in rodent control. If correctly carried out, fumigation is the most effective rodent control method (Meehan, 1984). It also has two main advantages over bait-poison use (Mason and Littin, 2003): secondary poisoning risks are minimal and the dependant young are killed together with their mother instead of being left in the nest to die.

Non-poisonous measures include traps and chemosterilants. Although the use of traps is generally labour-intensive (Meehan, 1984) and handling of (dead) rodents can lead to the transmission of pathogens (e.g., *Leptospira* or hantaviruses), traps have the advantage that the dead bodies can be easily collected and the unpleasant smell of decomposition prevented (Corrigan, 1998). Various types of traps exist: glueboards, electrocution traps, snap traps and

non-lethal curiosity traps, each with its own characteristics and effects on animal welfare (Mason and Littin, 2003). Fertility control of rodents using chemosterilants (e.g., synthetic steroids) is also an option (Gao and Short, 1993), although these drugs might have consequences for predator dynamics as well.

When the reasons behind the development of the three types of rodent control methods are considered, one sees different motivations (Table 8.1). The most important reason behind the development of bait poisons was effectiveness and/or safety. The drive behind the innovation of fumigant poisons was focused mainly on effectiveness, but also on reduction of the effect the method had on the environment. With regard to non-poisonous measures, the main focus of some methods was increased animal welfare or the reduction of animal suffering. The development of non-lethal curiosity traps during the last decades is a good example of this: residents can catch rodents in the traps and then release them somewhere where they will not cause problems. Another is the development of high-tech methods such as chemosterilants. It is rather difficult, however, to prove whether the welfare of animals is truly improved if rodent pests cannot fulfil their ecological duties (namely, to create progeny). More advanced lethal traps have also been developed in which rodents are caught more easily and hygienically.

In an excellent review, Mason and Littin (Mason and Littin, 2003) assessed the humaneness of all rodent control methods used in the UK and the USA. They showed that the methods that produce a quick death with minimal suffering are preferred from an animal welfare point of view. Snap traps or electrocution traps kill extremely rapidly if set properly (Broom, 1999) and are, therefore, considered relatively humane (Mason and Littin, 2003). Animal-welfare problems can occur, however, if the traps result in injury instead of death, which happens in about 7-14% of all cases (Mason and Littin, 2003). The humaneness of fumigants varies between the types and concentrations used. If properly applied, carbon dioxide can kill within minutes and causes unconsciousness before death. This method, therefore, is also judged as relatively humane (Mason and Littin, 2003). In contrast, ingested poisons, such as anticoagulants, are generally considered to induce animal suffering (Kirkwood *et al.*, 1994; Chambers *et al.*, 1999).

Rodents generally remain conscious between the time of poisoning and death, a period with an average duration of 7.2 days. During that time, haemorrhages in vital organs (e.g., lungs, kidneys) caused by anticoagulants can lead to serious discomfort through the accumulation of

blood (Broom, 1999). Moreover, because individual animals ingest the poison during foraging, dependant pups will die of dehydration and starvation when adult females are poisoned.

Table 8.1 Drive behind developments of the main types of rodent control methods

Rodent control method	**Development**	**Drive**
Bait poisons	*Acute rodenticides -> 1st generation anticoagulants*	• Effectiveness • Absence of antidote • Cruelty (in some countries)
	1st generation anticoagulants -> 2nd generation anticoagulants	• Effectiveness
Fumigant poisons	*Fumigants -> advanced fumigants*	• Effectiveness • Safety • Environment
Non-poisonous measures	*Lethal traps -> non-lethal curiosity traps*	• Animal welfare
	Lethal traps -> advanced lethal traps	• Effectiveness • Hygiene
	Chemosterilants	• Animal welfare

Finally, secondary poisoning may lead to lethal consequences for non-target species and the mode of action or dose of poison may lead to disturbances of the animal's own metabolic processes and increase the intensity of suffering. Glueboards and other restraining traps are also considered inhumane (Mason and Littin, 2003) because it takes longer than a few hours and sometimes days before the trapped animals die (Meehan, 1984; Randall, 1999). Their use can also result in panic: when the animal tries to escape, it can hurt itself through the forceful removal of hair, torn skin and broken limbs (Frantz and Padula, 1983). Rodents sometimes even bite through their own limbs to escape (Frantz and Padula, 1983). The use of glueboards is currently under debate. The National Animal Welfare Advisory Committee of New Zealand has concluded that glueboards can cause both physical pain and distress over a longer period of time. Although the Committee left the decision to abolish the use of glueboards to the government, it did conclude that frequent checking of glueboards and humane disposal of

trapped animals could produce a higher degree of acceptability. Although the use of glueboards in rodent control is forbidden in few countries (including the Netherlands), the method is still allowed in most (including the USA).

Criteria used in rodent control

The criteria mentioned by Mason and Littin (2003) for judging various rodent control methods are comparable to those cited by the Nuffield Committee in the case of animal experimentation: 1. the degree of pain, distress, or discomfort that is caused to the target animal; 2. the length of time during which rodents remain conscious after the method is applied; 3. the effects of the method on escaping and surviving rodents; 4. the effects on the rest of the population (e.g., on dependant pups) and 5. the effects of secondary poisoning (poisoning of non-target animals).

Despite the discussion about the criteria used to judge rodent control methods on animal-welfare grounds and the assessment of control methods, the methods being applied today have not changed. In practice, adherence to criteria cited in the literature appears difficult. Why? The reason is that the majority of the public does not have moral concerns regarding pest animals, the method of rodent control and the humaneness of the killing. There is a general desire to be as uninvolved with rodent control as possible. In fact, many people simply want a magic black box to take care of their rodent problems (Jackson, 1980). It can be concluded from Table 8.1 that effectiveness has been the leading principle underlying the development of rodent control methods. Effectiveness, hygiene and cost-efficiency are the key words for the public. As a consequence, the struggle for more animal welfare in rodent control does not come from society, but from the scientific world and (sometimes) policy. In contrast, animal experimentation has the full attention of the general public, which explains the amount of legislation on the use of animals in research. Even in the USA, federal regulations require that pain and discomfort among research animals be effectively minimised. Only in a few countries (many in the European Union) are there laws on how to deal with rodent pests and has the use of some methods been abolished on animal-welfare grounds. Unfortunately, legislation on this topic is still lacking in other parts of the world (including the USA).

Recommendations for ethical rodent control

Rodent control is a serious issue, since as many as 20 million rodents are killed each year in the UK alone (Fox and MacDonald, 1999). Rat and mouse control potentially affects the welfare of innumerable animals worldwide (Mason and Littin, 2003). In comparison, approximately 2.72 million animals (mostly rodents) were used in scientific procedures in the UK in 2003 (NCB, 2005). As noted above, even though many different techniques exist to control rodents, the application of relatively humane methods is often neglected. This provides food for thought.

The present analysis demonstrates an inconsistency in the human-animal relationship: the general public has demands about the way research animals are treated, while it shows apathy towards the methods of pest control. Apparently, the views humans have towards animals are not rigid, but vacillate and are context-bound. This seems rather illogical: animals of the same order of the Animal Kingdom (both pest and laboratory animals are classified as rodents) are judged depending on their context.

Two options are available to gain consistency in the matter. The first is to disregard the criteria cited by the Nuffield Council and start treating research animals in the same way we now deal with rodent pests. This means the abolishment of experimental animal legislation, i.e., we would again be allowed to use experimental animals in an inhumane way. The second option is to apply the criteria used in animal experimentation to rodent pests. If the latter option is chosen, then not only should a rodent control method be judged by its effectiveness, cost-efficiency and/or hygienic advantages, but the least invasive agent and most humane delivery mechanism available should be used. In other words, rodent control methods should not lead to intense pain or discomfort, the duration of any discomfort or pain should be minimal, and rodents that escape should still be able to lead a natural life.

Consistency also demands a shift in the public's perception about rodent pests and a shift in the methods currently used for rodent control purposes. In contrast to animal experimentation, consumers apply rodent control themselves and should, therefore, be informed, e.g., by means of labels, that rodent control methods can be inhumane. Professional pest controllers should also be warned that some methods can cause animal suffering. The use of sticky boards, for example, can result in serious, long-lasting discomfort and the application of second-

generation anticoagulants can lead to a slow death. Consumers should be advised that snap traps and electrocution traps do not usually require animals to first be captured and held (for potentially long periods of time), and thus produce a more acceptable level of pain or distress than other non-lethal traps. Finally, consumers should be made aware that a welfare-friendly trap design does not guarantee that pest animals do not suffer. Although rarely associated with pain, non-lethal curiosity traps can cause distress as a result of the animal being physically contained. If checked frequently, these traps are a good alternative and cause only limited amounts of discomfort.

