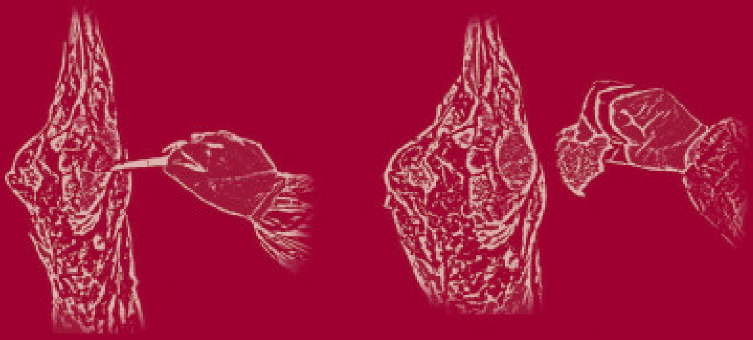

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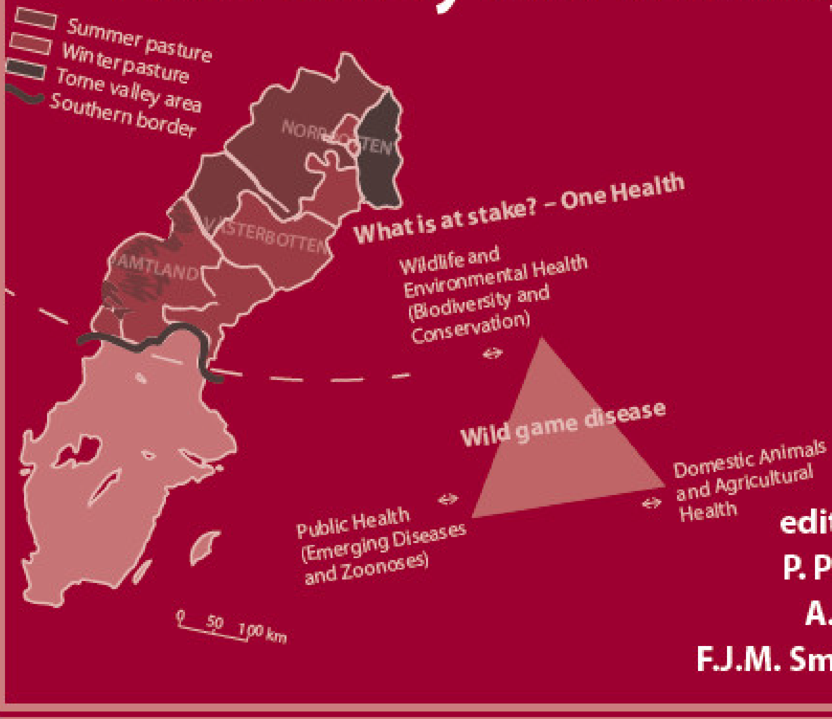
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VÄSTERBOTTEN
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Wild game disease
Domestic Animals and Agricultural Health
Public Health (Emerging Diseases and Zoonoses)
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edited by:
P. Paulsen
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Preface

'Game meat hygiene – Food safety and security' is the third volume in a series dedicated to highlighting current issues in the game meat production chain. The 19 contributions originate from authors and research groups not only from Europe, but also from the African and Asian continents, and consequently a broad variety of topics is covered in this book in addition to the more of game-meat-oriented issues.

Like its two predecessors, this volume comprises the contributions to a conference held in the framework of the 'International Research Forum on Game Meat Hygiene' initiative. Special thanks are due to Cristina Soare and Alex Seguino for the perfect organisation and for the hospitality the participants received at the Royal (Dick) School of Veterinary Studies Edinburgh, UK, where the conference took place. Selected contributions from this conference have been updated and been thoroughly reviewed before being collated into this volume.

The array of topics assures that not only readers interested in game meat production and safety will find relevant information, but also that those interested in zoonoses and emerging diseases in the wildlife-livestock-human interface will be attracted. We hope the readership will enjoy this book as did the contributors and reviewers in preparing it. May it serve as motivation/inspiration to get (or stay) involved in research in the field of game meat hygiene and safety, with the ultimate goal to assure safety, quality and sustainability of this particular food source.

We acknowledge the 'Verein Grünes Kreuz', Austria, for their financial support which made this publication possible, and we are grateful to the publisher for the continued support we have enjoyed over the recent years.

Vienna, December 2016

The editors

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Section 1

Biological hazards

1. African game meat and the safety pertaining to free-ranging wildlife: example of a wild suid in South Africa

Louwrens C. Hoffman[1*], Monlee Swanepoel[1,2] and Alison J. Leslie[2]
[1]Department of Animal Sciences, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch 7602, South Africa; lch@sun.ac.za
[2]Department of Conservation Ecology and Entomology, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch 7602, South Africa

Summary

Wildlife is the primary source of animal and zoonotic diseases and of major importance to the agricultural and public health sector since these diseases cause production and financial losses, including mortalities among livestock animals, while zoonotic diseases cause debilitating illnesses or death in humans. As the global wildlife-livestock-human interface is expanding and growing more complex, the possibility of pathogen transmission among these agents is expected to increase. Hunting and game meat consumption are important activities among many societies, with wild species often being translocated and introduced by humans for these purposes. However, intentionally introduced species may become naturalized and disperse within the surrounding area, providing pathways for potential disease transmission among hosts. The common warthog (*Phacochoerus africanus*) has been extra-limitally introduced to reserves and ranches across the country including the former range of the extinct Cape warthog (*Phacochoerus aethiopicus*). The species are popular for recreational and trophy hunting and have traditionally been hunted and consumed as game meat, similarly to the introduced feral pig. Warthogs are associated with a number of important animal and zoonotic diseases, such as African swine fever and bovine tuberculosis, with the potential to act as a wild reservoir. This raises serious concerns as the species are not restricted by standard fencing and move freely among natural and agricultural lands, with their distribution range expanding across South Africa. This review summarizes the pests and pathogens associated with common warthogs, current disease control measures in South Africa and the implications for human consumption of warthog meat.

Keywords: diseases, pathogens, pests, *Phacochoerus africanus*, warthog, wildlife, zoonoses

1.1 Introduction

1.1.1 Wildlife diseases

Wildlife may carry or be susceptible to a range of different diseases which affects domestic livestock or humans (Daszak *et al.*, 2000). Wildlife diseases are of major importance to the agricultural and public health sector since they cause production and financial losses,

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including mortalities among livestock animals, while zoonotic diseases cause debilitating illnesses or death in humans (Dhama *et al*., 2013; Kruse *et al*., 2004). Additionally, livestock may harbour exotic diseases that are transmissible to wildlife, who in turn act as carriers or spillover hosts, thereby maintaining the disease in the environment despite vaccination of livestock (Miller *et al*., 2013; Rhyan and Spraker, 2010; Siembieda *et al*., 2011). This bi-directional nature of animal diseases has important implications as the livestock-wildlife interface continues to expand and become more complex, with changing types of land use becoming more intertwined (Bengis *et al*., 2002).

Of all the known human pathogens 61% are zoonotic, and 75% are considered to cause 'emerging' diseases (Taylor *et al*., 2001). Human zoonotic infections occur mainly through handling of infected animals or carcasses and consumption of infected meat (Ahl *et al*., 2002). Hunting and game meat consumption is an important activity among many societies and it is therefore necessary to educate hunters on the risks associated with wildlife species, while the role of scientists is to detect and recognize diseases of significance. Hoffman and Cawthorn (2012) provide an extensive overview of the consumption of meat from wild animals on a global scale, with reference to the dependence of African peoples on the meat as protein source. The continent is host to a number of important zoonotic diseases which are highly infectious to humans and multiple animal species. For example, trichinosis and echinococcosis are intestinal parasites of humans obtained through ingesting infected, undercooked meat, of which game meat is a common source (Pozio, 2007). Both may cause debilitating diseases in humans and death in severe cases with important economic implications regarding public health and livestock production. The bacterium responsible for anthrax, *Bacillus anthracis*, has historically been responsible for devastating epidemics among domestic and wild animals in sub-Saharan Africa, with an outbreak in 1923 causing the death of an estimated 30,000 to 60,000 animals in South Africa (Sterne, 1967 in Hugh-Jones and De Vos, 2002). The disease is endemic to parts of southern Africa with both animal and human infections reported during the most recent outbreaks in 2013 and 2014 in Namibia, Zimbabwe and Lesotho (NICD, 2014). Human infections occur from contact with infected meat, mucosal membranes and damaged skin, inhalation of spores or ingestion of infected meat; the case fatality rate is however low (<1%).

Rabies is another disease that can infect all mammalian species, with the canine strain responsible for the majority of infections among animals and humans. The domestic dog (*Canis familiaris*), yellow mongoose (*Cynictis penicillata*), black-backed jackal (*Canis mesomelas*) and bat-eared fox (*Otocyon megalotis*) are considered the dominant maintenance hosts and readily transmit the virus inter species, while an inter-herbivorial outbreak among kudu in Namibia in the 1970's resulted in the loss of 30,000 to 50,000 animals (Bishop *et al*., 2003). Slaughtering and consuming raw meat from rabid animals have been implicated in human cases of rabies, but not for ingestion of cooked meat.

The main diseases targeted by the Disease Reference Group on Zoonosis and Marginalized Infectious Diseases (DRG6) (WHO, 2012) are presented in Table 1.1. All of these diseases have been recorded to infect humans in southern Africa while certain groups, including persons in animal-related occupations, hunters and poor and marginalized communities, are more vulnerable to contracting and spreading animal diseases. According to the Food and

Table 1.1. The zoonotic diseases targeted by the Group on Zoonosis and Marginalized Infectious Diseases (DRG6) (WHO, 2012).

1. Helminth infections	2. Protozoan infections	3. Viral infections	4. Bacterial infections
• taeniasis/cysticercosis	• cryptosporidiosis	• rabies	• brucellosis
• echinococcosis	• toxoplasmosis		• certain enteric bacterial pathogens
• food-borne trematodiases			• bovine tuberculosis
• zoonotic schistosomiasis			• anthrax

Agricultural Organization of the United Nations (FAO, 2013), livestock health is the weakest link in the global human health chain, and controlling animal and agricultural product movement is the most effective preventative measure for introducing and spreading animal diseases to a country and within its borders.

Poor and marginalized communities in particular are disproportionately affected by zoonotic- and vector-borne diseases due to their dependency on livestock and bushmeat (Bengis *et al*., 2002; Molyneux *et al*., 2011). Early detection of potential zoonosis and dissemination of this knowledge to these groups could help limit zoonotic infections, and contribute to improving public health among these communities. Anthropogenic activities such as transportation and introduction of species, provision of feeding stations and occurrence of feral animals have been identified as major facilitators of disease outbreaks among livestock and wildlife (Dobson and Foufopolous, 2001). In southern Africa, all of these are facets of the wildlife ranching industry which poses a unique situation for animal and human health. Additionally, feeding stations, including supplementary feeding or mineral licks, and permanent water points are typical features of livestock farms and intensive game farming operations, which may serve as an important source of disease spread as wild and domestic animals tend to aggregate around these points (Bengis *et al*., 2002).

In some cases, intentionally introduced species are able to become naturalized and disperse within the surrounding area (Forsyth and Duncan, 2001; Fraser *et al*., 2000), posing a disease risk as feral or free-ranging species. Feral pigs (*Sus scrofa*), for example, have been introduced onto every continent except Antarctica and are heavily persecuted for damage reprisal, but are also considered a popular game animal for sport hunting and meat production, the main reason they were originally introduced (Bengsen *et al*., 2014). The species are associated with, and may act as wild reservoir of economically devastating diseases such as African Swine fever and bovine tuberculosis (bTB), and humans have been infected with hepatitis E virus (HEV), *Trichinella* spp. and *Toxoplasma gondii* from ingestion of infected meat (Meng *et al*., 2009). Subsequently, there has been increasing concern regarding disease outbreaks among introduced/translocated populations as the contact between feral and domestic animals and human increases, and Kock *et al.* (2010) provide a global review of the risks and implications of species translocations/introductions and wildlife disease.

1.1.2 Background to game ranching

The production and utilization of wild animals as a food and revenue resource has become an increasingly popular industry among many southern African countries. Game ranching (also referred to as game farming), is a production system where wild animals are managed extensively or intensively on private or communal land, exclusively or in conjunction with other agricultural practices, including livestock rearing (Cousins *et al*., 2008). The establishment and success of the industry is attributed to the change in wildlife protection policies and private land- and wildlife-ownership during the 1960's and 1970's which encouraged wildlife protection and utilization on state-owned and private reserves (Benson, 1991; Luxmoore, 1985). The shift from livestock to wildlife based production was further encouraged by observations that indigenous wildlife are better adapted to the harsh conditions of marginal lands across the African continent, the high diversity of wildlife and the availability of land unsuitable for livestock production (Child *et al*., 2012; Lindsey *et al*., 2013).

It is estimated that currently more than 9,000 game farms covering 205,000 km^2 are operating in South Africa (Lindsey *et al*., 2013). The game farming industry generates revenue through eco-tourism, recreational (biltong) and trophy hunting, live trade and sales, taxidermy and meat production (Cloete *et al*., 2007; Van der Merwe and Saayman, 2003), whilst providing jobs and other socio-economic opportunities for local communities (DAFF, 2013a). Wildlife species that are valued as game animals are regularly introduced and/or translocated to farms and reserves as part of the game farming industry in South Africa. Species introductions are driven by the demand of tourists and hunters for certain species and a greater diversity of species, which is associated with increased economic activity (Barnes and De Jager, 1996; Castley *et al*., 2001). The practice appears to be confounded by the country's natural species richness and the lack of a national framework which governs the game farming industry (Cousins *et al*., 2010; Spear and Chown, 2009). This, together with increased stocking densities of domestic and wild animals on the same or adjacent properties (Ocaido *et al*., 1996), has raised legitimate concerns regarding the prevalence of animal diseases among wild and domestic animals (Bekker *et al*., 2012). Recently, the Department of Environmental Affairs has embarked on developing a national framework for the norms and standards of translocation of indigenous species (DEA, 2015).

1.2 The case of the warthog in South Africa

1.2.1 Warthog as an extra-limital species

The common warthog (*Phacochoerus africanus*) was introduced extra-limitally to provincial reserves in the Northern Cape, Free State and Eastern Cape provinces of South Africa, initially as part of re-wildling efforts of a warthog species (Cape warthog, *Phacochoerus aethiopicus*) thought to have gone extinct (Penzhorn, 1971). In South Africa, their known natural range includes the upper north-eastern parts of the country including the Limpopo, Mpumalanga and marginal parts of the Kwa-Zulu Natal/Mozambique border (Rautenbach, 1982; Skead *et al*., 2007; Skinner and Chimimba, 2005). Inland, warthogs also occur in the northern parts of North West province and the border of the Northern Cape and Botswana. These introductions were followed by introductions on private game farms and reserves across the country for eco-tourism and hunting purposes (Nyafu, 2009). Multiple introduction events coupled with a high reproduction rate has allowed warthogs to greatly expand their range and inhabit areas where they were historically absent.

Warthogs are a species not contained within the borders of a property by standard wire or wire mesh fencing and can be considered a ‘free-ranging’ species in South Africa. All three provinces (Northern Cape, Free State and Eastern Cape) where these introductions occurred are important agricultural producers utilizing the majority of land for farming purposes, and warthog presence has been positively associated with the occurrence of pastoral farms (Bamford *et al*., 2014). The Northern Cape covers an area of 363,389 km^2 of which 81% is utilized for agricultural purposes. The major agricultural activity is stock farming, including cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*), while crop farming comprises 2% of the total land use due to the aridity of the area. The Free State province covers 129,825 km^2 with agriculture accounting for 90% of the land use, of which about 57% is used for livestock farming and 33% for crop production. The Eastern Cape covers 170,616 km^2 of which 86% is used for farming (DAFF, 2013b). Game farming has become a major form of agricultural land-use in all three provinces, with game farms covering 48,520 km^2 (13.4% of total area) of the Northern Cape and 8,816 km^2 (5.2% total area) of the Eastern Cape.

Warthogs are a known agricultural pest and have become a managerial problem in traditional agricultural settings. Some farmers currently employ a shoot-on-sight strategy to control populations and mitigate negative impacts, while game farmers exploit the species for financial gain through hunting and tourism. The species have traditionally been hunted and consumed as bushmeat by rural communities (Martin *et al*., 2012), but is also a popular species for recreational and trophy hunters. In 2005, a total number of 994 warthogs were officially recorded as being hunted by international trophy hunters in South Africa (Van der Merwe and Saayman, 2005), which increased to 2,049 in 2009 and 3,849 in 2013, making warthogs the 2nd most often trophy hunted in South Africa (PHASA, 2014). These numbers, however, do not represent all the warthogs hunted in the country as it is expected that the majority of recreational and damage control hunting remain unreported.

Warthogs currently inhabit both private and public lands on which major agricultural activities are practiced in South Africa. Despite being associated with a number of important animal and zoonotic diseases, there has been almost no attention given to the prevalence and risk of disease among free-ranging warthog populations in South Africa. Warthogs are considered the major host of the *Ornithodoros* tick species which is responsible for the transmission and spread of African swine fever (ASF) in domestic pigs (Penrith and Vosloo, 2009). They have also been experimentally infected with the virus responsible for classical swine fever (CSF), and able to spread the disease to other warthogs (Everett *et al*., 2011). While the ASF and CSF viruses are unrelated, both cause devastating diseases in domestic pigs with severe economic consequences. Considering the potential implications the distribution and movement of free-ranging warthogs could have for livestock and game animal production, and for the consumption of warthog meat by humans, this review aims to summarize the most important diseases that have been reported for warthogs in sub-Saharan Africa.

1.2.2 Invasive diseases associated with warthogs potentially transmissible to animals and humans

The most important diseases associated with warthog that are potentially transmissible to animals and humans are presented in Table 1.2 and 1.3, respectively. Each disease is briefly discussed regarding the current state of knowledge on warthogs, the measures taken towards disease prevention and spread and the future avenues for research. A short section at the end provides an overview of other potential warthog diseases that could pose a future risk for animal and human health, but on which very limited knowledge is available.

1.2.2.1 African swine fever

ASF is arguably the most important disease associated with warthogs in Africa. Warthogs are considered to be the original host and carrier of the ASF virus, a unique DNA arbovirus indigenous to Africa. This highly contagious disease is of major concern for domestic pig production as it has a 100% mortality rate in pigs and its prevalence in sub-Saharan Africa continues to greatly impede the development of piggeries for pork production (Penrith and Vosloo, 2009). Following infection, pigs develop haemorrhagic fever and succumb within 5-15 days. Warthogs and argasid ticks (or tampans), *Ornithodoros porcinus* and *Ornithodoros moubata*, are the natural hosts that maintain the ASF virus in an ancient sylvatic cycle in eastern and southern Africa (Penrith *et al.*, 2004). The argasid ticks are mainly found in warthog burrows, but occasionally occur on adult warthogs where they are transported to grazing and farmed areas, coming into contact with domestic pigs (Gallardo *et al.*, 2011). Neonate warthogs become infected when bitten by infected ticks in burrows and develop detectable viraemia for 2-3 weeks but do not develop the disease. Although animals stay infected for life, no detectable viraemias have been found in older animals (Thomson, 1985). Therefore, ticks only become infected with ASF virus when feeding on neonate warthogs with elevated blood virus levels due to their limited immune systems.

Although Thomson (1985) found that all diagnosed cases of ASF among domestic pigs were in countries where warthogs occur, the co-distribution of warthogs and *Ornithodoros* spp. does not imply the existence of ASF in an area (Jori and Bastos, 2009). Other than the sylvatic cycle, the virus is also maintained in a cycle between domestic pigs and *O. porcinus* in pig shelters, and among domestic pigs without an apparent wild Suidae host or tampan vector (Costard *et al.*, 2009; Jori *et al.*, 2013). The movement of infected pigs and pig products has been considered the major route of ASF transmission in African countries where warthogs are absent (Penrith and Vosloo, 2009); all of the last outbreaks in South Africa since 1994 were caused by the movement of infected animals or animal products.

In South Africa the disease is limited to the Limpopo and parts of the Kwa-Zulu Natal and Mpumalanga provinces by the ASF control zone, where transmission and infection is primarily prevented by monitoring the movement of wild and domestic pigs and their products from and within the controlled zone. The most recent outbreak in 2011 in Gauteng was effectively contained within the infected pig population before it could spread (Penrith, 2013), which emphasizes the importance of maintaining strict biosecurity measures to protect against infection and spread. Infections outside of the control zone are treated by stamping

Table 1.2. Diseases associated with warthogs of importance to livestock and game animals.

Disease	Causative agent	Vector/host	Animals affected	Impact on production	Status in South Africa	Reference
Viruses						
African Swine fever (ASF)	ASF virus	*Ornithodoros porcinus* and *O. moubata* ticks, warthogs	domestic pigs, bushpigs	mortalities	confined to Limpopo and parts of Kwa-Zulu Natal and Mpumalanga by ASF control zone	Penrith and Vosloo, 2009
Classical Swine fever (CSF)	CSF virus	domestic pigs	domestic pigs, bushpigs	mortalities	confined to Eastern Cape province by CSF control zone	Penrith *et al.*, 2011
Rift Valley fever	*Phlebovirus*	mosquitoes: *Aedes* and *Culex* spp.	domestic and wild animals	high rates of abortion, neonatalities and mortality in young	occasional regional outbreaks	Britch *et al.*, 2013
Foot and mouth disease (FMD)	FMD virus, family *Picornaviridae*	African buffalo, cattle	domestic and wild hoofed animals	severe production losses	confined to the Kruger National Park (KNP) and parts of Limpopo and Mpumalanga provinces by FMD control zone	Bengis and Erasmus, 1988
Bacteria						
Bovine tuberculosis (bTB)	*Mycobacterium bovis*	notably cattle and buffalo	domestic and wild animals	animal production losses, mortalities	confined to the bTB control area around KNP	De Lisle *et al.*, 2002
Parasites						
Echinococcus granulosus		carnivores	domestic and wild herbivores	animal production losses, mortalities	low prevalence	Horak *et al.*, 1988
Nagana disease	*Trypanosoma* spp.	tsetse fly *Glossina* spp., cattle, elephants, wild suids	domestic and wild ruminants	animal production losses, mortalities	low prevalence, isolated to parts of Kwa-Zulu Natal	Claxton *et al.*, 1992

Table 1.3. Diseases associated with warthogs of importance for public health.

Disease	Causative agent	Human infections	Source of infection	Status in South Africa	Reference
Viruses					
Rift Valley Fever	*Phlebovirus*	potentially fatal	handling of infected carcasses	occasional regional outbreaks	Paweska, 2014
Rabies	*Rhabdoviridae* (canine strain)	fatal if untreated	exchanging mucosal material with rabid animal through bites, open wounds	high prevalence in Eastern Cape and Kwa-Zulu Natal	WHO, 2013
Parasites					
Echinococcosis (hydatidosis)	*Echinococcus granulosus*	can cause debilitating illness, death	consumption of raw or undercooked pork or warthog meat	low prevalence among domestic or wild swine	Wahlers *et al.*, 2013
Trichinosis	*Trichinella* spp.	intestinal infestation, can cause death	consumption of raw or undercooked meat from infected animals	no cases reported for domestic animals and humans in South Africa	Mukaratirwa *et al.*, 2013
Bilharzia	*Schistosoma* spp.	causes debilitating illness	Organism penetrates skin in freshwater, accidental ingestion of eggs	a re-emerging neglected tropical disease in Eastern Cape, Kwa-Zulu Natal, Limpopo and Mpumalanga	Horak *et al.*, 1988

out, where infected and in-contact herds are quarantined and slaughtered, since there is no vaccine or treatment for ASF. South Africa has been heralded as an example of how an ASF free-zone can be established and maintained within an endemic area (Costard *et al*., 2009). However, the illegal transportation of wild suid carcasses by humans and hunters remains a concern for spreading ASF in South Africa and across national boundaries, since the ASF virus can remain infectious for extended periods of time in meat and meat products (Penrith and Vosloo, 2009). South Africa is also unique regarding the diversity of pig production enterprises; ranging from free ranging rural herds on communal lands, to large scale commercial production systems, which increases the complexity of continued surveillance and protection programmes.

There is a paucity of information regarding the current prevalence and occurrence of ASF among *Ornithodoros* ticks in South Africa. Madder *et al*. (2013) indicated that the distribution range of the tampan follows that of the warthogs' distribution, which was historically limited to the more northern and eastern parts of South Africa (refer to http://www.itg.be/photodatabase/African_ticks_files/index.html for distribution of African ticks), and there is limited evidence that the distribution range of the tampan vector is changing in some parts of South Africa (Penrith and Vosloo, 2009). Arnot *et al*. (2009) and Boshoff *et al*. (2014) found no ASF viruses among *O. porcinus* ticks collected from warthog burrows in a game reserve within the ASF control zone and in Swaziland (respectively) which is adjacent to the ASF control zone. The findings of Arnot *et al*. (2009) are especially significant since ASF virus was detected, albeit at low prevalence (0.06%), in sampled tick populations in 1978, and since the warthog population had grown by 59% and tick infestation rate of warthog burrows had increased by 27%. The authors suggested that either the virus had disappeared from the area or that it was extremely localized. The prevalence and occurrence of ASF in *Ornithodoros* spp., and distribution of tampans and their hosts, are important areas for future research especially as the common warthog continues to expand its distribution in South Africa.

1.2.2.2 Classical swine fever

CSF is considered the most important disease of swine globally. It has been responsible for devastating economic losses across continents and in countries with notable pig production activities. The disease is caused by a small RNA virus which produces similar clinical symptoms and lesions to ASF in domestic pigs although the viruses are unrelated (Moennig *et al*., 2003). It is a highly infectious haemorrhagic disease which causes severe morbidity and mortality in domestic pigs in its acute form, but can also manifest in a subacute or chronic form (Moennig, 2000). This is attributed to strain variability, with moderately virulent strains responsible for chronic and protracted illness, while the associated immunosuppressive effect often results in mortalities from secondary infections. The disease is circulated and maintained among wild and domestic pigs through orthonasal transmission or contamination of the environment, most notably by swill (Edwards *et al*., 2000). Infected pigs begin to shed the virus during the incubation period when no physical symptoms have started to develop, and no combination of physical signs, symptoms or lesions can be used to predictably detect a suspected outbreak (Penrith *et al*., 2011).

It was successfully eradicated in South Africa after its initial introduction in the early 1900's, but was reintroduced in 2005 causing an outbreak among piggeries in the Eastern Cape. Large-scale pig slaughters (stamping out) and the implementation of strict regulations governing the transportation of live domestic and wild pigs and their products from the infected area has effectively brought the disease under control, and while there is still a control zone enforced, the disease is considered as eradicated in South Africa (Penrith, 2013; Penrith *et al.*, 2011). Currently, no live pig or pig product (domestic and wild) may be moved from the CSF control zone, and special permits are to be obtained if pig products are to be moved from or through the zone. The Eastern Cape province is host to large populations of rural pigs, warthogs and bushpigs (*Potamochoerus larvatus*), and there has been concerns that the disease might become established in wild suid populations, since experimental infection and intra-species transmission of CSF have been shown for both warthogs and bushpigs (Everett *et al.*, 2011). Penrith *et al.* (2011) suggested that warthogs might not play the same role in CSF epidemiology as with ASF, considering the ancient sylvatic cycle between warthogs and ASF virus tick vectors. However, future studies should aim to determine whether these wild suids may play a role in CSF epidemiology in South Africa.

Despite the existence of an effective vaccine, many countries including South Africa have abstained from vaccination as the difference between vaccinated and naturally infected serologically positive animals cannot be detected. Therefore the continued implementation of strict biosecurity measures is required to keep South African herds free from infections.

1.2.2.3 Rift Valley fever

Rift Valley fever (RVF) is caused by an arbovirus of the family *Bunyaviridae*, genus *Phlebovirus*. Endemic to sub-Saharan Africa, the virus was first diagnosed during an outbreak among infected sheep in the greater Kenyan Rift Valley in 1931 (Pepin *et al.*, 2010). The virus is carried and transmitted primarily by mosquitoes from the *Aedes* and *Culex* genera which infect animals through their saliva during feeding. The virus requires a vertebrate host in order to replicate and spreads by infecting other mosquitoes that feed on infected animals. Since host animals only remain viraemic for a couple of days, the invertebrate host may play a pivotal role in maintaining virus circulation among seasons. Periodic outbreaks (5-15 years) occur after heavy rains or floods where the accumulated surface water on shallow soil provides an ideal breeding ground for mosquito vectors (LaBeaud *et al.*, 2010). RVF causes a high rate of abortions and perinatal mortalities in pregnant ruminant animals, foetal malformation, and subclinical-to-fatal febrile illness in animals and humans.

There have been localized outbreaks of RVF in South Africa among domestic and wild animals since the 1950's (Pienaar and Thomson, 2013). Outbreaks have been widespread but are increasingly frequent in central, summer rainfall regions, one of the regions warthogs have been introduced to. During the 2010 epidemic, signs of RVF in a number of indigenous and exotic wildlife were reported for the first time. These included springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus dorcas dorcas*), bontebok (*Damaliscus pygargus pygargus*), waterbok (*Kobus ellipsiprymnus*), African buffalo (*Syncerus caffer*), sable (*Hippotragus niger*), greater kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasii*), gemsbok (*Oryx gazella*), fallow deer (*Cervus dama*), llama (*Lama glama*), alpaca (*Lama pacos*), Asian buffalo (*Bubalus*

bubalus) and ibex (*Capra ibex*) (Pienaar and Thompson, 2013). Although warthogs are not considered a wild host, Evans *et al*. (2008) discovered RVF antibodies in two warthogs in Kenya, and high levels of seropositivity have been found among populations also in Kenya, indicating that warthogs might be among the wild ungulates that play a role in the epidemiology of the virus (Britch *et al*., 2013). Other ungulates with seropositivity in this study were waterbok, impala (*Aepyceros melampus*), African buffalo, common eland (*Taurotragus oryx*), giraffe (*Giraffa camelopardalis*), gerenuk (*Litocranius walleri*), and also domestic camel (*Camelus dromedaries*).

There are still many gaps in understanding the epidemiology of RVF virus in the environment and the role of wild animals in maintaining and spreading the disease (Evans *et al*., 2008; Pienaar and Thompson, 2013). It has been suggested that wild animals, and certain domestic herds, maintain the virus through low-level circulation between periodic outbreaks, without the population manifesting any clinical symptoms (Pepin *et al*., 2010). The possibility of domestic or wild animals acting as spillover hosts in areas with a history of RVF outbreaks still requires investigation (Magwedere *et al*., 2012). Britch *et al*. (2013) suggested targeted serological surveillance among both livestock and wild ungulates to better understand the epidemiology of the virus, and possibly predict future outbreaks. Surveillance should be conducted before, during and after outbreaks as indicated by the authors to hopefully further elucidate the interepidemic maintenance and cycles of RVF.

If wild animals, and free-ranging warthogs, are potential maintenance host of the virus, continued vaccination of livestock in areas prone to RVF outbreaks could be the best method for effective disease management, while Oberem and Oberem (2011) suggested the application of pyrethroids on valuable game animals to prevent mosquito bites. Human infections with RVF are primarily through contact with infected dead animal tissue, including meat and mucosal membranes. There were a total of 302 laboratory confirmed cases of RVF in humans between 2008 and 2011 in South Africa of which 60% were farmers and farm labourers on animal farms (Archer *et al*., 2013). During this time period, 25 infections were fatal while contact with an infected warthog carcass (not confirmed) was suspected to be the source of infection in one fatality in 2008 (Paweska, 2014). This case fatality rate of 8% was higher than the estimated 0.5-2% among human cases in Africa and Arabian Peninsula given by Pepin *et al*. (2010). There is no treatment for RFV but prevention is possible through livestock and human vaccination and mosquito control. Educational efforts should aim to improve farmer and hunter knowledge on the risks associated with handling potentially infected animal tissue. Considering the long term persistence of the virus within mosquito vectors, the industry is yet to develop long term vaccines for humans and animals (LaBeaud, 2011).

1.2.2.4 Foot-and-mouth disease

Foot-and-mouth disease (FMD) is recognized as one of the most important livestock and game animal diseases globally. It is caused by a highly infectious aphtovirus of the family *Picornaviridae*, and although not fatal, greatly affects agricultural production including plant and plant product production (Thomson *et al*., 2003). The virus is epitheliotrophic with infected animals developing fever, lameness and vesicular lesions in the mouth and on the snout, feet and teats, which affects eating/feeding. It can cause myocarditis and death in young calves, lambs and piglets, and in some wild ungulate species (Arzt *et al*., 2011; Bengis and Erasmus, 1988). Endemic to many sub-Saharan African countries, transmission occurs through direct contact with infected animals, or mechanical transfer through contaminated animal feed, machinery, humans, animal and plant products, fomites or by airborne aerosols (Sellers and Gloster, 2008).

Essentially all members of *Artiodactyla* are susceptible to the virus but species and breeds may vary with degree of susceptibility, clinical manifestation of the disease and ability to infect (Arzt *et al*., 2011). Regarding domestic species, cattle (*B. taurus*) appear to be the main reservoir of FMD viruses globally, while in South Africa, the Cape buffalo (*S. caffer*) is the main host and long-term carrier of the South African Territories 1-3 (SAT 1-3) serotypes of FMD virus (Hedger, 1972; Thomson *et al*., 2003). The disease is maintained among buffalo populations in Kruger National Park (KNP) with periodic spillovers to other wildlife species, but wildlife has to date not been implicated in maintaining the disease independently.

Warthogs are reportedly highly susceptible to SAT 1 strains with reports of acute illness and death (Bengis and Erasmus, 1988). The species have experimentally been infected with SAT 2 and were able to infect other warthogs through contact, but did not become carriers (Hedger *et al*., 1972). This was also demonstrated for bushpigs, but there has been no evidence to date that either of these wild Suidae can become persistently infected. The SAT 2 serotype has been responsible for the majority of outbreaks among cattle and wildlife in and around the KNP (Dyason, 2010), affecting bushbuck (*Tragelaphus scriptus*), nyala, sable antelope (*Hippotragus equinus*) and roan antelope (*H. niger*). Kudu and impala have been implicated in transmitting FMD from infected buffalo populations to cattle (Hargreaves *et al*., 2004).

The FMD control zone encompasses the KNP and northern borders of Kwa Zulu Natal with the three World Organisation for Animal Health (OIE) control zones. The control zone monitors the movement of live cloven hoofed animals and their products from within the infected and buffer zones and the control measures are defined by the Veterinary Procedural Notice for Foot and Mouth Disease Control in South Africa. The fence is however subjected to extensive damage from primarily elephants and humans, which allows wildlife to enter surrounding farmlands and rural communities, and excluded cattle to enter the reserve (Jori *et al*., 2011). Warthogs were among the species observed to permeate fences and move between the reserve and surrounding areas. This impedes the effectiveness of veterinary fencing to separate domestic and wild animals and increases the likelihood of infectious diseases such as FMD being transmitted to naïve herds.

The last outbreak in South Africa outside of the control zone was in 2011, and resulted in the country losing its 'OIE FMD free without vaccination' status. In June 2012, the Directorate: Animal Health (DAFF, 2012) prohibited the movement of any live cloven hoofed animal from the FMD control zone in South Africa or from any area outside the country that is not recognized as free from FMD by the OIE. Strict implementation of these measures resulted in the country's 'FMD free without vaccination' status being partly restored in February 2014 and wholly restored in February 2015 (DAFF, 2015). Considering the massive economic implications FMD can have on countries with favourable livestock trade opportunities, it is a priority for South Africa to survey and maintain this status.

Although FMD is not considered a zoonosis, there have been recorded cases of human infection in the United Kingdom but these are extremely rare even among people that work in close contact with infected animals (Mayor, 2001; Prempeh *et al.*, 2001).

1.2.2.5 Bovine tuberculosis

The bacterium *Mycobacterium bovis*, the causative agent of bTB, is exotic to South Africa and was introduced through infected cattle imported by colonial settlers in the 1880's (De Vos *et al.*, 2001). The first confirmed case of infected wildlife was in greater kudu in the Eastern Cape in 1929, but the first widespread outbreaks occurred among buffalo populations in the KNP during the 1960's. Strong circumstantial evidence suggests that buffalo became infected from sharing pasture with infected cattle, as it is now known that transmission occurs through either respiratory or alimentary (sharing nourishment resources) pathways (Michel *et al.*, 2006). The typical clinical signs of bTB are weight loss, dyspnoea and swollen peripheral lymph nodes, with lesions forming on the lungs and lymph nodes of the head (Renwick *et al.*, 2007), but it may take years before infected individuals develop clinical symptoms (De Vos *et al.*, 2001).

Domestic cattle are considered the main reservoir of the disease while Cape buffalo are the most important maintenance host in South Africa. The disease has also been detected among a number of South African wildlife species (Table 1.4). Lions are especially vulnerable to the disease since buffalo are one of their preferred prey, while cheetahs and leopards possibly contract the disease from scavenging lion-buffalo-kills (Michel *et al.*, 2006). Warthogs and greater kudu appear to be important long-term maintenance hosts at high densities (De Lisle *et al.*, 2002; Kalema-Zikusoka *et al.*, 2005).

In South Africa the disease is monitored and controlled by the bovine tuberculosis scheme (DAFF, 2016), and the control area around the KNP prevents free movement of wildlife species

Table 1.4. South African wildlife species in which Mycobacterium bovis *infection has been confirmed to date (adapted from Michel* et al.*, 2006).*

Species	Species
African buffalo (*Syncerus caffer*)	Impala (*Aepyceros melampus*)
Blue wildebeest (*Connochaetes taurinus*)	Large spotted genet (*Genetta tigrina*)
Bushbuck (*Tragelaphus scriptus*)	Lechwe (*Kobus leche*)
Bushpig (*Potamochoerus porcus*)	Leopard (*Panthera pardus*)
Chacma baboon (*Papio ursinus*)	Lion (*Panthera leo*)
Cheetah (*Acinonyx jubatus*)	Nyala (*Tragelaphus angasii*)
Common genet (*Genetta genetta*)	Rhinoceros (unidentified spp.)
Eland (*Taurotragus oryx*)	Spotted hyaena (*Crocuta crocuta*)
Greater kudu (*Tragelaphus strepsiceros*)	Warthog (*Phacochoerus africanus*)
Grey duiker (*Sylvicapra grimmia*)	Waterbuck (*Kobus ellipsiprymnus*)
Honey badger (*Mellivora capensis*)	

between infected and disease-free zones. Control measures require that in confirmed cases of bTB all animals are quarantined and slaughtered, while buffalo populations in the KNP and Hluhluwe-Umfolozi Game Reserve are subjected to surveillance and monitoring programs. However, recent studies have found that the spatial distribution of the disease is expanding in southern Africa despite these control measures. Hlokwe *et al.* (2014) found bTB among cattle and wildlife in provinces that were previously considered free from infection, including Mpumalanga, Limpopo, KwaZulu-Natal, Free State and North West provinces. The study also confirmed *M. bovis* infection in blue wildebeest (*Connochaetes taurinus*) for the first time. De Garine-Wichatitsky *et al.* (2010) found limited evidence that the disease is spreading northward from the KNP possibly through transmission of buffalo or an unidentified wildlife species to local cattle herds. Spatial expansion of the disease poses an important threat to both animal and human populations in southern African countries, especially considering the prevalence of HIV among human populations in this region (www.unaids.org). Humans with HIV are increasingly at risk of contracting bTB due to the immune-suppressive effect of the virus (Grange, 2001). It remains unknown to what degree the prevalence of bTB among wild and domestic animals are responsible for human bTB infections. It is important to educate humans on the associated risks of contracting bTB from infected carcasses, and the relevant safety measures required in order to protect themselves from infection.

One of the greatest challenges of managing bTB is that the majority of infected wildlife shows no clinical symptoms, which limits the ability to detect the disease before infection becomes evident (De Lisle *et al.*, 2002). Furthermore, the spread and maintenance of bTB among free-ranging animals is confounded by high densities of maintenance and spillover hosts, and focal concentration of animals around supplemental feeding sites and artificial water points (De Lisle *et al.*, 2002). These are typical conditions present on a large number game farms in South Africa. The practice of species translocation and introduction for game ranching or conservation purposes has also been implicated in spreading the disease in South Africa (Hlokwe *et al.*, 2014). Hlokwe *et al.* (2014) suggested that wildlife should be screened for bTB before translocation or introduction as most ranched wildlife species have been infected, or in contact with infected species at some point. Current research on bTB control promotes increased screening efforts of domestic and wild animal populations, decreasing population densities of wildlife populations, the development and application of an efficient vaccine and improved diagnostics (Fitzgerald and Kaneene, 2013). Another potential strategy to increase surveillance efforts could focus on educating hunters and farmers on how to inspect for bTB *post mortem* in hunted wildlife, especially where hunting is a common activity.

1.2.2.6 Echinococcosis

A number of domestic and wild animals act as intermediate hosts for larvae of the dog (*Canis lupus familiaris*) tapeworm (*Echinococcus granulosus*) which causes the zoonotic parasitic disease echinococcosis (hydatidosis) (Otero-Abad and Torgerson, 2013). Adult tapeworms infest the intestine of the definitive carnivore host (domestic dog, black backed jackals (*C. mesomelas*), cape silver fox (*Vulpes chama*), spotted hyena, and lion) and produce oocysts, which are excreted into the environment and ingested by the intermediate herbivore host. Intermediate herbivore hosts include Burchell's zebra (*Equus burchelli*), Cape buffalo, greater kudu, hippopotamus (*Hippopotamus amphibius*) and impala (Bengis and Veary, 1997). The larvae occur as hydatid cysts on the liver or in the lungs of the intermediate host and are usually well tolerated, unless they develop on the brain, kidneys or heart, become large enough to damage adjacent organs, or burst, which causes anaphylactic shock and death (WHO/OIE, 2002).

Hüttner *et al*. (2009) found *E. granulosus* and *Echinococcus felidis* in warthog samples from the Queen Elizabeth National Park in Uganda, and suggested that they could be intermediate hosts to both species. The prevalence of *Echinococcus* among warthog populations in South Africa appears low; hydatid cysts were recovered from the lungs of only one of six sampled warthogs in the Limpopo province (Van Wyk and Boomker, 2011), one of 28 sampled warthogs in a reserve adjacent to the KNP (Boomker *et al*., 1991) and eight from 52 warthogs sampled within the KNP (Horak *et al*., 1988). Low prevalence among intermediate wildlife hosts is likely the result of the absence of larger predators, considering the importance of predator-prey relationship in sylvatic transmission of certain *E. granulosus* strains (Jenkins and MacPherson, 2003).

Seven strains of *Echinococcus* have been identified as infectious to humans, with the sheep strain (G1) of *E. granulosus* responsible for the majority of human cases. Wahlers *et al*., (2013) reported on the rising number of echinococcosis cases among humans in South Africa as recorded by the National Health Laboratory Service, but since cystic echinococcosis has a long latency period it is difficult to determine the actual prevalence of the disease. Interestingly, Wahlers *et al*. (2012) found that a high prevalence of the disease in animals does not necessarily correspond with a high prevalence in the human population in contact with infected animals and vice versa. Studies on the epidemiology of the disease is lacking in southern Africa in general and requires further research to determine its prevalence among animal hosts in the region, and the main routes of human infections (Wahlers *et al*., 2012).

1.2.2.7 Trypanosomiasis

Warthogs are one of the most important maintenance hosts of tsetse flies (*Glossina* spp.), the vector of *Trypanosoma* spp., which causes trypanosomiasis in humans or nagana disease in cattle (Claxton *et al*., 1992). While the parasite posed a serious threat to livestock production during initial colonization of South Africa in the late 19th century, the rinderpest epidemic which raged through the African continent from 1887-1897, had the unexpected benefit of also drastically reducing the southern African distribution range of the main vector *Glossina mortisans mortisans*. Together with intensive DDT use on farms and reserves, two tsetse fly species (*G. mortisans mortisans* and *Glossina pallidipes*) were eventually eradicated, and the impact of the parasite on livestock is limited to parts of Kwa Zulu Natal where small tsetse fly populations of *G. pallidipes austeni* and *G. pallidipes brevipalpis* still persist (Gillingwater *et al*., 2010).

1.2.2.8 Trichinosis

Trichinella spp. are internal parasites of wild carnivores and pigs, where the adult worm infests the intestines and the larvae occur as cysts in the muscle tissue. Domestic and wild animals and humans can contract *Trichinella* infections when ingesting raw or undercooked meat infested with *Trichinella* cysts, but only humans develop clinical trichinosis. The ensuing infection in humans can manifest as an acute and chronic disease with debilitation symptoms and cause death in severe cases. There has been a significant global re-emergence of trichinosis post 1970, which together with anthropogenic factors, is greatly exasperated by the maintenance and transmission of the parasites within a sylvatic cycle between wildlife reservoirs and domestic animals (Pozio, 2007).

As regards *Trichinella* species or genotypes, only *Trichinella nelsoni* and T8, a genotype of *Trichinella britovi*, have been found among certain large carnivore and small omnivore species in KNP (Marucci *et al.*, 2009). Warthogs infected with *T. nelsoni* have been reported from Tanzania (International *Trichinella* Reference Centre, www.iss.it/site/Trichinella), and there have been outbreaks of trichinosis in Senegal, Algeria, Ethiopia and Kenya after ingesting improperly prepared warthog and/or bushpig (*P. larvatus*) meat (Mukaratirwa *et al.*, 2013). Although freezing and cooking meat to a specific internal temperature are considered effective methods to kill the parasite (Gamble *et al.*, 2000), it has been shown that the causative agent of trichinosis in West Africa, *T. britovi*, is more resistant to freezing (Pozio *et al.*, 2006). Dupouy-Camet *et al.* (2009) reported on three cases of human infections after consuming warthog ham of which the meat had been deep frozen for several weeks before processing. Although not confirmed, *T. britovi* was the suspected infectious agent.

While studies on the prevalence of *Trichinella* infections among animals and humans is generally lacking in southern Africa, there have been no reports of *Trichinella* infections in domestic animals or humans in South Africa, Zimbabwe, Namibia or Mozambique (Pozio, 2007), and none among wild animals in South Africa outside of KNP. It should therefore be a continued effort to monitor for *Trichinella* infections in animals to prevent the introduction of the parasite elsewhere in South Africa.

1.2.2.9 Rabies

All mammalian species can contract rabies while carnivores are the major hosts of the canine strain, RABV, which accounts for the majority of humans and animals infections. The disease remains in circulation primarily among stray dogs and smaller wild carnivores in South Africa, with the highest prevalence amongst carnivores in Kwa-Zulu Natal. In South Africa, there were 12 confirmed cases of human rabies and 834 animal cases in 2012, of which 212 were among domestic animals and 114 among wildlife (WHO, 2013). While warthogs can become infected and transmit rabies, there were only two confirmed cases of infected warthogs between 1990 and 2009 in Namibia (Magwedere *et al.*, 2012), but no confirmed warthog cases have been reported in South Africa since 1990 (Bishop *et al.*, 2003).

1.2.2.10 Schistosomiasis

Schistosoma spp., commonly called bloodflukes, are prevalent among a number of wildlife species in sub-Saharan Africa, which act as intermediate or definitive hosts to different species (Horak *et al.*, 1988; Weyher *et al.*, 2010). Wild and domestic animals may develop chronic or acute schistosomiasis depending on the specific species involved, while human infections are caused primarily by *Schistosoma haematobium*, *Schistosoma mansoni*, and

Schistosoma japonicum. The ensuing infection in humans is commonly called bilharzia. It is one of the most prevalent but also most neglected tropical diseases globally among humans (WHO, 2010). *Schistosoma* eggs are excreted in the faeces or urine of infected animals or humans into freshwater sources where they hatch into miracidia, infecting freshwater snails and after multiplying through a number of cycles, and are released as free-swimming larvae called cercariae. Freshwater snails are the primary reservoir of parasitic worms. Animals and humans become infected through contact with infected water or by accidentally ingesting the eggs. Cercariae penetrate the skin of animals and humans and develop into schistosomula, which are transported by the lymph or blood system to internal organs or the urinary tract (WHO, 2016). The South African species, *S. mansoni* and *S. haematobium*, are found in parts of Mpumalanga, Limpopo, Kwa-Zulu Natal and Eastern Cape provinces. Horak *et al*. (1988) found a *Schistosoma* spp. in a warthog in KNP, but there is very little known as to whether the species may play a role in the parasite's epidemiology in Africa.

1.2.2.11 Other invasive diseases

Two probable cases of anthrax were reported for warthogs in the Serengeti ecosystem, Tanzania, between 1996 and 2009 (Lembo *et al*., 2011) and it has been suggested that members of the Suidae family are more resilient to the bacterium (Hugh-Jones and De Vos, 2002). There have been no reports of *Brucella* or *Clostridium botulinum* in warthogs, both important diseases for cattle and game animal production. Warthogs have been suggested as possible intermediate hosts of the pork tapeworm, *Taenia solium*, but evidence that the species plays a significant role in its epidemiology among suids and humans is lacking. *T. solium* is one of the most important zoonotic parasites globally as humans are both an intermediate and definitive host and become infected by consuming raw or undercooked meat infected with cysticerci, which is the larval form of the parasite (Sciutto *et al*., 2000). Domestic pigs and dogs are considered the main intermediate hosts (Ito *et al*., 2002). Considering its importance as zoonosis, research into the possibility of warthogs as potential intermediate hosts is required for South Africa and the Africa continent as a whole.

It is possible for warthogs as warm-blooded vertebrates to become infected with *T. gondii*, but no cases in wild suids have been reported in Africa yet (Hove and Dubey, 1999; Hove and Mukaratirwa, 2005; Riemann *et al*., 1975). The coccidian parasite, *Neospora caninum*, previously misclassified as *T. gondii* due to structural similarities, is responsible for causing neosporosis in wild and domestic animals. The disease causes abortions and neonatal mortalities in livestock and certain wildlife species. Lion, cheetah and white rhinoceros (*Ceratotherium simus)* have tested positive for seroprevalence in South Africa (Cheadle *et al*., 1999; Williams *et al*., 2002). Antibodies to *N. caninum* have been found in warthogs in Kenya but the sample size was small, making it difficult to extrapolate about the prevalence of the parasite in warthogs (Ferroglio *et al*., 2003). This study also found positive seroprevalence in zebra (*E. burchelli*), common eland, African buffalo, Thomson gazelle (*Eudorcas thomsonii*), impala, spotted hyena and in free-ranging cheetah. There is, in general, a lack of information regarding the prevalence of this parasite among domestic and wild animals in Africa, while no cases of human infections have been reported (Dubey *et al*., 2007).

Two species of *Sarcocystis* have been described for warthogs, *Sarcocystis dubeyella* and *Sarcocystis phacochoeri*; the latter forms visible cysts of about 4 mm (Stolte *et al*., 1998). *Sarcocystis* are protozoan parasites which occur as cysts in the cardiac and skeletal muscle of infected animals and humans. Livestock and wild animals rarely show clinical symptoms but cases of acute sarcocystosis have occurred among cattle in South Africa (Van der Lugt *et al*., 1994). While acute sarcocystosis may cause debilitating effects including abortion in animals, the most important impact, however, is the resulting financial losses as animal carcasses with visible cysts are likely to be declared unfit for human consumption. There is little known about the distribution of *Sarcocystis* spp. among warthogs and other wildlife in southern Africa, and their occurrence outside the KNP is yet to be determined.

It has recently been established that wild boars may serve as a source of hepatitis E infections in humans, through consumption of the meat from animals infected with the virus (HEV) (Meng *et al*., 2009). While it has been suggested that warthogs may carry the disease in South Africa and act as zoonotic agents (S. Korsman, personal communication), there has been no evidence found of this to date, while the authors are aware, and have contributed, towards ongoing research efforts to further illuminate on this possibility in the country.

1.2.3 Non-invasive biological hazards to warthogs

1.2.3.1 Internal parasites

The helminths that have been recorded for common warthogs in South Africa and Namibia are listed in Table 1.5. The most common helminth species recorded by several authors include *Oesophagostomum spp.*, *Probstmayria vivipara*, *Murshidia humata*, *Physocephalus sexalatus* and *Ascaris phacochoeri* (Boomker *et al*., 1991; Horak *et al*., 1983, 1988; Van Wyk and Boomker, 2011). Wyk and Boomker (2011) found a 100% prevalence of *Oesophagostomum mwanzae* and *P. vivipara* in warthog populations sampled from the Limpopo province, with warthogs having a mean helminth burden of 2,228 (excluding *P. vivipara*) compared to impalas (592), kudus and blue wildebeest (407), black wildebeest (588), gemsbok (184) and waterbuck (2,150). The mean burden of *P. vivipara* was 501,000. This nematode is considered a parasite of horses and may occur in large numbers, although no clinical symptoms of infection are observed. It has been suggested that more exploratory feeders have higher and more diverse burdens of internal parasites (J. Boomker, personal communication). Horak *et al.* (1988) also recovered nymphs of the pentastomid *Linguatula nuttalli* from warthogs in KNP. The nymphs are known to infect the livers of kudu and wildebeest without causing any significant damage. Humans can become infected by certain *Linguatula* pentastomids by ingestion of undercooked viscera of infected animals, acting as intermediate hosts, and developing visceral pentastomiasis (Tappe and Büttner, 2009), although no cases of human pentastomiasis caused by *L. nuttalli* have been recorded.

1.2.3.2 External parasites

Ecto-parasites that have been recovered from warthogs in southern Africa are presented in Table 1.6. Among these, *Rhipicephalus (Boophilus) decoloratus*, *Rhipicephalus evertsi evertsi*, *Hyalomma rufipes* and *Rhipicephalus simus* have been recorded to carry and

Table 1.5. Helminths species of warthogs in South Africa and Namibia (adapted from Boomker et al.*, 1991).*

Helminth species	Life stage present[1]	Location[2]	Record
Trematodes			
Gastrodiscus aegyptiacus (Railliet 1893)	A	KNP	Horak *et al*., 1988
Schistosoma spp.	A	KNP	Horak *et al*., 1988
Cestodes			
Echinococcus spp.	Cysts	KNP	Horak *et al*., 1988
	L	Hoedspruit NR	Boomker *et al*., 1991
	Cysts	Limpopo	Van Wyk and Boomker, 2011
Paramoniezia phacochoeri (Baylis 1927)	A	Limpopo	Van Wyk and Boomker, 2011
Moniezia/Paramoniezia	A (Scolices)	Namibia	Horak *et al*., 1983
	A (Scolices)	KNP	Horak *et al*., 1988
	A	Hoedspruit NR	Boomker *et al*., 1991
Taenia crocutae	Cysts	KNP	Horak *et al*., 1988
Taenia hyaenae larvae	Cysts	KNP	Horak *et al*., 1988
Taenia regis larvae	Cysts	KNP	Horak *et al*., 1988
	L	Hoedspruit NR	Boomker *et al*., 1991
Nematodes			
Ascaris phacochoeri (Gedoelst 1916)	3rd, 4th, A	KNP	Horak *et al*., 1988
	A	Hoedspruit NR	Boomker *et al*., 1991
Ascaris spp.	4th	Namibia	Horak *et al*., 1983
Cooperia hungi (Mönnig 1931)	A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Cooperia spp.	A	Namibia	Horak *et al*., 1983
Impalaia nudicollis (Mönnig 1931)	4th, A	Namibia	Horak *et al*., 1983
Impalaia tuberculate (Mönnig 1923)	4th, A	KNP	Horak *et al*., 1988
	A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Microfilaria spp. (*sensu* Neitz 1931)		Zululand	Neitz, 1931
Microfilaria		KNP	Palmieri *et al*., 1985
Murshidia hamata (Daubney 1923)	A	KNP	Horak *et al*., 1988
	L, A	Hoedspruit NR	Boomker *et al*., 1991
Murshidia pugnicaudata (Leiper 1909)	A	KNP	Horak *et al*., 1988
	L, A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Murshidia spp.	4th	KNP	Horak *et al*., 1988
	L	Hoedspruit NR	Boomker *et al*., 1991
Odontogeton phacochoeri (Allgrén 1921)		Natal	Allgrén, 1921 cited after Round, 1968

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Table 1.5. Continued.

Helminth species	Life stage present[1]	Location[2]	Record
Nematodes (continued)			
Oesophagostomum mocambiquei (Ortlepp 1964)	A	KNP	Horak *et al*., 1988
	L, A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Oesophagostomum mpwapwae	A	Namibia	Horak *et al*., 1983
Oesophagostomum mwanzae	A	Namibia	Horak *et al*., 1983
	A	KNP	Horak *et al*., 1988
	L, A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Oesophagostomum roubaudi (Daubney 1926)	A	Namibia	Horak *et al*., 1983
Oesophagostomum spp.	4th	Namibia	Horak *et al*., 1983
	4th	KNP	Horak *et al*., 1988
	L	Hoedspruit NR	Boomker *et al*., 1991
Physocephalus sexalatus (Diesing 1861)	3rd, 4th, A	Namibia	Horak *et al*., 1983
	3rd, 4th, A	KNP	Horak *et al*., 1988
	L, A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Probstmayria vivipara (Ransom 1911)	4th	Namibia	Horak *et al*., 1983
	4th	KNP	Horak *et al*., 1988
	L, A	Hoedspruit NR	Boomker *et al*., 1991
	A	Limpopo	Van Wyk and Boomker, 2011
Strongyloides spp.	A	KNP	Horak *et al*., 1988
Trichostrongylus falculatus (Ransom 1911)	A	KNP	Horak *et al*., 1988
Trichostrongylus deflextus (Boomker and Reinecke 1989)	A	Hoedspruit NR	Boomker *et al*., 1991
Trichostrongylus thomasi Mönnig 1932	A	KNP	Horak *et al*., 1988
	A	Hoedspruit NR	Boomker *et al*., 1991
Trichostrongylus colubriformis	A	Namibia	Horak *et al*., 1983
Trichostrongylus instabilis	A	KNP	Horak *et al*., 1988
Trichostrongylus spp.	A	Hoedspruit NR	Boomker *et al*., 1991
Trichuris spp.	4th stage	KNP	Horak *et al*., 1988
Pentastomes			
Linguatula nuttalli	N	KNP	Horak *et al*., 1988

[1] A = adults; L = larvae; N = nymphs.

[2] KNP = Kruger National Park; NR = nature reserve.

Table 1.6. The ecto-parasites of warthog in South Africa and Namibia (adapted from Matthee et al.*, 2013).*

Ecto-parasite	Life stages present[1]	Area[2]	Reference
Fleas			
Echidnophaga larina	–	Hoedspruit	Boomker *et al.*, 1991
	A	KNP	Horak *et al.*, 1988
	A	Namibia	Horak *et al.*, 1983
	–	Free State	Matthee *et al.*, 2013
Moeopsylla sjoestedti	–	Hoedspruit	Boomker *et al.*, 1991
	A	KNP	Horak *et al.*, 1988
Lice			
Haematopinus phacochoeri	N, A	Hoedspruit	Boomker *et al.*, 1991
	N, A	KNP	Horak *et al.*, 1988
	N, A	Namibia	Horak *et al.*, 1983
	–	Free State	Matthee *et al.*, 2013
Ixodid ticks			
Amblyomma hebraeum	L, N, A	Hoedspruit	Boomker *et al.*, 1991
	L, N, A	Swaziland	Gallivan and Surgeoner, 1995
Amblyomma marmoreum	L	Hoedspruit	Boomker *et al.*, 1991
Hyalomma truncatum	L	Hoedspruit	Boomker *et al.*, 1991
	A	KNP	Horak *et al.*, 1988
	A	Namibia	Horak *et al.*, 1983
	–	Free State	Matthee *et al.*, 2013
Hyalomma marginatum rufipes	A	Namibia	Horak *et al.*, 1983
Rhipicephalus simus	A	Hoedspruit	Boomker *et al.*, 1991
	A	KNP	Horak *et al.*, 1988
	A	Namibia	Horak *et al.*, 1983
	A	Swaziland	Gallivan and Surgeoner, 1995
	–	Free State	Matthee *et al.*, 2013
Rhipicephalus (Boophilus) decoloratus	L	Hoedspruit	Boomker *et al.*, 1991
	A, N, L	KNP	Horak *et al.*, 1988
Rhipicephalus appendiculatus	L	Hoedspruit	Boomker *et al.*, 1991
	N	KNP	Horak *et al.*, 1988
	A, N, L	Swaziland	Gallivan and Surgeoner, 1995
Rhipicephalus evertsi evertsi	N, L	Hoedspruit	Boomker *et al.*, 1991
	N, L	KNP	Horak *et al.*, 1988
Rhipicephalus evertsi mimeticus	A, N	Namibia	Horak *et al.*, 1983
Rhipicephalus zambeziensis	A, N	Hoedspruit	Boomker *et al.*, 1991
	N	KNP	Horak *et al.*, 1988
Rhipicephalus longiceps	A	Namibia	Horak *et al.*, 1983
Rhipicephalus oculatus	A	Namibia	Horak *et al.*, 1983
Rhipicephalus follis	A	Swaziland	Gallivan and Surgeoner, 1995

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Table 1.6. Continued.

Ecto-parasite	Life stages present[1]	Area[2]	Reference
Ixodid ticks (continued)			
Rhipicephalus masculatus	A	Swaziland	Gallivan and Surgeoner, 1995
Rhipicephalus muehlensi	A, N	Swaziland	Gallivan and Surgeoner, 1995
Rhipicephalus gertrudae	–	Free State	Matthee *et al.*, 2013
Argasid ticks			
Ornithodoros porcinus	N	Hoedspruit	Boomker *et al.*, 1991
	N	KNP	Horak *et al.*, 1988
	A	Swaziland	Gallivan and Surgeoner, 1995
Ornithodoros moubata	N	Namibia	Horak *et al.*, 1983

[1] A = adults; L = larvae; N = nymphs.

[2] KNP = Kurger National Park.

transmit *Anaplasma marginale*, the causative agent of bovine anaplasmosis (De Waal, 2000). Mutshembele *et al.* (2014) found that *R. decoloratus* and *R. evertsi evertsi* are the main species involved in *A. marginale* transmission in South Africa, and the prevalence of *A. marginale* is high across the country except in the arid North Cape province. Horak *et al.* (1988) considered *R. decoloratus* and *R. evertsi evertsi* (and *Hyalomma truncatum*) accidental parasites of warthogs; thus, their occurrence on warthogs is a reflection of their wide-spread occurrence, rather than an indication of host preference.

The tick *R. simus* is responsible for transmitting the parasite *Babesia trautmanni* which causes porcine babesiosis in domestic pigs. Warthogs are a wild host of the piroplasm and one of the preferred hosts of the tick species but develop no clinical symptoms (Stewart *et al.*, 1992). The distribution of *R. simus* does not include the drier central region of South Africa, where Matthee *et al.* (2013) found the tick among introduced warthog populations. This suggests that the tick was introduced with the introduced warthog populations. There has been a general low prevalence of *Babesia* parasites in the drier central parts of South Africa (Mtshali and Mtshali, 2014), but the introduction of *R. simus* outside its natural range might increase the distribution range of *B. trautmanni*. The tick typically has a low load of parasitism on individual animals, which reduces the risk of transmission and infection between wild and domestic suids, but high loads have been recovered from a healthy warthog (221), a sick lion (713) and a sick leopard (521) (Walker *et al.*, 2000). Certain *Babesia* spp. are known to infect humans but the condition is rarely severe, except in cases of immunocompromised or asplenic individuals (Gorenflot *et al.*, 1998; Gray *et al.*, 2010).

Warthogs are considered to be the major host of the tick *Amblyomma hebraeum* (Gallivan and Surgeoner, 1995; Horak *et al.*, 1988) the tick-vector of *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) the causative agent of heartwater in wild and domestic ruminants.

It is not known whether warthogs act as wild reservoirs for the bacterium, while suspected wild reservoir species include blesbok, black wildebeest (*Connochaetes gnou*), common eland, giraffe, greater kudu, African buffalo and sable antelope (Peter *et al.*, 1997, 2002). *A. hebraeum* is also the principle vector of *Rickettsia africae*, the bacterium responsible for most human tick bite fever cases in humans in southern Africa, which appears to be more common among non-African travellers than local habitants (Jensenius *et al.*, 2003).

Tick infestations themselves may cause sickness or physical damage. The toxins in the saliva of certain strains of *H. truncatum* and *R. evertsi evertsi* (and also *Rhipicephalus evertsi mimeticus*) cause sweating sickness in cattle and paralysis in lambs, respectively (Oberem and Oberem, 2011). Severe infestations of *Rhipicephalus appendiculatus* on an animal's ear may lead to loss of the ear.

1.3 Disease control in South Africa

The Animal Diseases Act No. 35 of 1984 (as amended; DAFF, 1991), Agricultural Product Standards Act No. 119 of 1990 (DAFF, 1990), and the Meat Safety Act No. 40 of 2000 (DAFF, 2000), are the legislative acts that govern the management of animal diseases and agricultural products, including meat, in South Africa. The country is also a member of the OIE, the intergovernmental organization responsible for improving animal health worldwide that is recognized as a reference organization by the World Trade Organization (WTO). DAFF, lists the following diseases associated with warthogs as notifiable controlled animal diseases in South Africa (in terms of Animal Diseases Act, Act 35 of 1984; DAFF, 1984): ASF, anthrax, CSF, FMD, trypanosomiasis, rabies, rinderpest, RVF and bTB. Controlled diseases require that the local/regional state veterinarian is notified of suspected or confirmed cases, who is in turn responsible for taking action according to the control scheme for the specific disease (in terms of Section 9 of the Animal Diseases Regulations Act; DAFF, 1986).

Highly infectious diseases such ASF, CSF, FMD and bTB are controlled through demarcated disease control zones in South Africa. The main aim of these control zones is to prevent contact between disease-free and infected populations, and the transmission of infected material from the control zone to disease free zones. Translocations of live cloven hoofed animals and their products from these areas are controlled through stringent surveillance measures, while still allowing the areas to benefit from livestock and game animal production. All live animals and animal product imports and exports, including introduction and translocation of live animals within the country, require permits to certify the animals are free from controlled diseases. Furthermore, active vaccination programs for livestock have been largely successful for controlling heartwater, bTB, anaplasmosis, and to an extent RVF, as well as vaccination of dogs for the rabies virus.

Despite stringent control measures, it is important to remember that South Africa is not immune to possible disease introductions from novel sources (Penrith, 2013), and continued disease surveillance among domestic and wild animals should be a priority for regional and national veterinary and associated authorities. Free-ranging species such as the extra-limital warthogs pose a unique problem as potential disease vectors as they are associated with a number of important animal and zoonotic diseases. This is an important avenue for future research, especially in countries such as South Africa, where the continued expansion of game farming, and combination game/livestock farming, often result in high stocking densities, close proximity of different animal species and frequent animal transportation (Bekker *et al.*, 2012).

In light of the significant shifts in wildlife management and production systems in southern Africa, it has been suggested that surveillance strategies be re-evaluated with the aim to develop more integrated and effective methods for animal and human disease surveillance (Vrbova *et al.*, 2010). Most current disease surveillance methods tend to focus on activities that overlap with livestock and human diseases and might not detect emerging or re-emerging diseases among wildlife (Grogan *et al.*, 2014). Mörner *et al.* (2002) stated that countries who conduct disease surveys among their wildlife populations are more likely to detect the presence of infectious and zoonotic diseases, and respond more swiftly with counteractive measures, and understanding the ecology of wildlife diseases is pivotal to understanding their epidemiology in animal and zoonotic infections. According to the 'Training manual on wildlife diseases and surveillance' by OIE (2010), the surveillance of wildlife diseases consist of four components; the detection of disease among wild populations, the correct identification of disease, the management of information pertaining to the disease and infected populations, and the analysis and communication of the data. The success of each component necessitates that all relevant stakeholders of the agricultural industry, veterinary authorities, and governance bodies work together to develop and implement effective wildlife surveillance strategies (Bekker *et al.*, 2012).

For example, regular serological surveillance surveys among wildlife could evaluate the occurrence and spread of pathogens such as bTB, *Trichinella*, and *E. granulosus* in southern Africa and identify wildlife hosts and specific cohorts of importance. This could aid in the early detection and subsequent management of diseases in wildlife and curb transmission to domestic animal and human populations. It could also prevent the introduction of *Trichinella* to domestic animals and humans, which to date have not been reported in the country. De Lisle *et al.* (2002) and other authors have referred to the difficulties in obtaining wild animals or animal samples for *post mortem* disease inspection. Here the hunting industry provides an ideal situation for robust sampling of wildlife, if hunters and veterinary associations can form partnerships where hunters provide specimens for disease inspection from the animals they hunt. It is however unlikely that the South African government has the financial and professional support to conduct such operations in the long-term.

As mentioned, there is evidence in support of changing distribution ranges of diseases and disease vectors in southern Africa (De Garine-Wichatitsky *et al.*, 2010; Hargreaves *et al.*, 2004; Matthee *et al.*, 2013; Penrith and Vosloo, 2009). The influence of climate change, increased human transportation/translocation of animals and animal products, and anthropogenic modification of natural environments have all been implicated in the emergence and proliferation of infectious diseases on a global scale (Patz *et al.*, 2000; Van Jaarsveld and Chown, 2001). The game ranching industry in particular requires improved control measures and compliance as the industry continues to expand with significant implications on natural and modified ecosystems and translocation/introduction of animals (Bekker *et al.*, 2012).

According to the OIE, adequate disease surveillance and education of parties involved in all aspects of animal production, conservation, veterinary care, game hunting and slaughter/processing should be conducted as education is the most effective method for detecting emerging diseases and the factors that govern their epidemiology.

1.4 Implications for utilization of warthog meat

While warthog meat is regularly consumed by rural communities and in informal markets, it is becoming more widely known and utilized in formal markets. Hoffman *et al.* (2005) found warthog to be a game meat regularly consumed in South Africa and South African restaurants. Similar to other game animals and game meat, warthogs have a high dressing percentage, low total intramuscular lipid content, high total protein and moisture content and a favourable fatty acid profile (Hoffman and Sales, 2007; Swanepoel *et al.*, 2014), and suitable organoleptic and technological properties for the use in processed product. Regardless of these findings, the meat from hunted carcasses is still relatively under-utilized by hunters and/or the commercial sector (Hoffman *et al.*, 2005), possibly due to the lack of information regarding the safety and preparation of the meat.

The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO, 2014) released a multi-criteria based ranking list of the most important food-borne parasites that pose a risk to humans. The parasites of warthogs that appear on the list in descending order of importance are *E. granulosus*, *Trichinella* spp., and *Sarcocystis* spp. All of these diseases are transmitted to humans when raw or undercooked meat from infected animals is ingested.

Although warthogs are able to carry and transmit diseases to humans, there is a lack of documented cases of this occurring in southern Africa. There are a number of potential reasons for this. It is very likely that zoonotic infections from warthog-associated parasites are underreported or misdiagnosed, or the role of warthogs as the source of infection is underappreciated in people who regularly consume bushmeat or meat from rural animals. Alternatively, it is also possible that the majority of warthogs are harvested from outside of known zoonotic distribution ranges, such as the KNP, and that potential zoonosis are effectively controlled through current disease management programs and control zones. In South Africa for example, *E. granulosus* appears to be limited to the northern bushveld region of the Limpopo province and KNP (Boomker *et al.*, 1991; Horak *et al.*, 1988; Van Wyk and Boomker, 2011), and *Sarcosystis* spp. associated with warthogs to the KNP (Stolte *et al.*, 1998).

If this is the case then warthog meat has the potential to become part of the formal game meat spectrum if sourced from disease free areas and/or processed according to regulations as set out in the internationally certified standard of Wildlife Ranching South Africa (WRSA) for the production of game meat in South Africa (Zerbst, 2015). To encourage the future safe utilization of warthogs in South Africa, it is suggested that hunters and butchers of game meat continue to equip themselves with knowledge on how to visually inspect warthog carcasses destined for human consumption for abnormalities in the organ tissues and the presence of visible parasites and cysts. Additional precautions would include abattoirs that specialize in the commercial production of game meat to regularly have their meat analysed by accredited laboratories for the presence of infectious agents (Mörner *et al*., 2002). Furthermore, disease surveillance surveys should continually provide information to farmers, hunters and game meat abattoirs on the prevalence of zoonotic diseases among wildlife populations outside of disease control zones.

1.5 Conclusions

1.5.1 What has been achieved?

Current research allows the identification of biological hazards carried by warthogs with relevance to human and livestock health. In combination with information on the spatial distribution of warthogs and of vectors, potential critical epidemiologic situations can be identified.

1.5.2 What has been neglected?

Information on the actual prevalence is not available for all biological hazards contained by warthogs, which will complicate risk assessment, both for epidemiological purposes and for assessment of meat safety.

1.5.3 What needs to be done?

Free-ranging species such as the extra-limital warthogs pose a unique problem as potential disease vectors, as they are associated with a number of important animal and zoonotic diseases. Future research should investigate the risk of introduced warthog populations in spreading and maintaining diseases, and more specifically the possible routes of disease transmission across their expanding distribution range in South Africa. This is necessary as warthogs will remain a popular species for recreation hunting and meat production purposes, with both trained and untrained persons handling and processing carcasses. Current disease surveillance and control among domestic and wild animals should be re-evaluated to include the risks associated with free-ranging wildlife and expanded distribution ranges of wildlife.

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2. A review of zoonotic disease of UK wild game

J. Paul Duff
Animal and Plant Health Agency Diseases of Wildlife Scheme (APHA DoWS), Penrith Veterinary Investigation Centre, Merrythought, Penrith, Cumbria, CA11 9HS, United Kingdom; paul.duff@apha.gsi.gov.uk

Summary

This chapter is a broad-based review of selected aspects of zoonotic and important diseases of wild game. The author's work for the Animal and Plant Health Agency, Diseases of Wildlife Scheme covers surveillance for wildlife disease in Great Britain. Surveillance is essential in understanding diseases of game. In simple terms, surveillance can be described as the collection of information (here disease information) with a view to acting on it in the future. Hunters, in particular, have an important role in disease surveillance of wild animals. This paper discusses surveillance for some European diseases; however, the principle focus is on zoonotic diseases of UK game species.

Keywords: surveillance, game species, wildlife disease, zoonotic disease

2.1 Introduction

Wildlife host a variety of pathogens and diseases that pose risks to the health of humans and domesticated animals, as well as having potentially negative impacts on biodiversity. Game species are a subset of wild animals in that they pose the same type of risks to the health of humans, livestock and biodiversity. To understand important diseases of game species we need to understand disease in all wild animals. To understand wildlife disease epidemiology a first step is the gathering of data elucidated from disease surveillance.

Surveillance helps us to assess and prioritise disease in British game species and how this is done in Great Britain (GB) is briefly described. We derive lists of what this author considers as important diseases of wildlife in Europe and information on the game reservoirs of these diseases is provided. The important diseases of GB game are listed. Finally we look at some recent challenges in GB game animal diseases.

2.2 Inventory of wildlife diseases

The main mammal and bird species hunted in GB are listed in Table 2.1. Traditionally, deer species and lagomorphs are hunted, and, more recently, free-living wild boar populations have established.

Table 2.2 provides a list of important diseases of wildlife in Europe. Admittedly, such lists are essentially subjective assessments, and depend largely on the point of view and the location

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Table 2.1. Important wild game species in the UK.

Mammals	Birds
• Deer, 6 species • Rabbit (*Oryctolagus cuniculus*) • Hare (brown hare, *Lepus europaeus*, and mountain hare, *Lepus timidus*) • Boar (*Sus scrofa*) – relatively recently established populations of escaped and now feral wild boar; hunted in the south of England	• Pheasant (*Phasanius colchicus*), partridge and grouse species • Waterbirds – ducks, geese • Smaller game birds – woodpigeon (*Columba palumbus*), snipe (*Gallinago* spp.), woodcock (*Scolopax rusticola*)

Table 2.2. Important wildlife (mammalian and avian) diseases in Europe.

Disease		Game species involved
Viral diseases	African swine fever (ASF)	boar
	Avian influenza virus (AIV)	geese, ducks
	Rabies (EBL)	–
	Phocine distemper virus (PDV)	–
	Bluetongue virus (BT)	deer
	Schmallenberg virus	deer
	Rabbit haemorrhagic disease (RHD)	rabbit
	Hepatitis E virus	boar?
	Filoviruses	–
	Congo crimea haemorrhagic fever	
Non-virus pathogens	Bovine tuberculosis (bTB)	deer, boar
	Brucellosis (Br)	boar
	Tularaemia (Tu)	all game
	Q fever (QF)	all game?
	Leishmania infantum (Li)	hare
	Echinococcus multilocularis (Em)	–

of the scientists making the assessment, as some diseases are geographically restricted. A comprehensive review of diseases of wildlife in Europe is presented in the appendices of Gavier-Widen *et al.* (2012). The list in Table 2.2 was based, to some extent, on EU-funded wildlife disease projects over the past 10 years, which prioritised important diseases, in addition to discussions with colleagues over the same time period. It is worth mentioning, that of the non-virus pathogens, only bovine tuberculosis and Q fever are found in the UK.

From a public health viewpoint, it is useful to narrow down the wildlife diseases to those transmissible to humans. Table 2.3 is a list of important zoonotic diseases of game species

Table 2.3. Important diseases of game species in the UK (Coburn et al.*, 2003).*

Pathogen/disease	Game species
Bovine tuberculosis	all UK game mammals potentially
Escherichia coli O157/VTEC infection	all UK game species potentially
Salmonellosis	all UK game species potentially
Yersinia pseudotuberculosis	all UK game species potentially
Botulinum intoxication	waterfowl
Campylobacter jejuni	all UK game species potentially
Chlamydia psittaci	game birds
Mycobacterium avium (Johne's disease)	deer, rabbit
Trichinella	boar
Lead shot toxicity (not infectious)	all shot game

in the UK; this was drawn up as part of an MSc thesis by a veterinary colleague (Coburn *et al*., 2003).

2.3 Discussion

2.3.1 General considerations on surveillance of diseases of wild game

The lists in Table 2.2 and 2.3 provide a framework to allow some underlying principles in game species disease surveillance to be discussed. The challenges of detection and prevention of disease in game populations and the ultimate challenge of preventing risks to human health from game species, all have disease surveillance as their central investigatory thrust. Wildlife disease surveillance therefore is fundamentally important to understanding the epidemiology of wildlife disease. Wildlife disease surveillance has been the main focus of the Animal and Plant Health Agency, Diseases of Wildlife Scheme (APHA DoWS) in England and Wales for the past 18 years. The principles of wildlife disease surveillance and game disease surveillance are similar and can be considered together.

Hunters deploy their sport in a wide range of natural, semi-natural and farmland 'habitats'; hunters are familiar with wild animal behaviour and are frequently the first to observe sick and dead animals in the wild. Many hunters are familiar with the more frequently occurring wild animal diseases and are therefore more likely to be appreciative of unusual, novel or new disease presentations in wildlife. As a consequence, hunters have a key role in identification, reporting and the collection of wild carcases brought to veterinary pathological laboratories, where *post mortem* and laboratory diagnostic examinations are performed.

Wildlife disease surveillance has not been developed along the same lines in Britain, to date, and while there are collaborations between veterinary diagnostic centres and hunting organisations, wildlife disease surveillance in GB has not originated, nor been developed, from hunting interests nor from hunters' concerns about the negative effects of disease on game and wildlife populations.

Many, if not most, countries in Europe have wildlife disease surveillance schemes that rely on close collaboration with hunters for collection of samples. In several continental European countries, it is the hunters that supply the majority of carcases for diagnostic examinations. The pivotal role of wildlife health and detection of wildlife diseases in a 'One Health' setting is shown in Figure 2.1.

Wild game disease, like wildlife disease, interfaces with three health spheres; the human health, livestock health and wildlife (or environmental) health spheres. There is potential for two-way movement of pathogens across these interfaces, and between each of the health spheres, through wild game: (1) from game to man and from man to game; (2) from game to livestock and from livestock to game; and (3) from game to wild animals and from wild animals to game.

It is recognized that potentially pathogenic agents can move from game, and wildlife, through these three health spheres, so the potential 'disease flow' through the three spheres, makes it important that we adopt a 'One health' approach when considering both game and wildlife disease transmission. In general terms, viral transmission (there are many exceptions) tends to be by animal to animal transmission, or by arthropod-vector transmission. Fomite, environmental and *post mortem* ('meat transmission') transmission of viruses occurs less frequently than for bacterial and parasitic pathogens. Viral disease is more likely to be host-species specific. New viral pathogens are more likely to be 'new-to-science' (e.g. Schmallenberg virus, rabbit haemorrhagic disease virus). New-to-science bacterial and parasitic pathogens tend to be less frequently found.

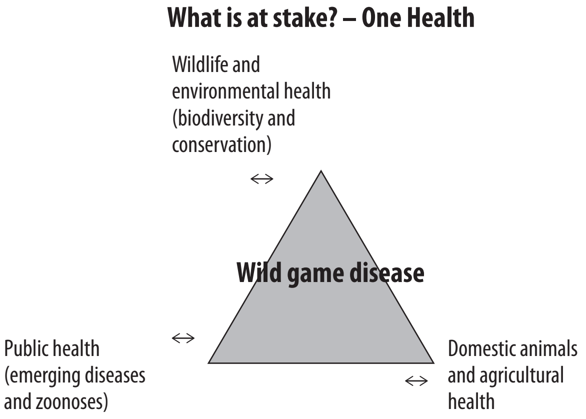
What is at stake? – One Health
Wildlife and environmental health (biodiversity and conservation)
↔
Wild game disease
↔
Public health (emerging diseases and zoonoses)
↔
Domestic animals and agricultural health

Figure 2.1. Wildlife and game interface with three health spheres – human health, wildlife and biodiversity health and livestock health (courtesy of the Wildlife Disease Association, Lawrence, KS, USA).

Bacterial and parasitic transmission may be direct, animal to animal, but may also be transmitted from environmental and fomite (inanimate substances e.g. plastic or vehicles) contamination by the pathogens. Some parasites may require a second host species to complete their lifecycle.

2.3.2 Surveillance of diseases of wild game in Great Britain

In Britain, currently there are several collaborations between veterinary diagnostic centers and hunters which could benefit from greater development, these are: (1) utilization of hunters as both observers and collectors of dead wildlife; (2) protecting hunters, through awareness, from disease acquired in the field through hunting and handling game; and (3) protecting hunters and the public from zoonotic disease acquired through the consumption of game meat.

In England and Wales, scanning surveillance has been the remit of APHA and its forebears since 1998. This surveillance covers all diseases (infectious and non-infectious, including toxicities/poisoning) in all wild terrestrial vertebrate species (including all avian and mammalian game species) and in all regions of the country. There is no specific prioritisation of surveillance of game species as is seen in several continental European countries. In general, when the monitoring of disease in wild and game species is opportunistic this is known as scanning surveillance; when the sampling is for specific diseases, in specified populations, this is known as targeted surveillance. In GB, we do however, encourage submission of found-dead wild deer and wild boar, and among birds, we encourage submission of water birds (for avian influenza virus (AIV) surveillance).

Surveillance is focused on three facets, these are: (1) new and re-emerging diseases in wild animals; this is important because these diseases must be assessed for their ability to cause disease in man, livestock and wild animals (Jones *et al.*, 2008); (2) zoonotic diseases which pose a threat to the health of man; this is also relevant to disease in game species, where there may be a threat to human health, from hunting, dressing in the field or from consuming wild game; and, (3) risks to livestock particularly where game species are in contact with livestock, and specifically where game species are in-contact with related farmed animal and bird species. For example, farmed deer in-contact with wild/park deer, domesticated pigs (especially in outdoor settings) in contact with wild boar, poultry in contact with wild gallinaceous game birds (pheasants, partridge and grouse), domesticated ducks and geese in contact with wild ducks and geese. Although, unrelated in taxonomical terms, poultry and wild water birds, in particular ducks and geese should be kept separated because water birds are the natural reservoirs of AI viruses (Irvine, 2013).

In 2009, following the England Wildlife Health Strategy (Duff *et al.*, 2010) surveillance for wildlife disease in GB became the remit of the GB Wildlife Disease Surveillance Partnership (GBWDSP). This, like the APHA DoWS, is funded by the Department for Environment, Food & Rural Affairs (Defra) and incorporates 7 government and non-government Partners. No game organisations are currently partners; however, the Forestry Commission England (FCE) and Wildfowl and Wetland Trust (WWT) partners both have strong links with game organisations.

Why do we undertake surveillance within APHA? It is primarily to identify and assess threats from new and emerging diseases (Duff *et al*., 2016). To do this we need a good knowledge of endemic disease in game and wildlife and to have and to maintain the necessary skill sets to diagnose, interpret and evaluate the findings accurately. The assessment pathway starts with examination of carcases, including game carcases, at a veterinary investigation centre to obtain a diagnosis. The diagnosis is assessed by the Species Expert Groups, for game, the avian and wildlife species expert groups will perform this assessment. Any threats identified at this stage of the process are elevated to the Veterinary Risk Group. The Veterinary Risk Group may undertake a risk assessment and the results of this, if considered to represent a significant threat in game species, would be shared with Public Health England and the Food Standards Agency and by these agencies advice would be directed back to the British game industry. It is not just about reaching a diagnosis; there is a defined process for initial assessing, risk assessment, prioritisation and mitigation actions.

2.3.3 Emerging diseases of wild game

Globally, some examples of new and emerging diseases threatening human and livestock health which have emerged from wildlife in the past three decades are given in Table 2.4. This is an arbitrary and non-exhaustive list, which has been selected mainly due to the prominence of the diseases in the public eye and through global press coverage. The diseases listed have caused human disease and human fatalities.

Among the new and emerging diseases, three have particular relevance as having reservoirs in European game species and therefore pose threats to hunters of game in Europe. Although only three such diseases are highlighted, hunters in Europe should not be complacent, new zoonotic diseases are regularly being found in European game. New diseases may be more likely to emerge in tropical countries outside of Europe (the validity of this point is debatable) nevertheless we stress that hunters should be aware of the possibility of new diseases of game in Europe. Awareness can be derived from scanning the scientific literature for reports of new conditions affecting game species. Several wildlife-related new diseases are arthropod-borne

Table 2.4. Diseases that have recently emerged from wildlife globally and which threaten human health.

Rabies[1]
SARS (severe acute respiratory syndrome)
MERs (Middle East respiratory syndrome coronavirus)
Hendra virus disease
West Nile virus (fever)
Bat Lyssa virus disease
AIDS (acquired immune deficiency syndrome)
AIV (avian influenza virus) infection[1]
Hantavirus infection (hantavirus pulmonary syndrome – HPS)
Nipah (Nipah virus (NiV) infection)
Ebola virus disease (infection from wild animals/fruit bats to humans)

[1] This agent has a reservoir in European game or commonly hunted wildlife.

and it could be argued that hunters are at no greater risk of these than any other people who work in the field. Suitable clothing to avoid arthropod-borne diseases, offering protection against the bites of mosquitoes and other insects, and ticks, are increasingly important with the expansion of the ranges of some arthropod vectors of disease.

Wildlife and wildlife disease is not confined to nature reserves and national parks, both are present in cities and areas of high human population density. If animal related disease, for example, AIV infection in gulls or feral pigeons, was a source of disease for man, then the prevention of transmission of AIV from pigeons/gulls, both common urban birds, to humans, with whom they live in close proximity, would be a significant urban health challenge. There is, however, no evidence of this scenario developing, at least not in Europe.

2.3.4 Specific aspects of selected diseases of game of recent concern in Great Britain

Diseases of game that were of recent concern include bovine tuberculosis (bTB; caused by *Mycobacterium bovis*), cryptosporidiosis in red grouse, and tularaemia.

bTB has been detected in many species of British wild mammals, including deer and wild boar and some rodents (Delahay *et al*., 2007). The badger (*Meles meles*) is considered the most important wildlife reservoir while the other species including deer and boar are considered spill-over hosts and less important in the epidemiology of the disease.

A recently discovered new game disease of red grouse is called 'bulgy-eyed disease in red grouse' and is caused by the parasite, *Cryptosporidium baileyi* (Coldwell *et al*., 2010). *C. baileyi* is mildly zoonotic and reported as causing limited disease in a very small number of immuno-compromised people. The parasite in red grouse produces a sinusitis leading to the inflammation of the infra-orbital sinuses of the head and swollen eyes with vision impairment in the affected birds. Grouse hunting in Britain is a valuable sport valued at millions of pounds sterling annually and it remains to be seen if the disease will cause significant financial loss to the grouse hunting estates. Grouse populations are at their highest in recent years since the sport started in the Victorian era (1870-1890).

One specific disease-related risk in the management of game populations which have known zoonotic disease, is that the concentration of game in areas around where they are given supplementary feeding, can lead to higher prevalence of disease than would otherwise be seen. Examples of this are seen with bTB in boar and deer in several areas of Europe, particularly in hunting estates.

The UK is now assessing, partially through surveillance by testing samples removed from hunted animals, the disease risks of the now reasonable populations of wild boar, particularly those in the South of England around the Forest of Dean. This disease surveillance in boar may become more important if the threat from African swine fever, currently in parts of Eastern Europe, continues to spread westwards towards Britain.

Tularaemia is an important disease of hunted game in continental Europe. The disease may affect almost all species of mammal but is most problematic in hares. In several countries hunters have become infected with this bacterial zoonosis when dressing shot hares. Tularaemia is not found in Britain. However, it is a disease that British hunters should be aware of and have some familiarity with the associated pathology, which is focal liver and lung lesions (Gyuranecz, 2012).

2.4 Conclusions

2.4.1 What has been achieved?

Surveillance schemes of wildlife diseases have been established, and have proven effective in the early detection of new disease threats. Results of such surveillance are utilized in formalized risk-based assessments of wild game diseases, with a view on their significance for human, domestic animal and wildlife health. It is recognized that hunters have an important role in helping to monitor for new disease in wild game and wildlife.

2.4.2 What has been neglected?

The excellent relationship between hunters and national wildlife disease authorities could be further developed to the benefit of all parties. Information on new diseases in European game is not always easily accessible to those involved in handling and hunting of game.

2.4.3 What needs to be done?

The development of close relationships between hunters and national wildlife disease surveillance schemes is important and we would encourage further, closer collaboration. Hunters and game meat inspectors should horizon-scan scientific and trade literature to be aware of new diseases reported in European game. Hunters and game meat inspectors need closer diagnostic laboratory support to submit, investigate and identify disease in game.

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3. Hepatitis E virus: the latest public health scenario

Cristina Soare[1] *and Kim Picozzi*[2]
[1]Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, United Kingdom; cristina.soare@ed.ac.uk
[2]Edinburgh Medical School, Biomedical Sciences, College of Medicine and Veterinary Medicine, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, United Kingdom

Summary

Although hepatitis E virus (HEV) was previously considered a disease restricted to humans, it is now demonstrated to have several animal reservoirs with increasingly complex ecology and molecular diversity. Currently, four HEV genotypes (G1-G4) are being broadly reported. Whilst infection with genotype 1 (G1) and 2 (G2) was only observed in humans, genotypes 3 and 4 were isolated from both humans and animal species. Zoonotic source hypothesis is supported by phylogenetic analysis which demonstrated similarities between gene sequences from patients with autochthonous HEV and animal sources, amongst which swine, wild boar, deer, shell fish and our understanding of the animal host species is still progressing. Animals are responsible for cross-species infections and for environmental maintenance. Human cases have been associated with handling of pigs, with consumption of raw or insufficiently cooked meat and with animal faecal contamination of water and environment. Increasing public health recognition of HEV infection and changes to our understanding of the pathobiology have prompted the current review which aims to summarize research findings in regards to HEV circulation, animal reservoirs, transmission routes and disease burden. These will be integrated into an appreciation of HEV public health significance to highlight control solutions. A special emphasis will be made on the wild animal population in the UK and Europe.

Keywords: zoonosis, foodborne virus, viral hepatitis E, Scotland, wild boar, deer, rabbit

3.1 Introduction

3.1.1 Hepatitis E virus: 36 years down the line, from the first occurrence report

The first report suggestive of a new type of hepatic viral infection came in 1980, following a large Indian epidemic during which 52,000 patients were affected by icteric hepatitis (Khuroo, 1980). Due to lack of sensitive diagnostic methods and knowledge of the virus pathobiology, it took the medical community 10 years to identify the novel entity (Reyes *et al.*, 1990), now known as hepatitis E virus (HEV). Until late 1990, hepatitis E was considered a disease affecting people in the developing countries in the form of waterborne epidemics due to unsanitary conditions. Accumulating research data and a recent world-wide seroprevalence study carried by the World Health Organization (WHO) show that sporadic, non-travel

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related HEV infections are increasingly common in industrialized countries (WHO, 2010) and many of these cases are zoonotic in origin.

3.1.2 Clinical presentation in humans from industrialized areas

The clinical features of autochthonous acute HEV infection depend largely on the immune status of the person exposed. Less than 5% infected humans (Said *et al.*, 2009) and more commonly middle-aged man develop signs of acute hepatitis, possibly due to greater likelihood of exposure to contaminated water and animals. Immunocompetent patients are less likely to seek medical attention as they rarely develop clinical signs and the disease takes a self-limiting course (Ijaz *et al.*, 2014). The most characteristic presentation translates into: high transaminases, jaundice, abdominal pain, headache, fever, nausea, vomiting, anorexia and hepatomegaly (Aggarwal, 2011). The disease manifests severely in organ transplant recipients, patients with previous history of liver disease or generally immunosuppressed who develop cirrhosis and subsequent liver failure, if untreated (Kamar *et al.*, 2013).

Extrahepatic manifestations of HEV infections have also been reported and can include: neurological problems such as Guillain-Barré syndrome, kidney injuries and haematological disorders (Bazerbachi *et al.*, 2016).

3.1.3 Clinical presentation in animals

The pathogenesis of HEV has mostly been studied in swine. Meng *et al.* (1997), who observed the disease in pigs for the first time, reported that infection is not associated with overt clinical signs or gross lesions but with mild microscopic lesions in the liver and hepatic lymph nodes. In experimental conditions Halbur *et al.* (2001) found that microscopic lesions include mild to moderate multifocal and periportal hepatitis, mild focal hepatocellular necrosis and lymphoplasmacytic enteritis.

Naïve pigs acquire infection via faecal-oral route, from contaminated feed, water or fomites. Acute infection can lead to transient fever during viraemia and faecal shedding due to virus primary replication in the liver (Schlosser *et al.*, 2015). In a recent study, naturally infected wild boars showed asymptomatic infection with HEV, similar to domestic pigs (Schlosser *et al.*, 2015).

3.1.4 Virus characterization

Molecular characterization of the HEV genome has enabled the development of phylogenetic analyses of human and animal strains which in exchange provide genetic evidence of the virus etiology.

The mammalian HEV is composed of a single protein capsid and a single stranded, positive sense RNA genome of a 7.2 kb in size. The genomic RNA encodes structural and non-structural proteins and consists of three open reading frames (ORFs) flanked by two short untranslated regions at its 5’ and 3’ termini. ORF 1, encodes a non-structural poly-protein of 1,693 amino acids and several putative domains, starting from the 5’ non-translated region, towards 3'. ORF 2 with a length of 660 amino acids, encodes the major capsid protein (pORF2) which contains the epitopes for neutralizing antibodies. ORF3 encodes a small nonglycosylated protein which is thought by some authors to be a 123-amino-acid protein (pORF3) (Sarin and Kumar, 2010), Overall, the HEV genome structures are disposed as: 5'-ORF1-JR-ORF3-ORF2-3', where ORF 3 largely overlaps ORF 2 (Cao and Meng, 2012).

3.2 Genetic classification of hepatitis E virus

HEV is the single member of the *Hepeviridae* family. Four genotypes of HEV (G1-G4) have been recognised in the past but recently new, highly divergent genomes (G5-G7) have been detected and added to the *Hepeviridae* family. Due to widening range of host species and new genotypes observed, the members of the International Committee on Taxonomy of Viruses recognized the need for a standardized taxonomical classification of *Hepeviridae* family in line with genus, species and genotype (G). Following reanalysis of current phylogenetic information, an updated classification is proposed (Smith *et al.*, 2014) which I adopted it in Table 3.1 with slight variation of the host species and genotype allocation.

The *Hepeviridae* family (Table 3.1) is divided into two genera: Orthohepevirus which includes all mammalian and avian isolates and Piscihepevirus that includes cutthroat trout virus.

Although the genome organization is similar for all HEVs, sequence variability exists between strains and thus further subdivision of genotypes into sub-genotypes/subtypes is necessary for accurate phylogenetic classification. The first comprehensive HEV phylogenetic analysis subdivided HEV genotypes 1-4 into a total number of 24 subtypes (Lu *et al.*, 2006). Due to increasing number of HEV sequences available in GeneBank and inconsistencies between research studies in subtype allocation, the Committee on Taxonomy of Viruses have proposed a new set of reference sequences to complement the hierarchy proposed by Lu *et al.* (2006) and to ensure consistent allocation of subtypes into their genotype (Smith *et al.*, 2016). Genotype 1 now includes subtypes a-f and genotype 2 is classified into two subtypes: a and

Table 3.1. Classification of the Orthohepevirus genus at the species and genotype level.

Orthohepevirus genus	Orthohepevirus A	humans	G 1, 2, 3, 4, 7
	(7 genotypes, divided in subgenotypes)	swine	G 3, 4
		wild boars	G 3, 4, 5, 6
		rabbits	G 3
		deer	G 3, 4
		mongoose	G 3
		camel	G 7
	Orthohepevirus B	birds	
	Orthohepevirus C	rats, ferrets	G C1
	(2 genotypes; C1, C2)	mink	G C2
	Orthohepevirus D	bats	
Piscihepevirus genus	Piscihepevirus A	trout	

b. According to the new subtype allocation, genotype 3 is the most complex, with multiple hierarchies. Subtypes 3a, 3b, 3c, 3h, 3i and 3j form one major clade and subtypes 3g, 3e and 3f form a different clade. HEV 3 rabbit sequences showed multiple levels of divergence and therefore these strains were classified within the 3ra clade but have not been assigned to a subtype. Genotype 4, now includes subtypes a-g and additional h and i. Genotypes 5-7 are recent additions to the GeneBank and in absence of sufficient isolates, only the first sequence of each genotype was assigned as subtype 'a' (5a, 6a, and 7a) (Smith *et al.*, 2016).

3.3 Epidemiology of hepatitis E virus

HEV is acknowledged as an important public health pathogen due to its worldwide occurrence and significant clinical disease burden, particularly in developing countries (Hughes *et al.*, 2010). The WHO estimates a number of 20 million hepatitis E human infections occurring every year worldwide, with over 3 million symptomatic cases and 56,600 hepatitis E-related deaths (WHO, 2015).

Recent understanding of global epidemiology of HEV infection reveals a distinct occurrence pattern between different world regions. Southeast and Central Asia, northern and western parts of Africa, India and the Middle East are considered hyperendemic (WHO, 2010). In these regions, outbreaks occur frequently, with peaks of disease lasting from several weeks to one year and with additional large proportions of acute sporadic hepatitis (Hughes *et al.*, 2010).

In most industrialized regions, such as developed Asian-Pacific, parts of Europe, HEV infections occur sporadically but are estimated to account for more than 25% of non-A, non-B acute hepatitis cases and thus hepatitis E is now considered an endemic disease (WHO, 2014).

Genotype 1 and 2 account for most of the hepatitis E cases in the developing countries and both appear to be anthroponotic (Hughes *et al.*, 2010)

Genotype 3 is highly prevalent in domestic and wild swine worldwide, also responsible for most autochthonous HEV infections in Europe, USA, and Japan (Kamar *et al.*, 2012) posing public health concerns. A special member of genotype 3 is the novel rabbit HEV, classified as a distant member due to 78-79% sequence identity with genotype 3 member strains (Smith *et al.*, 2014).

Genotype 4 has been recovered from both humans and domestic pigs with high genetic similarity between the two species which suggest possible zoonotic transmission. This genotype has been observed in eastern Asia initially, particularly in China and Japan (Liu *et al.*, 2012), but recently autochthonous human infections have been observed in Germany (Wichman *et al.*, 2008), France (Colson *et al.*, 2012) as well as it has been detected in swine populations in Italy (Monne *et al.*, 2015) and Belgium (Hakze-Van der Honing *et al.*, 2011) suggesting a local spread of HEV-4 in Europe, possibly through import of swine meat from Asia (Bouamra *et al.*, 2014). This genotype has also been isolated from a number of other animal species such as: wild boar, deer, yak, sheep but so far only swine, wild boars (Johne *et al.*, 2014) and deer (Choi *et al.*, 2013) have been involved in zoonotic transmission.

Genotypes 5 and 6 were isolated in single opportunities from wild boars in Japan (Takahashi *et al.*, 2011), but the zoonotic potential of these genotypes is not known yet (Li *et al.*, 2015).

Genotype 7 was isolated from camels during an epidemiology study carried by Woo *et al.* (2014). Limited data concerning consumption of camel milk and meat suggest G7 can be zoonotic (Lee *et al.*, 2015).

HEV serological exposure has been noticed in a number of other animal species (Pavio *et al.*, 2015) and with the advance of molecular biology techniques it is anticipated that our understanding of the host range as well as source attribution will improve and novel HEV strains will be identified from other animal species in the upcoming future.

3.3.1 HEV epidemiology in European wild boars

The prevalence and phylogenetic analysis of HEV obtained in different studies from industrialized countries is outlined in Table 3.2, aiming to capture most of publications which reported epidemiological data from 2010 onwards, in an attempt to understand the spread on HEV genotypes amongst wild boars as well as the subtypes reported. This in turn could allow determining the transmissions path to other animal species and humans.

As shown in Table 3.2, HEV is widely spread across wild boar populations in Europe but the seroprevalence can vary greatly, depending on the country investigated, as well as on the geographical areas within the countries. However, a meaningful longitudinal comparison of the serological data is not possible as different commercial serological tests were used (data not shown) and these tests might have different sensitivity and specificity.

Following molecular testing, different infection level was detected, for instance in blood samples HEV RNA ranged from 0 % in Italy (Caruso *et al.*, 2014) to 15 % in Germany (Schielke *et al.*, 2015). Although sequencing of positive HEV RNA samples was not always possible, as shown in the 'genotype' section of Table 3.2, evidence from several studies indicate that European wild boars have only been affected by genotype 3 to date, however considering the recent emergence of genotype 4 in human and pig population, it might be likely this will also spread to other species.

Phylogenetic analysis of HEV 3 sequences isolated from wild boars in Italy, the Netherlands and Sweden showed close relatedness to either locally isolated human or pig sequences, or both, suggesting that HEV can circulate between wild boars, pigs and humans. From the 11 subtypes of genotype 3 known (Smith *et al.*, 2016), most were isolated from European wild boars although a different subtype appears to affect each of these populations. However, in Belgium, Italy and Sweden the same subtype 3f has been observed (Caruso *et al.*, 2015; Thiry *et al.*, in press; Widén *et al.*, 2011).

3.3.2 HEV epidemiology in European deer

In a similar manner, data has been collated in Table 3.3 from European studies investigating HEV in deer. Lower infection rates appear to affect deer, when compared to wild boar, with

Table 3.2. A cross sectional summary of European studies reporting seroprevalence or detection of nucleic acids from a variety of wild boar samples.

Country	Samples	Assay	Results	Genotype	Reference
Belgium	383 serum	ELISA	34%	G3f	Thiry *et al.*, in press
	69 serum	PCR	5.79% RNA		
	61 liver	PCR	6.55% RNA		
France	86 liver	RT-PCR	5.8 RNA	–	Lhomme *et al.*, 2015
Germany	124 serum	RT-PCR	14.51% RNA	G3a, G3b, G3i, G3h, G3j	Oliveira-Filho *et al.*, 2014
	46 serum	ELISA	41% IgG	G3a, G3b-similar with strains from wild boar – Germany, pigs – the Netherlands; humans – Japan	Schielke *et al.*, 2015
		RT-PCR	15% IgG		
	22 liver	RT-PCR	18% RNA		
Italy (central)	64 serum	ELISA	56.2% IgG	G3, similar with local human isolates	Mazzei *et al.*, 2015a
	64 stool	RT-PCR	9.4% RNA		
Italy (NW)	594 serum	ELISA	4.9% IgG	G3e, G3f strains homology with local domestic pigs	Caruso *et al.*, 2015
		RT-PCR	0% RNA		
	320 liver	RT-PCR	3.7% RNA		
Italy (NW)	372 liver	RT-PCR	1.9% RNA	G3e, G3c, G3f	Serraca *et al.*, 2015
the Netherlands	1,029 blood	ELISA	12% IgG	G3c, similar with human and pig isolates from the same area	Rutjes *et al.*, 2010
		RT-PCR	5% RNA		
	93 stool	RT-PCR	2% RNA		
	73 liver	RT-PCR	2%RNA		
	39 muscle	RT-PCR	0% RNA		
Portugal	80 liver	RT-PCR	25% (livers)	G3e	Mesquita *et al.*, 2014
	40 stool	RT-nPCR	10 (faeces)		
Slovenia	288 serum	ELISA	30.21% Ig	–	Žele *et al.*, 2016
		PCR	0.35% RNA		
Spain	108 serum	ELISA	57.4%	G3	Kukielka *et al.*, 2015
	158 serum	RT-PCR	10.12 RNA		
Sweden	159 serum	RT-PCR	8.2% RNA	G3f, similar to local human strains	Widén *et al.*, 2011
Switzerland	303 serum	ELISA	12.5% IgG	–	Burri *et al.*, 2014

no significant differences between breeds. Both roe and red deer, which are the most common European breeds, developed HEV-antibodies at a relatively low detection level.

Scarce molecular data is available showing that strains collected in Italy and Spain were similar to human and pigs sequences (Boadella *et al.*, 2010; Di Bartolo *et al.*, 2017). The studies from Belgium (Thiry *et al.*, in press), Italy (Serraca *et al.*, 2015), the Netherlands (Rutjes *et al.*, 2010) and Spain (Kukielka *et al.*, 2015) reported presence of HEV infections in both wild boars and deer on a background of already demonstrated human and swine infections. These observations suggest HEV is able to adopt a dynamic transmission cycle between its multiple host species which, in turn will favour HEV survival. Considering the scarce molecular evidence, there is a need for further investigation to determine the role of

Table 3.3. Seroprevalence and molecular data of hepatitis E virus in deer: European reports 2010-2016.

Country	Species	Samples	Assay	Results	Genotype	Reference
Belgium	red deer	189 serum	ELISA	1%	Only one RNA identified and classified as G3f	Thiry *et al.*, in press
		29 liver	PCR	1 RNA		
	roe deer	235 serum	ELISA	3% IgG		
		27 liver	PCR	0% HEV RNA		
France	wild(?) deer	62 liver	RT-PCR	2.32% RNA	–	Lhomme *et al.*, 2015
Germany	red deer	161 serum	ELISA	2% Ig	–	Neumann *et al.*, 2016
		163 serum	qRT-PCR	1.9% RNA		
		101 liver		0% RNA		
	roe deer	154 serum	ELISA	6.8% Ig		
		46 serum	qRT-PCR	0% RNA		
Italy (NW)	roe deer	30 serum	RT-PCR	0% RNA	G3e-homology with local human and swine strains	Serraca *et al.*, 2015
Italy (central)	red deer	235 serum	ELISA	13.9% IgG 11% RNA		Di Bartolo *et al.*, in press
the Netherlands	red deer	38 blood	ELISA	5% IgG	–	Rutjes *et al.*, 2010
			RT-PCR	15% RNA		
	roe deer	8 blood	ELISA	0% IgG		
			RT-PCR	0% RNA		
Spain	red deer	70 serum	ELISA	12.85% IgG	–	Kukielka *et al.*, 2015
		81 serum	RT-PCR	16.05% RNA		
	red deer	968 serum	ELISA	10.4% IgG	G3-similar with Spanish pigs and 1 French patient	Boadella *et al.*, 2010
		81 serum	RT-PCR	13.6% RNA		

deer to the occurrence of HEV infection as well as to establish the risk factors associated with this species.

3.3.3 Hepatitis E virus epidemiology in European rabbits

Studies of the European rabbit have been carried mainly in France (Izopet *et al.*, 2012; Lhomme *et al.*, 2015) were also human autochtonous HEV-3 infections are frequently occurring. Essentially, the virus was found in 7.0% of the farmed rabbits and in 23% of the wild rabbits. Importantly, rabbit strains showed genomic homology with a locally isolated human strain suggesting zoonotic potential (Izopet *et al.*, 2012). In addition to the French study, Jirintai *et al.* (2012) successfully transmitted rabbit HEV virus to human cell lines, strengthening the hypothesis of zoonotic potential. A retrospective phylogenetic study of rabbit serum collected in Germany in 1989 discussed close relationship between rabbit sequences from France and Germany proving a long-established circulation of these HEV strains in Europe (Eiden *et al.*, 2016) and it might be possible that human cases could also be linked with transmission from rabbits. Evidence that rabbits carry potentially zoonotic HEV strains and limited information available for this species warrants further investigation to establish its role in HEV transmission to humans in Europe and across the world.

3.4 Transmission pathways for zoonotic hepatitis E virus

The concept of zoonotic hepatitis E has emerged recently following isolation of closely related animal and human HEV strains in countries where sporadic cases were indigenously acquired. Although, meat consumption is the most common transmission route linked with animals, transmission pathways are more complex, as described in Figure 3.1 and can involve direct contact with infected animals or their meat or indirect routes via environmental contamination from animal droppings or farm run offs.

3.4.1 Foodborne transmission

Direct transmission of hepatitis E to humans via consumption of contaminated meat was observed on several opportunities by phylogenetic analysis showing identical or near identical sequence homology between infected patients and suspected food. The first food-borne HEV infection was reported in Japan after consumption of sika deer sashimi (Tei *et al.*, 2003). This was followed by other cases through consumption of wild boar grilled meat in Japan (Li *et al.*, 2005), raw pork liver sausages (Figatelli) in France (Renou *et al.*, 2014) and pork meat in Spain (Riveiro-Barciela *et al.*, 2015). Furthermore, several case control studies found that consumption of any offal or wild boar meat (Wichman *et al.*, 2008), wild boar raw bile juice (Kim *et al.*, 2011), uncooked pig livers (Colson *et al.*, 2010) are risk factors for acquisition of HEV infection. Indirect evidence of transmission via infected food products is brought by repeated detection of HEV RNA in pork retail chain, in some cases up to 30% of produce being found positive (Pavio *et al.*, 2014).

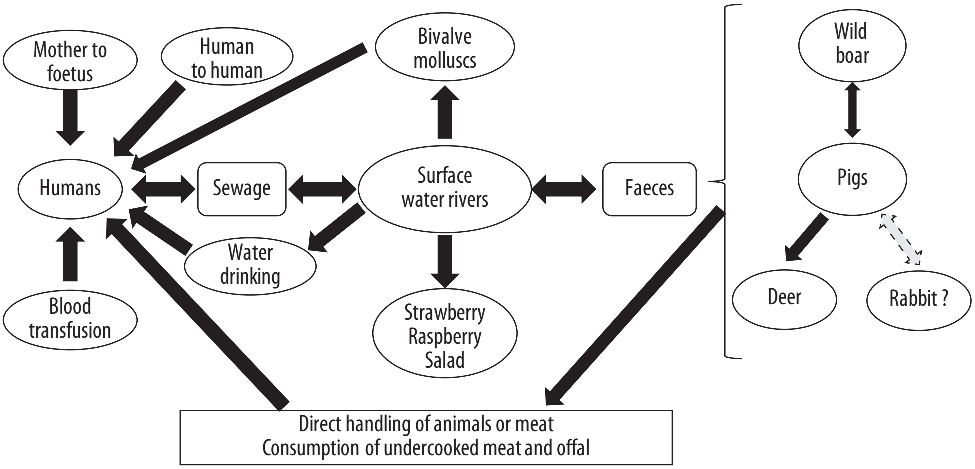
Mother to foetus
Human to human
Bivalve molluscs
Wild boar
Humans
Sewage
Surface water rivers
Faeces
Pigs
Water drinking
Deer
Rabbit ?
Blood transfusion
Strawberry Raspberry Salad
Direct handling of animals or meat
Consumption of undercooked meat and offal

Figure 3.1. Hepatitis E virus transmission pathways between humans, animals and environment; discontinued arrow reflect successful experimental transmission route.