Rodent control is, however, the last resort. Effective rodent management consists of three elements: prevention, monitoring, and control (Meerburg *et al.*, 2004). Prevention focuses on the exclusion of rodents or on reducing the attractiveness of their habitat by, e.g., minimising access to food and water. Removing specific habitat elements that function as hiding and nesting places (e.g., shrubs in gardens within 2 m of the family home, piles of rubbish), blocking rodent entrances to houses (e.g., ventilation shafts) with wire-netting (distance between the wires <5 mm), and reducing food and water access will lead to a significant reduction of rodent presence. Monitoring improves the decision-making process in the prevention of rodent infestations (Meerburg *et al.*, 2004). This approach to pest control is known as Integrated Pest Management, or IPM, and is applied as part of the quality procedures (e.g., HACCP) in many business sectors. Effective rodent management requires a thorough understanding of the ecology of "pest" species. Unfortunately, the importance of the first two steps (prevention and monitoring) is often overlooked in private environments (e.g., residential areas) and consumers usually apply rodent control.

Raising public awareness with regard to rodent management, therefore, is necessary, not only among consumers but also among animal-welfare organisations, policy makers, and researchers such as animal ethicists. There is much scientific literature available on the ethics of food-production animals, wild animals, or animals used in experiments, but limited information on moral concerns regarding those animals whose presence does not please us. This article is the first attempt to raise public awareness. Nevertheless, more systematic attention from the scientific world is necessary.

Conclusion and animal-welfare implications

This article has discussed a striking inconsistency in the relationship between humans and animals: animal welfare is a hot issue in animal experimentation, but almost completely ignored in pest control. People do not accord the same rights to pest animals as they do to experimental animals. This has led to a situation in which commonly used rodent control methods are inhumane and cause animal suffering. If the human-animal relationship is to be consistent, then either the same criteria must be applied to rodent pests and research animals, or the criteria used in animal experimentation must be abandoned. If the first option is chosen, which is most likely, then ethical rodent control methods should not lead to intense pain or discomfort, the duration of pain should be short, and escaped rodents should still be able to lead a natural life. In this case, both Replacement (prevention of rodent presence) and Refinement (to pick the most welfare-friendly alternative for rodent control) become important. This will consequently result in a shift in the design of rodent control methods: effectiveness will no longer be the main leading principle. It will have to share its position with animal welfare and humaneness. More awareness, therefore, must be created among consumers, professional pest controllers, policy-makers, animal-welfare organisations, and scientists in order to gain attention regarding moral concerns towards pest animals.

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Chapter 9

Towards Sustainable Management of Rodents in Organic Animal Husbandry

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Abstract

From 26 to 28 May 2004 an international seminar was held in Wageningen, The Netherlands, about current knowledge and advice on rodent management on organic pig and poultry farms in Western Europe. This paper summarizes the discussions. Rodent management is necessary to protect the food production chain from health hazards to livestock and humans. Some organic farmers prefer biological rodent control, but since rodents can also transmit diseases this bears certain risks for the production of healthy livestock and safe food. Effective rodent management requires a thorough understanding of the biology of the pest species concerned. These can be divided into two groups: field rodents, such as voles, and commensal rodents like house mice and rats. The objective of managing field rodents is to minimize livestock exposure to these vectors, and to regulate their populations in case their density is expected to grow dramatically. Infestation of livestock facilities with commensal rodents can be prevented, but once they are present, their eradication must be aimed for. General elements of rodent management are (1) the prevention of rodent infestations through strategic actions such as modifying the habitat or rodent proofing of the buildings, (2) monitoring their appearance and population density, and (3) rodent control measures. A number of possible management actions is described to provide a basis for examining the measures' social acceptability, their economic and environmental impacts, and their efficacy.

Introduction

From 26 to 28 May 2004 an international seminar was held in Wageningen, the Netherlands, about rodent control strategies on organic pig and poultry farms in Western Europe. This seminar was organized to address and discuss the issues of rodent control in relation to the principles of organic farming, food safety, animal health, efficacy, costs and animal welfare and suffering. The seminar was financed through the European Union Sixth Framework Programme 'Quality of Low Input Food'. This paper first presents the state of the art in the field of rodent management and then provides a number of recommendations following from the discussions at the seminar.

State of the art

The need for rodent management is imperative for farm production systems. Rodents can be divided into two main groups: native field rodent species (e.g. *Microtus, Arvicola, Apodemus*) that are part of the wildlife fauna, and commensal rodent species. Commensal rodent species (in Europe: *Rattus norvegicus, R. rattus* and the *Mus musculus-domesticus* complex) have

lived in association with humans for millenia, and their high reproduction rates and omnivory can lead to significant impacts (e.g. Meehan, 1984) by consuming or fouling stored agricultural produce, acting as disease vectors or destroying infrastructure. In many parts of the world, these commensal species are also able to live in fields and crops. In Europe, commensal rodents usually live in or near buildings, feed stations and shelters for farm animals. Major fluctuations in population density as have been reported for Australia and Asia (Singleton and Redhead, 1989; Hanski *et al.*, 2001; Jacob *et al.*, 2004) do not occur in Europe. Although commensal rodents in Europe, just like field rodents, respond to changes in food and shelter availability, the temporal variation in the human environment is smaller than the variation in field environments. Therefore, the population density of commensal rodents fluctates generally less than that of field rodent species. Under certain conditions, field rodent species can sometimes even show cyclic population dynamics. However, some field rodent species (e.g. *Apodemus*) do not show such pronounced interannual fluctations as e.g. *Microtines.* Control actions are necessary if a field rodent population is expected to reach a density that is of economic concern to a farmer.

Rodents can transfer pathogens and parasites (e.g. *Leptospira* spp., *Salmonella* spp*., Campylobacter* spp., *Trichinella* spp.) to animals and their products, to farmers and (indirectly) to consumers of animal products thus causing food safety problems (Le Moine *et al.*, 1987; Muirhead, 1993; Kapel, 2000). Also, at a broader geographic scale, rodents can be potentially hazardous because they can transfer contagious animal disease agents between farms. Examples are porcine parvo virus (Joo *et al.*, 1976), Aujeszky's disease virus (Maes *et al.*, 1979), Foot and Mouth Disease virus (Capel-Edwards, 1970; Epoke and Coker, 1991), *Listeria* (Iida *et al.*, 1998), avian influenza and leptospires (Le Moine *et al.*, 1987; Boqvist *et al.*, 2002). The consequence is that rats and mice cannot be tolerated in the food production chain, including livestock production, irrespective of the degree of infestation. Even a single rodent can be a vehicle for transmitting a disease between farms.

Rodents are often responsible for infrastructural damage such as gnawing on insulations. Besides, they can attract predators such as foxes (*Vulpes vulpes*) to intensive animal production units, which can result in high losses of young pigs and poultry. Rodents can cause a productivity drop (reduced weight gain and/or reduced breeding success) through harassing the farm animals (Caughley *et al.*, 1994). They can also be responsible for considerable feed losses and direct predation of young poultry and for wounding of livestock, especially the teats of lactating sows and the feet of chickens.

Trapping of rodents using snap-traps is often applied. Also rodenticides are frequently used, as they are among the most effective and least expensive measures for rodent control in intensive agriculture. Most of the time these rodenticides are anticoagulants, which act by interrupting the vitamin K cycle in the liver microsomes (MacNicoll, 1986). As a result, the maintenance of a number of clotting factors is hampered, resulting in fatal haemorrhages after a few days. Based on their toxicity, these rodenticides can be divided into two groups: the first-generation compounds such as warfarin, which have lower acute but higher cumulative toxicity, and the second-generation compounds, with a higher acute toxicity and developed in the 1970s and 1980s (Buckle, 1994). House mice and black rats are less susceptible to first-generation anticoagulants, whereas Norway rats are susceptible for these means, with the exception of certain restricted areas where resistance to one or several anticoagulants may occur in some populations. Here, knowledge of the resistance situation is a prerequisite for the choice of an active ingredient. In areas where resistance of rodents against one or different anticoagulant rodenticides exists (parts of Europe, North America, Australia and Japan), a rodenticide with higher potency should be used (Greaves, 1994). Unfortunately the second-generation rodenticides pose a higher hazard for primary or secondary non-target poisoning and so their use is limited. Four factors determine the uptake of a rodenticide bait (Klemann and Pelz, 2004): (1) whether the rodents are neophobic (fear of new objects) or neophilic (inquisitive of new objects), (2) the population structure of the target rodent population, (3) bait palatability and (4) habitat structure. However, control measures effective against one or a few rodent species should have a minimal effect on other species. Some of these other rodent species may even be part of the protected wildlife fauna (see Table 9.1).

For organic farmers, the presence of rodents on their farm is not always a problem, although they generally perceive rats as a bigger problem than mice (Leirs *et al.*, 2004). We therefore assume the threshold for starting rodent control to be higher among organic than among conventional farmers. However, in case a rodent problem emerges, traditional rodent control methods, such as application of rodenticides, do not really fit in with the philosophy of organic farmers, although in the European Union they are allowed by the regulations that apply to organic farming (Anonymous, 1991, 1997). A survey in the Netherlands showed that 100% of the conventional farmers used rodenticides, against only 69% of the organic farmers (Kijlstra *et al.*, 2004a). The organic farmers often preferred other methods such as the use of cats (Kijlstra *et al.*, 2004a). However, it is possible that the mice and rats caught by these cats are intermediate hosts for parasites such as *Toxoplasma gondii.* This parasite is transmitted

via the food chain and is known to alter the behaviour of infected rodents, making them less afraid of predators like cats (Berdoy *et al.*, 2000).