Due to its wide spread, genotype 3 is more commonly linked with food incidents but genotype 4 infection was also observed following consumption of roe deer meat (Choi *et al*., 2013) and wild boar bile (Kim *et al*., 2011).

Meat can become contaminated via excreta during processing, if handling procedures are not hygienic but may also be already contaminated because of infection of the living animal. The risk of HEV food-borne infection depends on the level of viral contamination and the extent of inactivation during food processing. Observation that some patients who ate a small portions of infected deer meat were not affected whilst others who ate higher quantities developed acute HEV (Tei *et al*., 2003), suggests there is a minimum viral load necessary to cause infection. To date, the literature is lacking information concerning the infectivity dose. Additionally, safe treatment procedures have not been extensively studied but it is apparent that refrigeration or freezing might not inactivate the virus and the effect of surface disinfectants such as chlorine need to be further elucidated (Cook and Van der Poel, 2015). Heat treatment of pig meat/liver at core temperature of 71 °C for 20 minutes could destroy the virus (Barnaud *et al*., 2012) but it is possible that other time/temperature treatment might be required for food products derived from other susceptible species. Investigating the effect of temperature, processing techniques and disinfection procedures will be of outmost importance to understand the full spectrum of public health risks associated with HEV infection.

3.4.2 Environmental contamination and subsequent transmission

HEV susceptible host species shed virus in their stool during viraemia and run offs from farms or land spread with untreated faeces can become a source of HEV pollution to the surface water, rivers or costal water with concomitant contamination of bivalve molluscs or crops which can become a further source of HEV transmission to consumers (Pavio *et al*., 2015). Transmission from faecal material is highly likely following observations of HEV-G3 in pig slurry of similar sequence identity to those circulating in humans from the same area (McCreary *et al*., 2008). Additionally, multiple variants of HEV-G3 strains were found in untreated sewage water from Edinburgh, Scotland (Smith *et al*., 2015). Accumulating information indicates that pig and human faeces can be contaminated with HEV but it is not clear how long the pathogen can survive into the environment once it has been shed.

Bivalve molluscs are known to accumulate viral particles during the process of filter feeding (Grodzki *et al*., 2014) and thus is not surprising that shellfish HEV positive samples have been found in China and the UK (Crossan *et al*., 2012; Gao *et al*., 2015). To date, two case control studies have identified shellfish consumption as a risk factor for transmission of HEV-G3 (Said *et al*., 2009) and HEV-G4 (Koizumi *et al*., 2004) to humans.

Data on possible association of HEV clinical infections from crops irrigated with contaminated surface water is rather limited, however HEV RNA isolated from strawberries showed high partial sequence identity with a strain previously isolated from a swine farm in Quebec with (Brassard *et al*., 2012). HEV has also been traced on raspberries (Maunula *et al*., 2013) and salad (Kokkinos *et al*., 2012) in the European supply chain.

3.4.3 Transmission by direct contact with infected animals

Abundant serological evidence was reported in different human population in direct contact with host species among which, forestry workers (Carpentier *et al*., 2012) and hunters (Schielke *et al*., 2015) following handling of wild boar meat, blood or derived products. While presence of HEV-antibodies in these populations does not necessary indicate presence of disease, it does identify risk factors of acquiring HEV and this route of transmission should be acknowledged. Further preventive measures such as protective gloves, working boots and adopting strict hygiene handling of meat (Chaussade *et al*., 2013) are necessary to reduce the HEV risk to professionally exposed individuals and to minimize the risk of introducing the virus into food.

3.4.4 Cross-species transmission

Swine were the first animal models to give a better understanding of the cross-species and zoonotic abilities of HEV. In experimental setting was shown that pigs seroconvert and develop viraemia following inoculation of both human HEV-G3 (Meng *et al*., 1998) and HEV-G4 (Feagins *et al*., 2008), suggesting these human genotypes can cross the species barrier and replicate in pigs.

Genotype 3, HEV has the widest host range which suggests that also has the natural adaptability for cross-species transmission. Genotype 3 of wild boar origin can transmit and affect domestic pigs in both experimental and natural conditions (Schlosser *et al*., 2015). Takahashi *et al*. (2004) noticed that deer can get infected with genotype 3 from wild boar in natural conditions and Matsuura *et al*. (2007) detected natural infection with genotype 3 in deer following natural transmission from domestic pigs.

A very important observation is that novel rabbit genotype also showed cross-species capability. On experimental challenge with rabbit HEV, cynomolgus macaques (*Macaca fascicularis*) developed all pathognomonic signs of an established infection (viraemia, elevated transaminases, virus shedding in stool and seroconversion) (Liu *et al*., 2013). These animal models are closely related to humans which indicates that rabbit HEV-3 may likely infect humans, same as other genotypes 3. Although human HEV infection was never directly linked to rabbits, in the given scenario, it can be speculated that consumption of insufficiently cooked rabbit meat, direct contact with rabbits or water contaminated with droppings may be additional sources of transmission. Studies to establish the zoonotic potential of rabbit HEV-G3 in natural conditions are awaited and would be beneficial if this could be translated into research in the UK where rabbit is one of the most common wildlife species which also enters into the food chain.

3.5 Diagnosis of hepatitis E virus in animal species

3.5.1 Sample collection and storage

Since animals do not manifest clinical symptoms, diagnosis is generally not necessary and only performed in research conditions. One of the challenges for obtaining accurate testing results is the type of sample collected, due to limited resources that are available in field conditions, particularly when collecting from wild animals.

Blood is a key testing specimen, enabling analysis of both antibodies through ELISA or for presence of HEV RNA by PCR. Sampling from wild game species where blood might be clotted can be done from the pool in the abdominal cavity or from the intracardiac clot. The vials must be transported and stored at refrigeration temperature until the serum is expressed and extracted by centrifugation, even if haemolytic (Rutjes *et al.*, 2010). Serum can be processed immediately or stored at -20 °C for short term usage or at -80 °C for long term use.

Faecal samples can also be collected, homogenised and HEV RNA retrieved from the supernatant. For instance, faeces from wild game were stored in Soya broth and 10% glycerol at -70 °C prior to resuspension in salt solution for extraction of viral RNA (Rutjes *et al.*, 2010). Tissue samples (liver, mediastinal lymph nodes or muscle) are also suitable for molecular diagnosis. Generally, the tissues are first chopped, homogenized, lysed and the RNA is purified from the lysate in a last step. Various kits such as QIAamp Viral RNA or RNeasy mini (Qiagen, Venlo, the Netherlands) are commercially available for the isolation of RNA from any sample type.

3.5.2 Methods for hepatitis E virus laboratory diagnosis

The virus can be detected directly by nucleic acid detection techniques or indirectly by serology which identifies host's specific immune response against the pathogen. Serological methods can target IgM, IgA, IgG individually or multiple antigens.

Generally, HEV-antibodies persist and can be detected for a longer period when compared to viral RNA (Aggarwal, 2013), thus increasing the detection window. Therefore, the research diagnostic protocol of HEV firstly uses the appropriate serological test for screening, which gives an indication of recent or past exposure. Positive results of recent exposure should be followed by a molecular method to identify the animals with current infection and thus likely to be shedding the virus (Figure 3.2).The advantage of the serological testing step is that offers information regarding acute infections, can be useful in establishing the seroprevalence and reduce the throughput of the samples processed by molecular analysis.

3.5.3 Serological testing

When electing the serological method, several features need to be considered, including the various antibodies which can be targeted, the time course of the disease, the antigen used and the different formats available.

It was observed that anti-HEV IgM detection systems respond better for the purpose of first line screening and using complementarily an anti-IgM and an anti-IgG EIA from the same producer could increase the sensitivity and specificity, providing an overall better screening performance (Wu *et al*., 2014).Such protocol has been suggested in Figure 3.2.

Natural course of infection in pigs often coincide with declining of maternal antibodies (Crossan *et al*., 2014). Infected swine seroconvert first with IgM anti-HEV which peaks between 12 and 15 weeks of age. The peak coincides with HEV detection in bile, liver, faeces, with occurrence of hepatitis and with viral shedding, followed shortly by seroconversion of IgG anti-HEV at around 16 weeks of age (De Deus *et al*., 2008). Whilst the presence of solely IgG indicates past infection (Figure 3.2), detection of IgM alone or in combination with IgA is specific of acute hepatitis (Takashaki *et al*., 2005).

In regards to the type of antigen used, all three HEV ORFs have shown to contain epitopes with antigenic properties (Khudyakov *et al*., 1999), however ORF 2 capsid is more commonly

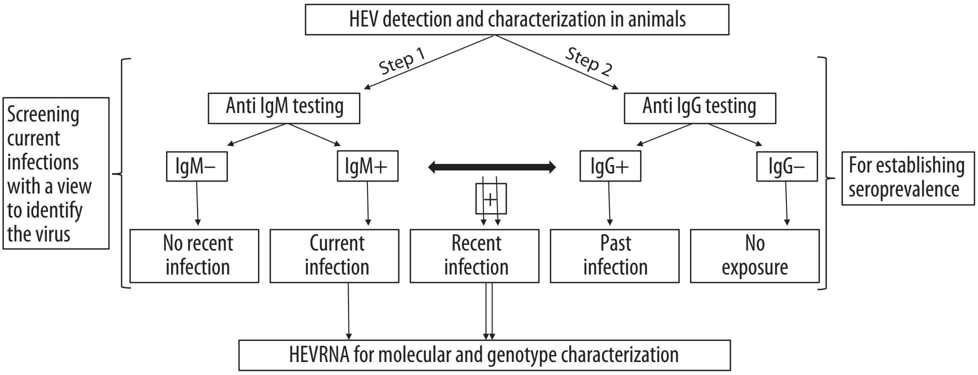
HEV detection and characterization in animals
Step 1
Step 2
Screening current infections with a view to identify the virus
Anti IgM testing
Anti IgG testing
For establishing seroprevalence
IgM−
IgM+
IgG+
IgG−
+
No recent infection
Current infection
Recent infection
Past infection
No exposure
HEVRNA for molecular and genotype characterization

Figure 3.2. Suggested protocol for detection and characterization of hepatitis E virus in animals.

Table 3.4. Host-independent commercial kits for serological detection of anti-hepatitis E virus antibodies.

Commercial kit	Antigens	Detects	Sensitivity	Specificity	Species detected
HEV ELISA, 4.0 MPB; MP Biomedicals Singapore	ORF2,a-a 394-660, of HEV-G3	IgM, IgA, IgG	100%	98.8%	swine (Hu *et al*., 2008), sika deer (Zhang *et al*., 2015)
HEV Ab-ELISA kit; Axiom, Bürstadt, Germany	ORF1, a-a 394-660, of HEV-G1	IgA, IgM, IgG	93%	89%	wild boars (Schielke *et al*., 2015), rabbits (Eiden *et al*., 2016)
ELISA kit HEV Ab; ULTRA, DIA.PRO, Sesto San Giovanni, Italy	ORF2.1, aa112-660, HEV-G1 and HEV-G2	IgM, IgG	100%	99.5%	hares, red deer, wild boar (Mazzei *et al*., 2015b)

selected as antigen in commercial kits because is more immunogenic and includes neutralization epitopes shared between different genotypes (Zhang *et al*., 2006). Most of the EIAs commercially available are for human use but few are species-independent and were used in veterinary studies as extracted in Table 3.4.

3.5.4 Hepatitis E virus molecular detection

Detection of HEV RNA is considered 'the gold standard for diagnosis' (Echevarría, 2014) as it confirms serological results as well as provides sequences for determining the genotypes which in turn offer further information concerning the zoonotic potential.

There are assays which have been designed and optimised to virtually detect the four mammalian HEV genotypes. These are based on methods such as quantitative real time polymerase chain reaction (RT-qPCR) (Gyarmati *et al*., 2007; Jothikumar *et al*., 2006), reverse transcription PCR (RT-PCR) (Cooper *et al*., 2005), nested RT-PCR (Johne *et al*., 2010), etc. Selecting a suitable testing protocol for scrutinising several species, potentially carrying different genotypes can be challenging as currently there is no validated or widely recognized method and thus, the molecular testing techniques differ widely between studies.

RT-qPCR, as described by Jothikumar *et al*. (2006) is assessed as effective for the seven genotypes classified within the *Orthohepevirus A* (Girón-Callejas *et al*., 2015) and the most sensitive HEV RNA detection method (Mokhtari *et al*., 2013). The majority of laboratories which took part of the working panel for establishing the International HEV-G3c RNA Standard used this technique (Baylis *et al*., 2013). However, due to inter and intra genotype variations it is suggested to use complementary test, such as the protocol described by Gyarmati *et al*. (2007), which targets a different genome region. Using both assays complementarily increases sensitivity and HEV RNA quantification (Vasickova *et al*., 2012).

To this end the high variability of protocols adopted between different veterinary studies highlights the need for standardization of genotype-independent nucleic acid techniques able of detecting all HEV genotypes from animal species.

3.6 A case scenario: hepatitis E in Scotland and the UK

Studies undertaken over the recent period have enhanced our understanding in regards to local occurrence of HEV infections. Whilst travel related cases continue to be observed, it is now well established that genotype 3 HEV can be acquired indigenously throughout the UK and has also recently been recognized as the most common cause of acute viral hepatitis in Scotland (Kokki *et al*., 2015) and England and Wales (Ijas *et al*., 2014). Data collected between 2003 and 2012, indicates more than half of HEV cases were indigenously acquired with a year on year a rise from 2010 onwards, correlated with emergence of a new genotype 3 subtypes c, h, and i (Ijas *et al*., 2014). In Scotland, through case studies it was established that 70% of autochthonous HEV-G3 are associated with risk factors such as consumption of pork or game meat (Kokki *et al*., 2015). This evidence on its own warrants further attention into the occurrence of HEV in different animal species.

The zoonotic potential of HEV was investigated on slaughter aged pigs in both Scotland (Crossan *et al*., 2014) and England (Grierson *et al*., 2015). In England, the seroprevalence observed was very high (92.8%) and HEV-G3 was detected in 20.5% of samples tested. The results confirmed that HEV-G3 is present but, importantly, the subtypes isolated from pigs (e, f, g) are different from those previously seen in humans (c, h, i) (Grierson *et al*., 2015). In Scotland, the overall seroprevalence was lower (61.4%) but HEV RNA-G3 was detected in a larger number of animals (44.4%). Phylogenetic analysis was not possible to perform and the circulating subtypes are not known. To this stage it can be discussed that: (1) studies carried thus far could not gather sufficient molecular evidence to support zoonotic transmission from slaughter age pigs to humans, although very strong epidemiological links point towards this transmission route; (2) autochthonous infections might be caused by imported pig meat or derived products; and (3) the source of human HEV infections might be attributed to other animal species.

These observations raise a very important question of what is the source of human HEV in the UK. Setting aside this question is not an option, given the ubiquity of infection in both pigs and humans. Further investigation into HEV infections in pigs but also in other animal species is needed to decide what would be a proportionate response to this zoonosis.

3.7 Conclusions

3.7.1 What has been achieved?

Studies undertaken over the last decade have significantly changed our understanding of HEV which is now recognized as a major global health problem due to its worldwide spread and the large number of human infections it causes yearly. New knowledge of the disease burden has also reinforced evidence that HEV circulates amongst pigs, wild boars and all deer species in Europe with the possibility of transmission to humans.

Hepatitis E has become a human notifiable disease in the UK through the Health Protection (Notification) Regulation 2010 (TSO, 2010). Ireland have also followed the decision under the Infectious Diseases (Amendment) Regulations 2015 (The Stationery Office, 2015). HEV is also a notifiable disease in Germany and France and most of the official organization throughout Europe and including the UK follow up confirmed hepatitis E cases to investigate those which are non-travel associated and to identify potential risk factors. Thus, increased awareness amongst medics has led to a rise in reporting of HEV infections in immunocompromised population and blood donors which led to an emerging concern over the blood safety. It is apparent that although people acquire HEV infection from animals, blood transfusion has been the transmission route which received the most attention, particularly in Western Europe. Following extensive discussions over the necessity of screening blood donors for presence of HEV (Anonymous, 2014), from 1st April 2016, HEV human patients subjected to transplants will receive blood which has been screened for hepatitis E (Anonymous, 2016a).

Concluding, the public health implications of autochthonous HEV have changed and progressed as investigations into the virus continued and now stretch one step beyond recognition of hepatitis infections. Whilst we continue to learn about the dynamics of this disease we should also try to find answers as to what is the appropriate response to prevent and control this zoonosis, whether this can be done through altering the husbandry practices to facilitate natural immunity at early age, through immunization of animals, changing the way in which meat is processed or perhaps advising patients to modify approaches of cooking and consumption.

3.7.2 What has been neglected?

Whilst the literature describes an increasing number of animal populations that might have a role in zoonotic HEV infections, in practice public health response is currently focused on blood transfusions due to increasing concern over the possibility of asymptomatic donors to contaminate the blood supply. Clinical data on human HEV is available in most EU countries, but is difficult to estimate the proportion of true zoonotic associated cases due to the relatively long incubation and largely asymptomatic course of disease in immunocompetent individuals.

In most industrialised countries, the potential of transmission from wild and farmed game species have been investigated, however the surveys were conducted on limited geographic areas and have included inconsistent sample types. Data extracted in Table 3.2 and 3.3 indicate a different infection level between countries, as well as between various regions of the same country and thus surveillance should be carried at national levels to be able to identify areas of concern. In Table 3.3 it is shown that none of the studies have included faeces samples from deer and considering that the virus is shed longer in faeces (Halbur *et al.*, 2001) perhaps this is a reason for which less molecular data could be retrieved from this species.

In the UK, the potential of transmission form wild or farmed game has been largely neglected. Even if game meat, particularly rabbit, is increasingly becoming part of the diet of the regular UK consumers (Brady, 2014), the potential of finding the virus into this species, or wild or farmed game in general, has not been investigated. In the current scenario, it becomes important to understand whether HEV human cases can be attributed to wild boars, deer or rabbits, even more when some of this meat can be commercialised in private settings, without passing though approved establishments where HACCP hygiene controls are adopted.

Environmental contamination is documented but it is unclear to what extent HEV can be transmitted via inadequately treated waste water, run off or faeces of wild animals. The pathobiology of the virus in animal species other than pigs, particularly wild boar and deer is not described and would also be of additional value to know how long wild life species are shedding the virus, to be able to assess their contribution to the environmental contamination. One particular aspect that should be considered is the length of time the virus actually survives into the environment, depending on the different climate conditions.

Public health prevention strategies available to the wider population (Anonymous, 2016b), are currently rather limited due to insufficient knowledge of the zoonotic species involved in transmission, the time/temperatures combinations to inactivate the virus or the susceptibility of HEV to disinfectants. Public health education is also a key factor to prevent current and future transmission, lowering the human burden and work should be carried in the direction of raising awareness among the general public of the emergence of HEV, the risk factors in relation to animals, water or food supply and of the safety measures that can be adopted. As future data will become available, public health strategies can be reviewed and updated as necessary to reflect the new information.

3.7.3 What needs to be done?

Due to the relatively recent emergence of HEV as a pathogen of public health importance, much work remains to be done to cover the gaps in knowledge. Recent emergence of genotype 4 into small clusters of human patients and pigs prompt us to keep monitoring animal species to identify possible further geographical spread or spill over to other susceptible animal hosts. Recognition of new different genotypes and subtypes pose further concerns as these might have different transmission routes, pathogenicity, animal reservoirs and capacity for cross-species transmission, thus epidemiological studies should be continued.

Considering the complex transmission pathways between pigs, wild boars, deer, rabbits and humans it is important to investigate in a more coordinated manner the potential public health risk from all the susceptible animal species. Analysing the animal-virus relationship will potentially elucidate the role played by each of the species in zoonotic transmission and the levels of HEV contamination of food supply chain. This in turn will help establish the extent of the issue for risk management of virus transmission.

Recent knowledge of wild life and farmed game should be used to redesign the monitoring and surveillance of HEV. Consistent and multiple sample collection, including faeces in addition to blood, liver and bile can bring additional molecular information. Previous observations of cross-transmission of HEV between the different species with zoonotic potential should be strategically integrated into study design with sampling collection prioritised from those regions where there is interaction between domestic pigs, wild boar, deer and ultimately rabbits. In the light of recent evidence showing there is shared sequence identity between rabbit and human strain, the zoonotic potential of rabbits must not be disregarded by future epidemiological studies. Finally, to be able to critically look at the different animal sources and at the food chain to evaluate the risk associated with zoonotic transmission, the HEV dose-response infectivity must be described, but in the absence of an effective culture system it seems difficult to find the appropriate answer.

Challenges of choosing the appropriate testing protocol for HEV RNA testing from different animal species might be addressed by the recently available HEV RNA standard. A comparison of already available species-independent molecular diagnostic tests against a set of samples from various animal species, using the HEV RNA as positive control, could help the research community to choose the most accurate test. However, ideally in near future, a validated, species-independent molecular technique will become available. This would enhance the value of surveillance by obtaining consistent molecular epidemiological data in different geographical locations which in turn will help understanding the sources of HEV infection.

Further work is also needed to establish how effective are the usual processing procedures for uncooked or ethnic products at inactivating pathogens such as HEV, to prevent spread through the food chain. Liver, blood, meat, intestines are notoriously described as food with risk for transmission of HEV and they can be consumed in separation or all together in one product such as sausages. It is important to know how safe these products are. Processing such as salting, light smoking, air drying are sometimes perpetuates for taste rather than as a necessity for preservation or inactivating pathogens. Can we continue using these techniques in future?

The complex and the numerous knowledge gaps remained to be covered suggest that HEV transmission and prevention from animal species cannot be dealt with distinguishably but extensive collaboration and integration of findings over different professional fields are required, resulting in a one health holistic approach.

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4. *Campylobacter* spp. carriage in wild game pheasants (*Phasianus colchicus*) in Scotland and its relevance to public health

Alessandro Seguino[1*] and Cosmin Chintoan-Uta[2]
[1]Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, United Kingdom; alex.seguino@ed.ac.uk
[2]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, United Kingdom

Summary

Campylobacteriosis in humans due to *Campylobacter jejuni* and *Campylobacter coli* is the most common food-borne bacterial diarrheal disease worldwide. Control measures currently focus on the reduction of *Campylobacter* in chickens, as 60-80% of human cases can be attributed to the poultry reservoir as a whole. However, *C. jejuni* and *C. coli* have also been reported in a range of livestock and wildlife species, including pheasants. Pheasants reach the consumer's table as a by-product of the shooting industry. Approximately 3.5 million game birds are shot in Scotland every year; however, only 700,000 are received at Scottish approved game handling establishments (AGHEs) for veterinary inspection. Despite this volume of wild game entering the food chain, there is a lack of information concerning the risk of campylobacteriosis in humans arising from consumption of wild game meat and the role wild game birds may have as a reservoir of infection. We carried out a study to determine the prevalence of *Campylobacter* in wild game pheasants processed in AGHEs in Scotland, identify the main sequence types (ST) present and evaluate their impact on public health. *Campylobacter* was found to be present in nearly 40% of the sampled birds and *C. coli* was found in higher numbers compared to *C. jejuni*. Positive caecal samples were subjected to a multiplex PCR for species identification and High Throughput Multi Locus Sequence Typing (HiMLST). The STs obtained in this study were compared to those available in PubMLST in order to assess the risk to public health and identify potential patterns of cross-transmission among livestock and pheasants. Samples that were successfully typed by HiMLST were also tested for phenotypic susceptibility to ciprofloxacin, erythromycin and tetracycline using the EUCAST disc diffusion method. The prevalence of antimicrobial resistance varied across sites, with one site being dominated by double or triple antibiotic resistance. The relevance to public health, in relation to the STs in which antimicrobial resistance was present, is currently under assessment. Our preliminary data suggest that wild game birds are a host for *Campylobacter* and a potential risk to humans through consumption of pheasant meat.

Keywords: *Campylobacter*, wild game meat, pheasants, antimicrobial resistance

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4.1 *Campylobacter* – general aspects

Campylobacter species are commonly found in nature and can contaminate drinking water but are more often associated with warm-blooded animals as commensal gastrointestinal organisms in livestock, domestic and wild animals or as pathogens in humans (EFSA, 2012). The two species of primary importance to public health are *Campylobacter jejuni* and *Campylobacter coli*, responsible for over 95% of *Campylobacter* infections in humans (Park, 2002). *C. jejuni* and *C. coli* can readily contaminate various foodstuffs, including meat, raw milk and dairy products, and, less frequently, fish and fishery products, mussels and fresh vegetables. Among sporadic human cases, contact with live poultry, consumption of poultry meat, drinking water from untreated water sources, and contact with pets and other animals have been identified as the major sources of infections (EFSA, 2013). Cross-contamination during food preparation has also been described as an important transmission route. Raw milk and contaminated drinking water have been implicated in both small and large outbreaks (EFSA, 2013). Other *Campylobacter* species, such as *Campylobacter upsaliensis*, *Campylobacter sputorum*, *Campylobacter hyointestinalis* and *Campylobacter lari* are present in mammals and birds in the UK, but are not generally considered of public health importance (Defra, 2013). The infective dose of these bacteria is generally low (500-800 cfu) (Janssen *et al.*, 2008) and the average incubation period in humans ranges from one to seven days (Blaser *et al.*, 1987; Wood *et al.*, 1992). Patients may experience mild to severe symptoms, most common ones including watery (sometimes haemorrhagic) diarrhoea, abdominal pain, fever, headache and nausea (as reviewed by Humphrey *et al.*, 2007). Usually infections are self-limiting and last only between 5 and 7 days. Extra-intestinal infections or post-infection complications, such as reactive arthritis and neurological disorders, can also occur. *C. jejuni* has become the most commonly recognised antecedent cause of Guillain-Barré syndrome, a polio-like form of paralysis that can result in respiratory failure, severe neurological dysfunction and even death (as reviewed by Humphrey *et al.*, 2007).

4.2 Epidemiology of campylobacteriosis in the UK

4.2.1 Campylobacteriosis in humans in the UK

Foodborne disease in the UK affects about 1 million people with 19,000 hospitalisations and 500 deaths each year (FSA, 2011). A recently published study estimated the cost to the UK economy of human campylobacteriosis alone at approximately £50 million in 2008-2009 (Tam and O'Brien, 2016). *Campylobacter* spp. are the most commonly reported bacterial cause of infectious intestinal disease in the UK (Tam *et al.*, 2012). The Second Study of Infectious Intestinal Disease in the Community established that the ratio of unreported to reported human *Campylobacter* infections is one in nine cases (Tam *et al.*, 2012). In 2014, there were 70,323 laboratory-confirmed cases of campylobacteriosis (Defra, 2015), it could be extrapolated that the total number of cases was approximately 700,000 in the UK. During 2014 in Scotland, the Health Protection Scotland (HPS) reported 6,636 laboratory cases of *Campylobacter* in humans, an increase of 472 (7.7%) compared to 2013 and the highest number of *Campylobacter* cases reported in the past ten years (HPS, 2015). During the same year, in mainland Scotland, the annual incidence rates of *Campylobacter* ranged from 81.2 to 162.8 per 100,000 population (HPS, 2015). A characteristic of *Campylobacter* epidemiology in humans is its marked seasonality in the UK and other European countries, incidence peaks in late spring/early summer (FSA, 2009). In Scotland, the annual peak is in late June-early July (Miller et *al*., 2004). There are discordant reports in the literature describing a possible correlation between ambient temperature and number of human cases but the general consensus is that there may be a weak association (Humphrey *et al*., 2007). Strachan *et al.* (2009) reported that in the Northeast of Scotland *Campylobacter* infection in young children living in rural areas was greater than in urban areas and it was linked to the direct contact with farm animals and contaminated water rather than consumption of poultry meat (Strachan *et al*., 2009). In the same study, the foodborne route was considered to be of primary importance in *Campylobacter* infection in the adult population, more than contact with animals and water (Strachan *et al*., 2009). Nonetheless, the poultry reservoir as a whole is still the main source of infection, with up to 80% of cases attributed to it (EFSA, 2011). The peak in human cases has been related to the fluctuations in carriage in poultry and other food-producing animals. Some studies reported that *Campylobacter* carriage rates in broiler chicken flocks (Wallace *et al*., 1997) and dairy cattle (Stanley *et al*., 1998a) peak in the spring and late summer, in contrast to lamb and beef cattle where such marked seasonal variation in carriage rates have not been observed (Stanley *et al*., 1998b). However, available evidence does not consistently support this hypothesis and the seasonal variation in the level of infection in humans and poultry has been proposed to be associated with 'a common, but unidentified, environmental source' (Meldrum *et al*., 2005).

4.2.2 *Campylobacter* carriage in animals

Thermotolerant *Campylobacter* spp. are widespread in nature (Kwan *et al*., 2008). The principal reservoirs are the alimentary tract of wild and domesticated birds and mammals. These bacteria are prevalent in food-producing animals such as poultry, cattle, pigs and sheep, companion animals (including cats and dogs), wild birds and in environmental water sources (Humphrey *et al*., 2007). Animals acquire infection mainly through the faecal-oral route from the contaminated environment and rarely show signs of disease caused by these organisms (Blaser *et al*., 1980).

4.2.2.1 Campylobacter *infection in food producing animals*

C. jejuni and *C. coli* are commonly found in cattle, sheep and pigs (Boes *et al*., 2005; Nielsen, 2002; Payot *et al*., 2004; Stanley and Jones, 2003). Intestinal carriage of *Campylobacter* in cattle can range from 0.8 to 89% and in lambs can be as high as 91% (Stanley and Jones, 2003). Most cattle and sheep are reared in outdoor systems where there will be frequent contact with the external environment and they may become infected with *Campylobacter* in those circumstances (Schaffner *et al*., 2004); however, infection can also be sustained within the herd by cycling between individuals (Humphrey *et al*., 2007). In 2000, *Campylobacter* infection in poultry was estimated to reach 60% of broiler flocks slaughtered in the UK (Berrang *et al*., 2000). A more recent analysis of the EU baseline survey of the prevalence of *Campylobacter* in broiler batches estimated that the UK prevalence in broilers at slaughter (based on caecal contents) was 75.3% (EFSA, 2008). The most recent survey by the UK's Food Standards Agency (FSA) described a prevalence of 73% over a one year survey carried out in 2014-

2015 (FSA, 2015). The prevalence of *Campylobacter* carriage in poultry during the summer months can reach 100% within a flock (Defra, 2009). Poultry can contract infection from their environment, via contaminated water or following breaches of biosecurity (e.g. poor cleaning and disinfection of poultry houses). Infection in poultry is mainly through the oral-faecal route or via vertical transmission from parent flocks (Humphrey *et al*., 2007). Chickens that are reared under extensive (free-range) systems are more likely to be *Campylobacter*-positive than housed animals (Heuer *et al*., 2001).

4.2.2.2 Campylobacter *infection in wild game birds*

Wild game birds, and in particular pheasants and partridges, are commonly reared in outdoor farms and may be exposed to *Campylobacter* infection from the environment and/or at a later stage when they are released in the field where they may share their immediate surroundings with other livestock (Dampney, 2009; Heuer *et al*., 2001). In live wild game birds the presence and prevalence of *C. jejuni* and *C. coli* has been reported in pheasants from studies conducted in Germany, Russia, Italy and the Czech Republic (Atanassova and Ring, 1999; Dipineto *et al*., 2008a, 2009; Nebola *et al*., 2007; Stern *et al*., 2004). One study conducted on live healthy pheasants sampled on a pheasant farm in the South of Italy reported a prevalence of 43.3% (n=240) with *C. coli* and *C. jejuni* found in 100% and 13.5% of the positive samples taken, respectively (Dipineto *et al*., 2008a). In the same study, the prevalence was significantly higher in adult pheasants compared to younger pheasants. This finding is consistent with *Campylobacter* spp. infection in chickens where younger birds in the second to the fourth week of life are less likely to be affected (Newell and Fearnley, 2003; Shreeve *et al*., 2000). There was no significant gender difference (Dipineto *et al*., 2008a). In another study Dipineto *et al.* (2008b) reported a prevalence of 86.7% (n=60) from cloacal swabs of live farmed pheasants; all positive samples were identified as *C. coli* and 19.2% of positive samples were also positive for *C. jejuni*. A study from the Czech Republic reported isolation of *Campylobacter* spp. from 502 caecal samples collected from adult farmed and wild pheasants (n=302 farm, n=200 wild). The prevalence of *Campylobacter* spp. in the intestinal contents of pheasants from the farm was 70.2% with 50.5% of isolates identified as *C. coli* and 41.4% as *C. jejuni* (Nebola *et al*., 2007). Farmed pheasants had a higher prevalence than birds shot in the wild (27.5% of cases) and this was linked to the fact that samples from wild pheasants were not taken immediately after they had been shot, while the samples from farmed pheasants were gathered within two hours of their death (Nebola *et al*., 2007). *C. jejuni* was more prevalent (58.2%) than *C. coli* (36.4%) in the wild birds. On the other end, studies from Germany and Russia reported an estimated prevalence of *Campylobacter* spp. in wild pheasants of 26% and 25% respectively (Atanassova and Ring, 1999; Stern *et al*., 2004). There are no data available in the literature on *Campylobacter* spp. intestinal carriage in pheasants in the UK.

4.3 The game meat supply chain in the UK and microbiological hazards to public health

4.3.1 Wild pheasants and the shooting industry

Phasianus colchicus is the most common species of pheasant in the UK (Canning, 2005), however, they are not innate to Britain. As reported by Pennycott (2001): 'They originated in parts of Asia, such as the Himalayas, Manchuria, Korea, Vietnam and Japan. They were introduced to the British Isles in the distant past by the Romans or the Normans but were certainly present in Britain by the 14th century'. Pheasants like a habitat that includes woodland or copses and hedgerows. There is a resident population of approximatively 8 million wild pheasants in the UK across the whole of England, Scotland, Wales and Ireland, except for the far north and west of Scotland and on the very high ground in England and Wales (Dampney, 2009). Wild pheasant meat reaches the consumer's table as a by-product of the shooting industry. Shooting is a sport that is worth approximately £1.6 billion to the UK economy and it is estimated that 600,000 people are involved in the provision of sporting shooting in the UK (PACEC, 2006). The vast majority of the income from shooting is gained from the actual sporting activity, itself worth £240 million in Scotland (PACEC, 2006). The meat from wild game species is thus a by-product of the shooting industry and the value of game birds sold is minimal. The shooting industry focuses mainly on pheasants, partridges and grouse. Within the UK in 2004 approximately 15 million pheasants, 3.6 million pigeons, 2.6 million partridges, 970,000 ducks, 400,000 grouse, and 250,000 woodcock and snipe were shot. Within Scotland, up to 2 million pheasants, 500,000 pigeons, 370,000 partridges, 140,000 ducks, 200,000 grouse, and 37,500 woodcock and snipe are shot *per annum* (PACEC, 2006).

4.3.2 Pheasant management on farm

The pheasant-shooting season closes at the end of January and approximatively 15 million pheasants are shot for sport in the UK each year (PACEC, 2006). This number of birds cannot be provided by the population of wild pheasants, of which there are approximately three million breeding birds each spring (Gibbons *et al.*, 1993), so the numbers are supplemented by artificially reared pheasants. On average, four-fifths (83%) of all shooting providers rely on released pheasants or partridges (PACEC, 2006). It is a common practice for pheasant-rearing sites to catch pheasants from the wild each year in February and March and transfer the birds to static or moveable breeding pens (Anonymous, 1993). Breeding pheasants will lay eggs from early March and the last eggs are placed in incubators in the middle of June. Incubation lasts 24 days. Hatching commences in the first week of May and finishes in the first week of July. Chicks will be transferred to brooder houses that provide heat, light and ventilation in controlled conditions. Heat is gradually reduced and space increased as the birds grow so that, by the time their feathers have developed, the birds can be given access to outside runs and become acclimatised to the outdoors. At 3 to 4 months old they will be mature enough to be released in the field until the shooting season starts at the beginning of October. Some gamekeepers by-pass this stage by buying birds at 6-8 weeks of age so they can be placed in outdoor release pens immediately. Others will buy at day-old and rear on. 15 million pheasants and 6.5 million partridges were reared and released for shooting in 2004 in the UK (PACEC, 2006).

4.3.3 Pheasant game meat processing and supply chain

The hunting season for pheasants in England, Scotland and Wales extends from October 1st to February 1st. Pheasant carcasses from large shoots are usually collected in larders and then given to game dealers, consumed locally or sent to approved game handling establishments (AGHEs). The EU Hygiene Regulations require that wild game meat for human consumption must be supplied to AGHEs and subjected to veterinary inspection (EC, 2004b); however, some derogations to the legislation are in force in the UK that allows pheasants from small shoots to be consumed locally by hunters, beaters and local householders, including restaurants, butcher shops and pubs. Retailers that operate on a national level (e.g. supermarkets or restaurants chains) can only source their game from AGHEs. Similarly, game bird carcasses in feathers or 'oven-ready' for the export market can only be sourced from AGHEs. A schematic representation of the pheasant game meat supply chain is shown in Figure 4.1.

Pheasant carcasses delivered to AGHEs are stored in batches in intake chillers at a temperature below 4 °C, waiting to be processed. The first step of the process is dry feather-plucking. Scalding of carcasses; as used for broilers in poultry abattoirs, is not common practice for small wild game; however, some AGHEs may immerse pheasants in hot wax after dry plucking to facilitate the removal of feathers. At this stage, damaged carcasses will proceed for breast and thigh meat removal and the rest of the carcass will be discarded. Well-presented carcasses will usually be manually eviscerated and then packed as 'oven-ready' product. Breast and thigh meat will be vacuum-packed in portions of different sizes according to customer

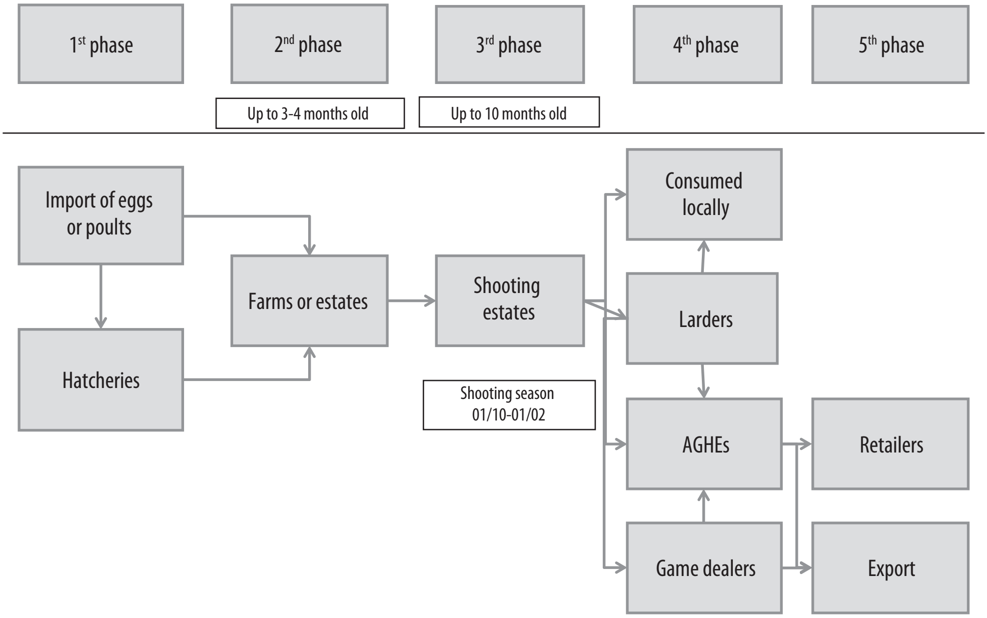

1st phase
2nd phase
3rd phase
4th phase
5th phase
Up to 3-4 months old
Up to 10 months old
Import of eggs or poults
Hatcheries
Farms or estates
Shooting estates
Shooting season 01/10-01/02
Consumed locally
Larders
AGHEs
Game dealers
Retailers
Export

Figure 4.1. Schematic representation of the pheasant game meat supply chain.

specifications. 'Oven-ready' products and breast meat can be stored in refrigerated conditions below 4 °C or frozen to extend their shelf life.

4.3.4 Official controls in approved game handling establishments and identification of microbiological hazards in wild game meat related to public health

EU food hygiene legislation is applied across the UK and has been implemented in Scotland by the Food Hygiene (Scotland) Regulations 2006 that came into force on 11 January 2006 (SSI, 2006). The legislation considers the hunting of wild game as primary production but private domestic consumption of wild game is unregulated (EC, 2004a). However, the EU Hygiene Regulations also require that wild game meat for human consumption must be supplied to AGHEs for veterinary inspection and must be passed fit for human consumption (EC, 2004b). The British Association for Shooting and Conservation (BASC) estimates that 90% of the wild game birds shot in Scotland are supplied directly from the shooting estates to the AGHEs (SRUC, 2012). However, for the shooting season 2010/2011, the Meat Hygiene Service (former executive agency of the FSA) recorded a throughput of small game birds and ground game by Scottish AGHEs of approximately 700,000 (SRUC, 2012) which is actually a much smaller proportion (approximatively one fifth) of the estimated 3.5 million game birds shot annually. Game meat can be offered directly to consumers without veterinary inspection because of extensive derogations from the EC Hygiene Regulations that have been applied in the UK allowing the direct supply of game meat from hunters or retail outlets to the final consumer (SRUC, 2012). However, concerns are raised by the fact that more than two thirds of game bird meat reaches the consumer's table without veterinary inspection and without being passed fit for human consumption. A Veterinary Laboratory Agency report on a qualitative risk assessment of wild game meat published in 2003 (VLA, 2003) stated that *post mortem* veterinary inspection in small wild game does not have any additional beneficial effect in identifying foodborne diseases and an effective hazard analysis and critical control point (HACCP) system should be able to detect and discard unfit meat. *Salmonella* spp., *C. jejuni* and *Escherichia coli* O157:H7 were considered the most important zoonotic hazards from small wild game species and they can pass undetected at veterinary inspection in AGHEs because they may not produce visible lesions in the carcass (VLA, 2003). The risk associated with these pathogens in small wild game meat inspected at AGHEs is summarized in Table 4.1 (Coburn *et al.*, 2005; VLA, 2003). *Campylobacter* was the pathogen that was considered to be of greater risk to public health from game bird meat.

4.4 Current status of knowledge on *Campylobacter* infection risk posed by pheasants in the UK

Despite approximately 700,000 wild game birds being consumed annually in the UK, there are no published data concerning the risk of campylobacteriosis in humans. As pheasants are the most widely consumed wild bird game meat (PACEC, 2006), we carried out a small pilot study to assess the risk posed by consumption of pheasant meat to public health. The study's main aim was to determine the prevalence of *Campylobacter* spp. in wild game pheasants processed in AGHEs in Scotland and to identify the species and sequence types (STs) of *Campylobacter* isolated from pheasants. Ongoing analysis of the data aims to provide

Table 4.1. Summary of hazards and risk associated with small wild game species.

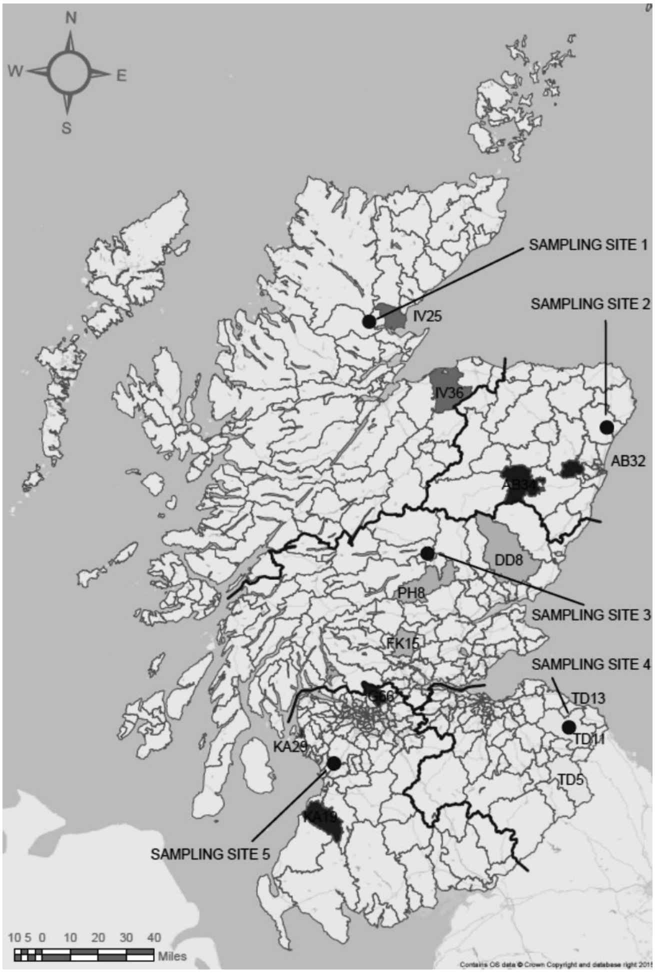
Hazard	Risk	Comments
Campylobacter spp.	Moderate: high prevalence of *Campylobacter* spp. in wild game meat	Survives well at refrigeration. Very susceptible to cooking temperature of 70 °C for a minimum of 2 minutes.
Salmonella spp.	Low: there is a low prevalence of *Salmonella* spp. in wild game meat	The absence of *Salmonella* in small wild game meat is not unusual and reported by other authors (Paulsen *et al.*, 2008). Susceptible to cooking temperature of 70 °C for a minimum of 2 minutes.
Escherichia coli O157:H7	Low: prevalence is considered low	Susceptible to cooking temperature of 70 °C for a minimum of 2 minutes.

a preliminary assessment of the relation of the STs of *Campylobacter* spp. from pheasants to those commonly present in humans and broilers in order to evaluate the potential risk to public health posed by pheasant game meat. Lastly, disc diffusion testing for susceptibility to ciprofloxacin, erythromycin and tetracycline identified the presence of antimicrobial resistance (AMR), including multiple resistant strains.

4.4.1 Preliminary study on *Campylobacter* carriage in wild game pheasants

Caecal samples were collected from pheasant carcasses in selected AGHEs in Scotland during the hunting season 2013/2014. The FSA records 11 AGHEs in Scotland that process small wild game (SRUC, 2012). Based on this information Scotland was divided into five geographical regions and a sampling site was selected in each region (Figure 4.2). The sampling site selection was made based on the size of the business, the ability to receive pheasants consistently during the hunting season and the capacity to receive birds from several estates within the catchment area.

In order to ensure a sufficiently large sample size to allow meaningful conclusions, a simple random sampling estimate was used to determine the sample size. The method was proposed by Thrusfield (2005) for a large, theoretically infinite population. This was used, as the pheasant population in Scotland is approximatively 2 million (PACEC, 2006). Assuming an expected prevalence of 25% in wild pheasants, inferred from relevant literature (Atanassova and Ring, 1999; Nebola *et al.*, 2007; Stern *et al.*, 2004), and a desired confidence level of 95% with an absolute precision of 5%, it was necessary to sample 58 birds per region. The total number of pheasants sampled for this project was 287.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SAMPLING SITE 1
IV25
SAMPLING SITE 2
IV36
AB32
AB34
DD8
PH8
SAMPLING SITE 3
FK15
SAMPLING SITE 4
TD13
G66
TD11
KA29
TD5
KA19
SAMPLING SITE 5
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Figure 4.2. The five Scottish regions for sampling are delimitated by a dark line, the location of the five sampling sites is identified by a black dot and estate sampled are highlighted (IV = Inverness, AB = Aberdeenshire, FK = Falkirk, PH = Perth, DD = Dundee, TD = Galashiels, G = Glasgow and KA = Kilmarnock).

4.4.2 Prevalence and level of carriage of *Campylobacter* in pheasants

4.4.2.1 Prevalence of Campylobacter *in wild game pheasants*

The survey indicated an overall prevalence of infection in pheasants of 38% (based on 287 birds). This is in line with previously reported prevalence levels, which were based on analyses of caecal content in hunted wild pheasants elsewhere in Europe, levels that ranged from 25 to 44% (Atanassova and Ring, 1999; Nebola *et al.*, 2007; Stern *et al.*, 2004). Prevalence was not uniform across the regions and it was significantly lower in one of the regions (data not shown). If this region were to be excluded the overall prevalence of infection would be 46%.

The lower prevalence in this region may be due to the time of collection as samples were collected at the nadir of *Campylobacter* carriage in poultry and of human cases or a genuine low prevalence of infection in this area.

The prevalence in this study is lower compared to farmed pheasants slaughtered on farms reported by Nebola *et al.* (2007), where the prevalence was as high as 70%. It is also lower than previous studies based on cloacal swabs from live birds where the prevalence reached almost 90% (Dipineto *et al.*, 2008b). However, these can only be taken as general indicators of possible trends as these studies were conducted in other countries. The discrepancy in prevalence between farmed and hunted pheasants has been attributed to the fact that samples from farmed pheasants are generally processed more quickly (Nebola *et al.*, 2007). In previous studies, while a kill-to-process time was not always specified, wild pheasant samples were not taken immediately after shooting, potentially compromising *Campylobacter* survival in the caeca (Nebola *et al.*, 2007). In this study, the time from shooting in the field to processing in the laboratory varied from two to seven days but there was no statistical relationship between the bacterial load in positive caecal samples and the kill-to-process time. If possible, further studies should include a comparison of samples taken immediately after shooting of the birds in order to determine whether cold storage of carcasses may have resulted in an underestimation of the prevalence and carriage levels. Even though no statistical differences between time of collection were detected in this study, it is possible that the majority of *Campylobacter* die off quickly, after which the bacteria that are able to survive the new conditions within the carcass are relatively stable. This pattern of bacterial loss was observed in chicken caeca stored at 4 °C from studies carried out in our labs (data not shown).

4.4.2.2 Level of Campylobacter *carriage in wild game pheasants*

The average bacterial load of positive samples (n=108) was 5.8×10^6 cfu/g (6.7 $\log_{10}$/g) and this was broadly in line with the bacterial load of extensively reared British-based poultry flocks surveyed in 2011 (Allen *et al.*, 2011). This study herein also found no difference in bacterial carriage means across and between Scottish regions and estates, even in the region where prevalence of infection was very low.

Although these results relate to caecal and not faecal content, they indicate a potentially high level of shedding of *Campylobacter* in the environment, which may increase the risk of infection to other pheasants and to humans. High levels of shedding increase the risk to the consumer through higher meat contamination during evisceration at AGHEs, while employees of pheasant farms may also be at increased risk through exposure to pheasant faeces.

4.4.3 Species of *Campylobacter* present

The results of a multiplex PCR, using the primers obtained from the PubMLST website http://pubmlst.org/campylobacter/info/primers.shtml, identified a dominance of *C. coli* (63%) over *C. jejuni* (37%) in the caecal contents of pheasants in Scotland. In a survey in Italy, Dipineto (2008a) reported that 100% (n=104) of cloacal swab isolates subjected to PCR were positive for *C. coli*, with 13.5% also positive for *C. jejuni*. In contrast, Nebola *et al.* (2007) reported that *C. jejuni* was more prevalent (n=54: 58%) than *C. coli* (36%) in wild pheasants in the Czech Republic, with mixed infection in 5% of birds examined. However, the same study also reported that in farmed pheasants (n=211) 51% of isolated strains were *C. coli* and 41% were *C. jejuni*. This discrepancy in results may reflect the varying sources of infection to which pheasants on different estates or farms are exposed. Cattle, sheep and chickens are major reservoirs and shedders of *Campylobacter* spp. and they are associated with different *Campylobacter* spp. (Sheppard *et al.*, 2009). *C. coli* was more widespread than *C. jejuni* in 4 out of 5 Scottish regions surveyed. In our study, statistical analysis confirmed that, across regions, there was a significant difference in prevalence of *C. coli and C. jejuni*, with the same pattern being evident at estate level (data not shown).

The results suggest that pheasants are either more susceptible to *C. coli* colonisation for yet unknown reasons or that there may be more contact between pheasants and ruminants than chickens. A larger scale study undertaken in Scotland in humans and livestock reported that cattle and sheep isolates were more likely to be genetically similar if they originated within rather than between farms (Rotariu, 2009). It has been shown that within-farm transmission is an important way of sustaining infection between individual animals (Humphrey *et al.*, 2007). This suggests that interactions with cattle and sheep may lead to introduction of *C. coli* on pheasant farms and that, following an initial introduction event, recycling of *Campylobacter* spp. within the farm or estate may lead to dominance of a particular species or strain on the farm. However, elucidation of which one of these mechanisms leads to dominance of *C. coli* in pheasants in Scotland would require experimental competitive infection under controlled conditions in pheasants. Further characterisation of the pattern of cross transmission between livestock and pheasants through a larger study that concurrently samples from livestock and pheasants would also help to clarify these aspects and would aid the development of control strategies.

4.4.4 Sequence types present and the risk to public health

The samples that were positive for *Campylobacter* were analysed by HiMLST as described by Boers *et al.* (2012), using the primers available on the PubMLST website. This identified the presence of STs in pheasants that are commonly isolated in clinical human infections. Amongst *C. coli*, ST 828 was isolated most often while amongst *C. jejuni* ST 19 was the most commonly isolated one. Both these STs are common human clinical isolates suggesting that *Campylobacter* strains carried by pheasants have the ability to infect humans. While this is an indication that consumption of pheasant meat may pose a risk to human health concerning campylobacteriosis, finer characterisation of this risk will be achieved through our current source attribution analysis of human infections that includes data collated from PubMLST.

4.4.5 Presence of antimicrobial resistance

The strains that were recovered during this study were subjected to antimicrobial sensitivity testing using the disc diffusion method described by EUCAST. The strains were tested for susceptibility to erythromycin, ciprofloxacin and tetracycline. There are the representative compounds recommended by EUCAST for testing susceptibility to the macrolides, fluoroquinolones and the tetracycline class of antibiotics. These three classes are the main classes used currently for treatment of campylobacteriosis in humans (WHO, 2011). Even though banned for use as growth promoters in food producing animals in the EU, antibiotics are still used as growth promoters in other countries. Quinolones are commonly used for this purpose in poultry and their use has been associated with an increase in AMR in both *C. jejuni* and *C. coli* in poultry and a subsequent rise in AMR campylobacteriosis in humans (Nelson *et al.*, 2007).

In our study when measured across the entire population sampled, we identified the presence of resistance to at least one antibiotic in 42% of the pheasants that carried *Campylobacter*. However, at individual estate level, the prevalence of AMR varied from 22% to 89%. More worryingly, the estate that had the highest level of antimicrobial resistance also had the highest level of multiple drug resistance (MDR), with 67% of samples having double antimicrobial resistance and 11% having triple antimicrobial resistance.

The risk of AMR *Campylobacter* infection posed by pheasants may be high within certain geographical areas. The high prevalence of AMR and MDR in certain establishments may be due to the status of some of the pheasant rearing establishments that allows them to continue the prophylactic use of antibiotics even in the UK. However, this data should be interpreted with caution as our analysis of the STs in which antimicrobial resistance was present and hence their risk of transmission to humans is ongoing.

4.4.6 Risk of campylobacteriosis posed to humans by pheasants

The vast majority of human campylobacteriosis cases are associated with *C. jejuni* and this tends to give an indication that pheasants may pose a lower risk for transmission of *Campylobacter* infection to humans. Compared to other sources of infection, a number of other factors are likely to also contribute to a potential lower risk of transmission of *Campylobacter* infection to humans from pheasants and they may comprise:

- A low level of human exposure to live pheasants and their meat products. This is due to the pheasant rearing industry being of a much smaller scale than the poultry industry and, likewise, a much lower level of pheasant meat consumption compared to poultry meat (estimated at 5 g per person per year in the UK).
- Although this study demonstrated that *Campylobacter* can survive at high counts up to seven days from the date of kill in pheasant caecal content, the chances of contamination of meat during processing and survival of *Campylobacter* spp. on pheasant meat could be reduced by applying strict HACCP controls on hygienic production (ACMSF, 2005; VLA, 2003) and a tight control of the cold chain since dry and cold conditions are deleterious to *Campylobacter* survival.
- Availability of pheasant meat to consumers mainly in wintertime as the hunting season runs October to February. This period includes the nadir in human campylobacteriosis cases and poultry carriage. Commonly, the notification rates in these months is decreasing or very low, giving an indication that the higher consumption of pheasant meat in these months, is not likely to contribute significantly to *Campylobacter* infection in humans.
- Availability of pheasant meat all year around is generally restricted to stored frozen products. There is evidence in the literature that freezing is detrimental to *Campylobacter* survival in food (Harrison *et al.*, 2013) and as such, the risk to public health from frozen

pheasant products is likely to be reduced. Regardless, consumers and public catering establishments should always be advised to cook meat thoroughly, in order to prevent any risk of infection through food.