Table 9.1 Potential pest, abundant and protected species in the Netherlands as considered by an expert panel

Species in the Netherlands		
Potential	Abundant, but no pest	Protected
Commensal species		
House mouse (*Mus domesticus)*		
Norway rat (*Rattus norvegicus*)		
Black, roof or ship rat (*Rattus rattus*)		
Wild species		
Common vole (*Microtus arvalis*)	Bank vole (*Clethrionomys glareolus*)	Northern vole (*Microtus oeconomus*)
Water vole (*Arvicola terrestris*)	Pine vole (*Microtus subterraneus*)	Beaver (*Castor fiber*)
Musk rat (*Ondatra zibethicus*)	Harvest mouse (*Micromys minutus*)	Yellow-necked mouse (*Apodemus flavicollis*)
Coypu (*Myocastor coypus*)	Field vole (*Microtus agrestis*)	Hazel dormouse (*Muscardinus avellanarius*)
Wood mouse (*Apodemus sylvaticus*)	Fat dormouse (*Glis glis*)	Garden dormouse (*Eliomys quercinus*)

These cats can then become the definitive host for the parasite, and excrements from infected cats can then pose a hazard to the health of farm animals and humans. *Toxoplasma* infection occurs if pigs and poultry accidentally ingest infective oocysts from the environment. Cats may shed more than ten million oocysts per day for 3 to 10 days after infection (Dubey *et al.*, 1995b). Apart from the health risk presented by cats, there is no sound evidence that cats regulate rodent populations.

One of the preconditions of organic animal production systems is access by farm animals to outdoor environments. Other characteristics of organic production systems are the use of organic feedstuffs, restrictions in medicine use, lower stocking densities, and, as in the case of pigs, a higher weaning age, straw bedding, and roughage in the diet. Access by organic pigs and poultry to the outdoors will lead to higher exposure to infective stages of both micro- and macro-parasites and to the transmission of disease through direct contact with wild fauna, compared with conventionally kept livestock. Cleaning and disinfection of the living

environment is more difficult for organic production systems. So an increase in organic animal farms could potentially lead to increased prevalence of *Toxoplasma gondii* and other pathogens.

To prevent emergence of *Toxoplasma* and other potential food safety hazards, there is a clear need for rodent control that is in line with the philosophy of organic farming. The seminar was organised to come up with recommendations for organic farmers how to organise their rodent management. This management should minimize welfare problems associated with rodent control measures, and the risk of environmental contamination or poisoning of non-target species must be negligible. Furthermore, slaughterhouse monitoring combined with on-farm prevention strategies and consumer education on preparation of organic meat products (e.g. Oosterom, 1991) are necessary to reduce potential infection risks and ensure food safety of products from farm animals raised within animal-friendly production systems (Kijlstra *et al.*, 2004a).

Proposed recommendations

Rodent pests belong to two different ecological groups. Field rodents, such as voles (*Microtus* spp.) are adapted to live in natural habitats. They become pests when appearing in crops and pastures, where they can be very prolific. The commensal rodents (house mice and rats) were brought into Europe with human settlement and the exchange of goods. With a few exceptions, they are adapted to live in artificial environments, such as feed mills and stables. The objective of managing field rodents is to minimize livestock's exposure to these vectors, and to regulate their populations in case their density is expected to grow dramatically. An infestation of livestock facilities with commensal rodents can be prevented, and in the case of their appearance, eradication must be aimed for.

On traditional farms a rodent management plan is preferably set up according to the principles of Integrated Pest Management (IPM), in which various management actions are integrated to assure effectiveness, cost efficiency and feasibility (Singleton, 1997). Very often these actions depend on the use of chemicals. Some of the basic principles of IPM (habitat management, control of rodent movements and control of the rodent population using physical measures) can be applied on organic farms. A more appropriate and efficient approach is ecologically-based rodent management (EBRM). EBRM is an extension of IPM and was developed as a formal description of a sound ecological basis for developing integrated management strategies for rodent pests (Singleton *et al.*, 1999; Singleton *et al.*, 2004a). During the seminar

it was proposed that a sustainable rodent management plan consists of three general elements: prevention, monitoring and control.

Prevention

On conventional farms exclusion of rodents is one option, because potential commensal rodent access routes into farm buildings can be blocked using physical barriers. Field rodents only occur outdoors, where it is impossible to exclude rodents, but their numbers can be reduced in order to reduce direct contact with farm animals if there is a reasonable understanding of the ecology of the main pest species. In general, all rodents often use specific habitat elements more frequently than the ones in a heterogenous landscape typical of animal farms. For example, piles of old material that the farmer stores on his property because he thinks he might use it again, are often important burrowing and nesting requisites for rats (*Rattus norvegicus*) (Endepols *et al.*, 2003). Removal of such piles in concert with other management actions, will decrease the risk of rodent infestation. Stacks of straw or hay may also form a good habitat for both rats and mice and thus form a potential source habitat for dispersal of rodents to other parts of the farm.

Some rodent species burrow or hide under low lying and ground-covering shrubs within the neighborhood of human dwellings (Colvin and Jackson, 1999). From a rodent management point of view it would be best to cut such shrubs down, but on the other hand these shrubs are needed as hiding places and shade for organic poultry, and may contribute to local biodiversity. We therefore recommend to remove vegetation only in a 2-m radius around the buildings and to place gravel, which deters rodents. Where rodents are an intractable problem, the removal of hedges within 100 metres of farm buildings or pigsties could be beneficial (Leirs *et al.*, 2004). These actions are likely to be effective because vegetation cover determines the perceived predation risk in small mammals. For example, house mice are known to adjust their feeding activity in farm environments according to this perceived risk (Ylönen *et al.*, 2002).

The presence of open drinking basins will attract rats, as do automatic feeders. Remnants of feed at outdoor feeding sites on organic poultry farms may be an important food-source for rodents, as is the case with silage. Practices that minimize spillage of feed and access by rodents to feed stations are recommended. The use of ultrasound and low frequency devices, chemical repellents, fumigants and non-poisonous chemicals are not recommended for rodent management on organic pig or poultry farms because there is little or no published evidence

supporting their efficacy in open environments. Moreover, ultrasound devices are known to disturb livestock (Algers, 1984).

Repellents may be effective under different farming circumstances, when used to protect seeds or young trees (if the items to be protected are treated directly with the repellent) and fumigants may be a useful method as part of an IPM-approach.

Monitoring

Monitoring improves the decision making process in the prevention of rodent infestations. The appearance and abundance of rodents can be estimated using a range of techniques such as trapping, ink pads, tracking plates with sand, non-poisonous baits or electronic devices (e.g. infrared cameras). The usefulness of the monitoring data is stengthened if a farmer is able to find out which rodent species is/are causing the greatest impact because each species has distinct behavioural profiles and ecological requirements. Such knowledge will therefore substantially influence the development of an effective rodent management plan (Singleton *et al.*, 1999).

Control

Even the smallest infestation with rats or mice may need to be treated to protect food safety and the health of livestock. However, farmers usually only take action if rodent population densities on their farm premises are above a subjective threshold. A rodent control plan is required to commence an effective rodent control, and to provide documentation as demanded by regulations, auditors or customers. As for the monitoring component, progress is contingent on knowing which species are the major pests, understanding their ecology and taking into account the farming system used by particular farmers. Also of importance are the behavioural peculiarities of particular rodent species, the production calendar, and the farm management within and between farms.

An example of rodent management on an organic pig farm: the Norway rat

During the seminar we used decision analysis techniques (Norton and Pech, 1988) to determine the most appropriate methods for managing an infestation of the Norway rat (*Rattus norvegicus*) on an organic pig farm (Table 9.2). This table provides an example of an approach to assess different management options for one target species, the Norway rat, which may be an important vector of diseases. Rodents have the ability to breed rapidly, so

effective control should lead to high levels of mortality in the resident rodent populations on the farm to avoid the populations from quickly returning to pre-control levels. Rodenticides are the most efficient way to control an existing high-density population of the pest species. The control of field rodents with cyclic reproduction is most effective if commenced as soon as indicators show the onset of a reproduction cycle. This approach can prevent the development of high population densities. Rodenticide usage thereafter can be minimized through strategic baiting, such as placement of bait stations in key places of the habitat (refuge and/or breeding places) (Table 9.2). Such a strategy requires a thorough understanding of the ecology of the major rodent pest species.

If an organic farmer does not want to use rodenticides, trapping seems the best alternative, although the labour needed to place traps around the farm premises and their checking may be costly. Other options are the use or encouragement of predators such as mustelids, cats and dogs. Unlike cats, dogs are less likely to be carriers of zoonotic diseases such as *Toxoplasma gondii*, although they may carry *Echinococcus multilocularis*. An extra action for organic farmers who do not want to use rodenticides, could be the placing of perches or nest boxes on their property to stimulate the presence of birds of prey and owls such as the common buzzard (*Buteo Buteo*), little owl (*Athena Noctua*) and kestrel (*Falco tinnunculus*). However, the evidence that predatory birds can effectively regulate rodent populations for extended periods is not strong. In case of organic poultry farms, attracting birds of prey could even result in predation of laying hens or broilers. The above suggestions of possible actions, either alone or integrated, are simply suggestions. Studies have demonstrated effective eradication of rodent populations on farms following intensive once-off rodenticide use (e.g. Endepols *et al*., 2003). However, whether effective, affordable and socially acceptable rodent management actions can be sustained under the conditions of organic farming has not yet been tested. Replicated field experiments on an appropriate scale are urgently needed.