Taking into consideration these aspects, the risk to public health from live pheasants and pheasant meat at the present time can be considered to be low compared to other sources. However, the high prevalence of AMR on some pheasant rearing establishments may pose an increased risk of transmission of *Campylobacter* antibiotic resistance to humans and to other farm animals. Further studies are required to better define the risk posed by these AMR strains to humans as they have the potential for a major impact on a local scale.

4.5 Conclusions

4.5.1 What has been achieved?

The current study represents, to our knowledge, the first survey of *Campylobacter* in wild game pheasants in the UK and suggests that consumption of this game meat may have the potential to transmit *Campylobacter* to humans. However, larger scale studies are required to quantify this risk. While the data suggest the possibility of cross-transmission of *C. coli* strains between pheasants and ruminants, further work involving longitudinal sampling from the same AGHEs and concurrent sampling from livestock would give a better characterisation of the direction and extent of this transmission. The study we undertook was limited to Scotland and similar studies in other regions of the UK would be desirable in order to establish whether the same pattern of *Campylobacter* infection in pheasants is present in those regions.

4.5.2 What has been neglected?

As antimicrobials are still used on some pheasant rearing establishments, the risk of transmitting AMR campylobacteriosis to humans may be higher compared to that from livestock. Our preliminary study identified AMR in *Campylobacter* in pheasants but larger scale studies using more powerful molecular methods would allow the tracing of development and transmission of AMR within pheasant flocks and transmission of AMR strains present in pheasants to humans following consumption of this game meat.

4.5.3 What needs to be done?

Larger follow-up studies could be undertaken to further characterise the risk posed by pheasants to public health and to determine the transmission of this pathogens amongst livestock and pheasants. This could be achieved by larger scale concurrent sampling of pheasants and livestock such chickens, cattle, sheep and pigs in order to allow inferences to be made on the direction and extent of transmission of this pathogen among different hosts. Such inferences could be drawn using molecular epidemiology methods, e.g. HiMLST and phylogenetic analyses. The elucidation of these aspects would allow targeted methods of control to be devised. Should wild pheasants be able to act as carriers of *Campylobacter* between livestock farms, appropriate control measures could be put in place. On the other hand, pheasants may prove to play a minor role in such cross-transmission, with wild flying birds that may more easily move across farms playing a larger role.

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5. Filarioid nematodes, threat to arctic food safety and security

Sauli Laaksonen[1,2*], Antti Oksanen[3], Susan Kutz[4,5], Pikka Jokelainen[1,2,6,7], Anniina Holma-Suutari[2] and Eric Hoberg[8]
[1]Faculty of Veterinary Medicine, University of Helsinki (FINPAR), P.O. Box 66, 00014 University of Helsinki, Helsinki, Finland; hirvi54@gmail.com
[2]Wazama Media Ltd, Nurminiementie 2, 93600 Kuusamo, Finland
[3]Finnish Food Safety Authority Evira (FINPAR), Elektroniikkatie 3, 90590 Oulu, Finland
[4]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Drive NW, Calgary, AB, T2N 4Z6, Canada
[5]Canadian Wildlife Health Cooperative, Alberta Node, Faculty of Veterinary Medicine, University of Calgary, 11877 85th Street NW, Calgary, AB, T3R 1J3, Canada
[6]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia
[7]Department of Microbiology and Infection Control, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark
[8]US National Parasite Collection, ARS, USDA, Animal Parasitic Diseases Laboratory, BARC East 1180, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

Summary

The nematodes *Setaria tundra*, *Onchocerca* spp. and *Rumenfilaria andersoni* appear to have emerged in Fenno-Scandinavian reindeer during the latter half of the 20th century, associated with microfilaraemia, peritonitis, necrotic granulomas and tarsitis. Filarioid nematode faunas now recognized in Fennoscandia are a mosaic assembled from disparate sources, over extended but recent time frames, through anthropogenic introduction, establishment and processes of environmentally driven geographic expansion. Geographic shifts linked to climate warming and host colonization to reindeer from sources in white-tailed deer, roe deer and red deer have structured this fauna, serving as precursors and drivers of emerging disease. Thousands of reindeer died in 1973 during emergence of *S. tundra* followed by recurrent outbreaks in 1989 among moose and in 2003-2006 and 2014 among reindeer leading to condemnation of carcasses during reindeer slaughter. Concurrently, chronic tarsitis and necrotic granulomas in liver and muscles, caused by *Onchocerca* spp., were increasingly common in reindeer and moose as revealed during meat inspection. In 2004-2006, previously unrecognized parasites were found in the lymphatic vessels of reindeer, and were identified, for the first time in Europe, as *R. andersoni* (Splendidofilariinae). In Finnish semi-domesticated reindeer, prevalence of *R. andersoni* was locally up to 95%. In moose, the observed prevalence was 10%, in wild forest reindeer 69%, white-tailed deer 15% and roe deer 3%. The impact of *R. andersoni* to cervid health and meat quality is unknown but visible changes were seen around lymphatic vessels. Our current data, including genetic comparisons of North American and Finnish isolates of *R. andersoni*, suggest this filarioid became established in Finland recently, coincidental with introduction of white-tailed deer from North America in 1935. As *R. andersoni* is found in all the four cervid species in Finland through host colonization,

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it can be anticipated to spread more extensively in Eurasia. Mosquitoes transmit *S. tundra* and black flies *Onchocerca* spp., whereas the vector of *R. andersoni* is unknown. Incremental and accelerating climate warming and extreme or ephemeral events of elevated temperature appear to interact to directly influence the overall limits on northern distribution and the potential for population amplification leading to disease emergence on local to regional scales for this nematode assemblage. We demonstrated that high mean summer temperatures exceeding 14 °C drive the emergence of disease outbreaks due to *S. tundra*, where morbidity manifests in the following summer, if conditions remain warm. This hypothesis was further supported in autumn of 2014 following 2 consecutive exceptionally warm summers, leading to the emergence of the most recent outbreaks of *S. tundra* and *Onchocerca* in Finnish reindeer. Although not zoonotic through meat consumption, Filarioids can cause significant morbidity, affecting the appearance, texture and quality of meat and organs, as well as impacting body condition, and in some cases, causing mortality. The consequent meat condemnation, and possible population level impacts (declines), have broader impacts on the food security for northern aboriginal people who depend on wild reindeer and caribou for food and income.

Keywords: climate change, Arctic, food safety, *Setaria tundra*, *Rumenfilaria andersoni*, *Onchocerca*, reindeer, cervids

5.1 Introduction

Worldwide, filarioses represent major health hazards with important medical, veterinary and economic implications (WHO, 2007). There is recent evidence documenting the range expansion of filarioid parasites of free-ranging ungulates to subarctic areas including Finland, along with an array of diseases associated with these nematode pathogens (Laaksonen *et al.*, 2007, 2009b). At northern latitudes species of several filarioid genera are known circulating among ungulate definitive hosts and various hematophagous insects as vectors (Laaksonen *et al.*, 2007). Each adult female filarioid worm produces thousands of larval stages, microfilariae (mf) daily (Nelson, 1966); for example, *Setaria labiatopapillosa* contains at least 50,000 (Nelson, 1966) and *Setaria tundra* over 200,000 mf (Nikander *et al.*, 2007) in the uterus. Microfilariae occur in the circulatory system or in the skin of an ungulate host where they are available to arthropod intermediate hosts (vectors) during blood meals; in the latter host, the microfilaria exsheathes, penetrates the gut wall, migrates to the haemocoel and develops to an infective stage. Vectors of different filarioid nematodes include most of the major arthropod groups known to feed on the blood of higher vertebrates, i.e. biting midges, blackflies, horse and deer flies, mosquitoes, lice, fleas, mites and ticks (Anderson, 2000).

In their typical spectrum of definitive hosts, most species of filarioid nematodes are often very well adapted and infections are well tolerated (Nelson, 1966). Specific pathology related to infections of filarioids is often related to the availability and timing or periodicity of infective stages in tissues (e.g. cutaneous lesions) or the circulatory system, maximizing the potential for transmission to arthropod vectors during blood-feeding (Anderson, 2000). Significant and debilitating disease in vertebrates is usually attributed to infections among incidental or suboptimal host species and from localities where multi-species assemblages occur in sympatry or in situations of invasion through geographic and host colonization leading to parasite translocation and introduction (e.g. Hoberg, 2010).

Filarioid nematodes and their impacts on wild and semi-domesticated cervid ruminants have been under intense interest since 2003, when there was a recognized outbreak of peritonitis in Finnish semi-domestic reindeer (*Rangifer tarandus tarandus*) caused by the nematode *S. tundra* (Solismaa *et al*., 2008). Over the past decade, emergence of 3 species/genera of filarioids, *S. tundra*, *Onchocerca* spp. and *Rumenfilaria andersoni*, have been observed among semi-domestic reindeer in the Fenno-Scandian reindeer-herding area. Occurrence of these pathogenic nematodes has been linked to a history of anthropogenic translocation or natural processes for invasion, with recent establishment and subsequent host colonization, now with northward geographic expansion in part related to accelerating climate warming and an increasingly permissive environmental setting (Laaksonen *et al*., 2015). Consequently, the contemporary fauna is a complex mosaic assembled from disparate sources through invasion and host colonization (e.g. Hoberg, 2010; Hoberg and Brooks, 2015; Hoberg *et al*., 2012). As an assemblage of vector-borne parasites, these filarioids cause the majority of condemnations of reindeer viscera during meat inspection and have the potential for disruption of food security with considerable socio-economic consequences (Laaksonen *et al*., unpublished data). Rehbinder *et al*. (1975) concluded that as the liver lesions and the peritonitis seem to appear concurrent with severe infections of *Onchocerca* sp. and *S. tundra* the possibility of a common biological and environmental connection cannot be dismissed. We explore the history of the filarioid fauna in Finnish semi-domestic reindeer and associated cervids (wild forest reindeer, *Rangifer tarandus fennicus*; moose or Eurasian elk, *Alces alces*; roe deer, *Capreolus capreolus*; red deer, *Cervus elaphus*, and white-tailed deer, *Odocoileus virginianus*) with respect to origins, biology, current host spectrum, and environmental determinants of distribution and disease emergence, as factors which influence our understanding of current and anticipated impacts to animal health, food safety and food security.

5.2 *Setaria tundra*

The subfamily Setariinae (Onchocercidae) includes 43 species that are normally found in the abdominal cavity of artiodactyls (especially Bovidae and Cervidae), equines and hyracoids (an endemic African mammalian group most closely related to elephants). Known vectors are haematophagous insects. Rather little is known about the routes of infection and migration for different species of *Setaria* in the definitive host (Anderson, 2000). *S. tundra* was first described in semi-domesticated reindeer from the Arkhangelsk region of Russia in 1928. It has also been reported in reindeer from the Baikal region (1980), and in roe deer from Germany (1975, 2000), Bulgaria (1973), Italy (2003) Denmark (2011) and Poland (2013) (reviewed by Enemark *et al*., 2011; Kowal *et al*., 2013; Laaksonen *et al*., 2007). Outside of northern Eurasia, other species of *Setaria* have been reported in reindeer including *S. yehi* from Canada (Fruetel and Lankester, 1989) and *S. labiatopapillosa* from China (Wang *et al*., 1989).

In 2003, an outbreak of parasitic peritonitis, caused by *S. tundra*, emerged in the Finnish reindeer population (Laaksonen *et al*., 2007, 2009b). This outbreak was the third to have been documented, following events in 1973 among reindeer and 1989 among moose. In the outbreak extending across 2003-2006 the prevalence and level of infection were very high in the calves and caused substantial economic losses to the reindeer herders in the region (Laaksonen *et al.*, 2007, 2009b). The most recent outbreak occurred during 2014, as the previous ones following 2 consecutive summers of elevated temperatures (Laaksonen *et al.*, unpublished data; Figure 5.1).

A perspective for changing patterns of infection and geographic distribution is evident subsequent to the initial discovery of *S. tundra* in Fennoscandia. During reindeer health monitoring in autumn 2015, following a cold summer, 4% of adult Finnish reindeer were carriers of *S. tundra* (Laaksonen *et al.*, unpublished data) based on the occurrence of microfilariae attributed to this species (smf). In contrast, during the height of the outbreak in 2004, prevalence based on smf was 35%. Earlier in 1997, prior to an apparent history of geographic expansion and emergent disease, smf were present at low density in 4% of samples, with infected definitive hosts being relatively localized in the southern part of the Finnish reindeer herding area (Laaksonen *et al.*, 2009b).

5.2.1 *Setaria tundra* and meat hygiene

Infections and disease have considerable consequences for the reindeer herding industry in Finland. The serofibrinous peritonitis outbreak was noticed by meat inspecting veterinarians in all the Finnish slaughterhouses handling reindeer originating from the southern and middle parts of the reindeer herding area in 2003-2004. The situation led to a marked increase of visceral organs being condemned in the southern parts of the Finnish reindeer husbandry area. The increase of parasite scars had clearly started already a few years earlier. In the season of 2004-2005, the outbreak appears to have somewhat settled down in Kuusamo area while expanding to the north and west into the South and Middle Lapland; the northernmost areas of Lapland remained free of peritonitis and liver lesions during this time frame (Laaksonen *et al.*, 2007).

In the endemic zone of the outbreak, meat inspection veterinarians reported that a high number of peritoneal and abdominal muscles of infected reindeer had to be discarded or

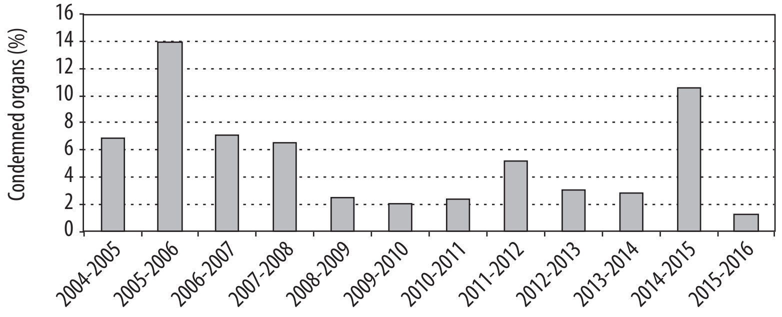

Figure 5.1. Reindeer viscera (%) condemned in Finnish reindeer abattoirs in 2004-2016 because of changes caused by Setaria tundra *(Laaksonen* et al.*, unpublished data).*

cleaned. Usually, no specific bacterial growth was present in ascites fluid, affected tissues, and organs or in muscles. *Corynebacteria* were not detected in any parasitic lesions or granulomas, in contrast to prior reports from Sweden (Rehbinder *et al.*, 1975). Even heavy *Setaria* infections seem to have had minimal influence on meat pH value and organoleptic evaluation in autumn and early winter. Thus, there is no reason to limit the normal use of meat-products in the human food chain or to heat treat carcasses as a precautionary measure, if there are no other contributory factors influencing food safety. Generally, in reindeer meat inspection, the removal of affected parts of the carcass and visceral organs are adequate measures but directly increase handling and processing time (Table 5.1; Laaksonen *et al.*, 2007).

The slaughter weights, back fat index and body condition of reindeer calves were lower during the slaughter season of 2003-2004, contrasting with 2004-2005, following the culmination of the outbreak in southern areas (Laaksonen *et al.*, 2007). It was obvious that heavy *S. tundra* infection had a pronounced influence on the welfare of reindeer and thus may have an impact on the meat quality. The degree of the influence appeared to be dependent on the intensity of infection, and was comparable to the abundance of adult living worms in the abdominal cavity. In contrast to the observations made in Sweden in the 1970's (Rehbinder *et al.*, 1975), no connection between dead and encapsulated worms and the degree of peritonitis was detected.

The impact of microfilaraemia on reindeer health is still unknown and difficult to separate from the impact of adult worms. Reindeer as hosts for chronic *S. tundra* microfilariosis appeared to suffer, exhibiting inexplicable symptoms resembling those described in long-term microfilaraemia attributed to species of *Setaria* in buffalo (reviewed by Laaksonen *et al.*, 2009b).

It is evident that intensity of infection may vary during outbreaks and may influence potential patterns of mortality in reindeer hosts. For example, in 1973 tens of thousands of reindeer died in the mountain herds from the Northern part of the Finnish reindeer husbandry area.

Table 5.1. Number of reindeer carcasses inspected and visceral organs condemned or partly condemned in Oulu and Lapland Provinces in the years 2000-2004 (data from the Finnish Food Safety Authority, Oulu, Finland). Data listed by calendar year (not by reindeer herding season) (Laaksonen et al.*, 2007).*

Year	Province	Reindeer inspected	Viscerals condemned	%
2000	Oulu	6,685	314	4.7
	Lappi	52,072	740	1.4
2001	Oulu	6,562	319	4.9
	Lappi	44,107	746	1.7
2002	Oulu	6,431	985	15.3
	Lappi	58,927	3,519	6.0
2003	Oulu	5,703	2,285	40.1
	Lappi	69,070	2,828	4.1
2004	Oulu	5,765	2,691	53.3
	Lappi	69,446	15,057	21.7

The mortality was associated with severe peritonitis and abundant *Setaria* worms (Laaksonen *et al*., 2007). Peritonitis caused by *S. tundra* was in 1973 also seen in Swedish forest herds while mountain herds appeared unaffected. The presence of *S. tundra* in the body cavities appeared asymptomatic except for focal areas of mild chronic peritonitis. The infection could be heavy, exceeding 1000 pre-adult and 100 adult worms (Rehbinder *et al*., 1975). In Norway, there was 6.6% prevalence of *S. tundra* infection based on the changes found during meat inspection in Kautokeino during the slaughter season of 1976-1977 (Poppe, 1977), and 4% in 1978/1979 (Korbi, 1982). Due to peritonitis, hepatitis and perihepatitis caused by the worms, a high percentage of livers and adjacent tissues had to be condemned (Kummeneje, 1980).

The fact that there was no reported mortality in the outbreak during 2003-2005 may be due to good nutrition and husbandry. Perhaps also the intensive application of ivermectin as antiparasitic treatment limited mortality (Laaksonen *et al*., 2008). In the southern part of the Finnish reindeer herding area, virtually all the reindeer are corralled and supplementary feed is provided during winter months (Laaksonen *et al*., 2007).

5.2.2 Sylvatic *Setaria tundra*

There is a previous report of a peritonitis outbreak in moose from Finnish Lapland in 1989 associated with *Setaria* sp. (Nygren, 1990). The causative agent was subsequently identified as *S. tundra* (Laaksonen *et al*., 2009b). During the outbreak in 2003-2005, mild peritonitis was also reported in moose. Six 1.5-year-old moose had moderate perihepatitis and 1 to 3 encapsulated pre-adult *Setaria* worms on the liver surface. In 18 moose samples a mild *Setaria*-type peritonitis or perihepatitis was diagnosed at Finnish Food Safety Authority in Oulu. In two cases, immature *Setaria* worms were detected (Laaksonen *et al*., 2007).

Of the 34 wild forest reindeer shot, 21 (62%) were suffering from peritonitis, perihepatitis or granulomas diagnosed as changes associated with *Setaria*. One roe deer had an adult *Setaria* worm encapsulated on the surface of the liver and the two roe deer autopsied fresh in the field had living adult *S. tundra* (2 and 4 worms, respectively) in the abdominal cavity without signs of present or previous peritonitis. In introduced white-tailed deer, no changes indicating *Setaria* infection were found (Laaksonen *et al*., 2007).

5.2.3 Transmission dynamics of *Setaria tundra*

5.2.3.1 Microfilaria

Transmission and circulation and sustainability of infections of *S. tundra* are dependent on predictable interactions between ungulate definitive and arthropod intermediate hosts and the availability of infective microfilariae (mf). Each adult female filarioid worm produces thousands of larval stages, microfilariae daily (Anderson, 2000) and those of *S. tundra* may each contain over 200,000 smf in the uterus. Smf are sheathed and occur in the blood circulation of the hosts, where they are available to arthropod vectors (Nikander *et al*., 2007).

Parasite circulation and transmission may be influenced by the timing and seasonal prevalence and intensity of infection in different age classes of definitive hosts. The mean prevalence of smf was higher in reindeer calves (56%) than in adults (35%) in 2004. Corresponding numbers in 2006 were 54 and 28%. Also the smf density was higher in calves. These results are consistent with findings from reindeer meat inspection about the distribution of *S. tundra* infection during the same period; severe peritonitis was more common in calves (Laaksonen *et al.*, 2007). In an intensively monitored naturally infected reindeer group, the peak period of high microfilaraemia was from the beginning of June to mid-September 2004, with the mean number of 950 smf/ml blood (range 62-4,000). In autumn, the intensity of infection for smf began slowly to decrease. In January, three experimental reindeer were free of smf, whereas another three reindeer maintained low microfilaraemia into the beginning of next summer. However, according to our results, it is evident that even a low prevalence and density of *S. tundra* in definitive hosts can maintain the infection in the reindeer population (Laaksonen *et al.*, 2009b).

Although smf are present in reindeer blood throughout the year, microfilarial production of *S. tundra* and smf circulation tend to be most intense in midsummer, a few weeks after the calving season, the time when passive immunity in calves is lowest (Orro *et al.*, 2006). The peak period of microfilaraemia coincides with the annual cycle of hair loss and hair-coat shift in adult animals following the winter, and with the mass appearance of several blood-sucking insects, all features favouring transmission. Räkkä-time (the appearance of mass insect harassment) is a stressful period for reindeer, since they are in constant movement round the clock trying to avoid mosquitoes, black flies, horse flies, stable flies, horn flies, warble flies, throat bot flies, biting midges, and other harassing insects. In exercise assessments, moderate movement of reindeer increased the density of smf on average to 130% (Laaksonen *et al.*, 2009a). The prepatent period of *S. tundra*, according to the temporal monitoring of reindeer slaughter batches, is about 4 months and the life span of female parasites in the definitive host is at least 14 months, probably much longer (Laaksonen *et al.*, 2009b).

5.2.3.2 Vectors

The smf develop in the arthropod into the infective stage (L1-L3) through two moults. When the intermediate host is feeding again, larvae break out and enter the tissue of the definitive host (Laaksonen *et al.*, 2009a). Mosquitoes, particularly *Aedes* spp. and to a lesser extent *Anopheles* spp., have an important role in the transmission of *S. tundra* in Finland. The vast majority of mosquitoes active in midsummer in Finland are species of *Aedes* (Utrio, 1978). Although *Anopheles* mosquitoes are also present, and can serve as vectors for *S. tundra*, their epidemiologic significance in Finland is likely limited because of their life cycle parameters and low numbers compared to *Aedes* spp. in reindeer herding areas (Laaksonen *et al.*, 2009a). The role of *Anopheles* mosquitoes may be more important in temperate areas or may increase in Finland coincidental with climate change and shifting environments. We suggest that *S. tundra* is not a very vector-specific parasite, and this may enhance its ability to expand its geographic range. Also, adult female *Aedes* spp. are vigorous round-the-clock feeders that can be infected with many infective *S. tundra* larvae (up to 70). This high larval abundance may, however, decrease vector efficiency and increase vector mortality (Laaksonen *et al.*, 2009a).

5.3 *Onchocerca* spp.

Parasites in the genus *Onchocerca* infect humans, ruminants, camels, horses, suids, and canids, with effects ranging from relatively benign to debilitating. In contrast to *Setaria*, species of *Onchocerca* nematodes produce microfilariae in subcutaenous sites and adults are most often localized in nodular lesions. Transmission is dependent on black flies (Simuliidae) and biting midges (Ceratopogonidae) rather than mosquitoes (Anderson, 2000; Schulz-Key and Wenk, 1981).

In North America, there are two species of *Onchocerca* reported infecting cervids: *Onchocerca cervipedis* (McFrederick *et al*., 2013; Verocai *et al*., 2012), and a recently identified species in white tailed deer, and even greater species diversity is suspected (McFrederick *et al*., 2013). At least five species of onchocercids may infect Eurasian cervids, including *Onchocerca flexuosa*, *Onchocerca jakutensis*, *Onchocerca garmsi*, *Onchocerca skrjabini*, and *Cutifilaria wenki* (reviewed by Santín-Durán *et al*., 2001). The geographic range of *O. cervipedis* extends into subarctic regions of western North America, where the host range was recognized to include caribou (*Rangifer tarandus granti*) (Verocai *et al*., 2012).

Onchocercinae were recorded in the USSR by Nikolaevskii (1961) and Mitskevich (1967). In northern Finland, Lisitzin (1964) found a species of *Onchocerca* in a subcutaneous nodule in the muzzle of a reindeer. Rehbinder (1973) and Rehbinder *et al.* (1975) recorded a high frequency of subcutaneous nodules in reindeer containing an *Onchocerca* sp. which was later identified as *Onchocerca tarsicola* (Bain and Schulz-Key, 1974); *O. tarsicola* was later synonymized with *O. skrjabini* by Yagi *et al*. (1994). Bylund *et al*. (1981) found these worms prevalent in reindeer in Finnish Lapland.

Species of *Onchocerca* have generally been considered of low-pathogenic potential, as dead worms in the subcutaneous tissues usually become calcified and surrounded by dense fibrous tissue. However, whilst causing little damage, they may also act as a focus for bacteria and abscesses that may develop in onchocercal nodules (Nelson, 1966). Different species localize and develop in precise anatomic sites of predilection in the host (Morandi *et al*., 2011).

Species attributable to the Onchocercidae are primarily recognized in nodules in subcutaneous tissues (Santín-Durán *et al*., 2001). The nodules are typically found in subcutaneous tissues of the legs. *O. cervipedis* generally affects subcutaneous tissues of the hindquarters from the tibio-tarsal joint to hoof, and thus is more commonly known as 'legworm' or 'footworm' (Verocai *et al*., 2012). Histological examinations of red deer infected with subcutaneous filarioids (*O. cervipedis* reported as *Wehrdikmansia cervipedis* and *O. flexuosa*) revealed microfilariae in several of these nodules (Dyková, 1970). Lizitzin (1964) described numerous viviparous adult nematodes and larvae, tentatively identified as *Onchocerca*, possibly the first description of such a lesion in a reindeer. In reindeer the worms were most often found in flat swellings or nodules of connective tissue in membranes surrounding the tendons of the tibiotarsal and radiocarpal joints (Bylund *et al*., 1981).

It is obvious that species of onchocercids inflict pathological changes which lead to condemnation of infected parts of carcasses in meat inspection of cattle and reindeer

(Rehbinder and Nikander, 1999; Solismaa *et al.*, 2008). Granulomas attributed to *Onchocerca* have been abundant findings since 1970's, often connected with corynebacterial growth (Rehbinder *et al.*, 1975), and can be a portal for necrobacillosis. Rehbinder (1973) and Rehbinder *et al.* (1975) recorded a high frequency of subcutaneous and subperitoneal connective tissue nodules containing specimens of *Onchocerca* sp. in Sweden; broader involvement included lesions in other organs for example in the abdominal wall, diaphragm and rumen. It is obvious that in heavy infections, *O. tarsicola* can manifest as granulomatous nodules in most organs, especially in the liver (Rehbinder and Nordkvist, 1983).

In Finland, according to reindeer meat inspection data, pathology attributed to *Onchocerca*, and the frequency of condemned tissues and livers, has increased. Initially it was observed that tarsal joints, and specifically the area of calcaneus, was prominently swollen and haemorrhagic in almost every adult reindeer taken to slaughter in the southern reindeer herding area (A. Välimäki, personal communication). In 2003-2006, during the *Setaria* outbreak, tarsitis was very common, so that in some slaughter batches, almost all adult reindeer had visible changes in the tarsal joint but lesions were very seldom seen in calves. In 2004 prominent tarsitis, with thickening and haemorrhage of the joint capsules and synovial membranes, occurred in 90% of adult reindeer and in 10% of fawns in the same area. In the latter samples, histological examination of the membranes revealed thick layers of several dead, calcified and living nematodes (Laaksonen *et al.*, unpublished data).

In other regions of Europe, the highest prevalence of *O. flexuosa* was found in adult red deer (49%), followed by yearlings (19%) and fawns (3%) (Santín-Durán *et al.*, 2001). Greenish granulomatous nodules have also been often found between muscle fasciae and surrounding the hip joint, and have been observed in other organs, for example in the abdominal wall, diaphragm and rumen. In Finland, during the most recent *Setaria* outbreak in 2014, liver nodules and condemnations were also due to infections by *Onchocerca* (Figure 5.2; Finnish reindeer inspection data, Laaksonen *et al.*, unpublished data).

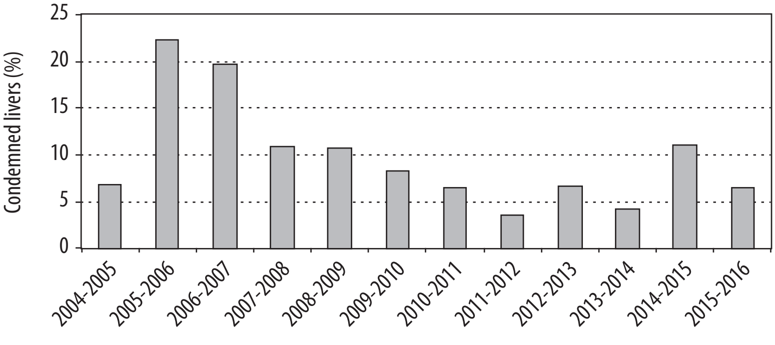

Figure 5.2. Reindeer livers (%) condemned in Finnish reindeer abattoirs in 2004-2016 because of necrotic granulomas caused by Onchocerca *spp. (Laaksonen* et al.*, unpublished data).*

The presence of *O. cervipedis* rarely causes clinical signs; however, massive infections can cause swelling and hoof damage in species of *Odocoileus*, which may increase susceptibility to predation. Clinical disease has not been reported in caribou, nor has associated histopathology been described from any of its multiple hosts (Verocai *et al*., 2012). In Finland, heavy infections, presumed to be *O. skrjabini*, have been associated with severe haemorrhagic tarsitis, which likely causes pain and lameness for reindeer and also for moose (Laaksonen *et al*., unpublished data).

5.4 *Rumenfilaria andersoni*

A filarioid nematode inhabiting the lymphatic vessels of the subserosal rumen and mesenteries associated with a high prevalence of its microfilariae in peripheral blood was observed in Finnish reindeer during 2004 and 2006. During the study period, geographic expansion and emergence occurred on a northward trajectory, coinciding with the outbreak of *S. tundra* (Laaksonen *et al*., 2007). This was the first recognition of the occurrence of the filarioid *R. andersoni* in Finland and Eurasia (e.g. Laaksonen *et al*., 2010b, 2015).

Subsequently, *R. andersoni* was identified from Finland in three endemic cervid species and in the introduced white-tailed deer. In 2004-2006 overall, microfilariae of *R. andersoni* (rmf) occurred in 64% of reindeer blood samples, with a mean density of 452 rmf/ml blood (range 1-19,400). The prevalence and density of rmf was higher in adults than in calves. Among both age classes of hosts, infection intensity decreased from the south to the north with the parasite apparently emergent on the periphery of an expanding geographic range extending into northernmost Finland (Laaksonen *et al*., 2015). Sampling during 2007-2009 demonstrated the presence of *R. andersoni* in all species of Finnish cervids examined: moose, 8%, white-tailed deer, 15%, roe deer, 3%, wild forest reindeer, 100%. Also, based on samples from 2010, one of 15 Swedish moose, near the Finnish border, was positive (Laaksonen *et al*., 2015). Patterns of seasonal transmission remain to be defined. The insect vector of *R. andersoni* is not known, but it is likely not a species of mosquito (see Laaksonen *et al*., 2009b).

During reindeer health monitoring in 2015, 71% of adult Finnish reindeer were carriers of *R. andersoni*, and the number of infected reindeer was similar to 2006 (see Laaksonen *et al*., 2015, unpublished). In contrast, based on archival blood specimens from 1997 (assessed after the initial recognition of the parasite in Finland), rmf were present at very low density in 8% of samples and only in the most southern part of the Finnish reindeer herding area (Laaksonen *et al*., 2015).

5.4.1 *Rumenfilaria* and meat hygiene

Infections of fully developed adult nematodes appear limited to the lymphatic vessels associated with the rumen, whereas rmf are entirely in the peripheral circulation of the host. Some of the nematodes (females 65 (40-100) and males 45 (30-55) mm long) were seen through the wall of the dilated vessels as thin white winding threads occluding the lumen of the vessel. Usually the reactions on the nematode infected lymphatic vessels were visible to the naked eye, especially in older reindeer. The typical gross pathological changes associated with the infection were the dilatation of the vessels, lymphoedematous swelling of the vessel walls around the living worm, and greenish or greyish granulomatous or fibrotic reaction in the foci containing dead worms (Laaksonen *et al*., 2010b).

The pathological impact of adult *R. andersoni* on cervid health and well-being remains unresolved, although macroscopic greenish inflammatory changes were frequently observed around the ruminal lymphatic vessels during reindeer slaughter in 2004-2007. The impact of microfilaraemia on cervid health also remains undetermined, although it is highly probable that substantial density of rmf observed in reindeer blood circulation may have negative systemic effects. The eosinophilic reaction in skin and in lymph nodes reveals that high counts of rmf in blood circulation also would be predicted to have a negative impact on overall cervid health (Laaksonen *et al*., 2015).

5.5 Drivers for distribution and patterns of emergence for filarioids

Drivers for emergence of parasites and disease are predicted to be complex, and for filarioids represent interactions among mammals, arthropods, and parasites where both direct and indirect effects of temperature and humidity can influence the outcome (Laaksonen *et al*., 2010a). Climate change, perturbation and the synergy between long term trajectories for incremental warming and embedded ephemeral and extreme weather events, is increasingly being recognized or anticipated as a primary factor linked to emerging diseases attributable to macroparasites and microparasites (including bacterial and viral pathogens) in these high latitude systems and globally (Handeland and Slettbakk, 1994; Harvell *et al*., 2002; Hoberg and Brooks, 2015; Hoberg *et al*., 2008a,b, 2013; Jenkins *et al*., 2006; Kutz *et al*., 2005, 2013; Laaksonen *et al*., 2009a, 2015; Patz *et al*., 1996; Ytrehus *et al*., 2008).

History, encompassing phylogeny, explorations of host-parasite coevolution, ecology and biogeography, reveals a foundation to elucidate the drivers and responses to perturbation that influence emergent pathogens in evolutionary and ecological time (Hoberg and Brooks, 2008, 2013). All of our knowledge starts with evolution, ecology and biogeography, as these interacting facets determine the history of biodiverse systems. Such insights provide a pathway for anticipating and mitigating the outcomes of accelerating change at regional to landscape scales and access to a nuanced history of geographic distribution, host association, and the intricacies of the host-parasite interface that influence the potential for disease and our capacity for effective control strategies in a rapidly changing world (e.g. Brooks *et al*., 2014).

5.5.1 Invasion, geographic expansion and host colonization

Filarioid faunas distributed in Finnish cervids constitute a complex mosaic of endemic and introduced species that have been assembled through processes of anthropogenic invasion or geographic expansion in association with changing patterns of distribution for definitive and intermediate hosts. Faunal mosaic assembly reflects differing scales of host colonization responding to environmental perturbation and a breakdown in ecological isolation for parasites and hosts, in the perspective of shifting distributions and contact for sympatric assemblages of cervid species from landscapes to regional settings (e.g. Hoberg, 2010; Hoberg *et al*., 2012; Laaksonen *et al*., 2015). A primary mediator of ecological disruption has been climate warming as a driver for alterations in the spatial and temporal extent of permissive environments for parasite transmission, reflected as an ongoing shifting balance, which drives the potential for substantial changes on the periphery of rapidly expanding geographic range (Hoberg and Brooks, 2015). Host colonization in the arena of ecological perturbation is central to understanding the contemporary distributions for filarioids among Finnish cervids. Colonization is the interaction between opportunity (in a simple sense defined by breakdown in ecological isolation) and capacity for parasites to utilize a particular spectrum of hosts or more significantly host-based resources within the context of ecological fitting (e.g. Agosta *et al*., 2010; Araujo *et al*., 2015; Combes, 2001; Hoberg and Brooks, 2015; Hoberg and Zarlenga, 2016). Ecological fitting facilitates translocation (geographic colonization and invasion), introduction and host-colonization, constituting an essential characteristic of faunal assembly on evolutionary and ecological time-scales (Agosta and Klemens, 2008; Agosta *et al*., 2010; Hoberg and Brooks, 2008, 2013). In this arena, not all hosts are equivalent or optimal, and thus may represent different contributions to the maintenance and persistence of parasites among sympatric and multi-species assemblages.

Considering *S. tundra* from this perspective, roe deer are regarded as the initial drivers of distribution in Fennoscandia, representing an optimal, apparently asymptomatic and highly vagile host with the potential to determine parasite occurrence and persistence over considerable geographic space. Initial recognition of *S. tundra* in Scandinavia appears to coincide with geographic expansion of roe deer from more southern latitudes of Western Europe; roe deer populations have subsequently expanded in northern Finland and are now present across the entire reindeer herding area. Host colonization to Finnish cervids is apparent, and adult reindeer have been suggested as the main contemporary source of infection for the calves. *S. tundra* now is established in broad circulation among roe deer, semi-domestic and wild forest reindeer and moose (Laaksonen *et al*., 2007, 2009b). A recent or contemporary expansion and events of host colonization are supported by molecular sequence comparisons of parasite populations. For example, *S. tundra* occurring in roe deer from northern Finland and Italy (Laaksonen *et al*., 2007) were demonstrated to be genetically similar to conspecifics from the 2003-2005 outbreaks in reindeer. Further, the haplotype of *S. tundra* found in roe deer from southern Finland was similar to the causative agent of peritonitis in moose from northern Finland in 1989 (Laaksonen *et al*., 2009b).

Exchange and circulation of *S. tundra* among wild forest reindeer and semi-domestic reindeer also may be significant with both serving as potential reservoirs for maintenance and persistence. That both serve as competent hosts for *S. tundra* is indicated by similar densities and prevalence for microfilaria between these two subspecies (Laaksonen *et al*., 2009b). The outbreak of *S. tundra* expanded from the south to the north, where reindeer and wild forest reindeer were in contact in the south. Concurrently, there was a crash in the wild forest reindeer population in Kainuu adjacent to a reindeer herding area, with a high percentage of peritonitis. Extensive mortality and peritonitis were not observed in the southern wild forest reindeer population where the smf density was also significantly lower. Whether the high *Setaria* prevalence of wild forest reindeer in Kainuu with signs of peritonitis (Laaksonen *et al*., 2007) was associated with the recent decrease of wild forest reindeer population (Anonymous, 2013), remains unknown.

Phylogenetic inference among ungulates and parasites indicates that host colonization with switching between bovid and cervid assemblages has been a prominent aspect of diversification during the evolutionary history of *Onchocerca* (McFrederick *et al*., 2013). *Onchocerca* sp. infection in cattle was fairly common in Finland, but the amount of pathological changes leading to condemnation of infected parts is low compared to the mf prevalence (Solismaa *et al*., 2008). Emergence of *O. skrjabini* (reported as *O. tarsicola*) in reindeer from Sweden during the late 1960's was hypothesized to be caused by range expansion of the putative primary host, red deer (*C. elaphus*) (Rehbinder, 1990).

In contrast to *Setaria* and *Onchocerca*, which may represent events of 'natural invasion', our data demonstrated that *Rumenfilaria* became established in Finland recently, coincidental with introduction of white-tailed deer from North America in 1935. Subsequent invasion and emergence in the past 70-80 years appears driven by climate-related factors (Laaksonen *et al*., 2015). Genetic comparisons and sequence identity of disjunct populations of *R. andersoni* from North America and those from reindeer in Finland support this hypothesis (Grunenwald *et al*., 2016). Among moose in Finland, the prevalence and intensity were substantially lower than levels observed among subspecies of reindeer. White-tailed deer had a relatively high prevalence and density of rmf, whereas our limited data for roe deer indicated that the nematode may not have been abundant. Subsequently, the density and prevalence of rmf in moose and white tailed deer suggested the nematode may be adapted to these species, and that these cervids may be among the primary hosts of *R. andersoni* and reservoirs for transmission in Finland; the historical range of *R. andersoni* appears limited and this filarioid is likely endemic to North America. Survey data for this parasite are sporadic in North America, although the parasite is now assumed to occur across most of the northern Nearctic. Originally described based on nematodes in a moose from Ontario, broad-based geographic survey has documented abundant infections in moose from Alaska (70% prevalence based on rmf), other locations in the USA, and in white-tailed deer from Minnesota (Kutz *et al*., 2012). Currently observations of *R. andersoni* in other Scandinavian countries or across Eurasia are lacking and the parasite is considered to be absent (Laaksonen *et al*., 2015). As *R. andersoni* is found in all four cervid species from Finland, however, it can be anticipated to have considerable potential for geographic expansion and host colonization in Eurasia.

The contemporary filarioid fauna in Fennoscandia is a mosaic including both endemic and exotic (introduced) species. Host colonization and circulation through haematophagous arthropods among an assemblage of cervids in zones of contact or in sympatry now serves to maintain the distribution of these parasites. A role for ecological fitting is apparent in establishing the capacity for ongoing faunal assembly. Significantly for *R. andersoni*, establishment in Finland reflects a process of multi-level ecological fitting involving independent colonization events initially to a novel array of arthropod vectors (facilitating persistence and maintenance of transmission) and shifts to a broadened assemblage of cervid hosts (see Laaksonen *et al*., 2015; Malcicka *et al*., 2015). An interaction with changing climate, as a multifaceted determinant of potential range expansion and emergent disease, may be a broader generality for northward trajectories now being observed for filarioids among Finnish cervids. Such drivers and patterns are not limited to Fennoscandia, but are consistent with ongoing distributional changes in ungulate host-parasite assemblages in other regions of the Arctic (Hoberg and Brooks, 2015; Hoberg *et al*., 2013; Kutz *et al*., 2013, 2014).

5.5.2 Climate change

Climate forcing, manifested in long term incremental change and short term extreme events for temperature and precipitation (IPCC, 2013, 2014), must be accounted for in anticipating responses in complex host-parasite systems. Regimes of perturbation that modify boundaries driving origins of new ecotones, sympatry among domesticated and free-ranging wild ungulates, and dissolution of mechanisms for ecological isolation in combination with expansion of permissive environments can be associated with amplification of populations, host colonization and emergence and disease (Hoberg and Brooks, 2015; Hoberg *et al.*, 2008a; Kutz *et al.*, 2014). Scenarios and models for substantial spatial and temporal alteration in patterns of temperature and precipitation associated with accelerated climate warming suggest complex responses (e.g. expansion/retraction, local extinction) with respect to geographic range and patterns of disease.

Potential downstream outcomes are directly influenced by the structure of host assemblages (both intermediate and definitive) and the factors that serve to limit or facilitate completion of life cycles and transmission. Specific parameters of resilience, tolerances, thresholds for development of larval stages in vector hosts, the coincidental timing of vector emergence and activity with parasite development and overlap with suitable cervid hosts are critical in establishing the limits for distribution. Filarioids in cervids are further buffered from short term shifts in temperature and precipitation, or adverse conditions that may extend over seasons and years, representing phases of climate variation, shifting balances influencing developmental rates for larvae and vectors and the relative spatial and temporal distribution of permissive environments (Hoberg and Brooks, 2015). Temporal buffers include extended longevity of adult parasites or microfilariae in definitive hosts, and longevity and vagility of cervid hosts, representing limiting factors that influence distribution over time, and especially capacities for persistence on the margins of rapidly expanding ranges that characterize conditions in Finland (Brooks and Hoberg, 2015; Hoberg, 2010; Hoberg *et al.*, 2008b, 2012; Laaksonen *et al.*, 2015).

According to Rehbinder *et al.* (1975) it seems obvious that the year 1973 was a watershed marking the appearance of *S. tundra* in Scandinavia after successive years with anomalously warm summers (Laaksonen *et al.*, 2010a). It has been demonstrated that at a mean temperature of 21 °C, *S. tundra* larvae develop in the mosquito to the infective third stage in approximately two weeks, while at a mean temperature of 14.1 °C, development is not completed (Laaksonen *et al.*, 2009a). The transmission of *S. tundra* is highly dependent on the life span of the female mosquitoes, with survival of adult mosquitoes depending in part on both temperature and humidity (Clements, 1963). There is unfortunately no information on the longevity of mosquito populations in Finland, although adult *Aedes* spp. survived approximately four weeks in a laboratory insectary at room temperature. Older females were a considerable part of the 'wild' mosquito population (Laaksonen *et al.*, 2009a). The initial outbreak documented for *S. tundra* in Finland and Sweden during 1973 was associated with the appearance of especially large numbers of mosquitoes (Rehbinder *et al.*, 1975), however, longevity of these vectors may be a critical control factor on the potential extent of transmission (Laaksonen *et al.*, 2009a).

A key factor promoting the transmission of *S. tundra* is warm ambient summer temperature. Within limits, this warmth improves development, reproduction, longevity and influences feeding habits of mosquito vectors, as well as, most importantly, the larval development of *S. tundra*. Warm summers, which may increase in frequency and duration (along with extreme events) as a consequence of climate change, also force the reindeer to flock and stay on mosquito-rich wetlands, behaviour which might increase the infection pressure (Laaksonen *et al.*, 2009a). Patterns of simultaneous emergence in cervids for *Onchocerca*, *R. andersoni* and *S. tundra* suggest a direct relationship between temperature (probably humidity) and incremental warming over the past 40-50 years. Such a threshold, or tipping point, unfolding in the 1970's relative to temperature, has been discussed in changing patterns of development, transmission, population amplification and geographic expansion for lungworm parasites among ungulates in the Central Canadian Arctic (e.g. Hoberg and Brooks, 2015; Hoberg *et al.*, 2008a; Kutz *et al.*, 2005, 2013).

5.6 Conclusions

5.6.1 What has been achieved?

Over the last several decades, there has been a substantial advance in our knowledge of the diversity and impacts of filarioid nematodes in northern and arctic ungulates, particularly in Fennoscandia. In high numbers, these parasites are causing significant morbidity and mortality and impacting food production systems (semi-domesticated reindeer industry) as well as the health of wild cervids and quality of meat. Substantial advances in understanding the transmission dynamics, and ecological drivers for epidemic and endemic disease have been made (Laaksonen *et al.*, 2007, 2008, 2009a,b, 2010a,b, 2015) and have demonstrated the close linkages between the parasite ecology and climatic conditions.

It is likely that climate change (IPCC, 2013, 2014) will continue to favour the northward expansion of filarioid nematodes, which might then become an even greater threat to arctic food safety and security. Direct evidence for such responses to warming is observed for *S. tundra* and *R. andersoni* in Finland with the progression of population expansion from the south to the north over the past decade coincidental with emergent disease in cervid hosts, especially semi-domestic reindeer (Laaksonen *et al.*, 2010a,b, 2015). Elsewhere in the Arctic, the increase and expanding distributions for some protostrongylid lungworms, a group of parasites that use gastropod intermediate hosts for transmission, has been attributed to warming. Initially, warming in the 1970's to late 1980's resulted in a tipping point from multi-year to single year transmission and amplification of parasite populations in core range; this was followed by subsequent range expansion to the north coincidental with continued warming and a shifting boundary (or balance) for permissive habitats facilitating larval parasite development (Kutz *et al.*, 2005, 2013; Hoberg and Brooks, 2015). These series of ongoing events serve to demonstrate the pervasive influence of climate warming on the distribution of complex host and parasite assemblages and emergent disease (Hoberg *et al.*, 2008a, 2013; Kutz *et al.*, 2014).

5.6.2 What has been neglected?

A robust understanding of the diversity, host and geographic range, ecology and history of these parasites is warranted, directly linking or integrating information from diverse sources for abiotic and biotic parameters of distribution. Translocation, establishment and invasion of otherwise exotic parasites continue in a regime of globalization serving as core drivers of disease emergence and socio-economic impact (Hoberg, 2010; Brooks and Hoberg, 2013; Hulme, 2014). Habitat perturbation, transitions, and shifting distributions due to accelerating climate warming are analogous (or equivalent) to historical episodes of climate fluctuation particularly through the Quaternary over the past 3 million years.

In contrast to tracking emergent parasites and disease after the fact, proactive assessments of diversity are necessary such as those outlined in the recently proposed Documentation-Assessment-Monitoring-Action (DAMA) protocols (reviewed in Brooks and Hoberg, 2013; Brooks *et al.*, 2014; Hoberg *et al.*, 2015) and in the Arctic Biodiversity Assessment (Hoberg *et al.*, 2013). DAMA promotes a proactive and collaborative capacity for biodiversity inventory and informatics, linking local or traditional ecological knowledge, with field collections, archived specimens, morphology and sequence data in museum resources, to understand, anticipate and respond to the outcomes of accelerating environmental change and globalization. Inventories at regional scales provide the mechanism to identify new or continuing pathways for anthropogenic invasion and climate-driven modifications, and to monitor host and geographic associations through shifting spatial and ecological boundaries. The development of timely and effective responses that mitigate emergent parasitic infections will directly depend on integrating knowledge across the past, present and the future of assemblages in northern systems in dynamic change (e.g. Dudley *et al.*, 2015; Hoberg *et al.*, 2013; Kutz *et al.*, 2014).

5.6.3 What needs to be done?

Globally, filarioid nematodes are a significant cause of morbidity and mortality in humans, domestic animals, and wildlife. Their transmission dynamics are intimately linked with climatic conditions, and recent emergences in boreal and Arctic regions have demonstrated the sensitivity of these parasites to climate. In fact, studying these parasites in the relatively simple Arctic ecosystem (Kutz *et al.*, 2009) can provide important and novel insights into the ecology and response of this group of parasites to climate with broader implications globally, particularly for those species that infect people and are an important part of the global burden of disease (e.g. *O. volvulus*).

In wild cervids at high latitudes filarioids are anticipated to become increasingly common, initially as epidemic disease associated with consecutive warm years, as illustrated by Laaksonen *et al.* (2010a), and then establishing in an endemic state as climate warming trends continue. While the effects of epidemic disease have been clearly illustrated, the more subclinical yet important impacts of an endemic state need further exploration, particularly with respect to impacts on population dynamics and sustainability. Additionally, as illustrated with *Rumenfilaria*, these parasites are able to colonize new regions, and new hosts, with potential detrimental effects as they establish in novel host systems. This highlights the critical importance of better defining the diversity of filarioids in cervids globally (e.g. Verocai *et al*., 2012; McFrederick *et al*., 2013).

Around much of the Arctic and boreal regions, caribou, reindeer, moose and other ungulates are critical components of the food system for aboriginal peoples. The appearance of new disease syndromes, such as tarsitis, peritonitis, hepatic granulomas, etc., can lead to decreased confidence in 'country foods' and consequent meat wastage and enhanced food insecurity. This is particularly true when there is no traditional knowledge about the syndromes. Concerted efforts are required to both anticipate (predictive modelling) and monitor range expansion and emergence, while simultaneously providing the appropriate public health messaging and communications. This is essential so that those harvesting wildlife for food are well informed about the parasites and able to 'inspect' their meat with confidence, and reduce meat wastage.

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6. Antimicrobial susceptibility of *Salmonella enterica* subsp. *enterica* serovar Choleraesuis strains from wild boar (*Sus scrofa*) in Italy

Chiara Donazzolo, Sara Turchetto, Martina Ustulin, Carlo Citterio, Gabriella Conedera, Denis Vio, Silvia Deotto, Tiziana Di Giusto and Monia Cocchi***
Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università, 10, 35020 Legnaro (PD), Italy; mcocchi@izsvenezie.it

Summary

An unusual mortality in wild boars (*Sus scrofa* Linnaeus, 1758) was observed in the Friuli Venezia Giulia region in north-eastern Italy, in 2013. Mortality was due to septicaemia caused by *Salmonella enterica* serovar Choleraesuis (*Salmonella* Choleraesuis), a swine-adapted serovar that may also lead to systemic infections in humans. In a public health perspective, wildlife can play a role at the *Salmonella*-animal-human-interface, since wild populations could act as a reservoir of this pathogen. Concerning *S.* Choleraesuis, some strains of this serovar, which may also be transmitted to humans by means of food, show resistance to different antibiotics with possible implications for human health, but currently, knowledge is still lacking. This study was aimed at assessing susceptibility to antimicrobials in the *S.* Choleraesuis isolates in wild boar from the cited outbreak (n=30), in order to gain more knowledge about the presence and distribution of antibiotic-resistant pathogens in wildlife of North-Eastern Italy. Tests used were disk diffusion (DD) and minimum inhibitory concentration (broth microdilution method, MIC). Moreover, an extended-spectrum beta-lactamases (ESBL) test was performed to identify ESBL-producing *Enterobacteriaceae*. Results indicated no resistance to beta-lactam antibiotics, while all isolates (100%) were resistant to spiramycin and tilmicosin by DD test, and 22/30 (73%) showed resistance to streptomycin by MIC. Since *S.* Choleraesuis may represent an emerging health problem for livestock and humans, due to both its pathogenicity and specific antibiotic-resistance, monitoring in sympatric swine and wild boars would be desirable.

Keywords: wild boars, Italy, *Salmonella* Choleraesuis, wildlife, antibiotic-resistance

6.1 Introduction

Salmonella enterica serovar Choleraesuis is a host-adapted, facultative intracellular pathogen, causing swine paratyphus, the source of which seems to be confined to carrier pigs. Swine may show clinical signs of enterocolitis and septicaemia (Field, 1958; Gray *et al.*, 1996; Reed *et al.*, 1986; Wilcock and Schwartz, 1992). Recently, the presence of *S.* Choleraesuis and *S.* Choleraesuis var. Kunzendorf was observed in slaughtered pigs in Spain and in three countries of Eastern Europe (Bulgaria, Poland and Slovakia) (EFSA, 2008). This serotype of *Salmonella* may also cause systemic infections in humans: in particular, it has been observed

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in different Asian countries, where transmission probably occurs from pigs to humans, leading to systemic infections without evident gastroenteritis (Chiu *et al.*, 2004; Su *et al.*, 2014).

Bacterial pathogens can show resistance to a various number of antimicrobial agents, representing a serious public and animal health concern (Carroll *et al.*, 2015). As an example, Carbapenemase-producing microorganisms, such as *Escherichia coli* and *Salmonella*, were reported in food-producing animals, livestock and companion animals, wildlife and environment (Fischer *et al.*, 2013; Guerra *et al.*, 2014).

Focusing on *S.* Choleraesuis isolated from wild boars between 2006 and 2008 during routine inspection at the regional laboratory of the federal state Thuringia (Germany), 24 *S.* Choleraesuis strains were isolated from 118 wild boars showing clinical signs (Methner *et al.*, 2009). Strains revealed different resistance profile against sulfamethoxazole and streptomycin (broth microdilution method, MIC): six isolates showed sensitivity to sulfamethoxazole and low resistance against streptomycin, while all other showed high resistance to both these antibiotic agents. Streptomycin resistance appeared also in European wild boar studied in Lazio Region (Italy): *Salmonella* isolates belonging to different serovars (1.8% were *S.* Choleraesuis) resulted resistant by disk diffusion (DD) test to streptomycin (18.5%), most were multidrug resistant (54%), while only 7.4% of the isolates appeared as susceptible to all antimicrobial agents (Zottola *et al.*, 2013).

Also Danish pig herds showed the presence of *S.* Choleraesuis between 2012 and 2013: four isolates from this recent outbreak were resistant to both streptomycin and sulphonamides (MIC test), while the three isolates of the previous outbreak (1999-2000) revealed a different resistance profile in which two of them were multidrug-resistant (Pedersen *et al.*, 2015). Finally, studies conducted in Taiwan reported high rate of chloramphenicol resistance in serotype choleraesuis isolated from swine (Chang *et al.*, 2002).

In the framework of human medicine, *S.* Choleraesuis isolates from patients at a university hospital in Taiwan showed an increasing prevalence of resistance to conventional antimicrobial agents (ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole), that implies empirical therapy with a broad-spectrum cephalosporin or a fluoroquinolone in patients with bacteraemia (Chiu *et al.*, 2002, 2004).

An unusual mortality of wild boars (*Sus scrofa* Linnaeus, 1758) was observed in the Friuli Venezia Giulia (FVG) region, North-Eastern Italy, and in particular in the Pordenone province in 2013 (Figure 6.1). Such a sudden and unexpected mortality triggered alarm for prioritary swine infections (as African and classical swine fever) and, since the wild boar population is free ranging between FVG, bordering Italian regions and bordering countries, regional and provincial sanitary authorities were involved in informing different stakeholders and increasing passive and active surveillance. Carcasses and organs, derived from both wild boars found dead in the field and hunted animals, were submitted to gross pathological inspection and ancillary tests. Laboratory tests provided evidence that, in most cases, the cause of death was a septicaemic form of salmonellosis due to non-typhoid Salmonella strains: namely *S.* Choleraesuis (Conedera *et al.*, 2014). *Salmonella* isolates from each positive animal

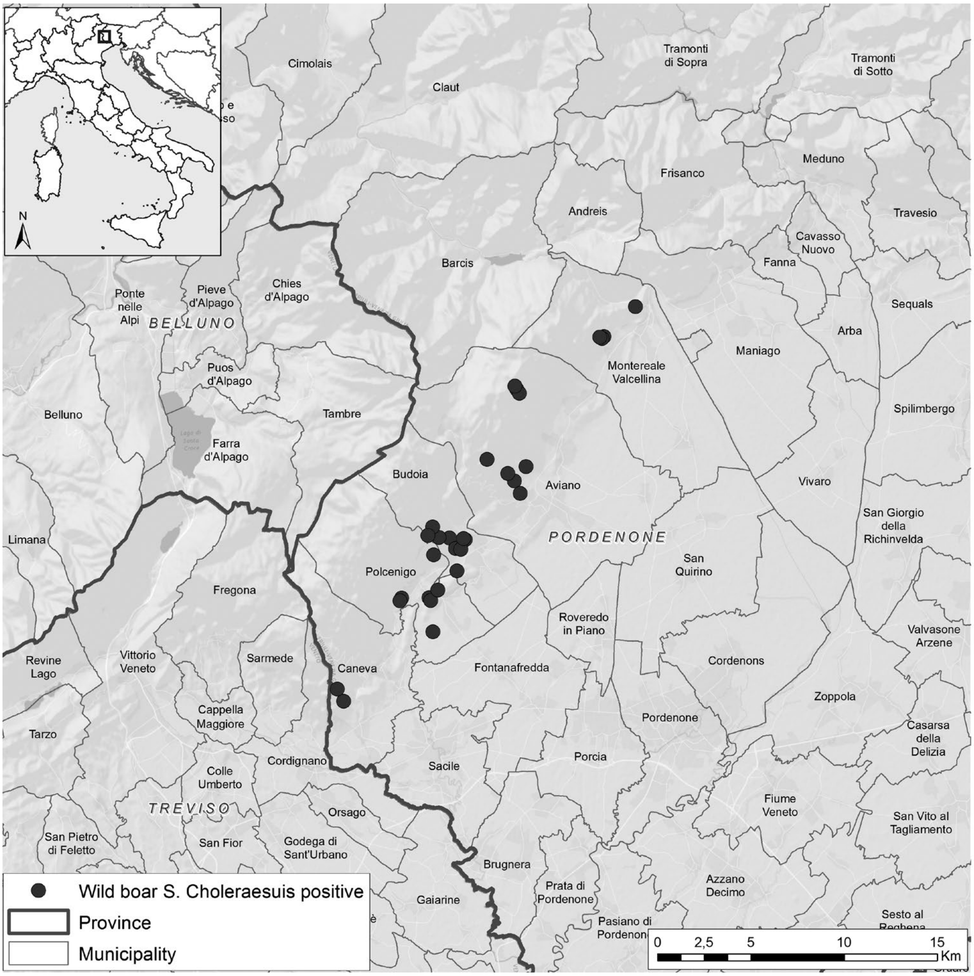
N
Cimolais
Claut
Tramonti di Sopra
Tramonti di Sotto
Meduno
Frisanco
Andreis
Travesio
Cavasso Nuovo
Fanna
Barcis
Sequals
Arba
Maniago
Ponte nelle Alpi
Pieve d'Alpago
Chies d'Alpago
BELLUNO
Montereale Valcellina
Puos d'Alpago
Spilimbergo
Belluno
Tambre
Farra d'Alpago
Budoia
Aviano
Vivaro
San Giorgio della Richinvelda
Limana
PORDENONE
San Quirino
Polcenigo
Fregona
Roveredo in Piano
Valvasone Arzene
Revine Lago
Vittorio Veneto
Sarmede
Caneva
Fontanafredda
Cordenons
Zoppola
Cappella Maggiore
Pordenone
Tarzo
Casarsa della Delizia
Cordignano
Sacile
Porcia
Colle Umberto
TREVISO
Orsago
Fiume Veneto
San Vito al Tagliamento
San Pietro di Feletto
San Fior
Godega di Sant'Urbano
Brugnera
Azzano Decimo
Prata di Pordenone
Gaiarine
Sesto al Reghena
Pasiano di Pordenone
● Wild boar S. Choleraesuis positive
Province
Municipality
0
2,5
5
10
15
Km

Figure 6.1. Area (Pordenone province, Friuli Venezia Giulia region, Italy) involved in the outbreak of Salmonella enterica *serovar Choleraesuis in wild boar.*

were biochemically characterised and sent to the National Reference Centre for salmonellosis (CNRS-IZS Venezie, Legnaro, Italy).

The aim of the present work was the assessment of antimicrobial susceptibility to different antibiotics in 30 *S.* Choleraesuis isolates from organs of wild boars collected during this mortality outbreak, in an ecosystem and public health perspective.

6.2 Materials and methods

All 30 *S.* Choleraesuis strains isolated from wild boars were tested for antimicrobial susceptibility by DD and minimum inhibitory concentration (MIC), in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2008). For the DD test, the following antimicrobials were used: ampicillin, cefotaxime, ceftazidime, colistin, florfenicol, gentamicin, nalidixic acid, tetracycline, aminosidin, amoxicillin and clavulanic acid, cefquinome, ceftiofur, enrofloxacin, spectinomycin, spiramycin, trimethoprim-sulfamethoxazole and tilmicosin. For the MIC, the following drugs were tested: ampicillin, cefotaxime, ceftazidime, colistin, florfenicol, gentamicin, nalidixic acid, tetracycline, ciprofloxacin, chloramphenicol, kanamycin, streptomycin, sulfamethoxazole and trimethoprim.

Finally, extended spectrum beta-lactamases (ESBL) pattern was investigated, as described in the CLSI guidelines (CLSI, 2008).

6.3 Results

Table 6.1 summarizes the results of the antibiotic-susceptibility tests: based on DD test, all 30 *S.* Choleraesuis isolates (100%) were resistant to spiramycin and tilmicosin; based on MIC, 22 out of 30 (73%) isolates showed resistance to streptomycin. Lastly, ESBL detection resulted negative for all isolates (100%), thus showing no resistance to beta-lactam antibiotics.

Table 6.1. Antimicrobial susceptibility testing in 30 Salmonella enterica *serovar Choleraesuis strains isolated from organs of wild boars in Pordenone province (Friuli Venezia Giulia region, Italy).*[1]

	Susceptible	Resistant
disk diffusion test	AMP, CTX, CAZ, CS, FLO, GM, NX, TE, AN, AC, CEQ, CEF, ENR, SPT, SXT	SP (30/30), TIL (30/30)
minimum inhibitory concentration	AMP, CTX, CAZ, CS, FLO, GM, NX, TE, CIP, CAF, K, SU, TMP	STR (22/30)
ESBL test	100% negative	

[1] AC = amoxicillin and clavulanic acid; AMP = ampicillin; AN = aminosidin; CAF = chloramphenicol; CAZ = ceftazidime; CEF = ceftiofur; CEQ = cefquinome; CIP = ciprofloxacin; CS = colistin; CTX = cefotaxime; ENR = enrofloxacin; ESBL = extended-spectrum beta-lactamases; FLO = florfenicol; GM = gentamicin; K= kanamycin; NX = nalidixic acid; SP = spiramycin; SPT = spectinomycin; STR = streptomycin; SU = sulfamethoxazole; SXT = trimethoprim-sulfamethoxazole; TE = tetracycline; TIL = tilmicosin; TMP = trimethoprim.

6.4 Discussion

In the framework of public health, wildlife may play a role at the animal-human-interface as a reservoir of important zoonotic bacteria, including *Salmonella* (Hilbert *et al*., 2012; Magnino *et al*., 2011; Paulsen *et al*., 2012; Vieira-Pinto *et al*., 2011a) and the presence of antimicrobial resistance traits would be a further risk factor for human health.

All *S*. Choleraesuis strains from our study in Northeastern Italy showed 100% resistance to spiramycin and tilmicosin (DD test), and most of them showed resistance also to streptomycin by MIC. On the other hand, although ESBL-Enterobacteriaceae in livestock have been recently detected (Stefani *et al*., 2014), all our strains tested negative for ESBL.

Interestingly, resistance to spiramycin and tilmicosin was not recorded by other studies, while streptomycin resistant strains, isolated from wild boar, have been reported by other Authors in Central Italy and Germany (Methner *et al*., 2009; Zottola *et al*., 2013).

6.5 Conclusions

6.5.1 What has been achieved?

Various bacteria, viruses and parasites typically cause zoonoses from a wildlife reservoir (Kruse *et al*., 2004). Moreover, wildlife can be involved in the ecology, environmental persistence and transmission of resistant pathogens through the interface with livestock and humans; in this perspective, wild boar may also act as an indicator for environmental contamination of various *Salmonella* serotypes (Chiari *et al*., 2013). Focusing on our results, the role of wild boar as a reservoir of antibiotic-resistant *Salmonella* strains is not inferable at the moment, although our data suggest they represent a possible hazard for human infection through: (1) direct contact (such as during hunting and carcass manipulation or by ingestion of contaminated meat products); (2) indirect contamination (e.g. of vegetables through faeces shedding); and (3) domestic pig herds that could come in close contact with wild boar (Decastelli *et al*., 1995; Paulsen *et al*., 2012; Vengust *et al*., 2006; Vieira-Pinto *et al*., 2011b; Wacheck *et al*., 2010).

6.5.2 What has been neglected?

A comparison between strains isolated from wild boars and domestic pigs is still lacking. Characterizing these strains from both the genetic and the drug resistance point of view would be essential to understand their ecology at the wildlife-livestock interface. Moreover, comparison with isolates from humans would be crucial to deepen the knowledge on this topic.

6.5.3 What needs to be done?

Considering all above, our results encourage further studies to fill the lack of knowledge about drug resistance in wildlife. First of all, the definition of epidemiological cut-off/

clinical breakpoint for pathogens isolated in wild animals and the usable methods would be necessary to allow specific interpretation of cases. Then, as a precautionary principle and in a perspective of education on meat hygiene and safety, it would be appropriate to further emphasize the importance of correct hygiene practices along the game meat chain. Finally, the implementation of passive surveillance programmes may be useful to monitor the spreading of these pathogens and possibly associated antibiotic resistance, as an indicator of ecosystem health. Focusing on the results presented herein, further data need to be collected and collated as a basis for the risk assessment of *S.* Choleraesuis and its antimicrobial resistance at the interface between wildlife, livestock and humans, including research on wildlife abundance and distribution, livestock farming methods, typical meat production, hunting activities and hygiene practices.

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7. Wild life status, consumption pattern and related possible risks to consumers in Pakistan

Kashif Nauman[1,2], Frans J.M. Smulders[2] and Peter Paulsen[2]*
[1]Department of Meat Science and Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan; kashifctn@gmail.com
[2]Department of Animal Production and Veterinary Public Health, University of Veterinary Medicine, Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Summary

Pakistan is rich in wildlife species. A number of species are protected, and the total area of national parks comprises more than 10% of the area of Pakistan. Hunting is not only done for the supply of (bush)meat, but also for by-products such as skin, teeth or as a part of pest management (i.e. wild boar). There is limited knowledge about the prevalence of zoonotic hazards in wildlife, but the extensive mode of livestock rearing indicates that pathogen transfer from wildlife to livestock and *vice versa* is likely. The absence of human disease caused by handling of wildlife or consumption of game meat could be due to the low prevalence of pathogens, to underreporting and/or the traditional meat preparation techniques involving prolonged cooking.

Keywords: Pakistan, wildlife conservation, hunting, bushmeat, zoonosis

7.1 Introduction

Pakistan is situated at the edge of the Arabian Sea in South Asia. Due to its profound blend of topography – including the world´s highest mountains and plain agriculture lands – it hosts a wide range of ecosystem/habitat types and associated biological diversity. Pakistan is rich in wildlife species. Some are local residents but many other are migratory, particularly birds, travelling thousands of miles from cold northern regions towards warmer regions of the world (Rizvi, 2004).

Nine major ecological zones are recognized in Pakistan, harbouring 195 mammal species, 668 bird species, 192 reptile species and 22 amphibians (SAWEN, 2014). Most famous wildlife species used as a meat source are deer, rabbit, quail, partridge, pheasant, wild goat, wild duck, wild geese, urial, ibex, markhor, houbara bustard, barking deer, hog deer, spotted deer, chinkara gazelle, blackbuck, grey goral, mouflon, nilgai and Marco Polo sheep (Sheikh, 2014). Interestingly, hyena meat is also consumed in Pakistan and it is considered Halal (Osborne, 2013).

Wildlife management is mainly targeted towards wildlife conservation, as the land use for agriculture, industry and housing increases with a rapidly increasing human population (Khan, 1991). In addition, environmental pollution (Festus and Omoboye, 2014), but also

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deforestation and disasters, such as floods and earthquakes, are changing the wildlife landscape every year.

7.2 Legal enforcement in wild life

After the decentralization of certain departments from federal government to provinces, wildlife affairs became a provincial matter in Pakistan. Every province has its own wildlife protection act; they are, however, quite similar and have been issued in the period 1973-1975.

As an example, the Punjab wildlife (protection, preservation, conservation and management) act of 1974 defines 72 species of birds and mammals that are protected throughout the year; hunting of nilgai or blue bull and urial requires a special permit, and 53 species of birds and mammals are allowed to be hunted in limited quantities during a defined season, while 21 species of wild birds and animals are not protected and could be hunted in any quantity any time.

For the protection and enhancing of endangered wildlife species on different locations of the country, wildlife national parks have been established. According to a biodiversity action plan for Pakistan, there is a network of 225 protected areas comprising 14 national parks (public and private), 99 wildlife sanctuaries, 96 game reserves, and 16 unclassified (private, proposed or recommended) protected areas in Pakistan. Not all these facilities are compliant with international standards due to which many major conservation projects are not rendering desirable results (Khan, 2012). The total area covered by these categories is 91,701.21 km^2 which represents 10.4% of the total land area (IUCN, 2000). After 2001, the number of national parks increased to 29 (Figure 7.1) to extend efforts for conservation and development of endangered and threatened wildlife species (http://tinyurl.com/zfat2d4).

The International Union for Conservation of Nature and Natural Resources' (IUCN) red list of threatened species lists 45 species as 'internally threatened animals' occurring in Pakistan. Of these, four are 'critically endangered', 12 'endangered' and 29 'vulnerable'. Out of these 45 species, 18 are mammals, 17 birds, nine reptiles and one fish. Several of these threatened species are found in northern areas. Although Pakistan is rich in wild caprines, at least 9 of its 12 subspecies are threatened, with five classified as 'endangered' or 'critically endangered' (IUCN, 2000).

7.3 Management and use of wildlife resources in Pakistan

7.3.1 Wildlife as a food source

As an Islamic country, most of the wildlife animals which are present in Pakistan are considered 'haram' (prohibited for consumption by Muslims). This explains the abundance of certain wild animals, such as wild boar, all over the country particularly near forests. Meat from wild game is usually consumed by the hunters themselves or presented as a gift to

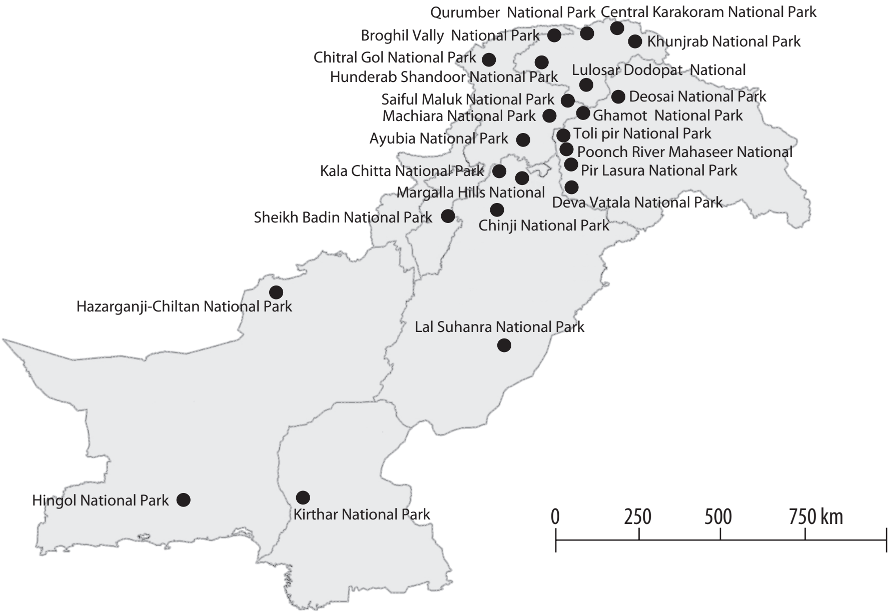
Qurumber National Park
Central Karakoram National Park
Broghil Vally National Park
Khunjrab National Park
Chitral Gol National Park
Hunderab Shandoor National Park
Lulosar Dodopat National
Saiful Maluk National Park
Deosai National Park
Machiara National Park
Ghamot National Park
Ayubia National Park
Toli pir National Park
Poonch River Mahaseer National
Kala Chitta National Park
Pir Lasura National Park
Margalla Hills National
Deva Vatala National Park
Sheikh Badin National Park
Chinji National Park
Hazarganji-Chiltan National Park
Lal Suhanra National Park
Hingol National Park
Kirthar National Park
0
250
500
750 km

Figure 7.1. National conservation parks in Pakistan.

people living close to the hunting area while marketing is not done. It is available, though, on personal level as a delicacy, where private sanctuaries are established for hunting purposes.

7.3.2 Other uses of wildlife

Wild animals are also hunted for their hides and skins, bones, fat and for certain specific body parts, as ivory (Festus and Omoboye, 2014), or to produce traditional medicine (Lee *et al.*, 2014); see also Table 7.1. However, most wildlife including wild animals and birds are hunted as a hobby and for collecting trophies. Around the country, particularly in rural areas, some parts of hunted animals are utilised for manufacturing traditional medicines (Lee *et al.*, 2014).

7.3.3 Hunting

Unlicensed wildlife hunting is illegal all over the country. In the hunting season, special permits are issued in limited quantity on special request and these are restricted to specific species; hunting of protected species is never allowed.

On different occasions – to control the hunting, conservation whilst involving the local community – different hunting schemes are introduced by the government. In this context, most prominent are community–based trophy hunting programmes, in which hunters are allowed to export the trophy. Markhor, ibex and urial are amongst the few major wildlife species that are found in different parts of the country and attract not only domestic hunters, but also hunters from abroad (Worboys *et al.*, 2015). These programmes (e.g. Chitral

Table 7.1. Human use of wildlife in Pakistan (IUCN, 2000).

Human uses	Species affected
illegal hunting	most ungulates, game birds, waterfowl
prosecution (in response to livestock/crop losses)	all predators (including brown bear, black bear, grey wolf, snow leopard, common leopard, leopard cat, wild pig, rhesus macaque)
falconry	saker
domestication – trade medicinal purposes	cranes, rhesus macaque, parrot and bear – rhesus macaque, bear, musk deer, dolphin, pelican and lizard
decoration (ornamental uses)	most felids, mustelids (fur), ungulates (trophies), crocodile, snake, (skins) turtle, (shells, oils) pheasant (feathers)

Conservation Hunting Program), a trophy hunting programme for markhor and the Torghar Conservation Project (TCP) work well as a conservation tool and help to generate income which is destined for supporting conservation park projects (Shackleton, 2001).

For certain species like wild boar, a hunting permit is not required as these are considered as a crop pest in agriculture areas. In Islamabad, this problem is most prominent due to the presence of vast forest area around the city.

In the winter season, when the migratory birds reach the country´s wet lands, hunting permits for slow breeding species like quail, partridge and grouse are issued with a maximum hunting quantity limit. Hunting of rare species (e.g. houbara bustard, crane, heron, pelican, stork, spoonbill, ibis, flamingo, marbled teal, cotton teal, spot bill duck, common shelduck, ruddy shelduck and woodcock) are strictly prohibited (Rizvi, 2004).

7.3.4 Legal and illegal trade of wildlife

Trade of wildlife and products thereof is a lucrative market demand globally. Legal and sometime illegal hunting and trade are common practice. For example, $60 million illegal shipment was intercepted at Karachi port (Anwar, 2015).

Trade of all species categorized as endangered is illegal under international environment agreements, i.e. CITES as well as by national legislation of Pakistan. Many other species (i.e. not endangered but hunted and traded through special permits) are sometimes exploited beyond quotas, which puts extra pressure on sustainability (AFP, 2015; Sheikh, 2014).

The National Council for Conservation of Wildlife (NCCW) is responsible for: (1) coordinating, formulating and implementing wildlife policies at the federal and provincial levels; (2) coordinating activities with international agencies; and (3) promoting conservation in general.

Musk deer (*Moschus leucogaster*), which is present in upper northern areas of Pakistan, is considered endangered by the IUCN and is listed on CITES Appendix I (CITES, 2016). It is hunted illegally not only for bushmeat, but also for its by-products such as musk pod, skin and teeth. Whereas musk pod is exported, skin and teeth are locally sold (Abbas *et al.*, 2015). Smuggling of endangered and protected species is another dilemma in this situation. Eagles, soft shell turtles and their meat are smuggled to Southeast Asian countries where they are sold as delicacy in restaurants.

Many snake varieties like *Naja naja* and *Vipera russelli* are captured by snake charmers for street performance and sometimes sold to health institutes for poison extraction for antivenom production. Many birds escaping the harsh winter season of Siberia migrate through Pakistan. These include the common crane (*Grus grus* L.), demoiselle crane (*Anthropoides virgo* L.) and Siberian crane (*Grus leucogeranus* Pallas). These birds are also captured and then smuggled to other regions of the world where they are esteemed for their beauty and soaring abilities (Perveen, 2012).

The monitor lizard (*Varanus* spp.) is mainly hunted for its leather; while its fat is used for extracting oil relied upon in different ayurvedic and traditional medicines for its assumed aphrodisiac properties (Auffenberg, 1989).

Pakistan banned the export of all reptiles and mammal species in the year 2000. But due to illegal trade on mass scale, many tortoise species are close to extinction, i.e. Indian narrow-headed softshell turtle (*Chitra indica*) (TCC, 2011). Twenty nine of the country's 229 amphibian and reptile species are listed in the CITES Appendices, including the Afghan tortoise (*Testudo horsfieldii*), spotted pond turtle (*Geoclemys hamiltonii*), Indian peacock softshell turtle (*Nilssonia hurum*) (CITES Appendix I, II; CITES, 2016), and also listed as vulnerable in IUCN's red data lists of 2008, 2009, 2010 (IUCN/SSC Tortoise and Freshwater Turtle Specialist Group; TCC, 2011), respectively. These tortoise species are also protected under the Pakistan Wildlife Protection Act and under the Balochistan Wildlife Act (Das and Singh, 2009; Rasheed, 2013).

According to CITES trade database 2015, Pakistan has exported wild animals to many countries including Germany, Denmark, Mexico, Russia, United States, Estonia, China, Canada, Lithuania, Turkey, Zambia, Tajikistan. They mostly include species such as markhor, dolphin, brown bear, urial, duck, wild goat, ibex and blue sheep for the sake of revenue (CITES, 2015).

7.4 Diseases in wildlife and transmission

Zoonotic diseases affect not only humans and domestic livestock, but also wildlife (Karesh *et al.*, 2005). The array of zoonotic diseases in Pakistan comprises tuberculosis (Khan *et al.*, 2010; Shahid *et al.*, 2012), leptospirosis (Saleem *et al.*, 2009), anthrax (IUCN, 2004), brucellosis (Shafee *et al.*, 2011), viral disease like Crimean-Congo haemorrhagic fever (Athar *et al.*, 2003; Saleem *et al.*, 2009), avian influenza (H5N1) (OIE, 2009), nipah virus (FAO, 2002) and many parasitic agents like *Fasciola* and *Trypanosoma* (surra) (IUCN, 2004), *Cysticercus* spp.

(Nauman *et al.*, 2013), *Toxoplasma* (Chaudhry *et al.*, 2014). Although there are few data on the specific occurrence of zoonotic diseases specifically in wildlife in Pakistan, there are several reasons to assume that circulation of pathogens in wildlife is a major issue:

Wild and domestic animals are mostly dwelling in close proximity of each other. In many cases, especially in hilly areas, they share the same grazing space. In this context, it is entirely conceivable that diseases in domestic animals could be transferred to wild animals and *vice versa*. Wild animals are more prone to zoonotic diseases in comparison to domestic animals, as the latter may have access to vaccination and other medication protocols, and spread of diseases is most probably indeed caused by wild animals. In particular, wild carnivores and wild boar are considered as a mixing vessel for many zoonotic agents.

Another trend which is seen in the hunting community is that offals and body parts are not disposed of properly, but rather left in hunting fields. These leftovers are then eaten by carnivores which can cause transfer of the disease.

In Pakistan, information on the quality and quantity of bushmeat consumption is lacking. Many disease or death causing agents are described as 'unknown disease' (Schaller and Mirza, 1974) due to lack of data.

In India, a neighbouring country with rather comparable rearing and processing conditions, two wildlife related outbreaks have been reported, e.g. an outbreak of anthrax (four fatal cases) in Mysore, Karnatka, India (which has been related to anthrax originating from deer meat; Ichhpujani *et al.*, 2004), and a trichinosis outbreak (18 cases hospitalized, 10 fatal) in Uttarakhand, where ingestion of wild animal meat was implicated (Sharma *et al.*, 2014). No reports of foodborne disease due to bushmeat or wild game meat consumption in Pakistan could be found. This may be due to underreporting, low prevalence of pathogens or, possibly, caused by the traditional meat processing techniques involving boiling or stewing meat at high temperatures, which would inactivate pathogens.

7.5 Conclusions

7.5.1 What has been achieved?

Recommendations of different international wildlife protection organizations and extinction profiling data from researchers have resulted in Pakistan´s federal and provincial governments to put a complete ban on endangered species by sanctioned by heavy fines and imprisonment.

7.5.2 What has been neglected?

Utilization of wildlife as source of meat and other by-products is only partially regulated. Until now, no outbreak or case has been reported that could be associated with handling or consumption of bushmeat. Yet, in many rural areas this is common practice. It is rather likely that underreporting, lack of expertise in source attribution and an underdeveloped reporting infrastructure are related to this finding.

Currently, very limited control options exist in the entire wildlife hunting and game meat consumption cycle in Pakistan. The only point where, to some extent, control measures can be implemented effectively appears to be the 'safe' meat preparation in the consumers' kitchen (Nauman *et al.*, 2016).

7.5.3 What needs to be done?

Community and trophy hunting schemes could be introduced, with a view to create more involvement of local people in protecting local endangered species, as government has insufficient resources to cope with all requirements and situations.

More knowledge on the abundance of zoonotic hazards in wild game should be generated, to assess not only the exposure of humans handling or consuming wild game or products thereof, but also the exposure of extensively-reared livestock, which ultimately could transfer pathogenic agents.

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Section 2

Meat inspection, sampling and health schemes

8. Reindeer – wild game *ante* and *post mortem*?

Arja H. Kautto[1*], Ivar Vågsholm[2] and Rauni Niskanen[1]
[1]National Food Agency, Food Control Division, P.O. Box 622, 751 26 Uppsala, Sweden; arka@slv.se
[2]Swedish University of Agriculture, Department of Biomedicine and Veterinary Public Health, P.O. Box 7036, 75007 Uppsala, Sweden

Summary

The official meat inspection (MI) in reindeer slaughter (*Rangifer tarandus tarandus* L.) has been under continuous development since it was implemented in 1968. Location of slaughter houses in areas of geographical constraints, dependence on weather, and reindeer with low grade of domestication create logistic and economic challenges. The aims of this study are to describe the MI results from reindeer slaughter during 2013-2015, and furthermore, to explore the possibilities for simplification of the *post mortem* inspection (PMI) without compromising food safety or animal health and welfare. In particular, we aimed to compare the results of inspection of abdominal organs (AO; liver and kidneys excluded) performed by skilled slaughter house staff with that carried out by official control staff. The economic comparisons between current procedure of PMI which is dependent on slaughter speed, and the two other independent alternatives are also calculated. The findings in MI mainly relate to non-zoonotic parasites naturally found in the reindeer ecosystem. Traumata are seen more often than at slaughter of domesticated animals. Condemnations are rare and mainly caused by emaciation. The comparison of results of the PMI of AO generated by official staff and by trained slaughter house staff showed that these procedures were at least equivalent. Skilled and trained slaughter house staff could perform the first visual inspection of AO under the responsibility of an official veterinary in a reindeer slaughter house with easier logistics and up to 87% reduction of costs without compromising food safety, animal health or welfare. This indicates a current comparative disadvantage for the reindeer meat industry, not justified by food safety.

Keywords: meat inspection, reindeer, cost, modernization, Sweden

8.1 Introduction

In Europe the reindeer husbandry is by tradition practiced in northern parts of Finland, Norway, Sweden and Russia. In Sweden it is an exclusive right for the indigenous Sami people with an exception of local farmers with few reindeer each in the Torne Valley, along the Finnish border. There are about 4,700 reindeer owners and just more than half of them, 2,500, get their main income from reindeer herding. Reindeer are semi-domesticated and graze extensively in the woodlands and mountains. There are 51 Sami villages, being the economical and administrative units with co-operative rights to use the land for reindeer herding in the northern part of the country, consisting of about 50% (200,000 km^2) of Sweden (Figure 8.1).

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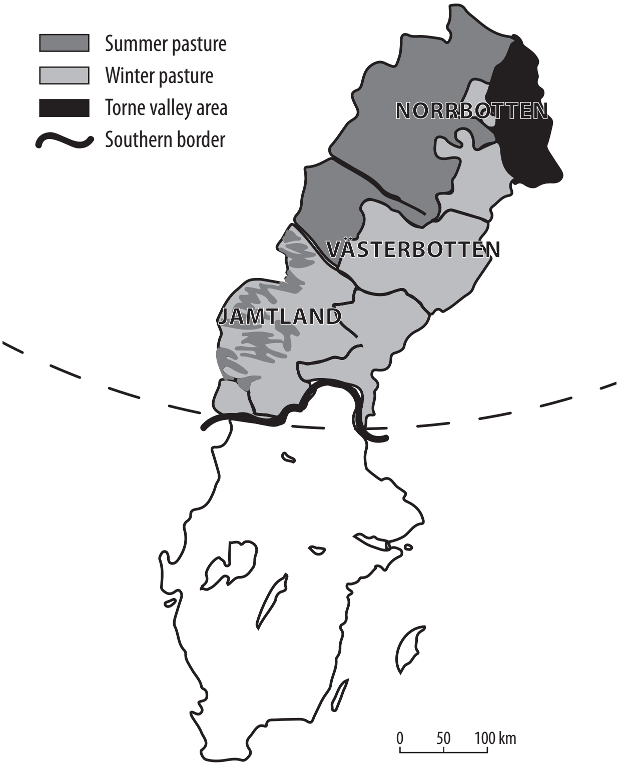


Figure 8.1. Reindeer herding area in Sweden is located above 62° latitude.

The Swedish reindeer population during winter time is about 250,000 reindeer. Calves are born in spring and early summer, mainly in western grazing areas. After earmarking they are released for free summer grazing. Prior to handling and slaughter selection in traditional corrals, reindeer are gathered in the autumn in grazing areas. The total slaughter numbers during September-March varies over years and was on average 55,000 reindeer per year in 2013-2015. This is equivalent to 1,430 tons of reindeer carcasses. More than half of the slaughter, about 70%, are calves. The weight of the carcass varies between 18 kg for calves and max 120 kg for bulls before the rut in September. The slaughter of reindeer is concentrated; 53% takes place in the northernmost county, Norrbotten. Half of the slaughter houses slaughtered 80% of all reindeer in 2014 and 2015. Calculated mean weight is 26 kg per carcass and price per kg carcass to the reindeer owner is about €9 (Sametinget, 2015).

Compulsory meat inspection (MI) of reindeer was implemented on the 1st of July 1968 by a Royal Act on reindeer MI (Anonymous, 1966). The new European Union (EU) legislation, Food Hygiene Package (FHP), was implemented the 1st of January 2006. The traditional slaughter for family members, friends and neighbours within the Sami community without official MI was discontinued. All reindeer are to be slaughtered in approved slaughter houses with the same standards as for domesticated animals except reindeer slaughtered for consumption in reindeer owners´ own households (5-6% of total slaughter; authors' estimation).

Reindeer slaughter houses are located in areas of geographical constraints and activities are dependent on the weather conditions. This causes a lot of logistical challenges for both slaughter houses and for the competent authority. Before implementation of the FHP the national legislation allowed slaughter house staff to make the first control of the abdominal organs (AO), including gastrointestinal tract, genitals, mesentery and spleen and excluding liver and kidneys, and only those with abnormalities were further checked by official staff. Today the whole MI is done during the slaughter because all AO are to be controlled by official staff in every case and there are no available chilling rooms to store hundreds of AO at the same time. Consequently, logistical challenges are even bigger and control costs of *post mortem* inspection (PMI) are very high, about six times higher than for pork.

Hence, the aims of this study are firstly to describe the MI results from reindeer slaughter during 2013-2015, and secondly, to explore the possibilities for simplification of the PMI without compromising food safety or animal health and welfare. In particular, to compare the results of inspection of AO performed by slaughter house staff with that carried out by official control staff. Thirdly, the economic comparison of the different procedures of PMI in reindeer slaughter is done.

8.2 Materials and methods

8.2.1 Meat inspection data

MI at reindeer slaughter complies with Regulation (EC) no. 854/2004 (EC, 2004b) and follows the same protocol as for sheep and goats. Official staff records all the findings, diagnostic codes and decisions concerning results in MI at National Food Agency's (NFA) central data system. The summaries for years 2013-2015 are collated from this system. The diagnostic groups of findings are presented in Table 8.1.

General slaughter statistics were retrieved from the NFAs data system and from the official statistics at Sametinget (2015) which is the national authority responsible for issues concerning the Sami minority in Sweden.

8.2.2 Comparison of *post mortem* inspection by slaughter house staff versus officials

Two MI procedures were run in parallel, without communication, to achieve blinding, on 11,836 reindeer, during 45 slaughter days between October 17th 2013 and March 10th 2014 at one reindeer slaughter house. The official control of AO (including gastrointestinal tract, genitals, mesentery and spleen and excluding liver and kidneys) was compared with the same control carried out by trained slaughter house staff. Actors were standing 10 meters apart from each other's on the line and registered their findings without communication. During the study the official control results always took precedence in terms of actual decisions. Both controls were visual according to the instruction.

Table 8.1. Findings, conditions and diseases, registered in system.

Group of findings	Includes	Comments
Parasites	Cysticercosis, onchocercosis, setariosis, dictyocaulosis, elaphostongylosis, *Hypoderma tarandi*, *Dicrocoelium dendriticum*	Small abscesses and other damages in liver as traces after parasites included here. Changes in lungs recorded here only when parasites visually confirmed. For cysticercosis, laboratory confirmation is required.
Traumata	Acute and chronic traumata	Acute: subcutaneous bleedings and fresh fractures in ribs or other bones, caused by humans or animals (goring) during handling of animals. Old traumata: healed wounds and fractures after accidents on roads/railroads, rutting fights between males, predator attacks
Emaciation	Emaciation	Animals declared unfit for human consumption because of wasting. Absence of epicardial fat (serous atrophy around arteria coronaria), absence of abdominal fat tissue, gelatinous bone marrow. Muscle atrophy.
Poor slaughter hygiene	Carcass not clean	Faecal or other contamination on the carcass visually confirmed. Some cases even after laboratory test. Incomplete bleeding not included.
High caesium-137 and caesium-134 content	Content of Cs-137 and/or Cs-134 in carcass exceed 1,500 Bq/kg	Measurement done externally and confirmed at the laboratory. Carcasses with levels of Cs exceeding 1,500 Bq (Becquerel) are declared unfit for human consumption.

Eight skilled reindeer slaughterers with at least 15 years' experience, employed by the slaughter house were selected to participate in the study. One-day-training prior to the project was done in order to calibrate assessments of deviations from the normal anatomy in reindeer and to highlight relevant epizootic diseases and zoonoses. The official staff consisted of two veterinarians and one auxiliary all with at least 15 years' experience in MI of reindeer. The trained reindeer slaughterers carried out an initial control of AO during evisceration. The official veterinarian or auxiliary controlled carcasses and all organs, including AO, on the slaughter line according to the normal procedure. Official staff registered PMI findings and results in the NFA system and results were retrieved from this database at the end of the project. Results from the slaughter house staff were sent as mobile phone SMS (Short Message Service) directly to the project leader and registered in data file (Microsoft Excel) on daily basis.

These two PMI inspection procedures were analysed and the results were compared with McNemar's Chi-square Test for paired comparisons (McNemar, 1947). The kappa values for the agreement between results were also calculated (Cohen, 1960).

8.2.3 Economic comparison of different *post mortem* inspection alternatives

Three different procedures were compared:

1. Current procedure (CP) for PMI where the whole PMI including AO is done by official staff. Slaughter house staff is not allowed to conduct parts of the PMI. Accordingly, official veterinarian or auxiliary staffs is present during all slaughter time.
2. PMI procedure with a new chilling room (CR). If a slaughter house want to have official staff to perform PMI only after slaughter all AO should be stored chilled until PMI is done. In reality, no chilling rooms of that size exist in reindeer slaughter houses today. Costs for an investment of a new chilling room are followed by less PMI costs because the official control is not dependent on the slaughter time. Anyhow, there is still time needed to inspect all AO. Running costs of a chilling room is not included in calculations.
3. Proposed PMI procedure (PP) includes an initial visual inspection of AO by trained slaughter house staff. Official control of carcasses and organs is done at the end of the slaughter day. Official PMI is not dependent on the slaughter time. Only AO with abnormities found by slaughter house staff are inspected later in the chilling room. The initial visual inspection procedure was in use during 1968-2005 in reindeer slaughter houses even if official staff was present most of the time. Small chilling rooms for just a few AO are already available in slaughter houses today.

The PMI costs for CP were calculated according to the current price per control hour (110 €/hour) and presence of official staff during slaughter because the official PMI is dependent on the slaughter speed. With heavy carcasses (45 kg) only slow speed (i.e. 20 reindeers/hour, total 900 kg/hour) was used. For medium size carcasses (26 kg), high speed (i.e. 60 carcasses/hour, total 1,560 kg/hour) was a realistic alternative. Moreover, two different volumes batches (100 and 300 reindeer per day) were assessed.

Costs of PMI in slaughter and game handling establishments in general were 0.033 €/kg carcass weight (0.3092 SEK/kg, 1 € = 9.4 SEK) during 2015 according to the NFA statistics (Livsmedelsverket, 2016). Industry expert opinion (K. Fredriksson, personal communication) suggests these costs were 0.019 €/kg pig carcass weight (total throughput under 3,300 tons per year) and 0.021 €/kg beef carcass weight (total throughput under 2,000 tons per year) during 2015.

According to expert opinions the cost of building a new CR for 300 AO was €44,200 (34 m^2 à €1,300) during 2015. The costs of PMI done by slaughter house staff are negligible because they perform evisceration and PMI simultaneously.

8.3 Results

8.3.1 Findings in meat inspection

In the NFA system, nearly all slaughters are documented. The improvement of documentation has been from 78% (2013) and 77% (2014) to 97% of total slaughter in 2015. There were findings at MI in 47, 60 and 53% of the carcasses, during 2013, 2014 and 2015, respectively. *Ante mortem* findings are rare (0.02% per year) and consist of general weakness and lameness. Different MI findings according to the diagnostic groups (Table 8.1) are presented in Figure 8.2. One reindeer carcass could have more than one finding.

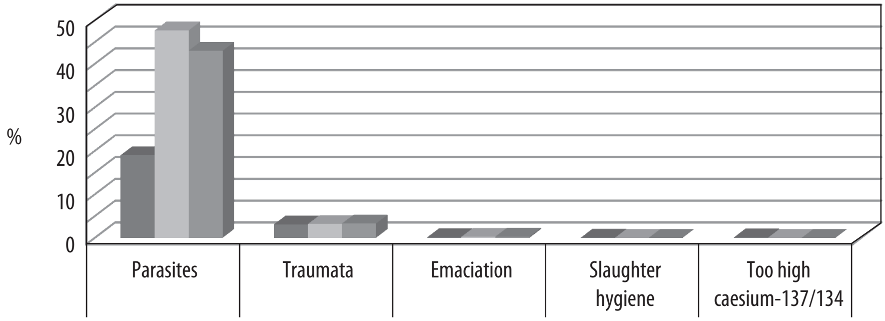
The causes for declaring reindeer carcasses unfit for human consumption were similar during 2013-2015 and emaciation and poor body condition with systemic problems were the most common reasons for this. Too high levels of caesium (Cs) led to the rejection of 33, 1 and 1 carcasses in 2013, 2014 and 2015, respectively. Overall the rate of carcasses unfit for human consumption was very low; 0.2, 0.3 and 0.2% for 2013, 2014 and 2015, respectively.

8.3.2 Comparison of *post mortem* inspection methods for abdominal organs

The level of agreement between the findings of slaughter house staff controls and official controls were very high (99.96%). There were no findings on AO by slaughter house staff and official control staff in 99.98% of cases, 11,830 animals (Table 8.2).

The slaughter house staff recorded five (5) findings while official staff recorded two (2) findings. Only one of the recorded findings referred to the same condition on the same organ. None of these findings were relevant for food safety. Assessment of AO did not differ between PMI done by slaughter house staff and official control staff (McNemar's χ^2=0.8, *P*=0.37).

Fair agreement could be seen (kappa = 0.29±0.008 SE) for the PMI of AO between official staff and slaughter house staff. The proportion of positive agreement was 0.29 and proportion of negative agreement 0.9998. Overall agreement was 0.9996, concluding that procedures are at least equivalent.



	Parasites	Traumata	Emaciation	Slaughter hygiene	Too high caesium-137/134
■ 2013	19	3.1	0.2	0.1	0.1
□ 2014	48	3.2	0.2	0.0	0.1
▨ 2015	43	3.3	0.3	0.0	0.0

Figure 8.2. Types and prevalence (%) of findings at reindeer slaughter during 2013-2015.

Table 8.2. Post mortem *inspection results of control of abdominal organs by slaughter house staff and official control staff.*[1]

		Slaughter house staff		Sum
		+	–	
Official staff	+	1	1	2
Official staff	–	4	11,830	11,834
Sum		5	11,831	11,836

[1] Positive finding (+) and absence of finding (–) per reindeer carcass.

8.3.3 Economic comparison of different procedures

The PMI costs for CP, CR and PP are presented in Table 8.3. The time for hygienic clothing, hand wash, knife sharpening and documentation was included in the time needed. Accordingly, a batch of 300 reindeer needed relatively less time than a batch of 100 reindeer. A batch of 100 reindeer, á 26 kg with producer price of 9 €/kg had a value of €23,400. With the current practice, PMI of this number of reindeer cost €278 (2.5 hours á 110 €) and this is equivalent to 1.2% of the carcass value. With the proposed new practice it would be €110 and equivalent to 0.5% of the carcass value. Reduction is around 60%. Moreover, the cost reduction with implemented PP was around 87% compared to the CP in the *post mortem* control of 300 heavy reindeer (0.122-0.016 €/kg = 0.106 €/kg).

Table 8.3. The post mortem *inspection (PMI) costs in three alternative procedures.*[1]

Variable	CP[2]	PP[2]	CR[2]
PMI hours per batch of 20 reindeer á 45 kg (h)	1		
Cost of PMI €/kg, 20 heavy carcasses (weight 900 kg)	0.122		
PMI hours per batch of 60 reindeer á 26 kg (h)	1		
Cost PMI €/kg, 60 medium carcasses (weight 1,560 kg)	0.071		
PMI hours per batch of 100 reindeer (h)		1	1.5
Cost PMI €/kg, 100 heavy carcasses (weight 4,500 kg)		0.024	0.037
Cost PMI €/ kg, 100 medium carcasses (weight 2,600 kg)		0.042	0.063
PMI hours per batch of 300 reindeer (h)		2	3
Cost PMI €/ kg 300 heavy carcasses (weight 13,500 kg)		0.016	0.024
Cost PMI €/ kg 300 medium carcasses (weight 7,800 kg)		0.028	0.042

[1] Control cost: 110 €/hour control time.

[2] CP = current procedure; PP = proposed procedure; CR = procedure with a new chilling room.

8.4 Discussion

8.4.1 Meat inspection method and results

Data in MI of reindeer in Sweden has become more reliable with less variation in quality and a higher grade of coverage. Quality assurance is done by calibrations and supervisions continuously. The main findings are various parasites which are a natural part of the ecosystem the reindeer live in. These parasites are not causing food borne zoonosis. Most abundant parasites are subcutaneous larval stages of reindeer warble fly (*Hypoderma tarandi*). Even traces of various nematodes were identified in many reindeer. It has to be noted that there are no findings *of Echinococcus* sp. in this dataset. Three cases of *Echinococcus granulosus* were verified at the national reference laboratory in the middle of the 1990s (SVA, 2015). Like in Finland (Oksanen and Lavikainen, 2015) the typical synanthropic cycle involving herding dogs and semi-domesticated reindeer has vanished in Sweden because of just few herding dogs left and their regular antiparasitic treatment.

The main part (71%; Sametinget, 2015) of reindeer slaughtered is calves under one year old and this group is more sensitive to circumstances causing animal welfare problems (bad pastures, handling, etc.). The monitoring focuses on high risk groups in this aspect. Thus, MI could be a good sentinel and screening for epidemiological indicators for animal welfare problems. Prevalence of traumatic injuries recorded was about 3% of all reindeer slaughtered and was quite constant during 2013-2015. This is a bit higher than the prevalence in slaughter of domesticated sheep and cattle in Sweden (A.H. Kautto *et al*., unpublished). Reindeer are carefully handled but only a few times a year and the grade of domestication is low. Acute subcutaneous bruises were the most common findings. More severe consequences as acute fractures or open wounds, seen already in AMI, were rarely seen.

Most of the carcasses declared unfit for human consumption are emaciated, e.g. old animals with bad teeth and calves with rumen villi not yet fully developed during the first winter are suffering most during harsh winters and bad pasture. These carcasses could have traces of parasites and have systemic illness. Whether these are causes to – or consequences of – emaciation cannot be assessed by MI data. The dynamics between host and parasites are very complicated (Irvine, 2007) and should be analysed separately. The number of reindeer declared unfit for human consumption because of too high Cs-137/134 is close to negligible today, e.g. one reindeer declared unfit during 2015.

Tuberculosis, caused by *Mycobacterium bovis*, could be harmful for both human and reindeer, but is not seen at all at PMI of reindeer in Sweden, Finland (EVIRA, 2016) and Norway (Godfroid *et al*., 2014). Moreover, other sources of information, like export testing and necropsies, strengthen this conclusion (A.H. Kautto *et al*., unpublished). Performance of MI is highly correlated with presence of clinical and/or pathological signs in affected animals and early or subclinical cases are likely to be non-detectable in slaughter (Stärk *et al*., 2014). Because affected wild animals weakened by clinical symptoms are easily taken by predators, it should be possible to see *M. bovis* in wild animals. Nevertheless, *M. bovis* is not found in surveillance of wild animals in Sweden (SVA, 2015).

There are many challenges concerning health and diseases in reindeer in Fennoscandia to meet in the future (Tryland, 2010). Some efforts to define baselines in the reindeer populations in Fennoscandia have been done for parasites, viruses and bacteria (Hänninen *et al*., 2002; Helle, 1980; Kautto *et al*., 2012; Kemper *et al*., 2006; Lahti *et al*., 2001) but this information neither covers the whole spectra of findings nor directly concerns food safety. The European Food Safety Authority (EFSA, 2013) has pointed out *Salmonella* sp. and *Toxoplasma gondii* as the most relevant hazards to be covered by MI of farmed game from a public health point of view, while not a problem in reindeer. These and many other hazards relevant for public health and animal welfare, as recently discovered chronic wasting disease in *Cervidae* in Norway, cannot be detected either by conventional or visual only PMI in slaughter (Hill *et al*., 2014; Stärk *et al*., 2014).

MI of reindeer covers the main part of animals slaughtered every year and should be able to detect animal welfare problems, zoonosis and notifiable diseases in the reindeer population in Sweden. Although many relevant food safety and animal welfare findings have low prevalence at population level the MI of reindeer has its strength in the big number of animals inspected. To use professional trained slaughter house staff to perform the initial visual inspection of AO could support beneficially the PMI. Nevertheless, the reindeer population is more like wild ungulates, and the fallen stock includes individuals dying in the wilderness. In fact, this actually weakens the status of MI of reindeer as a suitable source of data collection for monitoring of diseases and conditions (Vågsholm, 2014). Reindeer live on free pastures and veterinary interventions are extremely rare. Consequently, comparisons with alternative surveillance components directly on the reindeer are not an option.

8.4.2 Initial visual inspection of abdominal organs performed by slaughter house staff

Comparison of PMI on AO performed by slaughter house staff contra official control staff shows a very high level of agreement. Hence trained slaughter house staff can perform initial visual inspection of these organs equivalent to the control done by official auxiliary or veterinarian.

Slaughter house staff control of AO has been used in Sweden in reindeer slaughter at all times until the 1st of January 2006. No public health problems related to reindeer meat had been identified at that time. Bonde *et al*. (2010) conclude that the regular MI in general lacks sensitivity and underestimates the prevalence of some conditions and diseases. Actually, it may be easier for slaughter house staff to see pathological findings and parasites during evisceration than a visual inspection on the table after evisceration would reveal. Many of the slaughter house staff are used to handle viscera because they are often experienced hunters and even certified trained persons for first inspection of wild game (EC, 2004a). Their ability to find abnormalities can give higher sensitivity for PMI. Nevertheless, specificity for relevant findings is still dependent on the competence of official control staff. Consequently, this fact supports the possibility of keeping a high level of meat safety even with initial *post mortem* control of AO performed by slaughter house.

It has to be noted that prevalence of all findings is generally very low. This makes it even better that PMI is a part of the evisceration. Handling of the organs on the inspection table by the AO raises even concerns on hygiene and cross-contamination.

The PP is quite similar to the system with trained persons making the first inspection of hunted wild game according the current legislation. Highly skilled slaughter staff creates a reliable surveillance tool for reasonable cost in reindeer slaughter when totally omitting the PMI of AO is not an option.

8.4.3 Economic analyses

According to the NFA statistics (Livsmedelsverket, 2016) the average cost of MI for all slaughtered animals was 0.033 € per kg carcass year 2015, including domesticated ungulates, poultry, reindeer and wild game MI. Our calculations show that the current system for PMI of heavy reindeer costs about four times more (0.122 €/kg). Furthermore, the cost is more than doubled (€0.071) during winter slaughter. These costs are caused by the need of official staff to be present during all slaughter time in order to be able to perform PMI of all AO. This fact indicates a comparative disadvantage for reindeer meat industry, not justified by food safety.

The cost of PMI in a medium sized Swedish slaughter house was about 0.019 €/kg pig carcass weight and about 0.021 €/kg beef carcass weight during 2015. The cost in reindeer slaughter can reach the level of 0.016-0.028 €/kg if the PP is implemented. In this manner, the costs of official PMI in reindeer slaughter could be optimized and more equal to costs of PMI of domestic ungulates without compromising food safety and animal welfare.

8.5 Conclusions

8.5.1 What has been achieved?

The findings in MI of reindeer are rare and mainly relate to different non-zoonotic parasites. Only a few carcasses are declared unfit for human consumption and mainly because of emaciation. The skilled and trained slaughter house staff could perform the initial visual inspection of AO under the responsibility of an official veterinary in a reindeer slaughter house without compromising food safety, animal health or welfare and with up to 87% lower costs and easier logistic. Omitting PMI of AO is not an option because lack of other alternatives to the national monitoring.

8.5.2 What has been neglected?

MI results as such are not systematically used as an integral part of the national monitoring and surveillance system of reindeer population in Sweden today apart from the notifiable conditions.

8.5.3 What needs to be done?

Visual-only PMI of AO done by especially trained slaughter house staff under the responsibility of an official veterinary in a reindeer slaughter house would not compromise public health, animal health or welfare. Consequently, the provisions of Section I, Annex III in Regulation (EC) no. 853/2004 (EC, 2004a) are considered to be inappropriate to be applied to the production and placing on the market of meat from semi-domesticated reindeer. The coming risk based legislation in European Union should be more precise concerning the variety of hazards and risks in different species and regions including MI of reindeer. Systematic temporal and geographical analysis of results of MI of reindeer should be implemented.

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9. European Community food safety regulations taking effect in the hunted game food chain: an assessment with stakeholders in the Netherlands

Jolianne M. Rijks[1], Margriet G.E. Montizaan[1], Hans Dannenberg[2], Lenie A. Algra-Verkerk[2], Delphine H. Nourisson[1,3], Andrea Gröne[1] and Martin Hennecken[4]*

[1]Dutch Wildlife Health Centre, Yalelaan 1, 3584 CL Utrecht, the Netherlands; j.m.rijks@uu.nl
[2]Netherlands Food and Consumer Product Safety Authority, P.O. Box 43006, 3540 AA Utrecht, the Netherlands
[3]Università di Firenze, Dipartimento di Biologia, via Romana 17, 50125 Firenze, Italy
[4]Dutch Ministry of Economic Affairs, P.O. Box 20101, 2500 EC The Hague, the Netherlands

Summary

From 2006 onwards, the EU hygiene regulations were implemented in the hunted game food chain in the Netherlands, and national law was amended accordingly. This study evaluated the progress made in putting this new legislation to practice, and the method used was an interpretative qualitative assessment with stakeholders. Subjects for improvement were identified, and an international comparison was made to learn how some other European countries were dealing with these issues. Initial examination and declaration by a 'trained person' (TP) are required for all marketed hunted game in the Netherlands. A quarter of the Dutch hunters were TPs in 2013, having completed a specific training programme based on the legal requirements. The declaration may be filled in digitally for large game in some Dutch provinces, facilitating traceability. Based on the assessment, it appears that awareness of the TPs concerning proper cooling and hygienic handling of game for good quality game meat was significantly raised through the TP training, and that the decision to place game on the market is taken more consciously. Effort to comply with the regulation changes was found to be high for hunted game obtained from large hunting areas and/or destined for game-handling establishments. Conversely, it was low in local trade, in particular when small game or sales to restaurants were concerned, and this requires more attention. To further enhance game meat food safety, several stakeholders, especially in the local market, need more and clear information and feedback on legal requirements and on good practice for food safety. To further enhance self-responsibility, the Dutch hunter training and TP training must be adjusted. The assessment also identified a strong wish among stakeholders for refresher courses and accredited practical training opportunities. It would be a great step forward to develop these trainings through collaboration at European level.