Table 9.2 Analysis of actions for managing the Norway rat on an organic farm in the Netherlands. This table is an adapted example of a species-specific decision analysis of rodent control strategies

Action					**Acceptability**			**Practical**[1]
Type	*Place*	*Time*	*Efficacy*	*Cost*	*Organic*[2]	*Social*[3]	*Environmental*[4]	
1st generation rodenticide	Key habitats	If high numbers are present	++[5]	Low	+/-	+	+	+
2nd generation rodenticide	Key habitats	If resistant to 1st generation and not close to waterways	++	Low	+/-	+/-	-	+
Trapping	Key habitats	If high numbers are present	+/-	Very high	+	+/-	Live traps: + Snap traps: +/-	+
Shooting	Upon sight	Dusk & dawn	-	Medium	+/-	+/-	+/-	+/-
Cats	n.a.[6]	Always	-	Very low	+	+	-	+
Dog (e.g. fox terriers)	n.a.	Always	-	Very low	+	+	+/-	+
Nest boxes & perches (birds of prey)	Fields	Always	+/-	Low	+	+	+	+
Domestic mustelids	n.a.		-	Low	+	+	+	+

[1] Practical = whether a farmer can readily integrate the action into his farm management calendar.
[2] Organic acceptability = whether the action fits into the organic farming philosophy.
[3] Social acceptability = whether the action is accepted by society in general.
[4] Environmental acceptability = whether the action has detrimental effects on other species in the vicinity.
[5] ++ = very good; + = good; +/– = reasonable; – = very low.
[6] n.a. = not applicable, impossible to appoint a specific place for this strategy.

Conclusions

Food safety is an important issue in animal production systems. In organic animal husbandry, farm animals will have closer contact with rodents and other wild animals than in conventional systems where animals are not allowed access to an outdoor area. Also, because of outdoor access, it is more difficult for organic farmers to prevent rodents from entering their buildings. This may potentially cause problems with contagious animal diseases and parasites that can be harmful to the health of both humans and animals. This alone should be reason enough to control rodent populations, but their significant damage to farm infrastructures and feed storage facilities makes rodent management even more necessary. Effective rodent management requires a thorough understanding of the ecology of the main pest species. Based on this knowledge rodent management should consist of three elements: prevention, monitoring and control. From an organic perspective, most effort should be invested in prevention and monitoring. Organic farmers should select a solution that guarantees food safety and healthy livestock and that fits in best with their own farming philosophy. However, farmers should be aware that the often-applied use of cats as rodent control measure, can threaten animal health and food safety. A suite of possible management actions is described but the socio-economics of these actions need to be examined. Methods of economic analyses for management of field rodent populations have recently been described (Stenseth *et al.*, 2003; Singleton *et al.*, 2004b). We strongly recommend that ecologically-based actions be tested in a replicated study through farmer participation using an adaptive management framework.

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Chapter 10

General Discussion

Introduction

One can make a distinction between two routes of pathogen transmission from rodents to humans. The first route is direct contact between humans and rodents (or their excrements), which could e.g. cause leptospirosis or infection with hantaviruses. However, in this thesis the second route of transmission was the main object of study: some pathogens can be transferred indirectly to humans via consumption of livestock products. Prevention of food safety hazards is a priority throughout the food chain. Consumers nowadays demand that the food they consume is safe. Food producers and processors have the responsibility to satisfy this demand, e.g. by implementation of hygiene guidelines such as Hazard Analysis on Critical Control Points (HACCP). The government then controls whether companies fulfill the legal requirements. Control or elimination of these zoonotic pathogens in the first part of the food chain (farm-level) is therefore a priority.

Over the past decades, animal farming in Western Europe has changed drastically. Total indoor animal production systems emerged to increase production efficiency and animals were separated from their outdoor environment. In order to minimize the transfer risk of pathogens, hygienic measures such as obligatory disinfections of boots, clothes and equipment became established. The negative side-effects (e.g. on animal welfare) of these indoor farming systems have led to the classification “bio-industry” among the general public. As a result of public dislike, a demand among consumers for products from alternative and more animal-friendly production systems became gradually apparent. However, the uptake of these new animal-friendly and organic farming practices could conceivably lead to increased risks for the introduction of these pathogens in the first part of the food chain (on-farm), as there is more risk of contact of livestock with (zoonotic) pathogens present in the farm environment. Wild and domestic animals (e.g. birds, insects, rodents and cats) can be reservoirs or vectors of agents that cause disease in humans and food animals. These vectors can transmit pathogens that cause contagious animal diseases (e.g. Classical Swine Fever, Foot and Mouth Disease), others are foodborne pathogens and have consequences for human health.

The main aim of this thesis was to obtain better understanding of the health risks of rodent presence on such agricultural premises. In our model, we have chosen to incorporate three

important foodborne pathogens: *Salmonella*, *Campylobacter* and *Toxoplasma gondii*. In this chapter the results are discussed and put into a broader perspective.

Salmonella and *Campylobacter* prevalence in wild small mammals

The potential risk of the transmission of infection with the zoonotic bacteria *Salmonella* and *Campylobacter* is probably greater in organic than in traditional farming systems because close contact between small mammals and food animals is more likely to occur when the latter are reared outdoors. Furthermore, although allowed by organic regulations, most organic farmers are not willing to use rodenticides because these do not fit within their ecological farming philosophy. So far, the prevalence of zoonotic bacteria in wild small mammals commonly found on organic farms has not been investigated. The purpose of our study was to determine the levels of *Salmonella* and *Campylobacter* colonization in rodents and insectivores on organic farms in the Netherlands. These two pathogens were primarily selected because of their implications on the human health: salmonellosis and campylobacteriosis are two of the most frequent gastrointestinal diseases in the industrialized world. They are responsible for 24% of foodborne diseases caused by known pathogens in the United States (Mead *et al.*, 1999; Tauxe, 2002). Generally, environmental sources are believed to be important reservoirs for infection in domestic animals, although some studies claim that the importance of wildlife as infection reservoir is limited (Petersen *et al.*, 2001). From earlier studies we already knew that rodents captured in woodlands or alpine areas frequently carried *Salmonella* and *Campylobacter* (see also Chapter 2). In an urban context, 8% of the fecal samples of rats were positive for *Salmonella* (Hilton *et al.*, 2002). However, no *Salmonella* had been detected in the limited studies that were performed in rodents in an agricultural context. In their study of 259 wild rats caught on farm premises in the UK, Webster and MacDonald revealed that these rats carried 13 zoonotic and 10 non-zoonotic parasite species (Webster and MacDonald, 1995), but no *Salmonella* sp.

The results of our field study performed on organic pig and broiler farms, in which the colonization levels of rodents and insectivores with *Salmonella* and *Campylobacter* were measured (Chapter 3), showed that house mice (*Mus musculus*) are capable of carrying *Salmonella*, but numbers of infected animals are limited. However, *Salmonella* prevalence may be higher when small mammals are caught close to a *Salmonella*-positive herd or flock. More worrisome, house mice and the Norway rat (*Rattus norvegicus*) carry *Campylobacter* in

greater extent. In all other rodent species and insectivores (shrews) neither *Salmonella* nor *Campylobacter* could be detected.

Habitat differences might form an explanation why house mice and Norway rats were contaminated with *Salmonella* and *Campylobacter*, while other species were not. Several studies have shown that domestic animals (pigs, cattle) housed in production facilities with a high individual-to-area ratio may carry *Campylobacter* for longer periods of time (Weijtjens *et al.*, 1997; On *et al.*, 1999). House mice and Norway rats are commensal rodents and for them livestock farms provide unlimited amounts of water and food, and also shelter. As a consequence, they forage close to the livestock and can come e.g. into contact with infected manure of domestic animals. If infection in these rodents occurs, a new problem emerges. As a result of their allocoprophagic behaviour (consumption of feces of other individuals) other individuals can easily acquire infection with the same pathogen (Hirawaka, 2001), thus resulting in a continuous infection loop within the rodent population. This long-term source of infection was demonstrated in poultry in a previous study: three-week-old broiler chicks acquired infection via mice that had been artificially infected with *S. enteritidis* two and five months before (Davies and Wray, 1995b).

Other rodent species we encountered in this study have different main habitats (see also Chapter 3, Table 3.1), are generally more dispersed in the landscape and are therefore less in contact with (infected) manure of domestic animals. This might limit their carriage rate for *Salmonella* and *Campylobacter*. However, we have learned from earlier studies that also these other rodent species can in principle acquire infection (Chapter 2). In our study, we chose to analyse the intestinal contents of rodents and insectivores instead of using the simpler and non-invasive technique of culturing fecal droppings (Davies and Wray, 1995b; Pocock *et al.*, 2001). We did this for two reasons. Firstly, fecal droppings may be contaminated with *Salmonella* from the environment, (e.g. from dust), and their testing is therefore less reliable. Secondly, fecal droppings are a very unreliable sample source for *Campylobacter* because it is unstable and very susceptible to dry conditions.

Transmission pathways of *Salmonella* and *Campylobacter* are difficult to determine. Although we tried using molecular techniques (AFLP), we were unable to link the *Campylobacter*-strains in rodents to strains present in the pig manure (Chapter 3). Exact transmission patterns therefore still remain uncertain. Three possible transmission routes exist: 1. pigs acquire their infection from rodents; 2. rodents acquire their infection from pigs,

or 3. both rodents and pigs acquire infection from a common external source. Common sources could be e.g. wild migratory birds, the uptake of contaminated surface water (Buswell *et al.*, 1998), contaminated feedstuffs or contact with other wild (e.g. hedgehogs) or domestic mammals (e.g. cats). Additional investigations of the *Salmonella* and *Campylobacter* prevalence and type distribution in wildlife and their immediate environment would further elucidate infection pressure on food animals. Sampling of a greater number of farms is required to find an answer on this topic. Experiments under laboratory conditions are also needed for more meaningful risk assessments and the determination of transmission pathways (e.g. bioassay).

Interestingly, the Dutch government wants poultry meat to be free of *Salmonella* and *Campylobacter* from 2007 onwards, while in other member states of the European Union this will be from 2011. As transmission pathways of these pathogens between external sources and food animals are currently not clear, it is rather questionable whether 100% of the meat produced can be free of *Salmonella* and *Campylobacter*. Problems with *Salmonella* and *Campylobacter* will presumably occur in organic animal production, due to the relative open production system. Therefore it is doubtful whether the demand of the government for meat that is free of *Salmonella* and *Campylobacter*, is in line with the propagation of organic production methods.

Toxoplasma gondii prevalence in wild small mammals

Toxoplasmosis is still one of the most common parasitic infections in the world and is caused by *Toxoplasma gondii*. The parasite is the most important cause of infectious and sight threatening uveitis (intraocular inflammation) worldwide. Generally, it was thought that hygiene improvements and the introduction of total indoor farming had reduced the problem in Western-Europe. Due to social pressure however, the bio-industry was urged to reintroduce outdoor housing of food animals. Outdoor access of food animals could have consequences for the re-emergence of the protozoan parasite *Toxoplasma gondii*, especially in countries where high numbers of cats are kept as pets. In the Netherlands currently 3,300,000 cats are kept as pets (Chapter 5).

A previous serological study (Kijlstra *et al.*, 2004a) showed that about 3% of the pigs raised with outdoor access were infected, while the pathogen was absent in “indoor” pigs. Besides provision of goat whey to pigs and presence of more than 3 cats (Chapter 5), the level of pest control can be considered one of the main risk factors for *Toxoplasma gondii*-infection of food animals (Chapter 4). Therefore, we decided to investigate the *T. gondii* contamination

level of rodents and insectivores. Presence of *T. gondii* was assessed in the rodents by use of two methods: serological and molecular analyses based on the B1-PCR (Chapter 6). Serologically, *T. gondii* was demonstrated in 6.4% of the wild small mammals, and infection rates showed variation between species. White-toothed shrews for example were very often infected, which can be a result of their habitat (proximity to cats), feeding pattern (ingestion of sporulated oocysts from the environment or by uptake of infected earthworms/beetles) or pathogen sensitivity (e.g. immune response).

Molecular analysis of heart tissue of rodents and insectivores showed a much higher overall prevalence than we expected: over 70% of the small mammals in the study were infected (Chapter 6, Table 6.2). An earlier study (Marshall *et al.*, 2004) reported a prevalence of 59% for house mice, although another PCR detection method (SAG1) was used. The differences between serological and molecular detection can be e.g. the result of differences in antibody production patterns after infection with distinct *T. gondii* strains, the dosage of the pathogen, the age of the rodent or perhaps the infection pathway (congenital transmission versus acute infection). On the other hand, some authors question the specifity of the B1 PCR test due to co-amplification of genomic host sequences (Chabbert *et al.*, 2004; Kompalic-Cristo *et al.*, 2004). Thus, further optimization of molecular diagnosis of toxoplasmosis is needed (e.g. by mean of sequencing PCR products or confirmation tests using hybridisation with gene specific probes).

Toxoplasma gondii prevalence in slaughter pigs

Because of this difference of results between serology and PCR in rodents and insectivores, we decided to also have a look at fresh pig meat from both conventional and animal-friendly (with outdoor access) farms (Chapter 7). Consumption of undercooked meat of pigs is considered a hazard for *T. gondii* infection in humans (Dubey, 1994). All edible parts of infected pigs can contain *T. gondii* cysts and the meat of one pig is consumed by a large group of people. Therefore, infection of pigs can result in a serious public health threat. *T. gondii* prevalence in pork diaphragm was assessed by a PCR that focused on the B1 locus (Chapter 7). This test is often used to identify *T. gondii* in human clinical specimens. Positive B1 PCR results were obtained in 29.6% of the conventional pork samples (n=186), and in 55.2% of the animal-friendly raised pork samples (n=192).

On basis of serology we expected a much lower incidence. This difference between serological and molecular detection can be caused by 1. higher sensitivity of PCR in

combination with a low humoral response, 2. cross-reactions of the parasite with other closely related protozoa, 3. different serological responses after infection with different strains or 4. the possibility of amplification of a genomic DNA product instead of parasite DNA. More research on optimization and standardization of detection techniques is required in order to determine true *T. gondii* prevalence in pork.

Rodent management

Thus, rodents in agricultural environments can carry pathogens (*Salmonella* sp., *Campylobacter* spp., *Toxoplasma gondii*) that can have serious consequences for food safety and thus public health. In order to guard the biosecurity of farms (protective measures designed to protect plants and livestock against the entry and spread of diseases), minimal exposure of livestock to rodents should be promoted. This is the most difficult at organic farms, as food animals in these farming systems can roam outside and rodents have more easily access to farm buildings.

Effective on-farm rodent management is needed on these farms to protect the food production chain. Also, rodents can cause economic losses, spoilage of food and lead to structural damages. Chapter 9 describes the three elements of on-farm rodent management: 1. modifying rodent habitat & proofing, 2. monitoring appearance and population density of rodents, and 3. rodent control measures. Chemical compounds for rodent control should only be used if their use is unavoidable. One should keep in mind that too frequent and unnecessary use of anticoagulant poisons can induce genetic resistance to these poisons in rodent populations (Pelz and Klemann, 2004). In order to maintain the possibilities for effective control, management of this resistance (e.g. alternating use of anticoagulants with non-anticoagulant rodenticides) is useful (Kerins *et al.*, 2002). Furthermore, use of poisons can have serious consequences for animal welfare of target (Mason and Littin, 2003) and non-target species (Chantrey *et al.*, 1999). A problem for organic farmers is that use of poisons often does not fit in their farming philosophy. However, use of cats which many farmers prefer (Kijlstra *et al.*, 2004b) as rodent control measure should be avoided as well, because of potential excretion of *T. gondii* oocysts. Organic farmers could conceivably use a rodent management strategy that is more in line with organic principles such as Ecologically-Based Rodent Management (EBRM), which focuses more on the prevention and monitoring of rodents than on their control by the application of rodenticides or the use of traps.

Chapter 8 contains an ethical approach to rodent control in the family home. We have seen the need of rodent control in earlier chapters (economic losses, disease risks) at farming premises, but the same also applies at home. Therefore, residents kill these pest animals. However, many of the rodent control methods that are applied cause animal suffering (Mason and Littin, 2003), whilst the general public demands high standards of welfare in food production and animal experimentation. Public attitude thus reflects differences in the way we treat various classes of animals (e.g. laboratory animals vs. pest animals) and biological similarity does not guarantee the same ethical approach to individual animals. We have not only discussed the need for education for the general public, but also for professional pest controllers. This will hopefully lead to better use of currently available humane rodent control methods.

Conclusions and recommendations

The results indicate that in the Netherlands at least some rodent species can carry pathogens that can result in foodborne illnesses. Moreover, direct contact between humans and wild rodents should be minimal, as it is known that several zoonotic agents can also be directly transmitted from wild rodents to humans, e.g. by rodent bites, by skin contact with an infected, diseased, or dead rodent, by breathing in aerosols or dust containing rodent excreta (Kruse *et al.*, 2004). Moreover, during epidemics of Classical Swine Fever (CSF) and Foot and Mouth Disease (FMD) in this country, rodents were also frequently mentioned as disease vectors (Elbers *et al.*, 2001; De Vos *et al.*, 2003). Farmers should be aware of these aspects and take as much measures as possible to guard the biosecurity of the farm and prevent direct contact between livestock and wild animals in order to prevent horizontal transmission (Chapter 9). Especially commensal rodents (rats, house mice) are a threat because of their behaviour (close to livestock) and reproduction capabilities. Other rodent species (e.g. common voles/bank voles/wood mice) can of course also be infected with pathogens (Chantrey *et al.*, 1999). Nevertheless, the current intensity of farming systems which often includes use of monoculture grasses, high input of nutrients and intensive mowing/grazing regimes has resulted in habitat loss of many of these species. Huijser and colleagues (Huijser *et al.*, 2001) expect that these conditions and activities have a negative influence on the common vole populations and keep them from reaching high densities. This artificial predator-prey relation will also be valid for other non-commensal rodent and insectivore species and will ensure reduced contact risks between these species and livestock. We have constructed a simplified hypothetical model of interactions for rodent-borne zoonotic diseases

(Figure 10.1). This model could form part of an integrated public health response to emerging rodent-borne zoonotic diseases. This model consists of several components.