Keywords: food safety, hygiene regulations, hunted game meat

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9.1 Introduction

At the turn of the century, Europe revised its strategy for securing food safety of products, including those of animal origin (Berthe *et al.*, 2013). This resulted in new European laws collectively referred to as the hygiene regulations. These include the European Regulations (EC) nos. 178/2002, 852 to 854/2004, 882/2004 and 2075/2005 (EC, 2002, 2004a,b,c,d, 2005). These hygiene regulations provide the legal framework for food safety in the production and the marketing of hunted wild game in-fur or in-feather (Atanassova *et al.*, 2008; Deutz and Fötschl, 2014), in this chapter shortly referred to as game.

In the hygiene regulations, hunting products are defined as primary products. This entails that the hunter is considered as a producer of primary products, if the product is marketed (sold or given away). These products must be traceable throughout the food chain, to enable withdrawal (EC, 2002). For game, the function of 'trained person' (TP) was introduced. To become TP, a hunter must complete a training accredited by the competent authority. This training must deal with the subjects detailed by law (EC, 2004b). The TP has to undertake an initial examination for all game marketed to an EU-approved game handling establishment (GHE). The TP function was not assigned to hunters before these new regulations were implemented in 2006. The European regulations do not regulate the local market, i.e. the direct supply by hunters of small quantities of game, or the supply of game meat, directly to the final consumer or to local retail establishments directly supplying the final consumer. It is national law that regulates the local market. This national law must, however, comply with the objectives of the hygiene regulations (EC, 2004b).

The implementation of the hygiene regulations has been described to some extent for Austria (Deutz and Fötschl, 2014; Winkelmayer *et al.*, 2011), Hungary (Herényi, 2014), Italy (Ferri *et al.*, 2014) and Portugal (Vieira-Pinto *et al.*, 2014), but has not yet been published for the Netherlands. In Austria, a network of hunters called auxiliaries had been trained in the period 1994-2005 and was already performing tasks in line with those defined for TPs in the hygiene regulations, so that only minor adaptations had to be made in the procedure of handling game (Winkelmayer *et al.*, 2011). Conversely, in most other countries, including the Netherlands, more significant adaptations had to be made to comply with the regulations.

The primary aim of this study was to evaluate the progress made for game meat food safety since the implementation of this legal framework in the Netherlands, and to identify future directions to enhance it. The evaluation focused on the wild game food chain, in particular on the role of TPs in ensuring food safety and how the hygiene regulations were implemented and perceived. This study also examined how traceability of game was assured in the food chain. In addition, information was collected on how a number of other European countries had addressed the points identified by this study for improvement.

9.2 Materials and methods

The study consisted of three parts: the collection of background data, an assessment with the different stakeholders, and an international comparison. It was performed late 2014-early 2015.

9.2.1 Background data

The collection of background data included the documentation of the changes in legislation, procedures and responsibilities for ensuring food safety in the game food chain, i.e. the rules before and after the implementation of the hygiene regulations in the Netherlands in 2006. These data were obtained from expert knowledge and legislative documents. A distinction was made between large and small game, as defined in the regulations.

The collected background information also included numerical data on the game food chain at the time of the study. These numbers provide insight into economic importance and are relevant for the interpretation of the assessment. The sources of the numerical data were published data (not peer-reviewed) and data obtained from the provincial game management units (GMUs), the Royal Hunter Association (KJV), the Netherlands Organisation for Hunting and Land Management (NOJG), the Hunter Training Foundation (SJN), the Dutch Association of Game Meat Dealers (NBPW) and the Royal Dutch Catering Business Association (KHN).

9.2.2 Assessment with stakeholders

The assessment with the stakeholders focused on understanding the level of ownership of the changes implemented. This is relevant to understand perceptions and compliance within the food chain.

The assessment was made using a qualitative method. In brief, interviews (n=37) were held with persons belonging to different categories of stakeholders from the hunted game meat food chain. All interviews were held with single persons, except one interview which was with two persons. The interviewed persons (n=38) were selected progressively using theoretical sampling. The interviews were semi-structured and face-to-face (except one per telephone), and occurred between November 2014 and March 2015. The topic list was identified after the collection of the background data (Table 9.1). Questions were asked open-ended and answers were probed if needed. All interviews were performed by the first author. Informed consent was obtained for recording, transcribing and analysing the interviews anonymously. Duration was on average an hour. Analysis was interpretative, based on grounded theory (Boeije, 2010). Quotes were translated to English by the first author. The analysis led to suggestions to further enhance game meat food safety in practice.

Table 9.1. Topic list.

Topic	Question(s)
Responsibility	What has changed for the stakeholders? How is this change experienced?
Trained persons	What is the effect of introducing TPs? Are there enough (why)? Is the training sufficient?
Traceability	What has changed? Has it improved? Does electronic reporting diminish work load?
First examination	How is the difference between normal and abnormal made (before/after shot)?
Game hunted abroad	What was perceived by Dutch stakeholders of the implementation of the regulations in other European countries?

9.2.3 International comparison

The international comparison was directed towards examining how a number of neighbouring European countries had addressed the points identified by the study as possible future directions to further enhance game meat food safety. The data were obtained from expert knowledge, legislative documents and websites/hunter training documents. The selection of the countries was convenience-based (geographical proximity and official documents in English, German, Dutch or French): Belgium, France, Germany, Luxembourg, and the UK. For France the focus was on Metropolitan France, and the specific legislation of the Crown Dependencies was not considered for the UK.

9.3 Results and discussion

9.3.1 Background data/context

9.3.1.1 Marketing hunted game: legislation and responsibilities before and after 2006

Before 2006, the European Directive 92/45/EEG provided the legal framework for the production and marketing of game to a GHE (EC, 1992). The directive was worked out in Dutch national law by the regulation 'Regeling keuring en handel dierlijke produkten' (LNV, 2005a) and the decrees 'Besluit productie and handel vlees van vrij wild' (VWS, 2002) and 'Warenbesluit vlees, gehakt en vleesproducten' (VWS, 2001). All game in large quantities, game from abroad, and game intended to supply the international market had to be delivered to a GHE. At the GHE, game was visually inspected and examined *post mortem* by the competent authority. Every individual piece of large game had to be investigated, while in small game only a sample of the delivered animals was examined. *Trichinella* testing was required for each wild boar delivered at a GHE. Small quantities of game could be delivered to the local market. Testing for *Trichinella* in wild boar delivered to the local market was not mandatory, but it was legally specified that *Trichinella* should not be demonstrable in 10 g of wild boar meat (VWS, 2001). When game was hunted for own consumption, i.e. not marketed, there were no legally determined hygiene requirements or *Trichinella* testing requirements.

The new hygiene regulations are in force as from January 1st 2006. The complementary national legislation was from 1/1/2006 up to 1/1/2013 the regulation 'Regeling vleeskeuring' (LNV, 2005b), and from 1/1/2013 onwards the decree 'Besluit dierlijke produkten' (EZ, 2012). Collectively, these laws specify that, in the Netherlands, game must have undergone an initial examination by a TP to be marketed (including given away). The TP writes the results of the initial examination on a declaration. This declaration specifies amongst others the unique identification number of the animal (large game) or batch of animals (small game), and has to be delivered with the marketed product that bears the same identification. The TP must keep a copy of it for three years. Additionally, all marketed wild boar must be tested for *Trichinella* in an accredited laboratory. The TP may take the sample for testing. The test results must be negative and delivered with wild boar specimen, in addition to the declaration. As was the case before 2006, game in large quantities, game from abroad, and game intended to supply the international market must be delivered to a GHE. Game delivered at a GHE is still subjected to *post mortem* inspection by the competent authority. However, if no abnormalities were detected by the TP, large game carcasses are no longer accompanied by organs when delivered to the GHE. Marketed small game is generally not eviscerated before being placed on the market in the Netherlands. Game in small quantities shot in the Netherlands may be delivered directly by the hunter to the local market, including as game meat in an unprocessed condition, provided no abnormalities have been detected by the TP. The local market consists of the final consumer, a restaurant or local retail establishment (local retailer) supplying the final consumer. If abnormalities are detected or hazards are identified, and hunter or TP consider that the animal should not be placed on the market, they have the choice of leaving it behind in the field, moving it for disposal at a rendering plant, or submitting it – provided not eviscerated – for *post mortem* examination to the Dutch Wildlife Health Centre (DWHC). In case of doubt, they may also request an inspection of the body and organs by the Dutch Food and Consumer Product Safety Authority (NVWA), but due to costs this is uncommon (Figure 9.1). As was the case before 2006, when game is not marketed but hunted for own consumption, there are no hygiene or *Trichinella* testing obligations set by law.

Because in the beginning of the new hygiene regulations the number of Dutch hunters with a TP certificate was low, there was a transition period from 2006-2008, during which the initial examination by the TP could still be performed at the GHE, and the declaration by the TP was not required for game delivered to the local market. During this transition period, the Dutch regulation simply specified that game had to be delivered in a clean state, such that

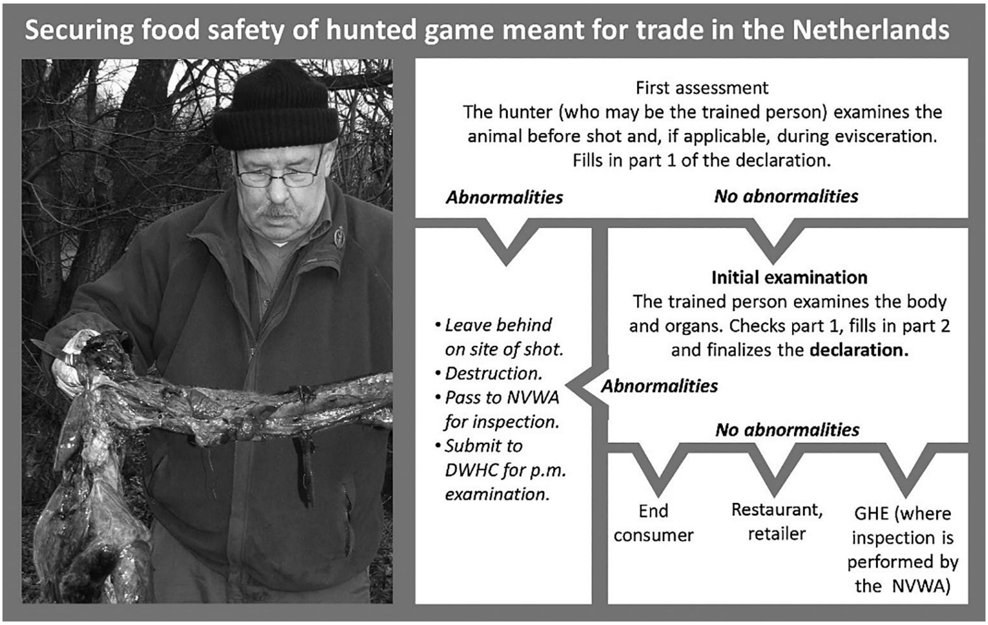
Securing food safety of hunted game meant for trade in the Netherlands
First assessment
The hunter (who may be the trained person) examines the animal before shot and, if applicable, during evisceration. Fills in part 1 of the declaration.
Abnormalities
No abnormalities
• Leave behind on site of shot.
• Destruction.
• Pass to NVWA for inspection.
• Submit to DWHC for p.m. examination.
Initial examination
The trained person examines the body and organs. Checks part 1, fills in part 2 and finalizes the declaration.
Abnormalities
No abnormalities
End consumer
Restaurant, retailer
GHE (where inspection is performed by the NVWA)

Figure 9.1. Since 2008, all marketed hunted game undergoes an initial examination by a trained person (GHE = EU-approved game handling establishment; NVWA = Netherlands Food and Consumer Product Safety Authority; DWHC = Dutch Wildlife Health Centre).

no contamination had taken place at levels potentially harmful to humans, and such that organisms harmful to humans could not multiply.

In conclusion, the hygiene regulations introduced the TP and made traceability of game delivered to GHEs mandatory. These measures, supporting self-responsibility for food safety in the hunted game food chain, were extended in the Netherlands to the local market. Consequently, nowadays all game placed on the market should have been examined by a TP for absence of abnormalities and be traceable.

9.3.1.2 Numbers and volume of hunted wild game species

In 2013, there were approximately 27,500 hunters in the Netherlands (KJV, unpublished data), of which 6,793 (24.7%) had completed the training for TP (SJN, unpublished data). The TP training course had a success rate of 99.3% for the period 2006-2014 (SJN, unpublished data). After peaking with over 2,000 hunters being trained in both 2008 and 2009, the demand for the course gradually declined to 164 hunters by 2013, and is now stabilizing (SJN, unpublished data). Few of the TPs have volunteered on the websites of two Dutch hunter associations to be available for an initial examination upon request (ca. 5%; websites accessed February 2015). However, given the decline in demand for TP training, presumably a workable field situation has been reached with a quarter of the hunters trained as TP, at least for the current levels of compliance (see Section 9.3.2.3 and 9.3.2.4).

Common game species present in the Netherlands and their estimated hunted numbers in 2007/2008 are detailed in Table 9.2 (Montizaan and Siebenga, 2010). The Dutch large game hunting bag is relatively small compared to other countries, even after taking into consideration country size (Deutz and Fötschl, 2014; Ferri *et al.*, 2014; Vieira-Pinto *et al.*, 2014; Winkelmayer *et al.*, 2011). However, the small game hunting bag is substantial, for example when compared to Austria (Winkelmayer *et al.*, 2011).

Overall, large game hunting bags have increased and the small game hunting bags decreased in the past years in the Netherlands (Table 9.2). Changes in species management and hunting permissions generally explained the increase in large game hunting bags (Montizaan and Siebenga, 2010). The same factors, as well as changes in legislation and various environmental factors, explained the small game hunting bag trends. For the European rabbit (*Oryctolagus cuniculi*), the decline in the numbers harvested was also associated with the introduction of viral haemorrhagic disease in 1990 (Montizaan and Siebenga, 2010). The described trends appear to continue. Specifically, in 2010/2011 the hare hunting bag was estimated to be 125,000 and the mallard hunting bag 175,000 (Montizaan *et al.*, 2013).

Hunted game originating from the Netherlands represents only a small proportion of game meat consumed in the Netherlands annually. One study mentions that approximately 12,000 tons of farmed and wild game meat are consumed in the Netherlands annually, and that game hunted in the Netherlands provides approximately 600 tons (Op de Beek, 2012). The remaining 95% of game meat would be farmed game and wild game imported for approximately one third from elsewhere in Europe and for two-thirds from outside Europe (Op de Beek, 2012). Based on hunting bags and weights, our estimation is that large game

Table 9.2. Data on hunted game bags (based on Montizaan and Siebinga, 2010).

Wild game type	Species[1]	Scientific name	Estimated no. of heads hunted in the year 2007/2008	Comparison to estimated no. of heads hunted in 1980/1981
Large	red deer	*Cervus elaphus*	1000	increased ++
	fallow deer	*Dama dama*	300	increased +
	roe deer	*Capreolus capreolus*	16,000	increased +++
	mouflon	*Ovis aries musimon*	<50	
	wild boar	*Sus scrofa*	1,200-4,000	increased +++
Small	brown hare	*Lepus europaeus*	170,000	declined +
	European rabbit	*Oryctolagus cuniculi*	75,000	declined +++
	wood pigeon	*Columba palumbus*	500,000	increased +
	mallard	*Anas platyrhynchos*	250,000	declined ++
	pheasant	*Phasianus colchicus*	75,000	declined +++

[1] Geese species and other waterfowl hunted in this period in the context of damage control are not included in this overview.

hunted in the Netherlands provides less than 200 tons of meat and small game around 750 tons, and part of these will be for own consumption.

In conclusion, a quarter of the hunters have been trained as TP. The numbers of game hunted in the Netherlands is relatively small compared to numbers hunted in other European countries, in particular for large game, and supplies only a fraction of the quantity of game meat consumed in the country.

9.3.2 The outcome of the assessment with the stakeholders

The new legal framework modified some of the roles, and thus the responsibilities, of stakeholders in the hunted game food chain (Section 9.3.1.1). The stakeholders currently involved in the production and marketing of game are the hunters, TPs, the restaurants and local retailers supplying game meat to the final consumer, and the GHEs (Figure 9.1). The interpretative analysis revealed four themes: (1) pathology; (2) contamination; (3) traceability; and (4) context. The first three are food hygiene pillars (Figure 9.2), and pertain directly to the stakeholders' (level of) ownership of the food hygiene responsibilities given to them by the new legal framework. The first theme concerns the capacity to identify health problems in the animal (pathology). The second concerns the ability to handle the animal after shot in such a way that contamination is minimized (contamination). The third relates to measures allowing identifying and recovering game meat that may affect human health after consumption, but that has reached the market despite the previous efforts, or could do so in the future (traceability). Finally, the fourth theme 'context' groups aspects relating to the context in which Dutch game is hunted. Results for 'context' are not presented as a separate section, but integrated into the results per pillar and identified by the postponed word 'context' in italics.

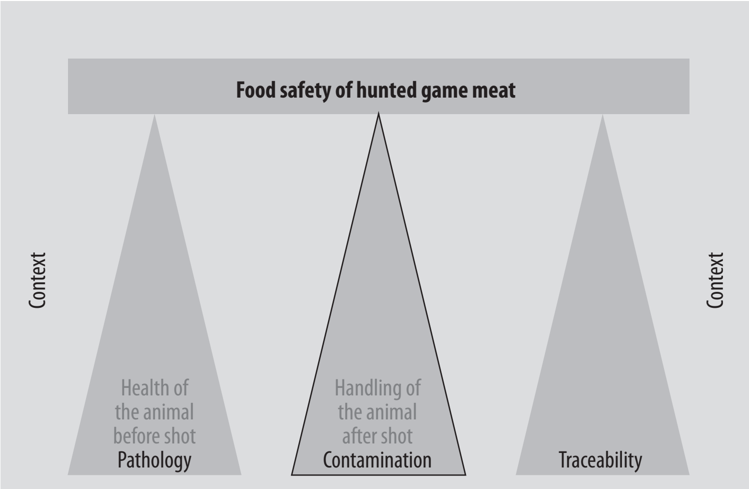
Food safety of hunted game meat
Context
Context
Health of the animal before shot
Pathology
Handling of the animal after shot
Contamination
Traceability

Figure 9.2. The four themes identified through the interviews. The highlighted food safety pillar is where the implementation of the hygiene regulations in the Netherlands appeared to have had the greatest impact.

9.3.2.1 Pillar 1 – pathology

In their first reaction, hunters and TPs tended to downplay the effect of the new legal framework on their role in ensuring food safety of hunted game, except for the increase in administration. Firstly, hunted game was conceived to rarely display visible abnormalities (<1% of specimens), because diseased animals would retreat to die, or get caught by predators. Additionally, the interviewed TPs considered that they had always assessed the hunted animal's health and judged whether it could be placed on the market for human consumption, i.e. they had assumed this responsibility well before the hygiene regulations took effect. An emaciated animal or an animal with abnormal behaviour would not be considered fit for human consumption. Such judgement was based on common sense and experience, and the deployment of the new legal framework (TP training) had not changed this.

Upon probing however, hunters and TPs often conceded that they discussed more often about wildlife health and payed more attention to the organs of large game and the exterior of small game, since some of them had been trained as TP:

> I must say that I think that we now look with more scrutiny at the organs, what do they look like, is it [the animal] fit for consumption? Yeah, I guess so, at least that's what I see around me. (quote TP 17)
>
> What I know about the introduction [of the hygiene regulations], is that there were training courses and that we then discussed together when we had shot [a hare], asking if the animal was healthy. (quote hunter 19)

The TPs qualified the TP training course as 'brief', 'mandatory', 'cash in on hunters', and considered the certificate quite easy to obtain. Most but not all TPs were aware that they were only expected to distinguish between normal and abnormal, and that they were not performing diagnoses. Some lesions could use more decision-making guidelines to determine when they should be considered a reason to not place game on the market. An example is a swollen joint. TPs estimated that most of the acquired knowledge had faded after a year and were in clear demand of non-obligatory refresher training, with accredited practical sessions and updates on legislation. The practical training given to a few TPs in a pilot project was considered extremely useful for improving the TP's ability to judge organs (Figure 9.3). It was also stressed that diseases considered most important in terms of zoonotic risk, e.g. *Trichinella* spp., ought to be clearly specified and regularly brought to the attention of all hunters, not only TPs. This because hunters consume many of the hunted animals themselves and not all are trained as TP.

Initially, the EU-approved GHEs had to insist on obtaining the declarations with the delivery of game, but meanwhile it has become routine. The GHEs find some TP still lacking experience in judging organs. A TP may occasionally place large game on the market for human consumption, while mentioning an unusual feature on the declaration, e.g. lean carcass. In such situations, some GHEs would like to see the organs, or at least find more detail on the declaration, such as a statement saying none of the organs presented abnormalities. GHEs, local retailers and restaurants insisted that game with abnormalities should be excluded from the food chain. In particular, restaurants are considered to be poorly informed of the current regulations applying to hunted game.

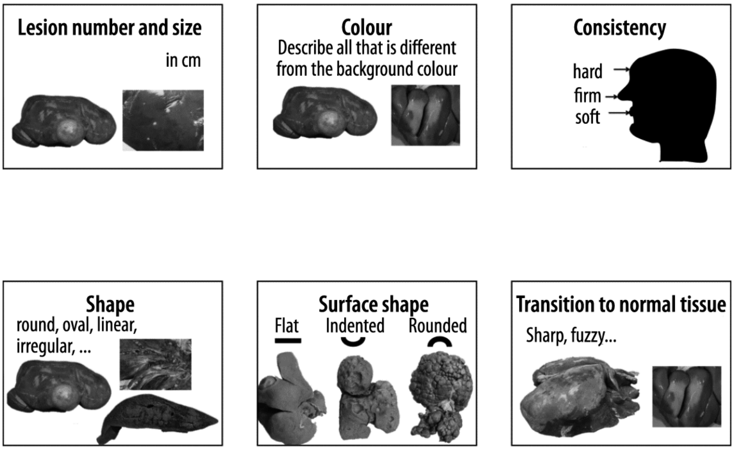
Lesion number and size
in cm
Colour
Describe all that is different from the background colour
Consistency
hard
firm
soft
Shape
round, oval, linear, irregular, ...
Surface shape
Flat
Indented
Rounded
Transition to normal tissue
Sharp, fuzzy...

Figure 9.3. How to describe an abnormality. This was a practical in the context of a pilot project that aimed at obtaining insight in the nature of the abnormalities that lead trained hunters to decide that hunted game should not be placed on the market.

9.3.2.2 Pillar 2 – contamination

All interviewed stakeholders were of the unanimous opinion, that – since the hygiene regulations had been implemented and the TP training existed – there was increased awareness about the importance of proper handling of shot game (transportation, evisceration, cooling…) as it relates to good meat quality:

> Since the TP training exists, we are more conscious about handling game hygienically. The importance of cooling, rapid evisceration, and so, for good meat quality. Yes, that is not dealt with enough in the basic hunter training. But the TP training pays much more attention to these points and that is much better, because I must say, there were quite a few points that I just didn't know. (quote TP 27)

> Of course, one knows subconsciously, that meat is perishable in warm weather and bacteria will grow fast. But once you see the statistics [during the TP training], you think, gosh, that goes fast…and the next time you do take action faster… it [the TP training] increases your awareness. (quote TP 29)

There has been an increasingly strong focus in the Netherlands on hunting for game population management and for game damage control, especially in large game (*context*). Some GHEs and restaurant-owners considered that the importance of hygienic handling of game was such an eye-opener for some hunters because hunting for food had been progressively alienated.

The general view was that all hunters, not only TP, should have knowledge of best practice in game-handling, i.e. it ought to be dealt with in the basic hunter training. It was also thought that the more common proper game-handling practice becomes, the more likely will hunters (together with other stakeholders) be able to organize access to the required logistics, e.g. quick access to a refrigerating facility. From an economic perspective, this is quite a challenge in the Netherlands, because most hunts and hunting grounds are small (*context*). Currently, sufficient refrigerating facilities and infrastructure exist predominantly on large hunting grounds. Evisceration techniques are learnt not only from family or colleagues, but also via demonstrations at meetings or via YouTube films found on internet. More guidance on best practice would be welcome, and some GHEs and local market suppliers indicated they would be willing to be involved in providing hands-on training.

It was mentioned that hunters do not always take enough care in bringing game they handled to the market. The GHEs said poorly handled animals were refused, but sometimes a hunter would then shift to supplying the local market, which would have less stringent standards. The GHEs complained that veterinary inspection takes place at GHEs, but hardly ever at restaurants or local retailers. This is in part due to the fact that two different divisions of the NVWA are responsible for these inspections. The competent authority supervising the inspection at GHEs is the Veterinary and Import Division (V&I) and the Consumer and Safety Division (C&V) is responsible for the control of the local market.

> The down-side [of the implementation of the hygiene regulations for GHEs] is that more and more game is delivered directly to restaurants, because we penalize hunters that deliver poorly handled specimens. I see that because I also process game for restaurants and I see that a roe deer that is correctly shot comes here, and poorly shot contaminated ones go to restaurants because restaurants hardly control for that. (quote GHE 18)

> Well listen, GHEs don't accept it all… [so] you go to the local retailer who will buy it all. (quote hunter 36)

Complying with the infrastructure requirements under the new hygiene regulations can be an economical challenge for local retailers or restaurants, and this has sometimes resulted in a change in business plan. For example, a local game restaurant stopped with processing game into meat and changed to be a collection centre for local game. The collected specimens are delivered in batches to a GHE, cut to meat there, and then returned to the restaurant. By serving as a collection centre for local game, the restaurant is assured the wild game meat it serves is indeed locally produced.

9.3.2.3 Pillar 3 – traceability

When asked why traceability was necessary, some respondents mentioned poisoning or environmental pollution hazards, others *Trichinella* sp. infestation, and one Shiga-toxin producing *Escherichia coli* (STEC). Interestingly, most persons did not produce an answer. This is a problem because perceiving the usefulness of a measure precedes compliance.

There are several dynamics discernible with regard to delivery of game with a declaration. While providing a declaration with each game delivery has become common practice for large game and delivery at a GHE, compliance is more questionable on the local market, in particular for small game. The added-value of providing a declaration is not perceived for the local market, and restaurants or retailers do not always request it.

> I guess in the EU-approved trade, around 95% is with papers [declaration and, if required, *Trichinella* test results]. Maybe even 100%, because the recognized trade cannot permit itself otherwise at the moment, they are under heavy control. In contrast concerning delivery of game to a friend or a private client, well that percentage is maybe even zero. And in between there is an area, well, that more or less depends on the species delivered… (quote TP 34)

Nature management in the Netherlands was recently decentralized to the 12 provincial governments (*context*). A growing number of these provinces use a digital registration programme for collecting data from hunters on game populations and shot game. In this programme, digital declarations can be made (at the time of the study, only for large game). All the information required in the declaration (except the observations made by the TP with regard to the health and handling of the animal) are already put in the system by the hunter. Hence, only the information from the TPs needs to be filled in. The declaration (which does not include the *Trichinella* test result) can be printed and provided with the animal.

But it may also be viewed on-line by the competent authority, or by GHEs with a license for the programme (only one GHE had a licence at the time of the study). Large game hunters perceived the tool as very practical, and it had facilitated the competent authority in providing feed-back to TPs.

> In handwritten declarations, TP contact details are not always complete, telephone numbers often lack. When declarations are made via the digital registration system, they are easy to trace if necessary. Several times this year we made use of that to contact the TP when there were discrepancies between the submitted sample and the declaration. (quote competent authority 10)

The above mentioned digital system is work in progress and many wondered how it would work for small game.

GHEs, local retailers and restaurants have entry and output registers. Declarations are part of the entry register. GHEs generally considered there was only a very small chance of recall for game, and output batches of small game (meat) in particular could span the production of an entire week.

Traceability of game hunted abroad and imported into the Netherlands is an issue. Many Dutch hunters regularly hunt elsewhere in Europe (*context*). The hunter cannot register game from abroad in the previously mentioned digital registration programme. A hunter may bring back one piece of large game hunted 'intracommunity' for own consumption. But if there is more, it has to be delivered at a GHE, where there is a veterinary *post mortem* inspection. The issue is surrounded by confusion or by non-compliance:

> Even this week I talked to a hunter who hunts in Germany and who hasn't delivered us [the GHE] a single head of large game specimen throughout this autumn. Well, they had brought them to restaurants. I said that restaurants are not allowed to accept specimens hunted abroad. Because game hunted abroad has to be delivered to a GHE, an exception is made for single specimens for own consumption. He can't give it to another hunter or deliver it to a local retailer. Because that is punishable. However, local retailers don't know this. Restaurants don't know it either. (quote GHE owner 35)

Trichinella sp. tests are performed at two accredited laboratories. In two of the 12 provinces, sample submission and test result returns are logistically coordinated by a provincial wild boar coordinator, which provides the laboratory with a direct and constant interlocutor. In principle, the accredited laboratories only perform the tests on Dutch wild boar. However, in the intracommunity trade of wild boar, it happens that GHEs in the Netherlands obtain properly traceable wild boar from elsewhere in the European Union without obtaining documented proof that the *Trichinella* sp. tests were negative (in some German regions, the hunter is only phoned if the result is positive). To avoid having to dispose of the wild boar for that reason, it has been temporarily accepted that in such cases, the accredited laboratories in the Netherlands can perform the *Trichinella* testing.

Restaurant owners indicated that certain consumers demand local products from nature, specifically products of known origin and composition, and that have a low ecological footprint (*context*). These customers demand clarity on the nature and origin of the products they consume. Most customers have not been preoccupied by such questions yet, but this could change in the future. This can increase the value of locally produced game, with positive effects on compliance and ownership.

9.3.2.4 Conclusions of the assessment – stakeholder suggestions for the way forward

It was concluded from the assessment that there were differences in the impact the hygiene regulations had had on the various aspects of stakeholder self-responsibility. The greatest impact appeared to be on game handling. The stakeholders expressed that the TP training had substantially increased hunter awareness of the effects on meat quality of good marksmanship and properly handling of game. But more guidance was demanded than currently offered in the TP course on best handling practice. Experience with best practice elsewhere and assessments of handling practices in relation to meat (microbial) quality are found in the literature (Atanassova *et al.*, 2008; Avagnina *et al.*, 2012; Coburn *et al.*, 2005; Deutz and Fötschl, 2014; Ferri *et al.*, 2014; Gill, 2007; Membré *et al.*, 2011; Vieira-Pinto *et al.*, 2014). There was also the request to deal with proper handling of game in the basic hunter training rather than the TP training, because most of the critical actions (proper shot, eviscerating, cooling) are undertaken by the hunter (Table 9.3).

In contrast to the strong impact on game handling awareness, stakeholders did not feel that the current TP training had significantly increased the capacity to assess animal health. Nevertheless, the stakeholders did consider that the decision to place game on the market was taken more consciously since the hygiene regulations. This was ascribed to an effect of mass (a quarter of the Dutch hunters trained in eight years, making game health and hygiene subjects of discussion) and the obligation to fill in the declaration. To increase the TP's capacity to assess animal health, accredited short refresher courses were suggested, including practical (non-theoretic) courses. These refresher courses should remain accessible for a large group of hunters (possibly through e-learning modules). Advanced courses on animal health should apply only for TPs seriously interested in disease surveillance and motivated to act as link between hunters and animal and public health services at GMU level (so-called 'TP monitoring' or TPM). Finally, it was felt that diseases presenting a serious public health risk should be known to all stakeholders, not only TPs.

In terms of compliance, eight years after the start of the implementation of the new legal framework, it seemed quite well ensured for large game delivered to GHE. Declarations made through the digital system made contacting the TP easier for the competent authority, so that it has increasingly facilitated providing TP with feedback to further improve performance. This feed-back was appreciated by all.

However, compliance to the new legislation was less for game supplied to the local market, in particular small game. Stakeholders were asking themselves how serious the government was assuring compliance on the local market level, in particular because there were hardly any controls to enforce the legislation changes, so that non-compliance in the local market seemed

Table 9.3. Hunter and trained person actions and responsibilities based on EU and Dutch national legislation, and the food safety pillars to which they apply.

Actions and responsibilities	Pillar
Hunter	
1. Judges the behaviour of the animal before shot. Informs the TP about any abnormal behaviour observed before killing and mentions it on the initial declaration, part 1.	pathology
2. Large game: Takes a good shot (abdominal shots give contamination).	contamination
3. Large game: Removes the stomachs and the intestines as soon as possible. Bleeds if necessary.	contamination
4. Large game: Removes the organs hygienically.	contamination
5. Large game: Judges stomachs and intestines, and odour if eviscerated in the field. Informs the TP about abnormalities and mentions these on the initial declaration, part 1.	pathology
6. Large game: Takes care that the organs can be linked to the carcass, and that they remain with it.	traceability
7. Identifies the animal for traceability. The unique (batch) number is reported in the initial declaration, part 1. Large game: A unique number is inserted into the carcass. Small game: Small game hunted on one day at one site can be grouped under a unique batch number.	traceability
8. Takes care that the animal is cooled as soon as possible.	contamination
9. Fills in the initial declaration part 1. Preferably in the digital registration system. Keeps a copy in his/her administration for 3 years.	traceability
Trained person	
1. Takes note of part 1 of the declaration.	pathology
2. Large game: Judges the carcass and the organs (except stomachs and intestines if eviscerated in the field).	pathology
3. Large game: Judges soiling.	contamination
4. Wild boar: Takes a sample for *Trichinella* testing. Sends a sample to the laboratory. Wild boar remains at sampling site until results are known.	pathology
5. Small game: Assesses the general appearance, samples (exterior) to confirm the impression obtained.	pathology
6. Fills in part 2 of the declaration. Preferably in the digital registration system. Keeps a copy in his/her administration for 3 years.	traceability

of no consequence. It was suggested that just a bit more control at local retail and restaurant level would suffice as a statement to change that perception. Local retailers and restaurants must also be better informed about legislation regarding game, in particular concerning game origin (local versus hunted abroad). They need to be provided not only general information on traceability, such as provided by Britt *et al*. (2013) but also specific examples of where it is useful in the wild game food chain.

In summary, it seems that to move forward, both the basic hunter training and the TP training need to be adjusted and developed further (refresher courses, accredited practical sessions). In addition, to consolidate current achievements, compliance on the local market level must now be addressed, through both information and control.

9.3.3 The outcome of the international comparison

To obtain perspective and to learn from experiences elsewhere, we investigated how neighbouring countries designed the basic hunter and TP trainings, and how they had structured the delivery to the local market in their national legislation. The number of hunters in the Netherlands (28,170) in 2010 was comparable to the numbers in that year in Belgium (23,000), more than the number in Luxembourg (2,000) and less than the numbers in Germany (351,000), France (1,331,000) and the United Kingdom (800,000) (FACE, 2010). The data obtained for Luxembourg were too limited to include, except the training of hunters.

9.3.3.1 Training of hunters

Hunters must pass a hunting license exam in Belgium, Germany, France, Luxemburg and the Netherlands, but not in the UK (Anonymous, 1991, 1998, 2009b, 2011; Bundesministerium der Justiz und für Verbraucherschutz, 2015; Direction Générale opérationnelle de l'Agriculture, des Ressources Naturelles et de l'Environnement, 1994; RSHCB, 2015b). The exam is preceded by a mandatory training in France, Luxemburg, the Netherlands and 11/16 states in Germany. The basic hunting training focuses largely on a safe and good shot, and handling of game after shot is hardly dealt with in Belgium, France and the Netherlands. But in Germany, the basic hunter training also includes all TP training subjects specified in Regulation (EC) no. 853/2004 (EC, 2004b), and new hunters automatically become TP if they pass the basic hunting exam. Generally, the legislation related to the basic hunter training does not refer to hygiene and food safety knowledge, except for the German and Dutch hunting legislation.

Training all hunters as TP is not envisaged in the Netherlands, which makes it difficult to directly match the hunter training in the Netherlands with those in Germany. However, some German states require field visits or practical training (e.g. on evisceration) for sitting the exam, and the experience gained in this can be of interest for amending the Dutch hunter training. Also, lessons may be learnt from the mentorship systems that are in place in parts of Germany and Luxembourg, in which aspiring hunters learn from mentors.

9.3.3.2 Training of trained persons

Overall, the TP training contact time elsewhere was short, comparable to the two evenings in the Dutch training: it was two half-days in Belgium, three hours-three days in France, and generally one day in the UK (Anonymous, 2009a; NGO, 2015; EZ, 2012; FSA, 2015; IJO, 2015; RSHCB, 2015a; The British Deer Society, 2015; Lantra Awards, 2015). In Germany, the TP training is nowadays included in the basic hunter training as previously described (Section 9.3.3.1), but the hunters trained before 2006 follow a training of one evening. In France, a legally formalized two-step approach to training TPs was adopted: TPs are trained by TP trainers ('formateurs référents'). These TP trainers follow a more extensive course (3 days, program specified in national legislation) than the TPs (3 hours), and are registered at national level while TPs are registered locally, at departmental level. This set-up could be of interest if the concept of 'TPM' (Section 9.3.2.4) is pursued in the Netherlands.

Only the Flemish 2008 TP training manual was accessible through internet at the time of study (Giffroy *et al.*, 2008). The manual is organized into four chapters corresponding to the four training requirement points for TPs mentioned in the Regulation (EC) 853/2004 (EC, 2004b). The manual is as elaborate as the Dutch manual. However, there was a much stronger focus on abnormalities rather than disease than in the Dutch manual, and this could serve as inspiration. Also in France the focus is strongly on the distinction of normal versus abnormal, with legislation specifying that the focus in the TP training should be on abnormality and not on specific diseases, and pictures should be of healthy organs (Anonymous, 2009a,c).

No evidence was found in 2015 to indicate that Belgium, Germany, France or the UK had formally adopted a system for TP refresher courses, or had distinct accredited practical training opportunities for TPs. However, in France the possibility of refresher courses is foreseen in legislation (Anonymous, 2009a). Also, based on literature, it appears that accredited refresher courses and practical sessions are common in Austria (Fettinger *et al.*, 2011), and that there is experience with e-learning based TP training, e.g. Portugal (Vieira-Pinto *et al.*, 2014).

9.3.3.3 Traceability, and rules for local market delivery and consumption in hunters' households in other countries

In all countries there were systems in place for traceability. In Belgium and France, this implied the use of unique numbers (tag numbers and/or declaration numbers; AFSCA, 2015a; Anonymous, 2009a).

The rules for the delivery by the hunter of small quantities of game or game meat to the local market differed among countries. In addition, in Belgium and France there were differences in rules for selling directly to the end-consumer or for selling to a local retailer (AFSCA, 2015b; Anonymous, 2005, 2006a,b,c,d, 2009a, 2010, 2012; BMELVG, 2007a,b; BT, 2013; EZ, 2012; FSA, 2009, 2015). The hunter can deliver game in-fur or in-feather or as game meat in Germany, the UK and the Netherlands, but only as game in-fur or in-feather in France and Belgium. In Belgium, the hunter can only deliver to a local retailer if a GHE is annexed to this retail business, and veterinary inspection can be performed. In France, game may be stored in collection centres with the required cooling facilities while awaiting delivery to the local market, but these centres are declared and registered by the competent authority. Small quantities were defined in Belgium and Germany, and in France for delivery to local retailers. 'Local' was defined in terms of kilometres in the larger countries, e.g. in Germany and the UK, and in France for delivery to local retailers. Initial examination and declarations were required for delivery directly to the end-consumer and to the local retailer in Belgium, Germany, and the Netherlands, and only for delivery to the local retailer in France. In the UK, they were recommended but not required. Finally, *Trichinella* testing in susceptible species as wild boar is mandatory in all countries, with two exceptions. One exception is direct delivery to the end-consumer (other than for a meal of an association or hunter group) in France, in which case the end-consumer must be informed of the risk. The other is delivery to the local market in the UK, in which case *Trichinella* testing is voluntary but free of charge. TP may take samples in Belgium in case of delivery to the end-consumer, in France in case of delivery to a local retailer, and in the Netherlands in both cases. In France and the Netherlands, the negative test results must be delivered with the animal. In Germany, it is possible to transfer the obligation for *Trichinella* testing to the consumer or retailer to whom the wild boar is delivered.

For game meat intended for self-consumption by hunters, it is not required to perform an initial examination and fill out a declaration, except in Germany. In Germany, both are required and if abnormalities are detected, these have to be reported to the authorities. Similarly, *Trichinella* testing of wild boar that will be auto-consumed is mandatory in Germany, but only recommended elsewhere.

Many Dutch hunters hunt in other European countries and observe how elements of the hygiene package are implemented in other European countries. They compare the measures applied abroad to those required in the Netherlands. This is usually enriching, but in this case may also be confusing, and contribute to downplay the relevance of adhering to the national rules.

9.4 Conclusions

This study evaluated the progress made for game meat food safety since the new EU legal framework for food hygiene was deployed in the Netherlands in 2006, and identified future directions to enhance it, using stakeholder perceptions as starting point.

9.4.1 What has been achieved?

TP training has been set-up and followed by a quarter of the Dutch hunters since the new EU legal framework for food hygiene was implemented in the Netherlands. The training has significantly raised hunter awareness concerning proper cooling and hygienic handling of game for safe and good quality game meat. This has led to logistical changes, in particular in large hunting areas. It has also become clear that a number of food safety responsibilities lie at the level of the hunter rather than the TP, and can be dealt with at that level.

For traceability of game put on the market, a numbered declaration is required, and, in case of wild boar, also proof of a negative *Trichinella* test. The declaration links to the individual (large game) or batch of animals (small game). The hunter, TP and buyer conserve it for three years. In practice, traceability in this manner seemed quite well ensured for large game, in particular from large hunting areas and/or destined for GHEs. In some provinces, declarations for large game can be filed digitally, which has facilitated feed-back to TPs by the competent authority.

Overall it seems that, since the TP training and the obligation to fill in a declaration, hunters take the decision to place game on the market more consciously and are in demand of more training to adequately meet their new responsibilities. To implement the changes, the focus has been on the GHE and their suppliers, and this is also where the most progress has been made.

9.4.2 What has been neglected?

Compliance is less strong in the local trade, in particular concerning small game. The added-value was not really perceived as such, and there were no signals to show the government found it important. However, operators on the international market gave examples of how this void of attention to the local market destabilizes the food hygiene achievements reached at their level.

Guidance in further education has also been neglected. Food safety training through the current basic hunter training and the TP training are minimal and theoretical. Hunters take to internet to complement their knowledge, but this does not always transfer best practice. Also the different points of view within Europe to what is best practice may work counterproductive.

9.4.3 What needs to be done?

Stakeholders need to be provided more background information and feedback on legal requirements, with further clarification of concepts and grey areas. The importance of traceability in the local market and for small game needs to be clarified, and the buyer on the local market properly informed of the rules, including those for specimens hunted abroad. The Dutch basic hunter training should be revised to elaborate more on the importance of proper cooling and hygienic handling of game. The basic TP training should be revised slightly to be oriented more strongly towards abnormalities, as is the case for some of the neighbouring European countries. Refresher courses and accredited practical training opportunities should be identified or developed for hunters and TP. European cooperation on these issues could be advantageous, as could be further harmonisation of best practice when practice is not related to local hunting conditions.

In addition, there needs to be more feedback by the different divisions of the competent authority to encourage proper food safety practices. The digital declaration system can support this, but for optimal use it needs to be further developed and more stakeholders need to participate. The GHE have been instrumental in chartering change in food hygiene practice, but the local market circuit is now lagging behind, and suggestions to better align compliance dynamics in both markets included making a statement in the local market through the occasional control. Collectively, these measures should further support stakeholders in the game food chain in taking self-responsibility.

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10. Challenges relating to the collection of high quality field samples and how to overcome them

Johanna Baily
Institute of Aquaculture, University of Stirling, Pathfoot Building, Stirling FK9 4LA, United Kingdom; j.l.baily@stir.ac.uk

Summary

The collection of high quality samples from wildlife or wild game species in the field is challenging. Sampling of free ranging species can be complicated by decay, predation and open to non-random selection. In addition, correct storage and expedient transport of samples is often fraught with technical difficulties due to inaccessibility of sampling sites and adverse environmental conditions. This chapter will expand on these challenges and propose methods to optimise sample collection and transportation for diagnostic and research material in order to get the most out of these valuable resources. Such challenges should not be seen as limiting factors to good research projects but carefully factored into the study design, data analysis and result interpretation. Topics covered will include in-field sampling for histopathology, bacteriology, virology, cytology, serology, toxicology, parasitology and genetic analysis. In addition, the emergence of new diagnostic tools applicable to the field situation and how best to future-proof samples will be discussed.

Keywords: in-field, practical advice, diagnostics, transport, histopathology, tissues, fluids

10.1 Introduction

Whether one is sampling on a remote alpine ridge, deep in a Scottish glen or off the edge of a moving boat, obtaining high quality samples is fraught with challenges. Perhaps the biggest challenge inherent to this type of sampling is due to the delay in accessing a suitable laboratory. This will be compounded by environmental factors such as high humidity of aquatic environments, the low humidity of deserts, coupled with extremes of temperature, often fluctuating throughout the day and night. In addition, correct storage and expedient transport of samples is often fraught with technical difficulties due to inaccessibility of sampling sites and adverse environmental conditions.

Sampling of wildlife is often relatively opportunistic given the difficulty of accessing wild animals, introducing bias into many studies. The researcher is also not always equipped with an ideal set of sampling gear to take optimal samples and must improvise with what is to hand.

Sterility is often a major concern in remote locations with difficulties arising when one needs to acquire a sample suitable for bacteriology in a partially predated or decomposed specimen. This same decay and predation can also lead to cross contamination of nucleic acid material.

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Finally, it is important to consider the ethical concerns of disturbing a group of wild animals in order to obtain a research sample, the health and safety of the operator and, of course, cost of the entire process.

This chapter aims to expand on these challenges and by combining personal experience and a review of recognised field sampling techniques, propose practical methods to optimise sample collection and transportation for diagnostic and research material in order to get the most out of these valuable resources. Such challenges should not be seen as limiting factors to good research projects but carefully factored into the study design, data analysis and result interpretation.

Following this, the chapter will provide some practical advice and tips for in-field sampling for histopathology, bacteriology, virology, cytology, serology, toxicology, parasitology and genetic analysis. In addition, the emergence of new diagnostic tools applicable to the field situation and how best to future-proof samples will be discussed.

10.2 General issues on taking biological samples

10.2.1 Choice of sample

What samples one chooses to take will primarily depend on the aim of the research project in question. Is the intention to perform disease surveillance or more targeted research? Does the project involve capture/re-capture? Whatever the goal, study design and good preparation are essential. Strict inclusion criteria for all samples included in a study are fundamental. It is tempting to want to use all material one can possibly obtain, and in some studies, this would be perfectly acceptable, but in others, an untrustworthy sample may not be better than none. For example, if a sample is known to have been collected without aseptic technique or the storage details of the sample cannot be confirmed, the risk of misleading results precludes inclusion in the study.

10.2.2 Preparation prior to sampling

Preparation prior to sampling is key. As far as possible, preparing replicates of easy to use, individual sampling packs with pre-prepared labels and user-friendly record forms goes a long way in limiting both frustration and errors in the field (Figure 10.1). Anything that can be facilitated for the operator in the field should be to allow energy to be spent on the numerous other considerations they will have. Whatever can be prepared in the lab before a sampling expedition should be: pre-fill sampling pots; try to colour code different sample pots if feasible to avoid errors; either pre-affix labels to pots or prepare labels suitably big enough to be handled with gloves and take into consideration that mud, rain or other undesirables will interfere with anything that seemed straightforward in the safety of the lab. Labels are key as these will be the only means of identifying valuable samples once back in the lab. Choose labels suited to the container and the conditions one will be working in. Assume they will rub off, so plan to double label each tube with labels ideally inside each tube as well as on the outside. Never only label the lid of a pot lid as these get readily mixed.

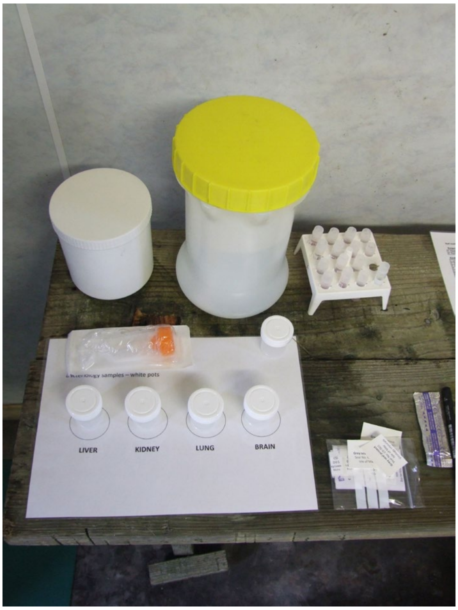

Figure 10.1. Pre-filled, pre-labelled sample pots for each specimen will both save valuable time and avoid labelling errors.

Design sampling packs so they can be easily transported. A hard carry case may seem a good option initially but a small backpack with multiple compartments is most often more suited to crawling around a hillside. Remember to carry small containers to collect sharps (again, these do not need to be large).

If samples are to be sent via postal service to particular laboratories, prepare pre-labelled and pre-paid envelopes prior to departure with ice packs or adequate insulation depending on the sample collected. Light weight hydrated 'ice sheets', designed for the food industry, can be a good alternative to standard ice packs if weight is a concern. These paper thin sheets containing a powdered polymer rehydrate rapidly, absorbing water and increasing in size to then be chilled or frozen depending on your need.

Coordinate sample collection with the laboratory so they can be prepared to deal with samples promptly and have the adequate equipment or reagents necessary for analysis. A very large number of samples arriving unexpectedly can add unexpected delays. Be prepared around weekends and bank-holidays for extended transit times and avoid these if at all possible.

It is a good idea to prepare a checklist of equipment, sampling locations (if feasible) and sampling kits prior to departure.

Remain realistic about what is achievable. It is better to have a small number of high quality samples than a very large number of questionable or poor quality ones.

Finally, although I will not elaborate further on this, before any study gets underway, suitable sampling permits should be obtained and ethical board reviews should be completed. Many wildlife species are protected, even after death, and any research involving live sampling of animals would fall under control of the UK Home Office or the local equivalent organisation.

10.2.3 Traceability and metadata

Obtaining relevant metadata and archiving of both data and samples is crucial. A field notebook is an essential piece of kit. This could be an electronic device or a paper notebook. If electronic, prepare suitable back-up of the data each day. If paper, prefer a hard-bound type over loose-leaf to avoid misplacing data and store the entire notebook in a safe place after sampling. Waterproof notepads are extremely handy, if costly, and waterproof paper can easily be pre-printed as individual data sheets.

For traceability of samples, the best approach is to use a pre-defined numbering system for any sample collected. This number could be pre-defined prior to sampling (used in sequence) or generated using a combination of unique identifiers relevant to the sample.

For example a numbering system may effectively combine:

> date-time-site-animal age group-unique number if several samples collected together
> Ex: 20160101-1025-IOM-LP-01
> (animal sampled on 1st January 2016 at 10:25 am on the Isle of May – live pup – sample number 1)

By introducing unique elements such as time or GPS location, one minimises any chance of having 2 samples with the same identifier.

Pre-defined sample numbers can be less complex and have the advantage that each sampling pack or tube could be pre-labelled. This can save a lot of labelling and hassle in the field but remember to exclude the entire sample pack and corresponding number if one chooses to exclude that sample/animal from analysis for any reason.

Whichever method one chooses, each sample will thus possess a unique identifier to which one may cross reference any metadata in a suitable spreadsheet or data storage system.

Remember that metadata is cheap and easily collected but very difficult, if not impossible, to recover retrospectively. For each sample record as much standardised information as possible, which may have relevance to future analysis. Record ALL parameters for ALL samples or one automatically limits your sample size for that parameter. Also record this promptly as memories fail rapidly… Standard record forms for each sample are a good idea to avoid omitting crucial data.

For wildlife studies, important metadata include details such as sex, age, species, weight, length, location, time, date, clinical signs, weather, etc. The list is endless and should be adapted to the study in question. For location, GPS coordinates are ideal but in certain circumstances, these can be gleaned retrospectively if a record of location is taken on a map. If at sea, GPS coordinates should be taken promptly. Record also the method of obtaining all metadata, and the imprecision of the tool used for this purpose.

10.2.4 Sample selection

Even in the best scenarios, sampling of wildlife as a whole tends to be opportunistic. Simply accessing particular animals is often almost impossible. Debilitated animals will be easier to catch or they may feed in areas more exposed to human activity and thus bias can be introduced into the sampling process. Similarly, sampling only dead or moribund animals introduces a similar bias. The exceptional nature of this material means that samples are often collected haphazardly when carcasses are stumbled upon. Nusser *et al.* (2008) address such considerations for readers who wish more detail on this particular topic.

10.2.5 Minimising time between collection and analysis

Limiting delay between sampling and transport to the lab is often a key to success. One could plan regular up-lifts of samples or consider posting samples next-day delivery to a suitable laboratory. This way, one can remain several days or weeks in the field without compromising the quality of samples. I recall one occasion when although no seat was available on a flight returning from a sampling trip, my precious samples were allowed in the cargo hold to be collected several hours later from the destination airport. I was less essential…

If delay is unavoidable, limiting deterioration of the sample is the next best step. If feasible, use freezers, portable fridges, dry ice or cool boxes but bear in mind the intended use of the sample (see below). Also be creative and ambitious – kind hotel owners may allow the use of freezer or fridge space; generators can be used to power a minus 80 °C freezer if finances allow, but then consider feasibility of transport from the freezer to the lab.

Consider one´s sampling equipment carefully. For example, the choice of bacteriology swabs or viral transport media can limit what types of pathogens can be realistically isolated from samples and time can be critical depending on the organism targeted.

Pathogens such as *Campylobacter* spp. are notoriously sensitive to delay between sampling and culture as well as to culture conditions. A relatively recent sampling expedition detected *Campylobacter* species in Antarctic fur seals (García-Peña *et al.*, 2010) which was a fascinating finding in its own right. However, the true prevalence of this bacterium was difficult to truly assess given that swabs were stored between 4 and 8 °C for between 96-124 days prior to culture.

10.2.6 Sterility in the field

Sterility is one of the biggest challenges in the field and indeed in many sampling situations. To obtain sterile samples, the skin or surface of an organ can be either seared using a red-hot scalpel blade or superficially disinfected using a solution of 10% neutral buffered formalin or 70% alcohol and left to dry. Once disinfected, the organ can be sectioned using a sterile instrument and swab or bacteriology loop inserted into the opening, avoiding the disinfected surface, for sample collection.

The use of disposable instruments in this situation is recommended although an alternative method consists of dipping metallic instruments into alcohol, before exposing them to a flame burning the alcohol off. Commercially available, battery powered automatic Bunsen burners using a gas-canister can be incredibly useful for this purpose.

Some researchers recommend placing tissue samples in 10% neutral buffered formalin (NBF) for 10 minutes to reduce surface contamination of the sample. This technique is not ideal as the formalin fumes may preclude growth of the organism you wish to culture.

Fluids such as pus or effusions can be collected using a sterile needle and syringe inserted through a previously disinfected surface.

When a specimen is markedly putrefied, organs such as bone marrow and brain are less likely to be affected by *post mortem* invaders so may be useful sampling sites. Collection and freezing of whole small bones is also possible to preserve intact bone marrow without risk of contamination (Rowles *et al.*, 2001).

Record the state of carcass decomposition if relevant at time of sample collection. This will enable the researcher to assess the validity of results obtained from each specimen and limit certain analyses to fresher animals.

10.3 Sampling for specific types of testing

10.3.1 Sampling for histopathology

Autolysis occurs rapidly at body or room temperature so samples for histopathology should be taken as quickly as possible and fixed in 10% neutral buffered formalin. If delay in sampling is unavoidable, it is much more preferable to sample chilled rather than frozen specimens as the ice crystals formed during freezing rupture cell membranes, exacerbating autolysis. Larger animals and animals with a generous fat/blubber layer tend to decompose faster due to retained body heat. Removing the fleece or blubber layer can slow down this process if sampling is to be delayed.

The degree of autolysis also depends on the tissue sampled with tissues of the digestive tract (intestine, pancreas) containing abundant digestive enzymes which rapidly break down host tissue after death. Tissues which cool rapidly such as skin or tissues from the extremities tend to be better preserved. Heart and skeletal muscle is often well preserved even in cases where other organs are markedly autolysed.

Autolysis will also depend on the species examined. Cold water fish left at room temperature tend to autolyse very rapidly (within minutes). Smaller animals, such as mice and tropical fish, will autolyse faster in warm environments.

Remember that excessive handling or crushing with forceps can lead to artefacts that will hinder diagnostic interpretation and that, where histopathology is concerned, small is best. Formalin penetrates tissues at approximately 5 mm every 24 h so keep at least one dimension of the specimen under 10 mm thick to aid fixation (Figure 10.2). Ratio of formalin to sample should be in excess of 10:1 with a suitable size of container. Do remember than formalin is a recognised carcinogen so make every attempt to protect you from fumes and leakages and that formalin fumes damage live cells and smears in shared packaging.

If no NBF is to hand, what else could be used? Industrial formalin (38% formaldehyde) can be diluted with 9 parts seawater in a crude approximation of NBF. Whilst alcohols are not ideal fixatives for histopathology, leading to shrinkage and brittle samples, a researcher with no access to formalin could consider attempting fixation with alcohol, again, buffered with a small volume of sea water, if the sample would otherwise be lost. I have processed samples of fish sent in by clients which had previously been fixed in vodka and sea water which, although having unusual morphology, were of sufficient quality to obtain a diagnosis.

Formalin is a carcinogen and many postal services have limitations on volumes they are willing to transport. Larger specimens can still be transported using the following method: fix sample entirely on site; wrap fixed specimen in formalin soaked towels; place specimen and soaked towels in two successive sealed plastic bags.

The brain and spinal cord are exceptions to the small sample size rule as these are very fragile tissues, subject to artefacts with too much handling. The brain should be fixed in its entirety for at least 48 h before being sectioned coronally at 1-2 cm thick slices. The spinal cord can be cut into segments once fixed.

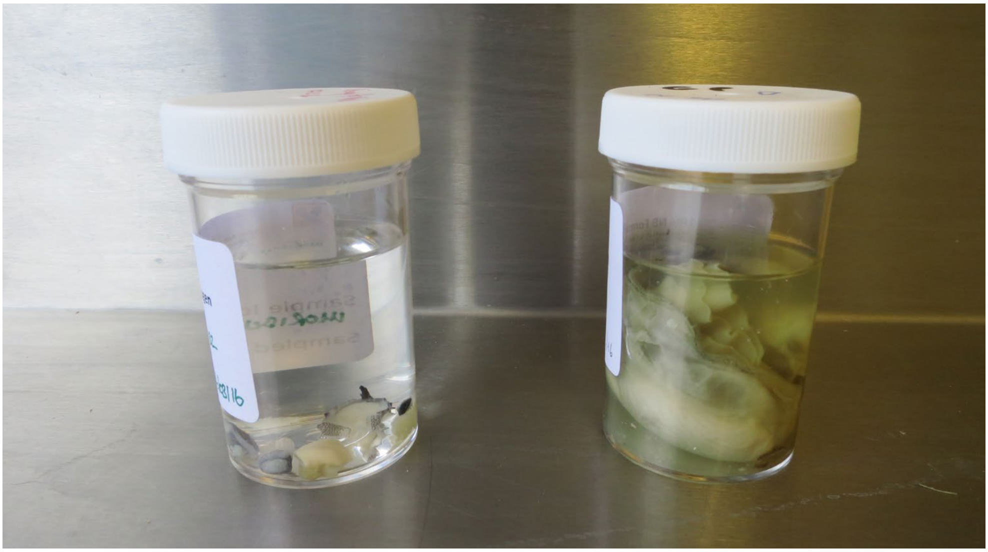

Figure 10.2. Pot containing a suitably sized sample with adequate volume of formalin (left side of image) and pot with inadequate amount of formalin for the tissue submitted (right side of image).

Samples intended for immunohistochemistry should ideally be fixed in formalin for a maximum of 24 h prior to processing, before transfer to 70% alcohol or industrial methylated spirits. In many situations this is unrealistic but with clever use of antigen retrieval methods, there is no reason why prolonged fixation in formalin should preclude immunohistochemistry.

Tissue samples can alternatively be snap-frozen in liquid nitrogen. Sections similar to histological sections can be cut using a cryotome and a wider range of immunohistochemistry can be performed on these samples as the cross-links formed by formalin fixation do not occur.

10.3.2 Transmission electron microscopy

Ideally small specimens should be fixed in 2.5% glutaraldehyde. This is a highly toxic substance that researchers are unlikely to have readily available but collecting samples for transmission electron microscopy is still a possibility. Fixation in 10% neutral buffered formalin, followed by post fixation in glutaraldehyde at a later date is a recognised sampling method. Similarly, our lab has had good results retrospectively dissecting formalin fixed paraffin embedded fixed tissues from wax blocks (Figure 10.3).

10.3.3 Parasitology in the field

Details regarding collection of individual parasite groups and species should be sought prior to sampling as accurate identification of parasites is often hindered by inadequate fixation

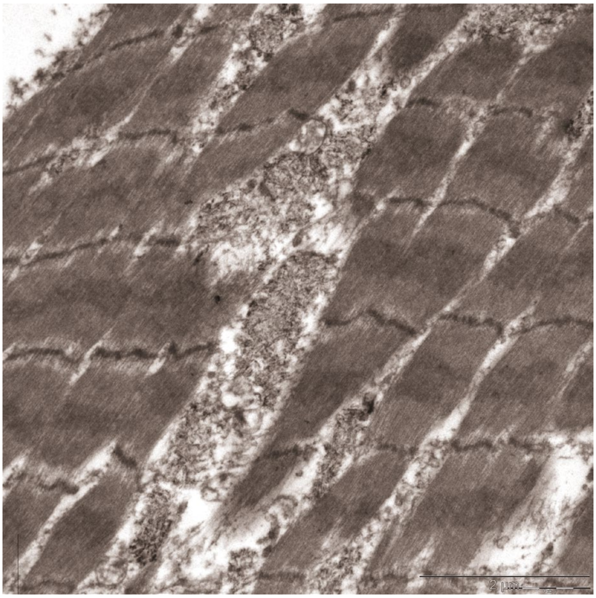

Figure 10.3. Pilot whale skeletal muscle with polysaccharide inclusions. Transmission electron microscopy obtained from paraffin embedded formalin fixed tissues.

methods. For example, optimal preservation of cestodes, trematodes, and acanthocephalans is obtained after letting them relax in saline or tap water at 4 °C for several hours prior to fixation in hot acid formalin alcohol (Dailey, 2001).

If time and handling prevent detailed analysis on site, the entire intestinal content can be stored in 10% neutral buffered formalin to fix for identification at a later date. This technique will not be optimal for collection of many samples but may represent a good compromise overall in certain studies where perhaps quantification is more important than specific identification.

Individual metazoan parasites can often be stored in 70% alcohol if no specific fixation methods are to hand. Do be careful when labelling pots as alcohol will erase any traces of marker pen which can be frustrating. Use pencil to label pots or place a label inside the pot instead.

10.3.4 Bacteriology

As a rule, fresh swabs in a suitable transport medium such as Amies charcoal medium can preserve samples for relatively prolonged periods (several days) if chilled. However, this is very dependent on the pathogen targeted and inoculation of samples straight onto specific agar media may be more rewarding if feasible. Agar plates are bulky but can be transported chilled. As mentioned, some pathogens such as *Campylobacter* are exquisitely sensitive to delay between sampling and culture. For these, establishing a field laboratory to incubate samples would be essential to avoid false negative culture results.

Again, depending on the pathogen suspected, large samples of tissue (>2 cm^3) can be frozen (-20 or -80 °C) and transported back to the laboratory for isolation and identification at a later date. This technique can be very rewarding as it tends to result in less loss of bacteria than swabs and permits delay in processing at an already busy time once returning from the field.

Samples should be collected as aseptically as possible (see 'Sterility in the field'). If bacteriology samples are taken, remember to place samples away from formalin or alcohol fumes during transport as these can inhibit bacterial growth.

10.3.5 Virology in the field

Several techniques are used in virology investigations: PCR, culture, serology and electron microscopy. The ideal sample for virology would be fresh tissue samples placed into viral transport medium. If not processed rapidly, storage at -80 °C would be required. Samples can be kept cool on ice for up to 12 hours before being transferred to a freezer. For PCR, frozen or fixed tissue can be used but freshly frozen tissue is ideal.

10.3.6 Toxicology

Toxicology sampling requirements are incredibly varied depending on the toxin suspected or targeted. Advice should be sought from the laboratory likely to process the samples prior to collection regarding type of container required, sample site and quantity of tissue required for analysis. Many laboratories require minimum sample volumes which can be surprisingly large (often >50 g of tissue or >3 ml serum or blood) and this will depend on the lab and the toxin suspected.

As a rule of thumb, if samples are not to be analysed within 24-48 h, samples should be frozen, ideally at -80 °C but -20 °C for a shorter time is generally acceptable. For persistent organic pollutants in blubber, samples should be wrapped in aluminium foil or Teflon to avoid leaching of unwanted compounds from plastic containers.

During collection, ensure that instruments, sampling boards or any objects coming into contact with the sample are unlikely to lead to contamination of the sample. Examples of contamination may include exhaust fumes if looking for oil derivatives or contamination by metallic paint or soil when investigating heavy metal burdens.

10.4 Sampling of specific tissues or cell compounds

10.4.1 Blood and fluids

Collecting blood from dead animals can be challenging. Basic equipment needed would be syringes or vacutainers. The heart chambers are often the best location to obtain whole blood or serum using a vacutainer system. Sampling from depressions of the axillae or groin on first incision at *post mortem* examination where blood pools rapidly is also useful in order to avoid obtaining a more contaminated sample later in the *post mortem* process. Another site where blood collection is facilitated is within the eye socket following removal of the eye. Blood may pool at the base of this cavity and is easily collected with a syringe. Depending on the analysis required, different blood collection tubes can be used with or without anticoagulant and centrifugation of whole blood is required for collection of serum. Remember that some parameters such as glucose require immediate separation as the glucose will can be consumed or secreted by red cells.

In field conditions without electricity, centrifugation can be problematic but this can be overcome using a hand or battery powered centrifuge. An alternative solution is to simply leave tubes to settle for a few hours and very carefully pour off the supernatant serum into a separate clean tube. Care should be taken to not disturb the coagulum so be conservative when recovering the serum or the sample will be rapidly contaminated.

As for most samples, storage of whole blood and fluids is optimal at 4 °C with rapid transport to a laboratory. Serum samples can, however, be frozen at -20 °C or -80 °C for analysis at a later date. In certain situations, particularly field conditions with high temperatures, this is not realistic and good results have been obtained using filter paper. Such filter paper can be stored at room temperature or frozen and studies are underway to assess specificity and sensitivity of these techniques (Curry *et al.*, 2014). Both blood samples and serum samples can be stored in this manner and are widely used in the fields of tropical medicine (Smit *et al.*, 2014). Main concerns regarding the use of such systems when designing sampling protocols include biosafety risks, contamination issues, test sensitivity and establishing quantitative cut off points for assays using such material (Smit *et al.*, 2014).

Increasingly, pen-side diagnostic tests such as snap tests using ELISA tests within laminar flow systems are being developed for specific pathogens and undoubtedly, their use and availability will increase dramatically in coming years.

Collection of serum in dead animals can be problematic due to a combination of desiccation, red cell lysis and bacterial contamination of blood. An alternative to serum can be obtained from 'meat juice' or fluid obtained from freeze/thaw of muscles or specific organs. This technique is widely used for investigation of serum titres in muscle of pigs or boars carrying *Trichinella* spp. Specific muscle groups are dissected, cut into small pieces, placed in a plastic bag and frozen at -20 °C. The bag is subsequently defrosted and a small corner of the bag cut off to collect the fluid exuding from the tissue. This fluid is deemed a suitable alternative to serum although analyses are run at a dilution ten times less than that of sera (10 times more concentrated).

10.4.2 Nucleic acids

If forensics scientists can extract nucleic acids from fossils why do we have to be careful when collecting nucleic acid samples? There are two reasons for this: (1) contamination; and (2) degradation.

As a rule of thumb, DNA is relatively stable, whereas RNA is much more labile. Degradation of nucleic acids occurs during autolysis, so sampling from locations that cool down rapidly after death such as the extremities (ears, limbs) is ideal rather than from visceral organs. The samples should be stored in tubes with a preservative, e.g. 95% ethanol, salt-saturated DMSO buffer, or RNA later. The latter is a concentrated salt solution which enables samples to be stored at room temperature for several weeks. Whatever the preservative, freezing at -80 °C in suitable tubes is the best long term method of preservation. Samples should be small ~5×5×5 mm and fully submerged in the preservative to avoid further degradation. Filter paper, as for serology, is a useful alternative to preserve DNA from fluids or blood in arid conditions and can preserve DNA for several years at room temperature. The downside of this technique is a risk of contamination and overall smaller DNA yield after extraction compared to tissues but this is a commonly used and cheap alternative in field conditions. FTA technology (fast technology for analysis of nucleic acids) represents an improvement on the simple filter paper approach with improved DNA fixation and inactivation of specific agents, increasing user safety (Ryser-Degiorgis, 2013).

Degradation is the second concern. As autolysis advances, through putrefaction, exposure to sunlight or repeated freeze-thaw cycles, chromosomes and DNA become increasingly fragmented. What samples are suitable for collection will depend to some extent on the intended use of the DNA. If one envisages whole genome sequencing, chromosomal analysis or transcriptomics, samples should be taken from very freshly dead animals and immediately stored in liquid nitrogen or a freezer. However, for population genetic or phylogenetic studies, which tend to target relatively short gene regions, more degraded nucleic acid may well still be suitable.

Table 10.1 details the methods of collecting suitable samples of DNA from different material types.

10.5 Transport of samples

Always check particular transport requirements for one´s needs. Within the UK, specimens consigned as diagnostic samples are classified as UN3373: biological substance – category B (Figure 10.4). Requirements for shipping this class of diagnostic samples are (IATA Regulations: PI 650). In this case, packaging is triple layered:

- primary receptacle (e.g. plastic container or blood tube);
- secondary packaging (e.g. plastic bag);
- rigid outer packaging (e.g. Esky® or box) clearly marked as UN3373.

Table 10.1. Methods of collecting suitable samples of DNA from different material types.

Sample type	Method
Dry solid (feathers, fur, skin, etc.)	Use sterile forceps to place in a sealed bag
Wet tissue (viscera, etc.)	Pare off surrounding tissue with sterile scalpel blade and place into RNA later
Fluid (saliva, blood, pus, etc.)	Sterile swab left to dry or placed in RNA later; filter paper

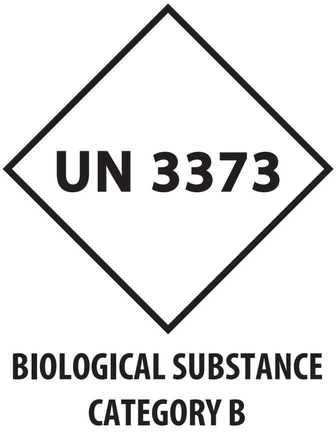

Figure 10.4. UN3373 label required for transporting diagnostic samples.

10.6 Future proofing concerns

Recent advances in molecular biology techniques illustrate the power of applying novel technologies to old samples. For example, we are now able to extract DNA of *Mycobacterium tuberculosis* in samples from Egyptian mummies (Nerlich *et al.*, 1997) and *Batrachochytrium dendrobatidis*, the agent of Chytridiomycosis has been detected in amphibians in China from samples collected in the early 1930's. This leads us to wonder what samples we should be taking and how we should be storing them to best preserve our samples for future generations of scientists.

Most progress seems to have been made by analysing nucleic acid sequences, a relatively well preserved structure, which encodes the proteins in each organism. Currently our best method of preserving nucleic acids on a long term basis is freezing at -80 °C. Samples would be relatively simple to store but would need to be small to reduce the energy needed for storage, and easy to access. We could consider cutting up samples at the point of storage to avoid multiple freeze/thaw cycles when accessing each sample. Similarly, it would be important to store the samples individually so as to avoid defrosting a whole batch of samples to access a single sample.

This may be the solution for DNA sample preservation but what technologies might appear in the next few years? We have recently seen the advent of deep sequencing, whole genome sequencing and epigenetics, predominantly focused on investigating nucleic acids but better methods of preservation will undoubtedly be developed to preserve the metabolome, transcriptome of each cell. Preservation of tissues samples in liquid nitrogen may be optimal as tissues freeze more rapidly than in dry ice forming fewer ice crystals. This technique will also allow cryosectioning with optimal morphology if no formalin fixed samples are available and allow immunohistochemistry as discussed above.

Regarding ease of sampling in the field, in field qPCR machines are now available and will continue to improve and reduce in size, time and sampling handling needs. Heat tolerant reagents for many will eliminate the need for refrigeration. One useful improvement would be the advent of novel and, in particular, safe fixation methods for tissues to decreased reliance on formalin.

Whatever the storage method or intended use of the sample, without good metadata or good initial sampling methodology, the sample is worthless. Rigor in aseptic technique during collection and maintaining full traceability of that valuable sample will go a very long way.

10.7 Conclusions

10.7.1 What has been achieved?

Concurrent with the progress in analytical methods, sampling techniques in the field have been refined. Modern logistics can ensure that the risk of invalidation of samples due to failures in storage and transport of samples is minimized.

10.7.2 What has been neglected?

Currently, fixation methods still largely rely on formaldehyde or similarly toxic chemicals. Sample preservation for future analyses with emerging or currently unknown methods is a challenge.

10.7.3 What needs to be done?

The use of toxic chemicals in sample preservation should be minimized. Development of portable analytical devices will reduce the need of sample preservation and storage/transport to laboratories. The following must be borne in mind:

> Learn from, but do not repeat, your mistakes. Any research or sampling effort can be improved. (Jessup, 2003)

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11. Health plans for wild gamebirds

Thomas W. Pennycott
St David’s Gamebird Services (Scotland), Midlothian Innovation Centre, Pentlandfield, Roslin, Midlothian EH25 9RE, United Kingdom; tomp@stdavids-poultryteam.co.uk

Summary

Large numbers of pheasants (*Phasianus colchicus*) and red-legged partridges (*Alectoris rufa*) are reared and released every year in the UK, to be shot for sporting purposes and for consumption of their meat. Since 2011, Codes of Practice for the Welfare of Gamebirds Reared for Sporting Purposes (the Gamebird Welfare Codes) Have been in place to safeguard the health and welfare of pheasants and partridges prior to their release. The welfare codes recommend that a veterinary health plan be devised and reviewed annually, and increasing numbers of pheasants and partridges are now reared under the protection of customised health plans. Health plans produced by one veterinary practice in Scotland include sections on performance parameters, disease profiles, medication, biosecurity, hygiene and welfare, and typically apply to the birds to the point at which they are shot for human consumption. Members of the general public consuming meat from farmed livestock are often concerned about the welfare of the animals or birds, the safety of the meat, and transparent traceability of the meat throughout the food chain, leading to the development of farm assurance schemes. Those consuming wild gamebird meat are likely to have similar concerns but currently there is no assurance scheme covering gamebirds. If the current health plans for pheasants and partridges could be extended to address these issues, consumers and retailers would have added confidence in the acceptability, safety, quality and sustainability of the meat produced, leading to greater consumption of pheasant and partridge meat. A similar approach could be developed for red grouse (*Lagopus lagopus scoticus*).

Keywords: pheasant, partridge, meat quality, safety, welfare, traceability

11.1 Introduction

Tens of thousands of pheasants (*Phasianus colchicus*), red-legged partridges (*Alectoris rufa*) and red grouse (*Lagopus lagopus scoticus*) are shot every year in the UK for human consumption. The great majority of the pheasants and partridges will have been artificially hatched and intensively reared before being released into a semi-wild environment, and their welfare prior to release is safeguarded by Codes of Practice for the Welfare of Gamebirds Reared for Sporting Purposes (the Gamebird Welfare Codes). The welfare codes recommend that gamebird rearers and their veterinary advisers prepare health plans to ensure the health and welfare of their birds, and customised health plans for pheasants and partridges are currently evolving. Red grouse incubate, hatch and rear their own chicks on managed grouse moors and are not included in the welfare codes, but in recent years there has been increasing reliance on the use of grit medicated with an anthelmintic to control the grouse caecal threadworm *Trichostrongylus tenuis*. All three species of gamebird are included in the ‘wild

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game' category of Annex 1 of Commission Regulation (EC) no. 852/2004 which deals with the hygiene of foodstuffs (EC, 2004a). The meat of pheasants, partridges and grouse is highly nutritious and contains more protein and selenium, and less cholesterol, fat and saturated fatty acids than lamb or beef (Anonymous, 2015), but makes up only a tiny fraction of overall meat consumption by the general public. Although there are farm assurance schemes ('farm to fork') for other sectors of the livestock industry, currently there are no such schemes for gamebirds. This paper will summarise the breeding, brooding and rearing of pheasants and partridges in the UK, describe the contents of gamebird health plans currently produced by one veterinary practice in Scotland, and explore the possibility of extending these health plans for pheasants and partridges to increase consumer confidence in wild gamebird meat.

11.2 Management of pheasants and partridges, welfare codes and health plans

11.2.1 Breeding, brooding and rearing pheasants and red-legged partridges in the UK

The housing of adult pheasants and red-legged partridges in the breeding season, the equipment used to artificially incubate the eggs, and the commonest methods of rearing pheasant and partridge chicks in the UK were reviewed by Pennycott *et al.* (2012) and are summarised here. Breeding pheasants may be kept in outdoor pens on grass, gravel or other substrates, or less commonly may be kept in elevated cages on wire floors. Group size can vary from seven females plus one male bird to much larger flocks of several hundred birds. The eggs are collected once to three times daily, sanitised and incubated artificially. Adult red-legged partridges may be kept in breeding groups but more often are kept in breeding pairs in small wire-floored pens. Incubation of pheasant and partridge eggs is carried out in a wide variety of setters and hatchers, some very modern and highly efficient but others old and poorly designed. As a result there is wide variation in hatching success, with hatchability ranging from under 50% to nearly 90%.

Although some of the pheasants and partridges reared in the UK are derived from eggs produced and hatched in the UK, Pennycott *et al.* (2012), describing 12 sites in Scotland rearing pheasants and 11 sites in Scotland rearing partridges, found that about two-thirds of the pheasants and three-quarters of the partridges were imported into the UK as hatching eggs or day-old chicks, mostly from France. Many chicks are reared in heated brooder huts, eventually being given access to unheated shelter or nursery pens and then grass runs. These huts and runs can be moved to fresh ground after one or two years. Other birds are reared in buildings that cannot be moved, and may be given access to outdoor gravel runs rather than grass runs. A minority of birds may be reared indoors with no access to outdoor runs during rearing. Pheasants usually remain in their rearing accommodation until they are 6-7 weeks old and partridges tend to be retained in the rearing pens until 8-14 weeks, after which they are transferred to larger pens and released in a controlled fashion. Group size in the rearing pens can vary from under 400 birds to over 1000. Heating is usually provided by gas brooders and is gradually reduced, and by the time the birds are five weeks old artificial heating will usually have been removed. Wood shavings, small squares of cardboard or chopped straw are commonly used as bedding material in the heated brooder areas, and food and water provided by a range of different types of feeders and drinkers.

When the pheasants are 6-7 weeks old and the partridges 8-14 weeks old they are transported to release pens. For pheasants these release pens can be several hectares in size, open-topped with a high perimeter fence, and may house over 1000 birds. Pheasant release pens have a mixture of ground cover, low shrubs, higher trees for roosting in and open areas for sunning and dust-bathing, and are usually only used for one batch of birds each year. Partridge release pens are usually much smaller, are stocked with fewer birds at any one time but may be used repeatedly in the same season. Feed and water is still provided artificially to the pheasants and partridges while in the release pens. Time spent in the release pens can vary from a few days to a few weeks, after which the birds are released into a semi-wild environment. This release takes place well in advance of the shooting season, and feed and water continues to be supplied to the birds.

During the breeding and rearing phases, and sometimes after release, different management devices and the use of medication may be required. Management devices in adult pheasants may include 'spectacles' and 'bumpa bits' which clip into the nostrils – the former impede the vision of the birds and the latter reduce the ability of the birds to peck, helping to reduce egg-eating, feather-pulling and more aggressive pecking. Breeding pheasants in open-topped pens may be fitted with a 'brail', a tape passed around one wing to prevent the birds flying out of the pens. In the rearing pens pheasants often have small 'bits' clipped into the nostrils, again to reduce pecking. These bits are usually applied around three weeks of age and removed prior to release, but some 'bio-degradable bits' can be employed that can be left in place until they fall off of their own accord. Strategic in-feed medication prior to release is common, including preparations to help control coccidia and other enteric protozoa, parasitic worms, and bacterial diseases. Live or killed vaccines may be given to birds prior to release, but more commonly such vaccines are administered to breeding flocks.

Unlike pheasants and partridges, red grouse are not bred and reared under controlled conditions but the grouse incubate, hatch and rear their own chicks on managed grouse moors. However grouse are susceptible to a parasitic nematode, the caecal threadworm (*T. tenuis*), and strategic medication of grouse grit with an anthelmintic is common practice.

11.2.2 Welfare codes and health plans

In 2008, the UK Farm Animal Welfare Council expressed concern about various aspects of the breeding and rearing of pheasants and partridges. After a number of consultations and revisions, Codes of Practice for the Welfare of Gamebirds Reared for Sporting Purposes (the 'Gamebird Welfare Codes') came into effect in England, Wales and Scotland in 2011 (Scottish Government, 2011). The welfare code applies to gamebirds bred and reared under controlled conditions for the purpose of release for sport shooting, and covers birds retained for breeding purposes, hatcheries, birds in rearing pens and birds in release pens. The welfare code does not apply to red grouse, or to pheasants and partridges once they can leave and re-enter the release pens voluntarily. Failure to comply with a provision of the welfare code is not an offence in itself, but it may be relied on as tending to establish liability where a person has been accused of an offence. Equally, compliance with the welfare code can help to establish that best practice has been followed. The welfare code says 'It is good practice to devise and review annually a flock health and welfare plan in conjunction with your veterinary surgeon'. Another code, the Code of Practice on the Responsible Use of Animal Medicines on the Farm (VMD, 2015), recommends that a clear animal health plan is drawn up between farmers and their veterinary surgeons. Accordingly, there has been increased interest in recent years in the production of health plans for pheasants and partridges, to take into account concerns about welfare and the use of medicines. Such health plans can also help to ensure the correct disposal of the carcasses of gamebirds that die in the breeding, rearing and release pens, in compliance with the EU Animal By-Product Regulations (EC, 2009). The possibility that health plans for pheasants and partridges can also be used to enhance food safety, traceability and consumer confidence in the final product, the gamebird carcasses for human consumption, should also be considered. Although the welfare codes do not apply to red grouse, in future there may also be merit in developing health plans for grouse in light of the widespread use of medicated grit to control the caecal threadworm *T. tenuis*.

11.2.3 What is currently included in health plans for pheasants and partridges?

The gamebird health plans prepared by St David's veterinary team in Scotland are customised for each site and currently include the following sections:

- client contact details;
- bird and feed details (current year and previous years);
- previous performance parameters and future targets;
- disease profiles (current year and previous years);
- hygiene, biosecurity;
- disposal of carcasses, etc.;
- use of management devices;
- use of medicines;
- further information about relevant diseases;
- records of veterinary visits;
- visitor records;
- medicine records.

11.3 Could the current gamebird health plans be used to promote the consumption of pheasant and partridge meat?

Farm assurance schemes such as assured food standards (red tractor logo) recognise the importance of animal or bird welfare and food safety to those consuming meat, milk, eggs or other products derived from farm animals, and the need for traceability throughout the food chain ('farm to fork'). Consumers nowadays are also concerned about the impact of the production system on the environment in general. Many of these issues are already included or could be included in gamebird health plans, and could help promote the consumption of pheasant and partridge meat by the general public. Possible areas of interest to consumers include the welfare of gamebirds during rearing and releasing; the welfare of gamebirds after release, including at the time of shooting; the bacteriological quality of the meat; the possibility of drug residues in the meat; the responsible use of antimicrobials during rearing, to reduce the risk of antimicrobial resistance that could impact on human health; levels of lead, other heavy metals and agrochemicals in the meat; the traceability of the end product; and possible adverse environmental effects arising from the rearing, releasing and shooting of the birds.

11.3.1 Welfare of gamebirds during rearing, including transport and preparation for release

Consumers often profess an interest in the conditions in which livestock are kept and the possible impact on the welfare of the animals and birds. There is currently an assurance in the health plan that there is a copy of the welfare code on-site and relevant personnel have been instructed to read it. Any management practices that do not comply with the welfare code must be discussed with the veterinary advisor and justified on a flock by flock basis.

In addition, there are specific details in the health plan about the use of management devices (applying bits and spectacles, beak trimming, brailing and trimming flight feathers) on the site. The welfare code states that these should not be used routinely, and where possible changes to the husbandry system should be considered to avoid the need to use such devices. If the use of these management devices is deemed necessary to safeguard the welfare of the birds or for management purposes, the welfare code advises that bits only be used in pheasants for short periods and be closely monitored, that 'bumpa bits', spectacles and beak trimming be used only in exceptional circumstances or in response to a specific problem, that clipping the outer primary feathers of the wings is permissible but not to trim actively growing blood quills, and that applying a brail to the wing of an adult bird should only be done with extreme care and the brail removed again before the birds are released from their breeding accommodation. As part of the gamebird health plan, the use of management devices on the site is reviewed and discussed, and where appropriate advice is given to reduce their use.

11.3.2 Welfare of gamebirds after release, including at the time of shooting

Members of the public have expressed concern about the actual shooting of the birds and the possibility that some birds are wounded and left to die over several days. The welfare code requires that gamebirds are healthy, fit for transport and hardened-off when sold for release, that suitable arrangements have been made for supplementary feeding, and that the release pens are well prepared before the birds arrive. Compliance with the welfare code is a requirement of the gamebird health plan.

There could in addition be an assurance in the health plan that there is a copy of the Code of Good Shooting Practice (BASC, 2012) on-site, and that relevant personnel have been instructed to read it. This code is overseen by a Steering Committee with representatives from the British Association for Shooting and Conservation; Countryside Alliance; Country Land and Business Association; Game Farmers' Association; Moorland Association; National Game Dealers' Association; National Gamekeepers' Organisation; Scottish Gamekeepers' Association; Game and Wildlife Conservation Trust; and Scottish Land and Estates. Any deviation from best practice in the code would need justification.

In particular, there should be compliance with best practice to ensure that birds are fed adequately after release, using compounded feed until the birds are fully grown; birds are killed cleanly and consistently; there is adequate provision to retrieve all wounded game whenever safe and practicable; any wounded quarry is humanely and swiftly despatched; and sufficient feed is provided for released birds remaining at the end of the shooting season, until natural food sources become available (usually in May).

11.3.3 Food safety – bacteriological quality

The carcasses of gamebirds are handled in a very different manner from the carcasses of livestock killed in abattoirs and processing plants. The consumer might therefore be concerned about the bacteriological quality of the gamebird meat, with regard to spoilage organisms but also potential food-poisoning pathogens such as *Campylobacter*, *Escherichia coli*, *Salmonella*, *Yersinia* and *Listeria*. *Campylobacter* is the commonest bacterial cause of food-poisoning acquired from chicken meat. Pheasants and partridges can also carry *Campylobacter*, including strains that cause human food-poisoning (Seguino and Chintoan-Uta, 2017). *Salmonella* food-poisoning from broiler chickens is exacerbated in part by the young age (often under six weeks old) at which broiler chickens are slaughtered. Pheasants and partridges can also be infected with *Salmonella*, but they are older when shot, reducing the risk that they are excreting *Salmonella*. In addition, gamebird health plans specifically include measures to control *Salmonella* in gamebird breeding flocks, hatcheries, and during rearing.

Unless supplied in-feather to family, friends or in small quantities directly to final consumers or local retailers, the supplier must comply with the initial handling requirements of wild game (EC, 2004b) and the general hygiene requirements for game larders (EC, 2004a). These Regulations require that game larders are large enough, adequately ventilated or refrigerated, protect the carcasses from pests and animals, and are kept clean and if necessary disinfected. For carcasses being supplied to approved game handling establishments (AGHE), the regulations require that the vehicles taking the gamebirds away from the shoot are clean and not contaminated by animals or pests, that the carcasses are not piled in heaps, and that the carcasses are chilled as soon as possible and are transported to the AGHE in a refrigerated vehicle.

Compliance with the hygiene requirements in Commission Regulations (EC) nos. 852 and 853/2004 should, therefore, help to protect the consumer from bacteria such as *Campylobacter*, *E. coli*, *Salmonella*, *Yersinia* and *Listeria* (EC, 2004a,b). The extended gamebird health plan could contain a statement confirming compliance with these regulations.

11.3.4 Food safety – drug residues

Consumers may be concerned that meat from pheasants and partridges might contain residues of medicines given to the birds prior to being shot. Meat from gamebirds is periodically tested for drug residues as part of the UK National Surveillance Scheme, as required by Council Directive 96/23/EC (EC, 1996). The health plan discusses the timing of medication, dose rates and withdrawal times for the different medications used in gamebirds on the site, to minimise the risk of drug residues in the tissues when the birds are shot for human consumption.

Because few of the drugs used are licensed for use in gamebirds, most are prescribed under the 'cascade system' and have a 28-day meat withdrawal period. The health plan details the records to be kept when medication is provided to food-producing animals such as gamebirds, and contains forms for recording medicine acquisition, use and disposal.

Extracts from the Code of Practice on the Responsible Use of Animal Medicines on the Farm (VMD, 2015) are included in the gamebird health plan. Topics in the code that reduce the risks of residues in the meat include: the importance of having a flock health plan produced in consultation with the veterinary surgeon who has the birds under his/her care; obtaining medication from authorised sources only, and not using or passing on medicines (including medicated feed) intended for use in other people's birds; record keeping, including withdrawal periods; proper administration of medicines; and strict adherence to withdrawal periods.

11.3.5 Food safety – antimicrobial resistance

Consumers may be concerned that the meat from pheasants and partridges might be contaminated with bacteria that have become resistant to antimicrobials used to treat diseases in humans. If these resistant bacteria then infect humans, or pass on the genes conferring resistance to bacteria already present in humans, it could be difficult to treat such diseases.

The gamebird health plan ensures that medicines are only used when appropriate, and where possible gives advice on strategies to reduce the use of antimicrobials. Such strategies may include greater use of probiotics instead of antibiotics, and changes to management and husbandry, for example improving site biosecurity, reducing group sizes or stocking densities, or increasing the numbers of feeders or drinkers. This will help to reduce the selection of bacteria in gamebirds that are resistant to one or more antimicrobials. Where antimicrobial use is deemed unavoidable to protect the health and welfare of the birds, the health plan ensures that antimicrobials are used at the correct dose rate and duration – under-dosing could help to select for resistant bacteria. The health plan also permits regular reviews of the effectiveness of the antimicrobials used on the site, detecting the emergence of resistant strains of bacteria.

Some antimicrobials are considered by the World Health Organization (WHO) to be critically important for human health, and there are fears that their use in animals or birds could contribute to the emergence of strains of bacteria resistant to these critically important antimicrobials. Four classes of critically important antimicrobials are classified by the WHO as being 'highest priority' – fluoroquinolones that are used to treat *Salmonella* and *E. coli* infections in humans; macrolides that are used to treat infections with *Campylobacter* in humans, especially children; third/fourth generation cephalosporins that are important in treating *Salmonella* and *E. coli* infections in humans; and glycopeptides that are essential for treating *Enterococcus* infections in humans (WHO, 2012). Certain fluoroquinolones and macrolides are sometimes used in gamebirds, on occasion with limited veterinary input. Developing and following a health plan offers the opportunity for controlling the use in gamebirds of these highest priority critically important antimicrobials, reducing the likelihood of the emergence of resistant strains of bacteria that could then spread to humans through contamination of the meat or environment. Ultimately, health plans might prohibit or severely restrict the use of such antimicrobials on certain sites.

11.3.6 Food safety – residues of lead, other heavy metals and agrochemicals

Consumers and retailers might be concerned about possible residues of heavy metals, most notably lead but also cadmium and mercury, or residues of agrochemicals applied to the environment. Unfortunately there is no agreed safe level for lead intake by consumers. A small number of people frequently eat game that has been shot with lead, and in 2012 the Food Standards Agency advised that such people should cut down their consumption of shot game, especially of small game. Toddlers, children, pregnant women and those hoping to become pregnant were considered to be especially vulnerable (FSA, 2012). Gamebirds may also be accidentally exposed to other potentially hazardous chemicals in the environment such as agrochemicals, and health plans could include a statement to the effect that the person tending the birds after release will liaise with other users of the countryside to ensure that the birds are not exposed to such hazards.

11.3.7 Food safety – traceability

Consumers and retailers will have greater confidence in the meat they eat or supply if there is a clear chain of traceability from 'farm to fork'. There is also a legal requirement for food business operators to maintain records of traceability throughout the food chain (EC, 2002). Health plans can add substance to the traceability records by offering further specific assurances about the medication used at different stages or a statement to the effect that the birds were reared in accordance with a gamebird health plan, relevant details of which could be obtained from the veterinary advisor.

11.3.8 Environmental concerns

Consumers might be concerned that the rearing, releasing and shooting of gamebirds could have an adverse effect on the environment. Such concerns could include the dangers of lead poisoning in waterfowl that pick up spent lead shot as a source of grit; general littering of the environment; and the consequences of releasing large numbers of gamebirds into a small area.

An assurance could be put into the health plan that there is a copy of the Code of Good Shooting Practice (BASC, 2012) on-site, and that relevant personnel have been instructed to read it. Any deviation from best practice would need justification. In particular, there should be compliance with best practice to ensure that: lead shot is not deposited in wetlands important to feeding waterfowl, and there is compliance with relevant legislation restricting the use of lead shot; all cartridge cases and other litter are removed after each shoot, and if possible cartridges with degradable wads are used; cover crops are used to enhance the environment; the Game and Wildlife Conservation Trust 'Guidelines for Sustainable Gamebird Releasing' (GWCT, 2007) are followed, to enhance habitats and wildlife biodiversity; and all predator and pest control is carried out lawfully and with due consideration to local residents and other countryside users

11.4 Conclusions

11.4.1 What has been achieved?

Gamebird health plans that have been customised for individual sites and regularly reviewed undoubtedly benefit the health and welfare of the gamebirds and encourage best practice by those breeding, rearing or releasing the birds. The health plans prepared by St David's Veterinary Team in Scotland for pheasants and partridges address disease and medication issues, hygiene and biosecurity measures, welfare concerns such as the use of management devices, and provide opportunities for performance benchmarking. This paper has explored the possibility of extending the health plans to include the final product, the pheasant or partridge meat destined for human consumption. Areas identified as being of potential added value to the health plan include assurances about the welfare of the birds after release and at the time of shooting; the safety of the meat in terms of bacterial load and residues of undesirable substances; extended traceability of the end product, equivalent to 'farm to fork' in other livestock sectors; and assurances about measures taken to limit adverse environmental effects arising from the rearing, releasing and shooting of the birds. It is hoped that these extended health plans would give consumers and retailers added confidence in the acceptability, safety, quality and sustainability of the meat prepared from pheasants and partridges to which the health plans relate.

11.4.2 What has been neglected?

To date, the customised gamebird health plans have been prepared for pheasants and red-legged partridges but not for red grouse. Although grouse are not reared and released in an intensive fashion such as occurs with pheasants and partridges, the consumer and retailer may welcome further information and assurances about the medication and management of these birds. The other main area not yet addressed is the possibility that extended health plans for gamebirds could provide the basis of a more formal assurance scheme such as those currently available for other sectors of the livestock industry.

11.4.3 What needs to be done?

We need to continue to develop the customised health plans for pheasants and partridges, and encourage those involved with grouse production to prepare similar health plans with their veterinary advisers. Further discussions must also take place with consumers, retailers and UK certification bodies to see if extended health plans could eventually be developed into more formal assurance schemes.

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Section 3

Chemical hazards

12. Reduction of lead contents in game meat: results of the 'Food safety of game meat obtained through hunting' research project

Christine Müller-Graf, Antje Gerofke, Annett Martin, Niels Bandick, Monika Lahrssen-Wiederholt, Helmut-A. Schafft, Thomas Selhorst, Ellen Ulbig and Andreas Hensel*
Federal Institute for Risk Assessment, Max Dohrn-Str. 8-10, 10589 Berlin, Germany;
christine.mueller-graf@bfr.bund.de

Summary

Due to its high toxicity, no safe alimentary uptake level can be defined for lead. Therefore, an effort has to be made to minimize lead intake. Game meat belongs to the foods with a comparatively high lead content. A research project was carried out to study the effect of lead-based ammunition as compared to non-lead ammunition on contamination of game (roe deer, *Capreolus capreolus* and wild boar, *Sus scrofa*) with lead. Results of the research project clearly show that lead-based hunting ammunition significantly increases the lead concentration in the game meat. The effect of the construction of lead ammunition was also studied. Unexpectedly, there was a tendency in roe deer for bonded bullets to show higher lead contamination than fragmenting bullets. No such effect was noted in wild boar. In roe deer the point of impact of the projectile appears to have an influence on the levels of lead contamination. Increased lead levels were observed when a bone hit was reported. For wild boar no significant difference in lead contamination between a bone hit or a non-bone hit was observed. Non-lead bullets in combination with suitable game meat hygienic measures can therefore be recommended to minimize the uptake of lead in order to protect the consumers.

Keywords: ammunition, human health, game meat hygiene, consumer protection

12.1 Introduction

Recent toxicological findings indicate that a minimization of lead intake via food and drinking water is necessary because of the high toxicity of lead (EFSA, 2010). Game meat is among those food items with potentially high lead contents due to the use of lead ammunition for hunting and other factors.

According to the risk assessment 'Bleibelastung von Wildbret durch Verwendung von Bleimunition bei der Jagd (Lead contamination of game by use of lead ammunition in hunting)' of the Federal Institute for Risk Assessment (BfR) of 3rd December 2010 (BfR, 2010), a health risk resulting from the lead-containing remains of ammunition in game meat is possible for 'extreme consumers' of game meat, such as hunters and their families.

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In order to acquire a knowledge-based background for political decisions, the project 'Food safety of game meat obtained through hunting' (German acronym: LEMISI project) was initiated by the Federal Ministry of Food and Agriculture (BMEL) and coordinated by BfR; the Federal States involved in the project were Mecklenburg-Western Pomerania, Lower Saxony, Saxony-Anhalt, Bavaria, Hesse, North Rhine-Westphalia, Hamburg and Bremen, further project partners were food and hunting associations, respectively. The project was already described in a previous volume of this book series, 'Trends of game meat hygiene' (Gremse *et al.*, 2014).

The main objective was to understand the contribution of lead ammunition to lead content in edible parts of game meat. It was also examined whether there was a difference in lead contamination between roe deer and wild boar.

Concerning game meat hygiene, factors that may also have an influence on lead contamination such as the choice of specific types of projectiles and the effect of the projectile – depending on the point of impact – were considered as well.

More specifically, the following questions were asked:
- In the case of lead ammunition, do the projectile's constructive characteristics lead to higher contamination with lead? Here, the hypothesis is that higher levels of lead are caused by using strong fragmenting (non-bonded) bullets, as compared to bonded constructions which may result in a lower lead content in game meat.
- Is there an association of the location of the wound channel in the carcass with contamination levels? Is there an impact when the bone rather than mainly the muscle tissue is hit by the bullet?

12.2 Material and methods

12.2.1 Project design

Animal species examined comprised roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*).

Six regions within Germany were chosen, according to the lead content of the top soil. This should allow to control for lead concentrations attributable to soil lead contamination in the (statistical) analysis. For each of the three lead content levels in top soil (i.e. low lead content, medium lead content, high lead content according to a geographical map indicating lead content in top soil – Bundesanstalt für Geowissenschaften 2004: http://www.bgr.bund.de/DE/Themen/Boden/Bilder/Bod_HGW_Karte_g.html), two regions were selected.

To elucidate the input of lead through hunting, different bullet materials were used in the project: lead ammunition and non-lead ammunition. To account for the lead distribution within the animals, from each carcass, three samples were taken, i.e. haunch, saddle and marketable meat close to the wound channel (Figure 12.1). Overall, a total of 1,254 roe deer (745 lead, 509 non-lead) and 854 wild boar (514 lead, 340 non-lead) were shot, resulting in 6,324 samples.

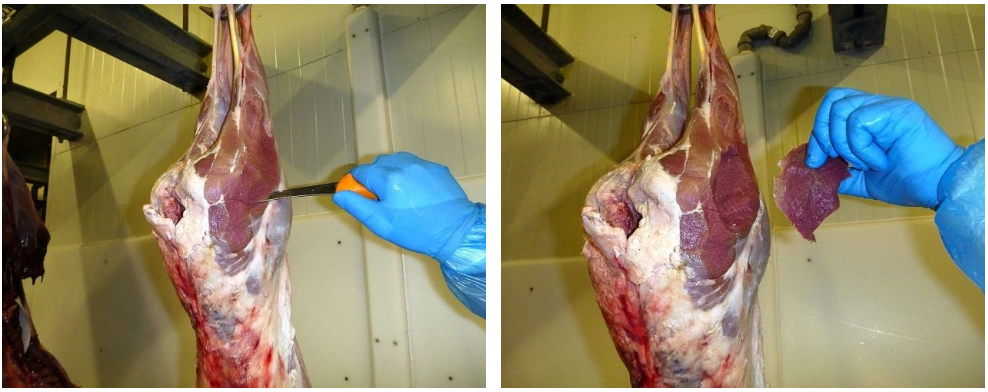

Figure 12.1. Taking a sample from the haunch (courtesy of European Poultry, Eggs and Game Association; http://www.epega.org).

12.2.2 Quality assurance

Quality assurance was a vital part of the project and quality assurance measures were integrated in all phases of the project.

12.2.3 Sampling and sample amount

Sampling was done by trained game traders. The sample amount was 100 g. Hunters had to give detailed information on how animals were killed in a specially designed data sheet (i.e. type of bullet material used, shooting distance/flight (escape) distance, location of the wound channel (entry/exit wound)) as well as to indicate the location (i.e. the site of entry/exit of the bullet) of the wound channel in a schematic drawing.

12.2.4 Statistical analysis

Beanplots were used to compare the lead content in the three edible parts of roe deer and wild boar hunted with non-lead or lead ammunition. In a beanplot, the shape is the estimated density and the short horizontal lines represent each data point. Wider lines indicate more duplicate values. The longer thick lines are the median for each sample. The plots were created with the package 'beanplot' (Kampstra, 2008) with the statistical software R version 3.2.3 (https://www.r-project.org).

The lead content of some of the samples was below the limit of detection (LOD) or the limit of quantification (LOQ); hence these are left censored data. For the descriptive beanplots, lead contents lower than the LOD or LOQ were replaced by half of the detection (or quantification) limit (middle bound).

To test significant differences in lead contents of meat according to bullet material (lead or non-lead), lead bullet construction (bonded or non-bonded), the location of wound channel (entry/exit wound) and bone hit (yes or no) and for determination of the geometric mean with 95% confidence intervals of lead content, the Tobit regression was used. Tobit regression is a statistical method for the analysis of censored data and allows consideration of different LOD's or LOQ's (Lorimer and Kiermeier, 2007). This method was executed with the function 'survreg' from the R package 'survival' (Therneau and Lumley, 2011) with the statistical software R version 3.2.3. LOD and LOQ may be specific for each laboratory and/or analysis method. To compare the lead content between different bullet constructions, different combinations of entry and exit for the wound channels (abdomen, thorax) and occurrence of a bone hit (yes or no), the geometric mean lead contents with 95% confidence intervals were presented in bar graphs created with Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). The significance level was set at $P=0.05$.

12.3 Results and discussion

12.3.1 Distribution of the lead content in game meat

A considerable number of the samples were found to be below the detection and quantification limit. The proportions of quantifiable lead contents were between 23% (haunch; non-lead ammunition) and 61% (around the wound channel; lead ammunition) in roe deer and between 25% (haunch; non-lead ammunition) and 62% (around the wound channel; lead ammunition) in wild boar. Lead contents in game meat from roe deer and wild boar basically exhibited a big variation when lead ammunition is used (Figure 12.2 and 12.3). Sporadically, extremely high values were found around the wound channel. These parts with high contamination then pose a problem for the consumer.

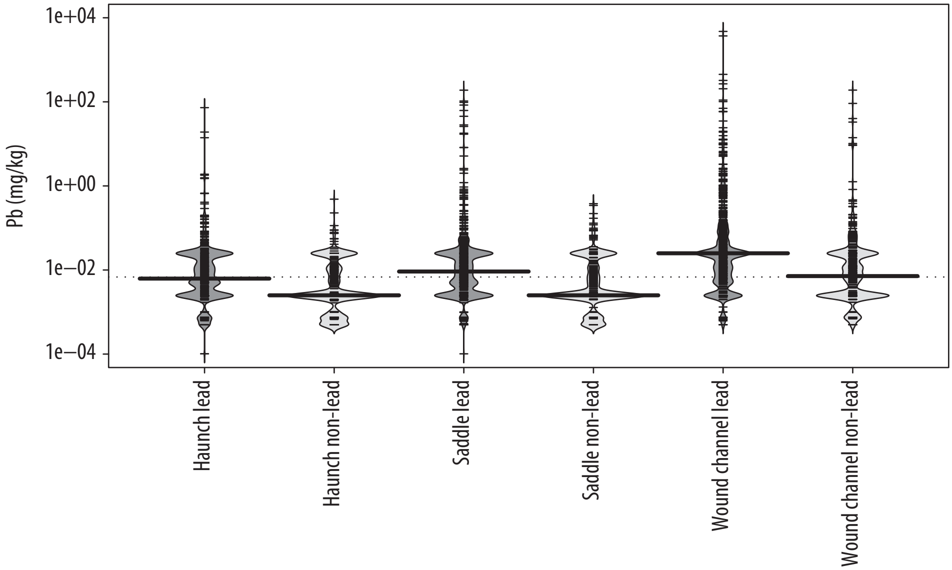

Figure 12.2. Beanplot showing lead (Pb) content in different edible parts of roe deer by bullet material (lead, non-lead).

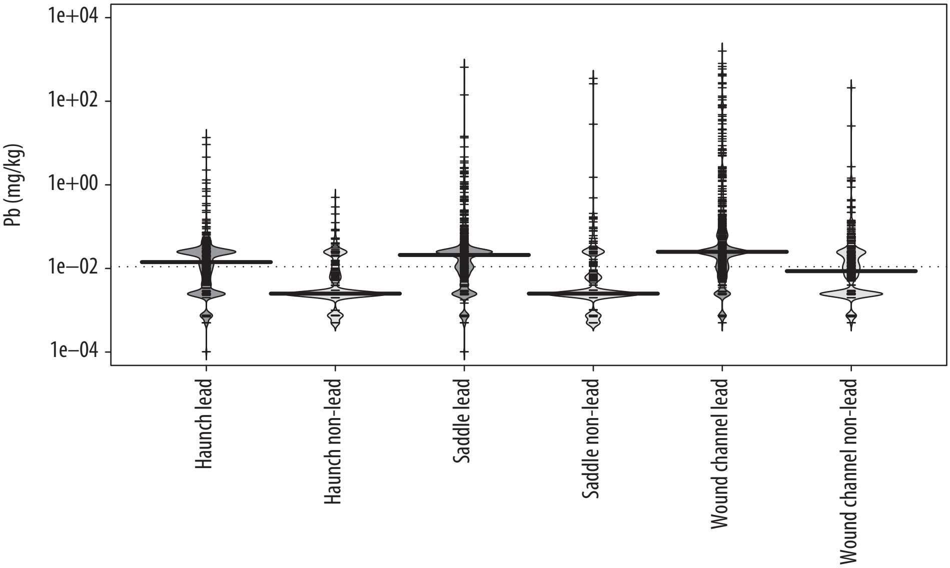

Figure 12.3. Beanplot showing lead (Pb) content in different edible parts of wild boar by bullet material (lead, non-lead).

Lead contents lower than the LOD (or LOQ) were replaced by half of the detection (or quantification) limit (middle bound). The shape of the plot represents the estimated density, short horizontal dots represent each data point. The median is indicated by a solid line for each and the overall median by a dotted line.

12.3.2 Dependence of lead content in tissues on type of ammunition used (lead vs non-lead)

The geometric mean by ammunition type and edible part was re-estimated using Tobit regression in order to account for the censored data (Table 12.1 and 12.2). In comparison to non-lead ammunition, the use of lead ammunition leads to a statistically significant increase (*P*<0.001) of the mean lead contents in all three edible parts of roe deer as well as in wild boar (Table 12.1 and 12.2).

This finding was further supported by the fact that the contamination around the wound channel was highest (Figure 12.4). This could be observed in wild boar (Figure 12.4) and roe deer (results not shown). Even though the wound channel was cut out, some lead fragments may have entered the edible section. Saddle and haunch were less contaminated, as had been expected because of the distance to the wound channel.

Even in the wild boar shot with non-lead ammunition, a certain amount of lead was found around the wound channel. This may be partly explained by the fact that even in the so called non-lead ammunition there may be some traces of lead in addition to some background

Table 12.1. Lead content in hunted roe deer (mg/kg) (LEMISI project).

Sample	Bullet	n	GM (95%-CI)[1,2]	Mean value[3]	Median[3]	Maximum
Haunch	lead	745	0.0028*** (0.0016;0.0051)	0.169	0.006	73.0
	non-lead	509	0.00074 (0.0006;0.0009)	0.010	0.003	0.48
Saddle	lead	745	0.0043*** (0.0022;0.0083)	0.968	0.009	189.29
	non-lead	509	0.00069 (0.0005;0.0009)	0.012	0.003	0.3781
Close to wound	lead	745	0.0138*** (0.0071;0.0265)	13.958	0.025	4,727.979
channel	non-lead	509	0.0027 (0.0020;0.0036)	0.807	0.007	190.4

[1] GM = geometric mean, based on Tobit model.

[2] *** = *P*<0.001: *P*-value indicates the difference between lead and non-lead per subsample.

[3] Values < limit of detection (LOD) or limit of quantification (LOQ) were set to 0.5 LOD or LOQ.

Table 12.2. Lead content in hunted wild boar (mg/kg) (LEMISI project).

Sample	Bullet	n	GM (95%-CI)[1,2]	Mean value[3]	Median[3]	Maximum
Haunch	lead	514	0.0040*** (0.0020; 0.0081)	0.086	0.014	13.517
	non-lead	340	0.0010 (0.0007; 0.0014)	0.0011	0.003	0.501
Saddle	lead	514	0.0067*** (0.0028; 0.0159)	1.716	0.021	650.100
	non-lead	340	0.0008 (0.0005; 0.0012)	1.904	0.003	351.932
Close to wound	lead	514	0.0219*** (0.0094; 0.0513)	14.302	0.025	1,582.060
channel	non-lead	340	0.0032 (0.0022; 0.0047)	0.733	0.009	209.00

[1] GM = geometric mean, based on Tobit model.

[2] *** = *P*<0.001: *P*-value indicates the difference between lead and non-lead per subsample.

[3] Values < limit of detection (LOD) or limit of quantification (LOQ) were set to 0.5 LOD or LOQ.

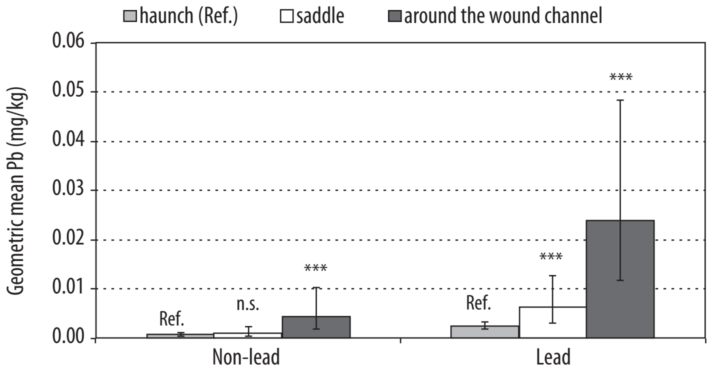

Figure 12.4. Comparison between the three subsamples (haunch, saddle, around wound channel) using Tobit regression in wild boar. Ref. = reference category; n.s. = not significant; ***P<0.001*, based on Tobit model.*

contamination. These findings were also statistically significant when taking into account the effect of regions. The effects were observed for the two species (BfR, 2014a).