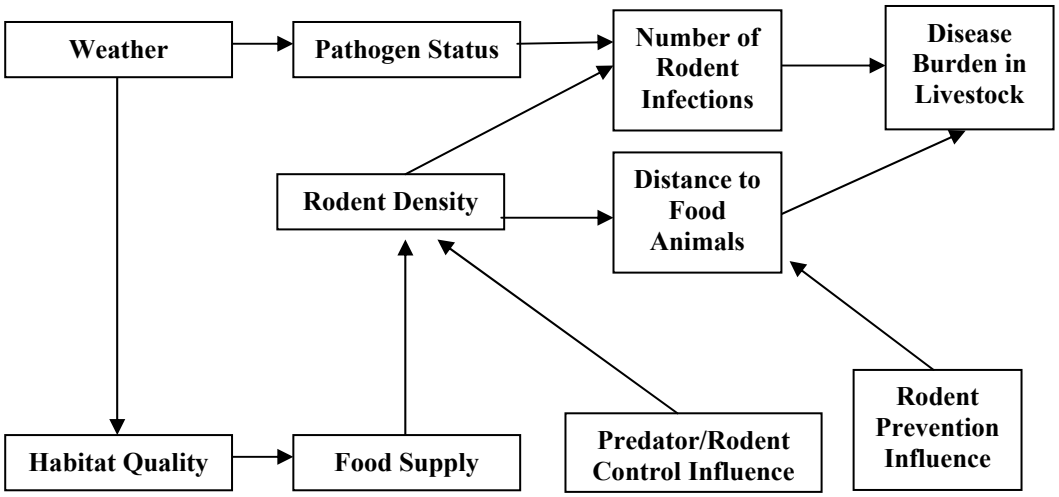

Figure 10.1 Hypothetical model for rodent-borne zoonosis and their transfer to food animals on farms

We assume that the risk of food animal infection (disease burden) is directly related to the rodent density and the status of the pathogen. Rodent density is influenced by biotic elements (food supply and habitat quality), that in their turn are influenced by the main abiotic components (weather: temperature, amount of sun or rain etc.). The weather also has its influence on the status of the pathogen, as some pathogens need warm circumstances to reproduce and some are killed under cold circumstances. This will have its influence on the level of infection in the rodent population at the farm. The presence of predators or rodent control methods can limit the amount of rodents on-farm, while preventive measures such as rodent proofing will increase the distance that is kept between rodents and food animals, thus decreasing the disease burden. Although not mentioned in Figure 10.1, distance is of course also related to the type of rodent species, e.g. commensal rodents live closer to livestock.

However, one has to bear in mind that with good farm hygiene management, it is still impossible to eliminate all rodent threats. Although their numbers might decrease, there is still a chance of pathogen spreading. And even if we are able to eliminate this zoonotic threat, other wildlife sources (e.g. birds) remain. Therefore, additional measures are also needed further in the food chain. In previous studies, consumer education was often mentioned as a solution: consumers should learn how to deal with food in a sensible way. In some countries

(e.g. Belgium), health education in combination with decreasing risk because of indoor animal production has led to a decrease in the seroprevalence of *Toxoplasma gondii* in humans (Breugelmans *et al.*, 2004). However, it remains uncertain to what extent health education was the factor responsible for this success (Jones *et al.*, 2003). Good kitchen hygiene and proper cooking of meat can prevent the problem of many hazardous pathogens. In case of *T. gondii* for example, meat should be cooked thoroughly at 65°C to destroy tissue cysts. Nevertheless, consumption of undercooked meat is the main risk factor for pregnant women to acquire infection with *T. gondii*. One case-control study in six European centres revealed that between 30-63% of the infections with *T. gondii* can be attributed to this risk factor, while soil contact only attributed to 6-17% of the infections (Cook *et al.*, 2000). Therefore, more awareness should be created among pregnant women that not only contact with soil or with the cat litter tray, but also consumption of improperly cooked meat can represent a hazard for *T. gondii* infection. Pregnant women are not the only group at risk, though. Primary *T. gondii* infection or reactivation of latent infection in the immunocompromised (e.g. AIDS patients, transplant recipients) can have severe consequences and is often life-threatening. This group of patients should better be informed about the risks of *Toxoplasma* infections and its transmission pathways. However, for patients that acquired *T. gondii* infection during an earlier life stage, the benefits of providing this information are low.

To diminish *T. gondii* infection rates in humans, actions could include measures to reduce infection in livestock and felines. A possible research direction for the near future (<10 years) will be the development of a vaccine which provides long-lasting protective immunity against *T. gondii*. During the development of such a reliable vaccine, one should take into account that development of protective immunity can be strain/or stage-specific, as was found in felines (Omata *et al.*, 1996). Currently, a live attenuated vaccine for veterinary purposes (e.g. cats) is available, but protection is only guaranteed for three years and the risk of reversion to a pathogenic form of the parasite makes this vaccine unsuitable for the management of human toxoplasmosis (Sonda and Hehl, 2005). Use of inactivated parasites, non-persistant strains or recombinant proteins have shown partial protection at best (Dubey *et al.*, 1991; Lunden *et al.*, 1997). Most promising seems the development of DNA vaccines: immunisation of animals with plasmid produce expressing *T. gondii* surface antigens can provide effective protection in mice (Nielsen *et al.*, 1999; Desolme *et al.*, 2000; Scorza *et al.*, 2003) although its protective value may vary between different surface antigens. DNA vaccines are also effective against

other intracellular protozoas, such as *Leishmania* spp (Gurunathan *et al.*, 1997) and liver-stage *Plasmodium falciparum* (Ballou *et al.*, 1987; Scorza *et al.*, 2005).

Further development in prevention of toxoplasmosis may also include DNA vaccination in humans. This type of DNA vaccination could be based upon: 1. causation of an protective immune response at the mucosal level as soon as *T. gondii* penetrates the intestinal barrier thus preventing invasion of the host and settling into tissue (Bout *et al.*, 2002), 2. inhibition of endogenous multiplication of *T. gondii* (formation of tachyzoites), and 3. prevention of their dissemination or the formation of *T. gondii* cysts (bradyzoites). However, much scientific progress needs to be made before we will completely understand the mechanisms of *T. gondii* and we will be able to use DNA vaccination in humans to elicit cell-mediated immunity.

As mentioned earlier, most infections in humans are caused by ingestion of infected meat (Cook *et al.*, 2000). Until DNA vaccination in humans becomes available, prevention of viable *T. gondii* cysts in meat is another solution that could contribute to the reduction of toxoplasmosis. Dubey (Dubey, 1988) has demonstrated that tissue cysts in pork can be rendered nonviable if they are kept at -12°C during 3 days. Freezing of meat products prior to cooking could also be beneficial to prevent other parasites, such as *Trichinella spiralis* and *Taenia soliu* (Gamble, 1997). Moreover, the viable number of other foodborne pathogens such as e.g. *Campylobacter* decreases if the meat is stored at low temperatures (Hänninen, 1981; Oosterom *et al.*, 1983; Dykes and Moorhead, 2001) of -15°C to -20°C, although in chicken meat *C. jejuni* still remained viable even at -70°C (Lee *et al.*, 1998).

Another measure to reduce many health hazards in meat (e.g. *T. gondii*., *Campylobacter jejuni*, *Salmonella*, *Listeria monocytogenes* and *E. coli* 0157 is food irradiation (Tauxe, 2001). Irradiation is a physical treatment of food with high-energy, ionising radiation from a linear particle accelerator. The high energy rays directly damage the DNA of living organisms, thus inducing cross-linkages that limit growth or reproduction of these organisms. Although it is allowed in the European Union, the use of this technique in practice is rather limited. Nevertheless, the potential health benefits of irradiation are large, although public concerns exist about the potential dangers of irradiation (Tauxe, 2001). More research needs to be performed in order to see whether these concerns are realistic.
In conclusion, rodents can impose risks to public health if they get into close contact with food animals. From a food safety perspective, this threat should be eliminated. However, it

will be impossible to eliminate all biosecurity threats at farm-level (e.g. birds, hedgehogs etc.), especially at "animal-friendly" farms, where food animals have outdoor access. In the previous section, a number of suggestions have been mentioned to keep food safety at retail level intact (e.g. by means of food irradiation and freezing), while still allowing food animals outdoors according the demand of the general public. Nevertheless, consumers should realise themselves that there is a continuous tension between food safety on one hand and animal-friendly production at the other.

Summary

Besides economic losses through spoilage of feed or structural damage, rodent presence on livestock farms can have serious consequences for public and animal health. Direct contact between humans and rodents can be a way of pathogen transmission, but rodents can also transfer (zoonotic) pathogens via livestock to consumers of livestock products. As prevention of food safety hazards is a priority throughout the food chain, the main aim of the work presented in this thesis was to obtain a better understanding of the risk of on-farm rodent presence in the Netherlands.

Contamination levels of wild rodents with several pathogens were investigated. In order to do so, we selected pathogens that are major causes of human foodborne disease (*Salmonella*, *Campylobacter* and *Toxoplasma*). In Chapter 2 the results of a literature review are presented about the role that rodents play in transmission from *Salmonella* and *Campylobacter*. Rodents can carry these pathogens, but until now most research was performed in natural environments and not in agro-ecological surroundings. However, as *Salmonella* and *Campylobacter* can cause serious health problems in humans, we think that elimination of these pathogens in the first part of the food chain should be a priority. Rodent control will form a crucial part of this process.

A field study on *Salmonella* and *Campylobacter* colonization in small mammals (both wild rodents and insectivores) is described in Chapter 3. On ten Dutch organic livestock farms (9 pig, 1 poultry) rodents and insectivores were screened for the presence of *Salmonella* and *Campylobacter spp*. *Campylobacter spp*. was isolated in 9/282 animals and *Salmonella sp*. in 1/282 animals. All infected small mammals were either house mice or Norway rats. In order to check for shared genotypes, manure samples of the livestock were collected at 6 farms. However, Amplified Fragment Length Polymorphism (AFLP) did not reveal any direct relationship between small mammal and livestock isolates.

Reintroduction of animal production systems with outdoor access of livestock (e.g. organic animal husbandry) as result of public dislike towards indoor farming/bioindustry can lead to re-emergence of diseases that were also present in the past. In Chapter 4, we specifically look at the risk factors on-farm for re-emergence of the protozoan parasite *Toxoplasma gondii*. This parasite can have serious consequences in humans, especially in pregnant women and immunosuppressed persons. The majority of human infections is caused by consumption of undercooked meat, thus control of this pathogen at farm-level is of vital importance. As main

risk factors for contamination at farm level on organic pig production facilities, we identified the number of cats present on farm premises and pigs drinking goat whey (Chapter 5). Unfortunately, the survey in Chapter 5 did not offer sufficient opportunity to study the role of rodents in transmission of *T. gondii* to pigs; this requires an intervention study. We concluded that adoption of relatively easy farm management procedures can contribute to a reduction of *T. gondii* prevalence in pigs.

In Chapters 6 and 7 respectively, the prevalence of *Toxoplasma gondii* among wild small mammals trapped on-farm and in fresh pork meat was assessed. Small mammals are often mentioned as a possible transmission route of this pathogen to pigs.
We found that a substantial percentage of small mammals on Dutch farms carried *T. gondii* and thus could play a role in transmission of this pathogen. We are the first to show a substantial *T. gondii* seroprevalence (9.1%) in the white-toothed shrew (*C. russula*). Overall, *T. gondii* was serologically demonstrated in 6.4% of the small mammal samples, while 71.1% displayed a PCR product.
In pork, we found a similar discrepancy between molecular detection and the results of earlier serological studies. These serological studies demonstrated that in Northern Europe *T. gondii* seroprevalence had declined during the last decades to below 5%. However, in this study a positive PCR reaction was encountered in 29.6% of the conventionally-produced pork and 55.2% in organically-produced pork. This discrepancy could be the result of: 1. higher sensitivity of PCR in combination with a low humoral response, 2. cross-reaction of the parasite with other closely related protozoa, 3. different serological responses after infection with different strains or 4. the possibility of amplification of a genomic DNA product instead of parasite DNA. We concluded that further optimization and standardization of detection techniques are required in order to determine true *T. gondii* prevalence in pork.

Chapter 8 contains an ethical analysis of rodent control. In Western societies animals are generally seen as objects of moral concern. Therefore, demands are placed upon the way we keep or use animals during food production or animal experimentation. In case of rodent pests however, often inhumane control methods are applied. Thus, there is inconsistency in the human-animal relationship. We claim that two options are open to solve this inconsistency. The first one is to let go of the criteria on animal experimentation. However, from an animal ethics perspective the second option is more preferable: application of the same criteria on rodent control. This implies that ethical rodent control methods should not lead to intense pain

or discomfort, have only a short duration and escaping rodents should be able to lead a natural live. Consequently, this will require a shift in the design of rodent control methods.

Because of the food safety and public health risks, prevention of contact between food animals and wild rodents is necessary. In Chapter 9 the state of the art rodent management and recommendations from an international seminar are presented. Decision analysis techniques were used to determine the most appropriate methods for managing an infestation of Norway rats on an organic pig farm. Rodenticides were classified as the most efficient rodent control method, but they do not always fit in the farming philosophy of organic farmers. In these cases, trapping seems the best alternative, although labour costs are high.
However, rodent control forms only one part of rodent management and is a final remedy. Continuous monitoring of rodent populations and prevention (e.g. proofing of farm buildings, removal of piles of old material, removal of habitat elements near the stables, limiting access to feed and water), will limit the development of high population densities on farms and thus contact opportunities between rodents and livestock will decline.

A general discussion of the work presented in this thesis is given in Chapter 10. Some rodent species can carry pathogens that may result in foodborne illnesses. Farmers should be made aware of this risk and should be encouraged to take measures to guard the biosecurity of their farm and prevent contact between livestock and rodents. Because of their ecology, especially commensal rodents (house mice and rats) will be a threat as they live close to livestock and have good reproduction capabilities. Figure 10.1 describes a simplified hypothetical model for rodent-borne zoonosis and their transfer to food animals.
One of the measures to reduce infection in livestock and felines and thus also in humans, is rodent management. Another option for the future is the development of DNA vaccination of animals or even humans. Until vaccination that provides long-lasting immunity against *T. gondii* becomes available, elimination of viable *T. gondii* cysts in meat could prove beneficial. Freezing of meat products or food irradiation are possible methods to reduce consumption of infected meat. Moreover, these measures could also be beneficial to prevent other foodborne pathogens. However, even with the application of these options, consumers should always be aware that there is a tension between animal-friendly production of livestock products on one hand and food safety on the other.

Samenvatting

Naast economische verliezen door verspilling van veevoer en structurele schade, kan de aanwezigheid van knaagdieren op veehouderijbedrijven ernstige consequenties hebben voor de dier- en volksgezondheid. Door direct contact tussen mensen en knaagdieren kunnen pathogenen worden overgedragen, maar knaagdieren kunnen ook (zoönotische) ziekteverwekkers via het vee overdragen aan consumenten van dierlijke producten. Omdat preventie van voedselveiligheidsrisico's een prioriteit is in de voedselketen, was het belangrijkste doel van het onderzoek dat in dit proefschrift wordt gepresenteerd om een beter begrip te krijgen van de risico's van aanwezigheid van knaagdieren op boerderijen in Nederland.

Besmettingsniveau's van wilde plaagdieren met een aantal ziekteverwekkers werden onderzocht. Hiervoor selecteerden wij pathogenen die belangrijke oorzaken zijn van humane infecties, veroorzaakt door voedsel (*Salmonella*, *Campylobacter* en *Toxoplasma*). In Hoofdstuk 2 worden de resultaten gepresenteerd van een literatuuronderzoek over de rol die knaagdieren spelen bij de overdracht van *Salmonella* en *Campylobacter*. Knaagdieren kunnen deze pathogenen bij zich dragen, maar tot nu toe was het meeste onderzoek uitgevoerd in natuurlijke omgevingen en niet binnen agro-ecosystemen. Echter, omdat *Salmonella* en *Campylobacter* serieuze gevolgen voor de gezondheid kunnen hebben bij de mens, vinden we eliminatie van deze pathogenen in het eerste deel van de voedselketen een prioriteit. Plaagdierbeheersing is een cruciaal onderdeel van dit proces.

Een veldstudie naar de kolonisatie van kleine zoogdieren (bij zowel wilde knaagdieren als insectivoren) met *Salmonella* en *Campylobacter* wordt beschreven in Hoofdstuk 3. Op tien Nederlandse biologische veehouderijbedrijven (9 varkensbedrijven, 1 pluimveebedrijf) werden knaagdieren en insecteneters gescreend op de aanwezigheid van *Salmonella* en *Campylobacter spp*. *Campylobacter* werd geisoleerd in 9 van de 282 geteste dieren en Salmonella in 1 van de 282. Alle geïnfecteerde kleine zoogdieren waren ofwel de huismuis of de bruine rat. Mestmonsters van het aanwezige vee werden verzameld op 6 bedrijven. Echter, toepassing van de Amplified Fragment Length Polymorphism (AFLP)-techniek bracht geen directe relaties tussen isolaten van kleine zoogdieren en vee aan het licht.

Herintroductie van dierlijke productiesystemen met uitloop van vee (zoals bijvoorbeeld in de biologische veehouderij) welke ontwikkeling het resultaat is van de publieke afkeer van de

bio-industrie en het binnen produceren, kan leiden tot het opnieuw opkomen van ziekten die ook in het verleden aanwezig waren. In Hoofdstuk 4, kijken we specifiek naar de risicofactoren op bedrijfsniveau voor herintroductie van de protozoaire parasiet *Toxoplasma gondii*. Deze parasiet kan ernstige consequenties hebben voor mensen, vooral bij zwangere vrouwen en bij personen met een lage weerstand. Het merendeel van de humane infecties wordt veroorzaakt door de consumptie van onvoldoende verhit vlees, en dus is controle van dit pathogeen op bedrijfsniveau van vitaal belang. Als belangrijkste risicofactoren voor besmetting op bedrijfsniveau op biologische varkensbedrijven, identificeerden wij het aantal katten met toegang tot de bedrijfsomgeving en het verstrekken van geitenwei aan de varkens (Hoofdstuk 5). Helaas bood het onderzoek uit Hoofdstuk 5 onvoldoende aanknopingspunten om de rol van knaagdieren in de overdracht van *T. gondii* naar varkens te bestuderen: hiervoor is een interventiestudie nodig. We concludeerden dat het toepassen van relatief eenvoudige bedrijfsmatige procedures een bijdrage kan leveren aan het verminderen van *T. gondii* prevalentie in varkens.

In Hoofdstukken 6 en 7 werd respectievelijk de prevalentie van *Toxoplasma gondii* in kleine wilde zoogdieren gevangen op agrarische bedrijven en in vers varkensvlees onderzocht. Kleine zoogdieren worden vaak genoemd als een mogelijke transmissie route van dit pathogeen naar varkens.

We vonden dat een substantieel percentage van de kleine zoogdieren op Nederlandse boerderijen *T. gondii* met zich meedroeg en dus mogelijk een rol spelen bij de overdracht van dit pathogeen. Voor het eerst toonden wij een substantiële *T. gondii* seroprevalentie (9.1%) in huisspitsmuizen aan. Over het geheel genomen werd *T. gondii* serologisch aangetoond in 6.4% van de monsters van kleine zoogdieren, terwijl 71.1% een PCR product liet zien. In varkensvlees vonden we een soortgelijke discrepantie tussen moleculaire detectie en de resultaten van eerdere serologische studies. Deze studies lieten zien dat in Noord-Europa de seroprevalentie van *T. gondii* de laatste decennia tot beneden de 5% is gedaald. Echter, in deze studie werd een positieve PCR reactie in 29.6% van het conventioneel geproduceerde varkensvlees gevonden en in 55.2% van het biologisch geproduceerde varkensvlees.

Deze discrepantie zou het resultaat kunnen zijn van: 1. een hogere sensitiviteit van de PCR in combinatie met een lage humorale respons, 2. een kruisreactie van de parasiet met andere sterk gerelateerde protozoa, 3. verschillende serologische responsen na infectie met verschillende stammen, of 4. de mogelijkheid van vermeerdering van een genomisch DNA product in plaats van parasitair DNA. We concludeerden dat verdere optimalisatie en

standaardisatie van detectie-technieken nodig zijn om de daadwerkelijke *T. gondii* prevalentie in varkensvlees te kunnen vaststellen.

Hoofdstuk 8 bevat een ethische analyse van plaagdierbestrijding. Over het algemeen worden in westerse maatschappijen dieren gezien als objecten van morele zorg. Daarom worden er eisen gesteld aan de manier waarop we dieren houden en gebruiken tijdens voedselproductie of dierproeven. Echter, in het geval van het optreden van knaagdierplagen, worden vaak inhumane bestrijdingsmethoden ingezet. Kennelijk is er hier sprake van een inconsistentie in de mens-dier relatie. Wij claimen dat er twee opties bestaan om deze inconsistentie weg te nemen. De eerste optie is het opheffen van criteria rond dierexperimenten. Echter, vanuit het oogpunt van de dierethiek is de tweede optie meer wenselijk: namelijk toepassing van dezelfde criteria op plaagdierbestrijding. Dit heeft tot gevolg dat ethische plaagdierbestrijdingsmethoden niet zouden moeten leiden tot intense pijn of ongemak, alleen van korte duur zijn, en dat een ontsnappend knaagdier in staat zou moeten zijn een natuurlijk leven te leiden. Dientengevolge vraagt dit om een aanpassing in het ontwerp van plaagdierbestrijdingsmethoden.

Preventie van contact tussen productie-dieren en wilde knaagdieren noodzakelijk, vanwege de voedselveiligheids- en gezondheidsrisico's. In Hoofdstuk 9 worden de huidige stand van zaken met betrekking tot plaagdierbestrijding en aanbevelingen van een internationaal seminar gepresenteerd. Beslissingsanalyse-technieken werden gebruikt om te bepalen wat de meest geschikte methode is om een bruine rattenplaag op een biologisch varkensbedrijf het hoofd te kunnen bieden. Rodenticiden werden geclassificeerd als meest efficiente methode van plaagdierbeheersing, maar ze passen niet altijd in de bedrijfsfilosofie van biologische boeren. In deze gevallen lijkt vangen het beste alternatief, hoewel de arbeidskosten hiervan hoog zijn.
Maar plaagdierbestrijding is maar een onderdeel van plaagdierbeheersing, en is het laatste redmiddel. Continue monitoring van knaagdierpopulaties en preventie (bijvoorbeeld het beperken van hun toegang tot bedrijfsgebouwen, het wegnemen van stapels oud materiaal, het verwijderen van habitatelementen nabij de stallen en het beperken van toegang tot veevoer en water) zal de ontwikkeling van hoge populatiedichtheden op boerderijen beperken en dus zullen contactmogelijkheden tussen knaagdieren en vee afnemen.

Hoofdstuk 10 bevat een algemene discussie over het werk dat in dit proefschrift gepresenteerd wordt. Sommige knaagdiersoorten kunnen pathogenen bij zich dragen die voedselgerelateerde ziekten kunnen veroorzaken. Veehouders moeten bewust worden gemaakt van dit risico en zouden moeten worden aangemoedigd om maatregelen te nemen om de veiligheid van hun producten te bewaken en het contact tussen vee en knaagdieren te voorkomen. Door hun ecologie zullen vooral commensale knaagdieren (huismuizen en ratten) een bedreiging vormen, aangezien zij dicht bij het vee leven en over goede reproductie-capaciteiten beschikken. Figuur 10.1 beschrijft een versimpeld hypothetisch model voor zoönoses die worden veroorzaakt door knaagdieren en hun overdracht aan productiedieren.

Een van de maatregelen om infectie in vee, katten en ook in mensen terug te dringen, is plaagdierbeheersing. Een andere optie voor de toekomst is de ontwikkeling van DNA vaccinatie in dieren of zelfs mensen. Totdat vaccinatie die langdurige immuniteit tegen *T. gondii* garandeert beschikbaar komt, zou preventie van het voorkomen van levende *T. gondii* cysten in vlees nuttig kunnen zijn. Het bevriezen van vleesproducten of het doorstralen van voedsel zijn mogelijke methoden om de consumptie van geïnfecteerd vlees te verminderen.

Bovendien zouden deze maatregelen ook hun nut kunnen hebben bij het voorkómen van andere voedselgerelateerde pathogenen. Echter, ondanks het toepassen van deze maatregelen, moeten consumenten zich altijd bewust zijn van het feit dat er spanning is tussen diervriendelijke productie van voedingsmiddelen van dierlijke oorsprong aan de ene kant en voedselveiligheid aan de andere.

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List of publications

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Curriculum Vitae

Bastiaan Gezelle Meerburg werd op 28 september 1978 in Utrecht geboren als zoon van Otto George Frans Gezelle Meerburg en Annemarie Aukje Overeem. In 1997 behaalde hij het gymnasium-diploma aan het Erasmiaans Gymnasium in Rotterdam. Later dat jaar begon hij met de studie Zoötechniek aan de toenmalige Landbouwuniversiteit Wageningen (tegenwoordig Wageningen Universiteit). In de doctoraalfase verrichtte hij onder meer onderzoek naar het effect van natuurbeheer op de kleine zoogdierpopulatie op agrarische bedrijven (afstudeervak Dierlijke Productie Systemen, onder begeleiding van Marcel Huijser), naar het voedselveiligheidsbeleid van de Europese Unie (afstudeervak Internationaal Bestuur) en naar de geschiedenis van coöperatieve kredietverlening in Nederland (afstudeervak Agrarische Geschiedenis). Stages werden doorgebracht bij Rijk en Cora de Jong in Tupiza, Bolivia (onderzoek naar de mogelijkheden van de vicuña, een lama-achtige, voor de rurale bevolking van de Andes), en bij de FAO (Food and Agricultural Organization of the United Nations) in Rome en Boedapest, waar de voedselveiligheidssituatie in Hongarije met het oog op toetreding tot de Europese Unie nader werd bestudeerd.

Na zijn afstuderen in maart 2002 trad hij in dienst als persoonlijk medewerker van europarlementariër Jan Mulder in Brussel. Het onderzoek bleef echter trekken en daarom kwam hij in november 2002 in dienst bij het toenmalige ID-Lelystad, dat tegenwoordig onderdeel uitmaakt van de Animal Sciences Group van Wageningen UR. Na een uitstapje in het gamma-onderzoek dat werd afgesloten met de AKK Kennisketen Award 2004 voor het wetenschappelijke essay “Maatschappelijk Verantwoord Ondernemen: een kans voor de keten!”, startte hij met het onderzoek dat in dit proefschrift beschreven wordt. Sinds september 2005 is hij tevens officieel VROM-erkend ongediertebestrijder.

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