12.3.3 Comparison of the game species

A comparison between the two species revealed that wild boar shows higher lead contamination than roe deer (Figure 12.5). That applied to the lead-containing bullets and all three subsamples. We hypothesize that this may be explained by the different body types of the roe deer and wild boar: roe deer has a significantly lower mean body weight than wild boar, thus the body mass of game could have impact on the target ballistic performance of a projectile, in the way that a body having a larger mass produces clearer changes in the material of the projectile compared to those with a lower mass. For roe deer with a lower body weight one would expect a smaller loss of bullet material and thus lower levels of lead as compared to wild boar. However, a detailed analysis of whether a larger/denser animal body produces clearer changes of the material of the projectile compared to a body with lower mass would include taking into account the shooting range, the type of rifle used as well as the specific construction of the projectile and some other factors. There was no difference in the lead content between the two species (for three subsamples), when hunted with non-lead bullets (results not shown).

12.3.4 Game meat hygienic aspects

12.3.4.1 Effect of bullet construction

The choice of the appropriate bullet will depend on various factors, like the type of hunting and hunted species. In addition to the emphasis on the killing potential of the bullet construction, the game meat safety aspect is another major challenge. It was expected that the use of bonded bullets – due to their construction – results in a lower contamination of meat, because these

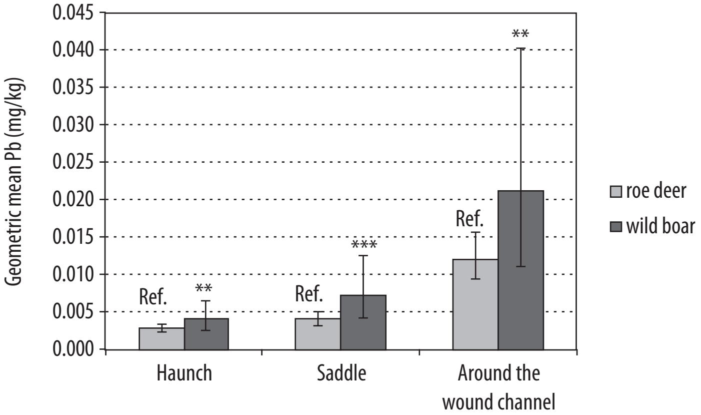

Figure 12.5. Comparison of lead (Pb) contamination of roe deer and wild boar shot with lead-containing bullets. Ref. = reference category; *P*<0.05,* ***P*<0.001, based on Tobit model.*

bullets are supposed to release less material to the surrounding tissue than fragmenting types. Fragmenting bullets react – by design – with heavy loss of material upon hitting the game's body and thus impart energy to the target.

Only lead containing ammunition was compared for this topic. Unexpectedly, the results showed that there was a tendency of higher lead contents in the saddle ($P<0.001$) and around the wound channel ($P<0.05$) of roe deer (Figure 12.6) when using bonded bullets (i.e. bullet types where a mass loss is not expected in the target media). These results cannot be explained at the moment. Perhaps the specific construction of the bonded bullet may play a more important role than hitherto assumed. A suitable analysis of the specific different subtypes of bonded bullets was not possible with the present data set because of the partly low and imbalanced number of the different bullet subtypes. In the edible tissues of wild boar no difference in lead content was observed between bonded and non-bonded bullets (mass loss expected in the target media). Also the observations in wild boar may partly depend on the specific type of bullet used. No differences between lead content of bonded and non-bonded ammunition constructions were observed when using non-lead ammunition (in all 3 edible parts and both species). Further research is needed on the possible effect of the different types of bullet constructions of bonded projectiles.

12.3.4.2 Location of wound channel

Here, the question was whether there was a specific effect of the entry and exit site of the bullet on lead levels. Descriptions of these sites and location of the wound channel were obtained from hunters' data sheets.

For roe deer, lower lead contents in the saddle ($P<0.05$) and around the wound channel ($P<0.01$) were observed when the wound channels were located in the abdomen, compared to entry and exit in thorax (Figure 12.7). No difference could be found for wild boar.

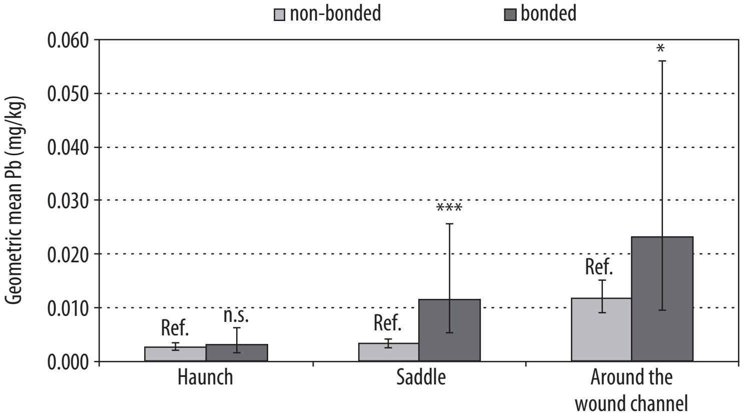

Figure 12.6. Comparison of lead (Pb) contamination of roe deer shot with bonded and non-bonded bullets. Ref. = reference category; n.s. = not significant; *P<0.05, ***P<0.001, *based on Tobit model.*

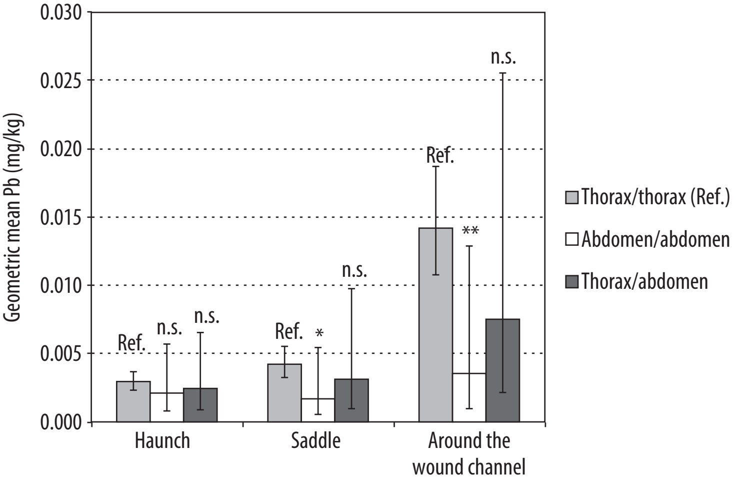

Figure 12.7. Lead (Pb) content as result of location of wound channel (entry/exit wound) and its angle (roe deer). Ref. = reference category; n.s. = not significant; *P<0.05, **P<0.01, *based on Tobit model.*

Compared to the relatively soft abdomen, the ribs of the chest provide a greater resistance when the projectile hits a bone (see also ‘Effect of bone hit’) and thus possible fragmentation of the bullet. This may result in the observed higher lead contents in marketable game meat from around the wound channel for roe deer hit with an entry and exit bullet in the thorax. The different build (body mass, weight, bone structure) of the wild boar compared to roe deer may offer an explanation for the different findings.

12.3.4.3 Effect of bone hit

The lead content in roe deer was also significantly increased when bones were hit ($P<0.01$ for all 3 subsamples; Figure 12.8). No differences could be observed for wild boar.

The density of the tissue/body mass has an effect on the loss of material from the projectile. For a bone hit the effect of body mass is less prominent. Here, the effect of a firm material such as bone has probably more of an impact. This may lead to a higher lead contamination in roe deer after bone hits, especially around the wound channel. In wild boar on the other hand, this could be less pronounced due to the larger and possibly denser body mass. Yet, there may be other factors, which could not be taken into account here, which may additionally play a role. Furthermore, there is considerable uncertainty due to reporting bias.

12.3.5 Consumer protection

The measured lead contents in edible/marketable meat of hunted game are in a similar range as the lead contents considered for the BfR risk assessment in 2010 (BfR, 2010, Table 7 p. 32). Some lead values measured close to the wound channel are on average significantly higher than values used for the 2010 exposure assessment. As pointed out here, there is also

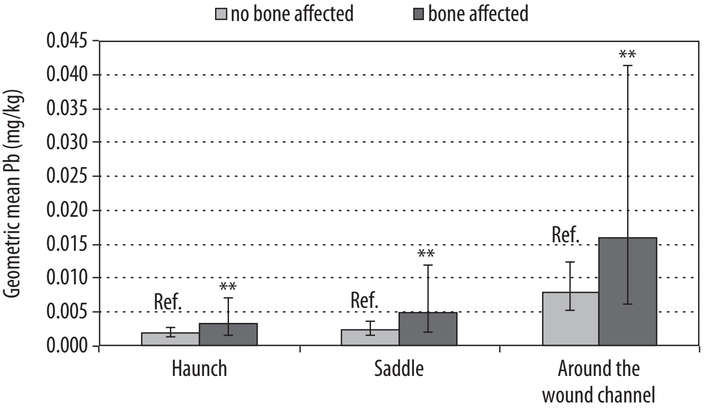

Figure 12.8. Comparison of the lead (Pb) contamination in meat of roe deer if a bone was hit or not, based on Tobit model. Ref. = reference category; n.s. = not significant; *P<*0.05,* **P<*0.01,* ***P<*0.001.*

a considerable heterogeneity in the amount of lead contamination, so that there is a chance that occasionally the consumer will eat game with elevated levels of lead. On the other hand quite a few samples had lead contaminations that were below the detection limit.

Since no uptake quantity of lead that can be regarded as safe to health can be established for lead, exposure to this heavy metal should be avoided to the extent that is reasonably achievable (ALARA principle). Overall, the intake of lead by all consumers via food is so high that adverse health effects are possible (BfR, 2014b). In contrast to adults, children reach or exceed already levels for developmental neurotoxicity (BfR, 2014b). Therefore, a reduction of lead intake is strongly recommended for this group. Due to its high toxicity for the developing nervous system, children, pregnant women and women of child-bearing age are advised not to consume the meat of game animals that have been shot with ammunition containing lead. For adults (excluding pregnant women and women of child-bearing age) the additional uptake of lead via average and even high consumption of game meat (women: 1 to 5 meals à 200 g per year; men: 2 to 10 meals à 200 g per year) can be neglected as compared to the lead uptake via consumption of other food groups.

For extreme consumers of game meat, i.e. consumers in hunters' households, the uptake of lead-containing hunted game meat can significantly add to the alimentary lead uptake (for this group, an average of 91 meals à 200 g per serving per year have been reported). For this group it is important to know, that different parts of the game meat show different lead concentrations and concentrations are significantly higher in meat around the wound channel, implying that in order to reduce a possible uptake of lead via consumption of game meat, different parts of the game should be consumed and different species if relevant.

12.4 Conclusions

12.4.1 What has been achieved?

The use of lead ammunition leads to statistically significant increase of mean lead contents in roe deer and wild boar meat compared to non-lead ammunition, even when effects of the region of origin were considered.

The marketable game meat around the wound channel shows on average a higher contamination than the saddle. The haunch was found to have the lowest lead values. Game meat from roe deer showed slightly lower lead levels than game meat from wild boar. Other factors such as the location of the wound channel and whether the projectile hit a bone may also have an influence. The role of the specific construction types of the bullets requires further investigation.

However, some lead content in game meat can also be due to geogenic exposure (background contamination) and alternative bullets may also contain some traces of lead. Using bullets made from alternative materials, i.e. copper and/or zinc do not appear to lead to concentrations of these elements which will present another health risk (D. Schlichting *et al.*, unpublished data).

Yet, it could clearly be shown that by using non-lead ammunition, a significant reduction of the lead content in game meat is possible. Combining this with suitable game meat hygienic measures and appropriate skills of the hunters would be 'state of the art' in consumer health protection!

12.4.2 What has been neglected?

Further research is still needed to study the effect of particular bullet construction types and alternative types of bullets as well as the effect of the place of impact of the projectile. Moreover, the effect of a particular type of rifle interacting with the bullet needs to be analysed.

12.4.3 What needs to be done?

The effect of the shooting range on lead contamination and animal welfare requires to be carefully looked at. Perhaps more detailed knowledge will allow elucidating other mitigating factors for lead contamination in game meat.

In general more research on the ballistic aspects of game meat hygiene appears to be necessary. A larger societal discussion on the aspects of hunting with lead or non-lead as well as a discussion of the animal welfare aspects would support finding an acceptable solution for the majority of hunters and consumers.

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13. Assessment of primary oxidation products in venison with embedded copper particles subjected to culinary processing

Peter Paulsen[1], Iris Schuhmann-Irschik[1,2], Manfred Sager[3] and Friedrich Bauer[1]*
[1]Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Veterinärplatz 1, 1210 Vienna, Austria; peter.paulsen@vetmeduni.ac.at
[2]Lower Austrian district government (veterinary services), Frauenhofner Str. 2, 3580 Horn, Austria
[3]Austrian Agency for Food Safety and Nutrition Ltd., Spargelfeldstrasse 171, 1220 Vienna, Austria

Summary

In pork-beef patties doped with up to 28 mg/kg copper (Cu) powder, levels of peroxide value (POV) increased after dry-heating and subsequent storage. Fat oxidation was retarded at higher Cu doses, similarly as observed in oils. The correlation of POV and thiobarbituric acid reactive substances (i.e. secondary oxidation products) was strong and positive. Results gave no indication that the release of Cu from embedded bullet fragments would lead to an increase of fat oxidation in venison, but on the contrary higher Cu could actually retard fat oxidation. In practice, the pro-oxidative effect of iron ions liberated from myoglobin during heat processing and species-specific differences in fatty acid patterns are the major drivers of fat oxidation.

Keywords: rifle bullet, copper, venison, curing, boiling, fat oxidation, peroxide value

13.1 Introduction

The increasing use of lead-free bullets to kill large game might result in deposits of copper (Cu) in venison. The extent of release of copper from such fragments embedded into meat (Irschik *et al.*, 2013), or into gastric juice (when fragments are ingested; Paulsen *et al.*, 2015) has been studied previously and also implications on oxidative spoilage (catalysed by Cu ions) have been explored. In a previous study, we demonstrated that the presence of embedded copper bullets in lean venison (red deer, fallow deer, wild boar) had no significant effect on the levels of secondary products of fat oxidation, i.e. on the levels of thiobarbituric acid reactive substances (TBARS) (Schuhmann-Irschik *et al.*, 2015). On the contrary, the mode of culinary processing significantly affected TBARS levels, as TBARS were higher after (1) sous-vide boiling without addition of water and (2) sous-vide boiling without addition of water plus subsequent storage compared to (3) boiling in brine or (4) barbecuing. The observation that TBARS levels would increase during storage of already heat-processed meat was confirmed with mixed meat patties (beef-pork) doped with up to 28 mg/kg Cu powder. This was not unexpected, since heat treatment will liberate iron ions which then act as pro-oxidants.

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Interestingly, increase of TBARS was retarded with increase of Cu doses (administered as powder) from 7 to 14 and 28 mg/kg. Similar observations have been reported for oils, and it is thought that Cu in fact might help to degrade peroxide radicals, i.e. the formation of hydroperoxides as primary oxidation products is then restricted (Janíček *et al.*, 1963; Pokorný *et al.*, 1963, 1967; Sedláček, 1971). Species differences in fatty acid composition and iron (heme) content have to be taken into account, as TBARS in pork-beef patties were lower than in those from wild boar (same percentage of crude fat).

In this chapter, we present the corresponding data for peroxide value (POV) to estimate levels of primary oxidation. We expected a low content of primary oxidation products in copper-doped samples. This could explain why contents of secondary oxidation products were comparably low. In addition, an experiment is described indicating that the physical state of copper (solid block, powder or as sulphate) had some effect on TBARS or POV levels.

13.2 Material and methods

13.2.1 Effect of copper content on TBARS content and POV of meat patties from game

For this experiment ('Experiment 1'), 3 kg meat from fallow deer (lean) and wild boar were combined to give a mix containing 10% fat. Meat was minced and after addition of 20 g/kg NaCl, the batter was divided into six 500 g batches. To batches 1 and 2, copper sulphate monohydrate (Merck, Darmstadt, Germany) was added corresponding to 7 and 14 mg/kg Cu, respectively, whereas batches 3 and 4 received copper powder (particle size <63 µm; CP21.1, suspended in 10 ml water; Roth, Karlsruhe, Germany), to give final contents of 7 and 14 mg/kg Cu, respectively. From each batch, meat patties of ca. 30 g weight were formed. Batch 5 was prepared by inserting a 0.223 inch diameter copper bullet (Barnes TSX®, Mona, UT, USA; 3.5 g weight) in each 30 g meat patty, whereas the sixth batch received no copper doping and served as blank. Patties were dry-heated at 175 °C for 30 min. (core temperature ≥75 °C), then left to cool to room temperature. Per batch, five patties each were tested for TBARS and POV values (1) immediately after cooling; and (2) after storage at 5 °C in a household refrigerator with a glass door to ensure exposure of patties to ambient light (samples wrapped in cling foil) for 7 and 14 days.

The oxidative status was determined as POV according to AOAC (1980) and as levels of TBARS (Witte *et al.*, 1970). Analytical reagents were obtained from Merck and Sigma-Aldrich (Darmstadt, Germany. All results refer to fresh weight.

13.2.2 POV in minced meat patties, as affected by levels of copper doping, heating and storage, and meat species

To minced beef-pork mix (10% fat), 2% NaCl were added. The batter was divided into four sections, three of which received copper doping (powder, particle size <63 µm; Roth CP21.1) at levels of 28, 14 and 7 mg/kg, respectively. Patties of ca. 30 g were formed and were dry-heated at 175 °C for 15 min. (to reach a core temperature of 75 °C), then left to cool to room temperature. Samples were wrapped in cling film and stored at 5 °C in a household refrigerator with a glass door to ensure exposure of patties to ambient light (samples wrapped in cling foil) (termed as 'Experiment 2'). In another experiment ('Experiment 3'), meat patties were formed from wild boar meat or beef-pork mix (10% fat) and 2% NaCl were added. From the batter, patties of ca. 30 g weight were formed, dry-heated and subsequently stored as described above. Details on the experiments are given in Schuhmann-Irschik *et al.* (2015). The oxidative status of the fat was determined as POV according to AOAC (1980).

13.2.3 Statistical processing

Significance ($P<0.05$) of differences was assessed in Experiments 2 and 3 by analysis of variance, with Scheffe's test to discriminate among means. Dependent factor was POV, independent factors were: copper levels and storage time post-heating (Experiment 2) and animal species and storage time post-heating (Experiment 3).

13.3 Results and discussion

The amount of copper powder added to the meat patties was based on the highest individual copper content found in meat cubes with an embedded 0.223 inch diameter bullet (Schuhmann-Irschik *et al.*, 2015), i.e. 7 mg/kg. To estimate worse conditions, also multiples of this amount of copper were considered.

In the first experiment, it was obvious that the time of storage post-heating exerted an influence on both POV and TBARS levels. Changes relative to controls (no copper doping and immediately after heating) are shown in Figure 13.1 and 13.2, respectively. The addition of Cu as powder or $CuSO_4$ was associated with higher POV and TBARS levels than a Cu solid body. This was not unexpected since the powder or sulphate allow a more even distribution of copper in the patties and the active surface is presumably increased. Since the flanks of a bullet passing through the body will be 'abrased' and deposit small metal fragments in the body tissues along the bullet path (Felsmann *et al.*, 2016), using Cu powder is a reasonable scenario to simulate bullet borne Cu contamination near to the wound channel. Thus, Cu powder was used in further experiments.

In pork-beef mix patties, immediately after heat treatment, average POVs decreased significantly with the increase of copper doping, whereas at day 14 there was a trend for POVs to increase with increasing Cu content. This differed from the findings for TBARS, where the inverse relation of TBARS and storage time was significant at day 14 only. During storage, POVs increased, but remained lower in Cu doped samples than in controls (Figure 13.3).

Correlation of TBARS with POV was positive and strong in dry-fried and subsequently stored meat patties without copper doping ($r^2=0.85$), but also in dry-fried and subsequently stored patties with copper doping (as solid particles, as Cu powder and as Cu-sulphate; $r^2=0.90$). As regards the effect of increasing Cu content (7, 14, 28 mg Cu/kg) in meat patties subjected to dry-heating, the correlation of TBARS and POV was also strong and positive ($r^2=0.92$). The

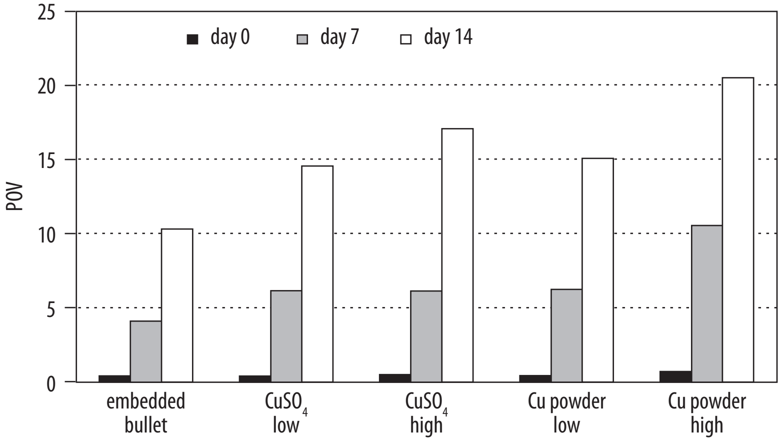

Figure 13.1. Changes in peroxide value (POV) during storage (0, 7, 14 days) of dry-heated wild boar/fallow deer meat patties (data are relative to non-copper-doped control samples at day 0 corresponding to '1' on y-axis); 'low' and 'high' correspond to 7 and 14 mg Cu/kg, respectively.

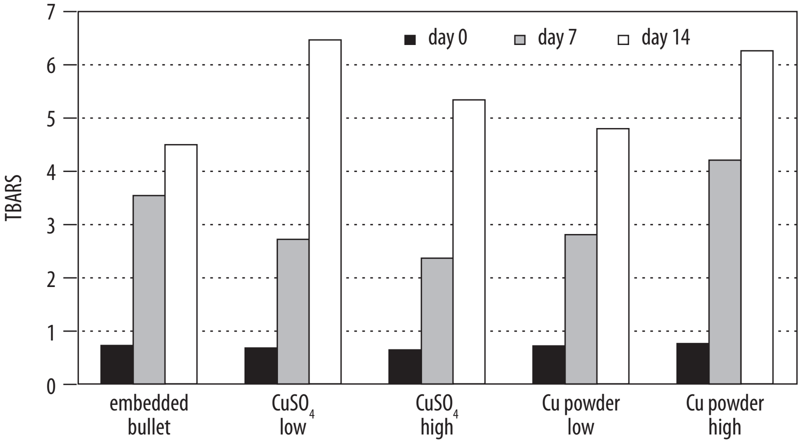

Figure 13.2. Changes in thiobarbituric acid reactive substances (TBARS) during storage (0, 7, 14 days) of dry-heated wild boar/fallow deer meat patties (data are relative to non-copper-doped control samples at day 0 corresponding to '1' on y-axis); 'low' and 'high' correspond to 7 and 14 mg Cu/kg, respectively.

latter results would support the hypothesis that increasing Cu contents would be associated with lower levels of primary oxidation products.

Clearly, more detailed studies need to be conducted to confirm and characterize a dose dependent effect of copper on fat oxidation in meat.

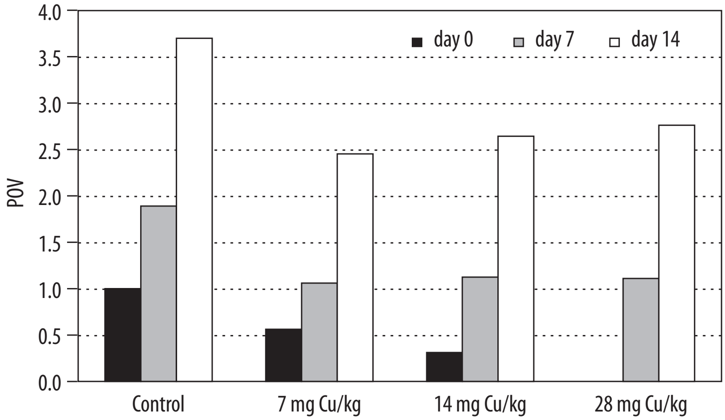

Figure 13.3. Changes in peroxide value (POV) during storage (0, 7, 14 days) of dry-heated beef-pork mix patties (data are relative to non-copper-doped control samples at day 0 corresponding to '1' on y-axis); with Cu-doses from 7 to 28 mg/kg.

However, the pro-oxidative effect of iron ions liberated from myoglobin during heat processing and species-specific differences in fatty acid pattern are likely to overshadow copper-mediated effects on fat oxidation, as demonstrated in Experiment 3, see Figure 13.4. For the purpose of food safety assessment, this means that processing of venison with copper contamination is unlikely to cause problems with fat oxidation.

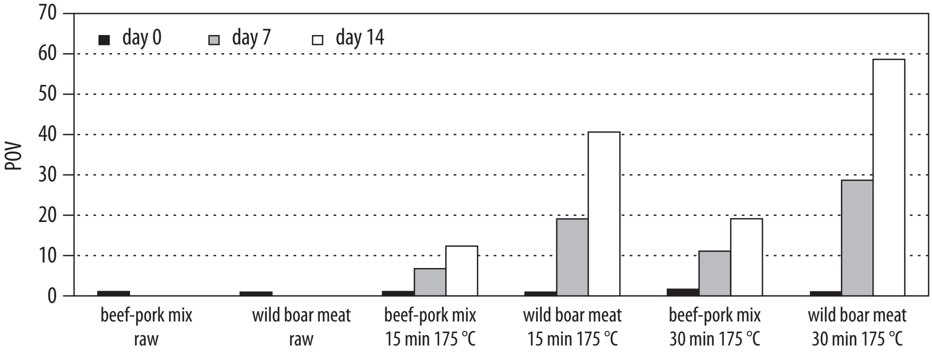

Figure 13.4. Changes in peroxide value (POV) during storage (0-7-14 days) of dry-heated beef-pork mix and wild boar meat patties (data are relative to non-copper-doped raw control samples at day 0 corresponding to '1' on y-axis).

13.4 Conclusions

13.4.1 What has been achieved?

Increasing awareness about negative effects of spent ammunition – either deposited in edible tissues or as an environmental contaminant – has fostered the introduction of 'lead-free' rifle bullets in hunting of large game. The contribution of ablated small metal flakes or bullet fragments embedded into venison to total metal content of venison has been studied in particular for copper, as regards dietary exposure and potential for triggering oxidation of fat. The use of deforming instead of fragmenting bullets can minimize the risk of embedded fragments, whereas ablated flakes near to the shot wound could be controlled only when the surrounding of the shot wound is excised and not used for human consumption.

13.4.2 What has been neglected?

Apart from solids consisting of 'pure' copper, alloys or bullet coatings may contain metals which are under debate because of low toxic threshold and/or already existing alimentary exposure close to tolerable levels. This, in particular, applies to nickel. Both bullet manufacturers and hunters should not only consider ballistics in terms of precision and energy transfer upon impact, but also extent and composition of bullet metal deposition in edible tissues. Knowledge transfer from science to end-users has obviously been hampered by the necessity of a multi-disciplinary approach and the fact that statistically based recommendations can in some cases, conflict with personal observations of end-users.

13.4.3 What needs to be done?

Although not considered as 'food contact material', bullets for hunting should be composed from metals with no or least minimal toxic potential to the ecosphere and to consumers. Deposition of bullet metals in tissues should be minimized. Combination of these requirements with good ballistic performance is possible but may pose a challenge in specific settings. This will require more awareness and action of hunters, which have relied in the last decades on a large assortment of well-proven lead-contain bullets.

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Section 4

Game meat hygiene

14. Microbial quality of springbok (*Antidorcas marsupialis*) meat in relation to harvesting and production process

Pieter A. Gouws[1*]*, Nompumelelo Shange*[1,2] *and Louwrens C. Hoffman*[2]
[1]Department of Food Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa; pgouws@sun.ac.za
[2]Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

Summary

Prevalent bacteria on springbok (*Antidorcas marsupialis*) carcasses were investigated. Twelve springbok carcasses were swabbed around the incision area after in-field evisceration; carcasses were swabbed again after skinning and after chilling at the deboning area. Swab samples taken after skinning portrayed a presence of *Escherichia coli* and *Enterobacteriaceae* counts. Springbok carcasses swabbed after chilling indicated a high level of aerobic bacteria, *Clostridium* spp. and lactic acid bacteria (LAB). In contrast, swab samples taken at the incision area tend to be lower in counts than samples taken at the processing plant. Previous studies have found a reduction in microbial counts as temperature decreases. This was not the case for this study, as both *Clostridium* spp. and LAB counts increased after springbok carcasses had been kept at 2 °C for 24 hours. Further investigation in the abattoir is warranted, as results obtained during the study indicate that contamination may be due to poor processing and hygiene practice in the processing plant.

Keywords: game meat, food safety, *Clostridium*, *E. coli*, cross contamination

14.1 Introduction

Animal carcasses can remain sterile when protected by the skin (Gill, 2007). However, the incision made for evisceration can expose the carcass to bacteria. Microbial load on carcasses can also be increased by cross contamination between the hide and carcass. The possibility of these contaminations occurring is exasperated in southern African harvested game species as these first steps occur in the field (Hoffman *et al.*, 2004; Van Schalkwyk *et al.*, 2011). Furthermore, during the deboning process, carcasses can come into contact with dirty equipment and hands. The initial and contaminating microbial load can consist of both spoilage and pathogenic bacteria. Spoilage bacteria can result in undesirable changes in the fresh meat and meat products, such as off-flavours and odours. Pathogenic bacteria can result in food poisoning or food-borne infection in humans.

This study was the first step in a practical view into bacterial load that may be found on game carcasses from point of field harvest to deboning at a game abattoir. Selected micro-organisms

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were identified using ISO methods, so that major sources of contamination during processing could be identified.

14.2 Materials and methods

Springbok carcasses were hunted at a privately owned game reserve, located 10 km away from Graaff-Reinet, South Africa. Springbok carcasses were shot in the head, bled out and eviscerated in the field. Incisions for evisceration on the stomach were made from the inside to the outside. Springbok carcasses were later taken to a field depot where the hooves and heads were removed (Hoffman *et al.*, 2004; Van Schalkwyk *et al.*, 2011).

Springbok carcasses were hung overnight in a cold truck to lower carcass temperature to <7 °C, whilst transporting the carcasses to an abattoir. Springbok carcasses were refrigerated until skinning commenced at the abattoir/breaking plant, the following day (day 1). Springbok were skinned and visibly contaminated flesh was trimmed off, carcasses were then chilled further at <4 °C for 24 hours and finally deboned in the deboning section of the plant, the next day (day 3 *post mortem*). The springbok carcasses were sampled at the incision area (after evisceration); this included swabbing both the surrounding skin as well as the muscles exposed with the incision, after skinning and after chilling (at the deboning plant). At each stage the same 12 carcasses were swabbed. The tip of the sterile swab (Lasec, South Africa) was removed from its sterile wrapping, moistened with peptone water. The moistened tip of the swab was placed on the surface of the carcasses, primarily swabbing the rump, flank, brisket, neck, back of the carcass surface as well as around the belly where the incision was. Swabs were rotated between thumb and forefinger during swabbing. After swabbing, swabs were immediately placed back into their individual plastic holding tube. Swabs samples were collected over the 3 days were tested for the presence of *Escherichia coli*, *Enterobacteriaceae*, *Clostridium* spp., lactic acid bacteria and aerobic bacteria. For the enumeration of the microorganisms, ISO methods were used, for aerobic plate counts the ISO 4833:1991 (ISO, 1991), for *Enterobacteriaceae* the ISO 21528-2:2004 (ISO, 2004), for lactic acid bacteria ISO 15214:1998 (ISO, 1998), for *E. coli* the ISO 16654:2001 (ISO, 2001) and for *Clostridium* the ISO 15213:2003 (ISO, 2003). Detection of *Salmonella* was done according to ISO 6579:2002 (ISO, 2002). Bacterial counts were converted to logarithmic form for statistical analysis.

14.3 Results and discussion

As seen in Figure 14.1, the *Enterobacteriaceae* counts found on the swabs used for the incision area ranged from 0.5 to >2.5 log cfu/cm^2 for the carcasses. After skinning, *c*arcasses had higher *Enterobacteriaceae* counts. After the carcasses were chilled in the cold room for 24 hours, 50% of carcasses had counts as high as 2.5 log cfu/cm^2. It is apparent that *Enterobacteriaceae* counts increased after skinning and after chilling (at the deboning plant). Therefore, *Enterobacteriaceae* contamination increased as the carcasses reached the processing plant.

Aerobic growth was witnessed for the three sampling points, for all 12 springbok carcasses (Figure 14.2). Incision area swabs and after skinning swabs had lower counts when compared

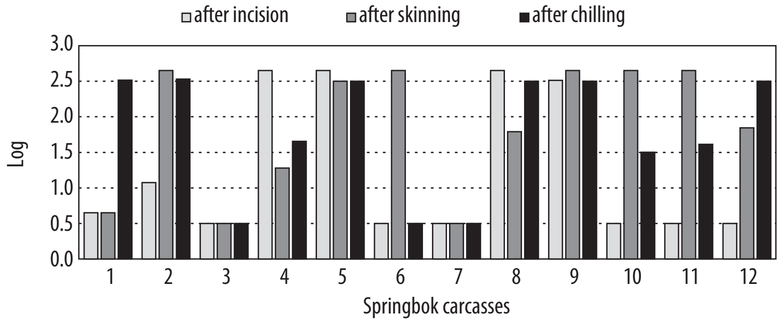

Figure 14.1. Mean log Enterobacteriaceae *counts per cm² in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling.*

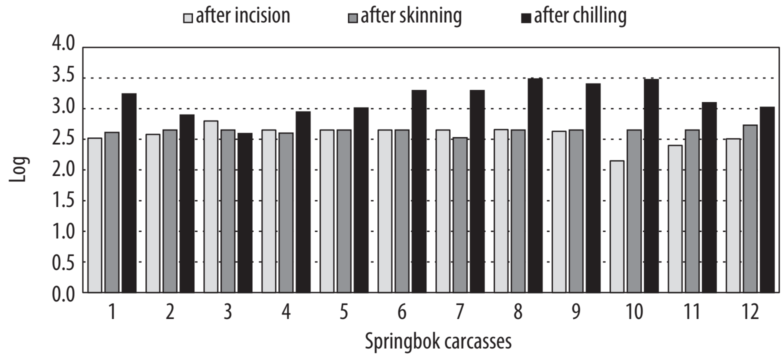

Figure 14.2. Mean log aerobic plate counts per cm² in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling.

to counts obtained after chilling. Swabs taken after chilling at the deboning plant showed an apparent increase in bacterial count.

An undeniable presence of *Clostridium* spp. was found on all 12 springbok carcasses at all three stages (Figure 14.3). *Clostridium* spp. counts found from swab samples of the incision area and after skinning reached log counts of <2.7 log cfu/cm^2. However, samples taken after chilling at the deboning plant exhibited a slight increase in *Clostridium* spp. levels. The highest count at this stage was ~3 log cfu/cm^2.

A consistent presence of lactic acid bacteria (LAB) contamination was found on the springbok carcasses for the three sampling points (Figure 14.4). LAB counts found from swab samples of the incision area ranged from 1.5-2.8 log cfu/cm^2. Swab samples taken after skinning were also contaminated with LAB, with LAB levels ranging from 2.1-2.6 log cfu/cm^2. However, samples taken after chilling at the deboning plant exhibit a slight increase in LAB levels, where counts reached ~3 log cfu/cm^2.

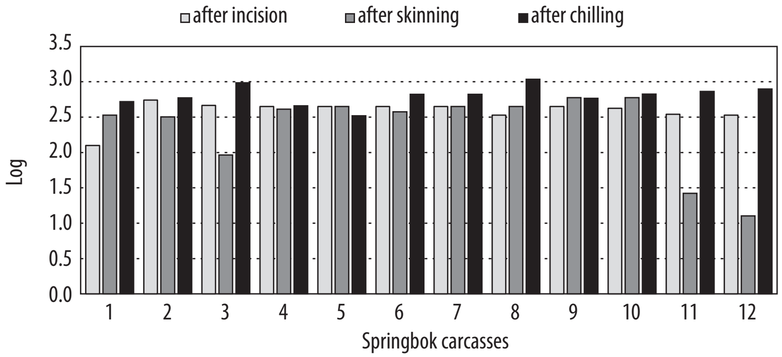

Figure 14.3. Mean log Clostridium *spp. counts per cm² in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling.*

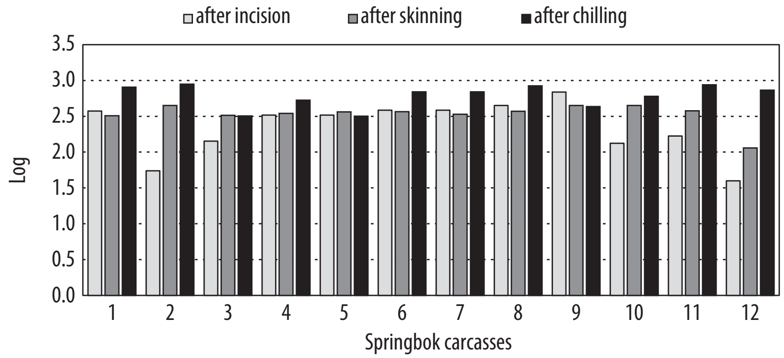

Figure 14.4. Mean log lactic acid bacteria counts per cm² in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling.

E. coli contamination was found on the springbok carcasses for the last sampling point (Figure 14.5), at least 42% of carcasses sampled were contaminated with *E. coli* after skinning. *E. coli* was detected only at the deboning plant after skinning, where counts for some of the carcasses reached >2.5 log cfu/cm².

From samples gathered after evisceration, at the incision area, *Enterobacteriaceae*, *Clostridium* spp., LAB and aerobic bacteria were enumerated. The presence of these microorganisms can be attributed to possible contamination from the skin, during the making of the incision, for evisceration of the white offal. Skin contamination can be from various factors such as animals coming into contact with their own or other animals' faeces (typically as found during allo-grooming commonly practised by ungulates), lying in their own faeces to contamination being aided by dust in the field created by wild angulates moving in herds (Bell, 1997). It must also be noted that the numbers of *Enterobacteriaceae* were quite variable, and sometimes as low as 0.5 log cfu/cm². *E. coli* is part of the *Enterobacteriaceae* family therefore the lack of *E.*

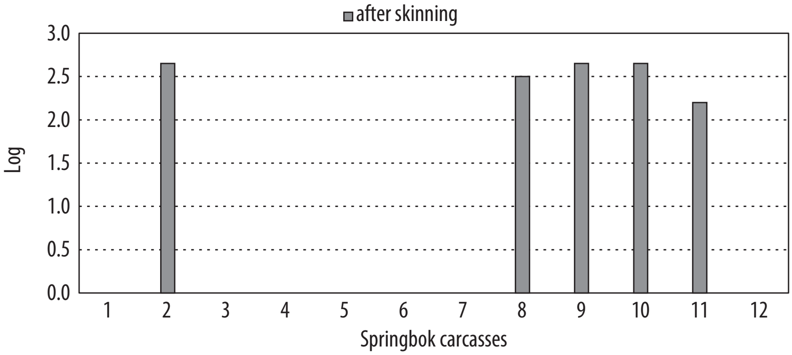

Figure 14.5. Mean log Escherichia coli *counts per cm^2 in springbok carcass swab samples collected from the incision area at the field after skinning.*

coli at this stage is no surprise (Cloete, 2010). After skinning of carcasses, samples showed presence of *Enterobacteriaceae*, *Clostridium* spp., LAB, aerobic bacteria and even *E. coli*. However, contamination of carcass surfaces can be associated with improper execution of the skinning technique. Van Schalkwyk *et al.* (2011) reported that the transfer of bacteria from the hide to the surface of the carcass is a possibility during skinning. These carcasses were chilled when the skinning proceeded and it is more difficult to skin a cold carcass than a warm one. Therefore, it would seem that the skinning technique is important in determining the bacterial load on game carcass surfaces. Contamination after chilling showed consistency and a slight increase in bacterial counts. Contamination can be due to frequent exposure and handling of carcasses by the time the carcasses reach this point (Cloete, 2010). Contamination at this point can be a reflection of the adherence to hygiene practises during the slaughter process, at the abattoir.

14.4 Conclusions

14.4.1 What has been achieved?

Swab samples taken after skinning portray a presence and an increase of *E. coli* and *Enterobacteriaceae* counts. Similarly, swab samples taken after chilling portray a presence and an increase in aerobic bacteria, *Clostridium* spp. and LAB.

14.4.2 What has been neglected?

This study monitored the dynamics of the microflora on the surfaces of springbok carcasses, but did not differentiate between poor processing within the plant and poor hygiene in the cold room as sources of contamination.

14.4.3 What needs to be done?

Further investigation of the abattoir is warranted; in particular more specific sampling of carcass areas is needed, to provide more clarity on how the contamination occurs.

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15. The microbial quality of black wildebeest (*Connochaetes gnou*) carcasses processed in a South African abattoir

***Pieter A. Gouws*[1*], *Nompumelelo Shange*[1,2] *and Louwrens C. Hoffman*[2]**
[1]Department of Food Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa; pgouws@sun.ac.za
[2]Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

Summary

The aim of this study was to investigate the microbial quality of black wildebeest (*Connochaetes gnou*) carcasses from the point of slaughter until the deboning process, allowing for the determination of possible points of contamination during the slaughter process. Carcasses were sampled at three processing points (skin on, skin off and post chilling) at four different carcass sites (rump, flank, brisket and neck). Before skinning, aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* were enumerated from hide samples, counts ranged from 0.92 to 7.84 log cfu/g. After skinning the same bacteria were enumerated on the carcass. Counts ranged from 0.93 to 6.12 log cfu/g. A decrease in bacterial counts was seen after chilling of the carcasses. Significant differences between sites' bacterial loads were seen. For aerobic bacteria differences were prominent for flank (after skinning) and neck (after chilling), whilst for *Enterobacteriaceae* and *E. coli* noticeable differences between sites were noted for flank samples after chilling. *Clostridium* spp. showed an increase in counts after skinning, but this was not statistically significant. All samples were negative for *Salmonella* spp. The results from this study allow identifying when and on which area of the carcass contamination events occur.

Keywords: game meat, food safety, *Clostridium*, *E. coli*, cross contamination

15.1 Introduction

Over the years game meat has become popular in South Africa. This popularity can be attributed to the fact that game meat is a nutritional source of high density protein. Hoffman (2003) indicated that at least 73% of Western Cape (South African province) consumers interviewed had eaten game meat. Furthermore, an increase in suppliers of game meat has been recognised, where more and more game farmers are emerging in South Africa. Game farming has been deemed the fastest growing agricultural industry in South Africa. Game animals from wildlife ranches contribute to ecotourism, hunting, breeding, selling of game animals and meat production (Cloete *et al.*, 2007). Van der Merwe and Saayman (2014) revealed the economic impact of game meat to be at least R2.6 billion in Limpopo province alone.

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The apparent interest in game meat and the industry by both local and international market requires attention to be focused on the hygiene and microbial safety of game meat. Hygiene and microbial safety of meat would normally be dependent on the slaughter process (Bell, 1997; Galland, 1997; McEvoy *et al*., 2004). However, for game meat, hygiene status and microbial safety is dependent on both the harvesting and slaughter process. Handling and processing during harvesting and slaughtering can result in the spreading of microorganisms. *Ante*- and *post-mortem* inspection are traditional methods aimed to detect contamination. However, visual inspection has shown short-comings along the years. Therefore, the implementation of programmes based on the hazard analysis and critical control point (HACCP) approach have now become the norm in the formal meat industry (Loretz *et al*., 2011; McEvoy *et al*., 2004). During the harvesting and slaughtering process, previously sterile muscle/meat can be contaminated by the hide and viscera (Bell, 1997; Gill, 2007). Additionally, equipment and personnel can aid the advancing of contamination.

This study investigates the microbial quality of game, namely black wildebeest (*Connochaetes gnou*) carcasses from the point of slaughter until the deboning process, allowing for the determinations of possible points of contamination. Additionally, through this study the differences in bacteriological load amongst sample sites are assessed.

15.2 Materials and methods

A South African game abattoir in Graaff-Reinet received chilled black wildebeest carcasses that were partially opened, unskinned and eviscerated; these animals had been harvested commercially utilising procedures described by Hoffman and Laubscher (2009). At the abattoir, black wildebeest carcasses were processed in the following manner: partially dressed carcasses were delivered hanging on one Achilles heel in a sealed cold truck (carcass temperature ≤7 °C) to the abattoir. For this study, six carcasses were chosen to be sampled pre-skinning and post-skinning and post-chilling (post-chill samples were collected the next day, during the deboning of the carcasses), these carcass were tracked, to ensure that the same carcasses were sampled at each point. On the first day, pre-skinning and post-skinning sampling of the six chosen carcasses occurred. Pre-skinning samples were taken in the following manner: whilst hanging and during the skinning process, 10 g of skin (an area of at least 100 mm^2 was excised to ensure that back up samples if analysis had to be repeated) per sampling position was aseptically removed at the four sampling sites. For post-skinning samples, the same six carcasses were sampled. Immediately after skinning, 10 g of meat (a sterile cork borer of 25 mm with a surface area of 5 cm^2 was used to obtain pieces of meat) were aseptically removed from the demarcated sampling sites. Post-chilling samples were collected in a similar manner from the same chosen carcasses; however, sampling took place on the second day, as carcasses were cooled overnight at ≤4 °C. Once carcasses arrived at the deboning plant, pieces of meat were aseptically removed from the demarcated sampling sites. For the enumeration of the microorganisms, ISO methods were used, i.e. for Aerobic plate counts the ISO 4833:1991 (ISO, 1991), for *Enterobacteriaceae* the ISO 21528-2:2004 (ISO, 2004), for *Escherichia coli* the ISO 16654:2001 (ISO, 2001) and for *Clostridium* ISO 15213:2003 (ISO, 2003). Detection of *Salmonella* was done according to ISO 6579:2002. Bacterial counts were converted to logarithmic form for statistical analysis. To show highly contaminated carcass sites at each processing point, log means of rump, flank, brisket and neck were determined and subjected to an analysis of variance (ANOVA).

15.3 Results and discussion

In this study the microbiological status of game, specifically black wildebeest carcasses processed in a South African game abattoir was evaluated. This sampling was conducted in October, i.e. in the winter season. The results report on total aerobic bacteria (Table 15.1), *Enterobacteriaceae* (Table 15.2), *Clostridium* spp. (Tables 15.3 and 15.4) and *E. coli* (Table 15.5).

Table 15.1. Aerobic bacteria enumerated from rump, flank, brisket and neck samples before skinning, post-skinning and post-chilling (log cfu/g ± SD).[1]

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	5.45 ± 0.282^{d} (4.82)	7.84 ± 0.204^{a} (7.21)	6.79 ± 0.421^{b} (6.16)	5.93 ± 0.787^{c} (5.30)
Post-skinning	5.3 ± 0.427^{d} (4.68)	6.12 ± 0.576^{c} (5.49)	4.00 ± 0.427^{ef} (3.37)	4.29 ± 0.460^{e} (3.66)
Post-chilling	3.75 ± 0.682^{f} (3.12)	3.76 ± 0.450^{f} (3.12)	4.09 ± 0.450^{ef} (3.46)	2.7 ± 0.398^{g} (2.08)

[1] Different superscript letters indicate significant differences in rows and columns ($P<0.05$). Values in brackets are mean values in log cfu/cm^2.

Table 15.2. Log mean Enterobacteriaceae *counts (log cfu/g ± SD) enumerated from rump, flank, brisket and neck samples before skinning, post-skinning and post-chilling.*[1]

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	3.25 ± 1.599^{cd} (2.62)	4.43 ± 0.426^{a} (3.80)	3.63 ± 0.535^{cb} (3.00)	4.22 ± 0.387^{ab} (3.59)
Post-skinning	1.64 ± 0.840^{e} (1.01)	2.78 ± 0.666^{d} (2.15)	0.93 ± 0.779^{f} (0.30)	3.04 ± 0.482^{cd} (2.41)
Post-chilling	1.73 ± 0.970^{e} (1.10)	0.13 ± 0.450^{g} (0)	2.62 ± 0.750^{d} (1.99)	0.74 ± 1.121^{fg} (0.11)

[1] Different superscript letters indicate significant differences in rows and columns ($P<0.05$). Values in brackets are mean values in log cfu/cm^2.

Table 15.3. Log mean counts (log cfu/g ± SD) of Clostridium *spp. enumerated rump, flank, brisket and neck samples before skinning, post-skinning and post-chilling.*[1]

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	1.97 ± 1.853^{bc} (1.34)	2.17 ± 1.435^{bc} (1.54)	2.11 ± 1.698^{bc} (1.48)	2.92 ± 1.688^{ac} (2.29)
Post-skinning	3.47 ± 1.130^{a} (2.84)	3.33 ± 0.449^{a} (2.70)	3.52 ± 0.212^{a} (2.89)	3.21 ± 0.524^{a} (2.58)
Post-chilling	1.83 ± 1.178^{c} (1.20)	0.79 ± 0.979^{d} (0.16)	3.04 ± 0.481^{ab} (2.41)	1.88 ± 0.672^{c} (1.25)

[1] Different superscript letters indicate significant differences in rows and columns ($P<0.05$). Values in brackets are mean values in log cfu/cm^2.

Table 15.4. Clostridium *spp. occurrences for rump, flank, brisket and neck samples collected in a South African game abattoir at three processing points/stages.*[1]

Cut	Before skinning	Post-skinning	Post-chilling
Rump	100% (6/6)	100% (6/6)	100% (6/6)
Flank	66.7% (4/6)	83.3% (5/6)	16.7% (1/6)
Brisket	100% (6/6)	100% (6/6)	100% (6/6)
Flank	66.7% (4/6)	83.3% (5/6)	16.7% (1/6)

[1] Percentage of positive samples for each sample area and processing point. Values in brackets are number of samples tested at each point that were positive for *Clostridium* spp.

Table 15.5. Escherichia coli *occurrences for rump, flank, brisket and neck samples collected in a South African game abattoir at three processing points.* [1]

Cut	Before skinning	Post-skinning	Post-chilling
Rump	66.67% (4/6)	100% (6/6)	83.33% (5/6)
Flank	83.33% (5/6)	100% (6/6)	50% (3/6)
Brisket	83.3% (5/6)	100% (6/6)	100% (6/6)
Neck	100% (6/6)	100% (6/6)	100% (6/6)

[1] Percentage of positive samples for each sample area and processing point. Values in brackets are ^{n}umber of samples tested at each point that were positive for *E. coli*.

Slaughter operations such as skinning can allow for contamination of sterile flesh (Abdalla *et al.*, 2009). Present work revealed that *Enterobacteriaceae* and *E. coli*, but also various other aerobic bacteria were found on the carcass after skinning, therefore the skinning process can be identified as source of contamination (Gill, 2007; Gill *et al.*, 1998). Bacterial counts on the surface of the carcass were lower than those reported from the skin. This is in agreement with the notion that bacteria transferred from skin to carcass surfaces during skinning is only a fraction of the bacterial load found on the skin of slaughter animals (Antic *et al.*, 2010). Further reduction of bacterial counts was seen from samples taken post-chilling. This is in agreement with the idea that temperature does affect further growth of bacteria and bacterial counts determined (Vaarala and Korkeala, 1999), since low temperatures are not conducive to growth of mesophilic bacteria transferred from skin or intestinal content.

In 72 samples which were evaluated, *Salmonella* sp. was not detected. Magwedere *et al.* (2013) also noted an absence of *Salmonella* sp. for springbok samples. The prevalence of *Salmonella* spp. in game meat has been reported to be rare. This was also confirmed by Van der Merwe *et al.* (2013) where the analyses of 162 game meat samples from three different abattoirs showed an absence of *Salmonella* spp. The distribution of aerobic bacteria, *Enterobacteriaceae*, *Clostridium* spp. and *E. coli* was also investigated. On partially opened carcasses (before skinning) aerobic bacteria, *Enterobacteriaceae*, *Clostridium* spp. and *E. coli* were enumerated on the skin at the rump, flank, brisket and neck. After skinning, it can be assumed that some bacteria were transferred onto the carcass surfaces; the neck had one of the highest *Enterobacteriaceae* and *E. coli* counts. Through visual observation, it was seen that the neck region was the bloodiest, hair deposit were also seen; this was most probably caused by in-field exsanguination. Immediately after being shot in the head, the animals are exsanguinated, usually with a two knife procedure. Visual observations agreed with Vaarala and Korkeala (1999) who reported that the neck area can be one of the dirtiest regions on a carcass. Furthermore, the carcasses are hung from the hind leg and thus the neck is the last region to be skinned, which can allow for accidental touching of the skin, due to vigorous movement of carcasses when the skin is pulled off.

Lastly, after chilling the brisket was high in aerobic bacteria, *Enterobacteriaceae*, *Clostridium* spp. and *E. coli*. After chilling contamination could be due to close stacking of carcasses in the chiller and/or due to the typical pushing and pulling of carcasses with the hand as it is easy to grab a carcass via the brisket to move it along the hanging rail. Also, more human contact with skinned carcasses when they were moved/carried from the chiller to the truck (during transportation to the deboning plant) could result in an increase in bacterial load.

15.4 Conclusions

15.4.1 What has been achieved?

This study indicated that there were significant bacteria deposited on the surfaces of fresh carcasses during the skinning and confirmed that proper cooling of skinned carcasses is able not only to prevent multiplication of bacteria on carcass surfaces, but even to reduce numbers.

15.4.2 What has been neglected?

This study focused on only one abattoir in South Africa (although this is the largest game meat processing abattoir in the country) and contamination of large game carcasses. Also, field samples were not included; the extent of contamination due to field tasks can only be speculated upon.

15.4.3 What needs to be done?

The results underpin that skinning should be executed with care so as to ensure minimum cross contamination, thereby improving the microbial quality of game carcasses. Further research in the microbiology of game carcasses from other abattoirs is required for a wider evaluation of the microbial quality of game meat, as the data present is still scanty; this study only provides a baseline. Furthermore, more pathogenic species in relation to game meat need to be studied. Future research should aim to start sampling in the field, to effectively determine points of contamination and short comings of field procedures.

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16. Determination of the microbial population of blesbok (*Damaliscus pygargus phillipsi*) meat in South Africa

Pieter A. Gouws[1*], Zandré Germishuys[1,2] and Louwrens C. Hoffman[2]
[1]Department of Food Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa; pgouws@sun.ac.za
[2]Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

Summary

The aim of this study was to investigate the microbial population and their prevalence on blesbok meat after slaughter and dressing of the carcasses. Animals were harvested from the University of Stellenbosch's experimental farm in the Western Cape, South Africa. Eight animals were shot and bled out in the field where after they were transported to the slaughter facility on the farm where dressing of the carcasses was done and two meat samples were taken. Surface swabs of the meat samples were used for analyses. All samples were tested for the presence of total coliforms, aerobic count, *Staphylococcus aureus* and *Escherichia coli*. Total aerobic count was in the range of 1.6-5.1 log cfu/cm^2 whereas all total coliform count was above 5 log cfu/cm^2. *E. coli* counts ranged from 0-2.19 log cfu/cm^2 and lastly *S. aureus* were all considerably low except for blesbok seven that was excessively contaminated. It was therefore evident to see that cross contamination took place during the slaughter process including during removal of the hide.

Keywords: game meat, food safety, tempo system, petrifilm, cross contamination

16.1 Introduction

The consumption of game meat is increasing worldwide due to consumers becoming more concerned about their health and the products they consume. Due to these health concerns, consumers have started adapting their lifestyles to incorporate more low kilojoule and low cholesterol products (Hoffman and Wiklund, 2006). One such products is game meat, known for its health benefits (Klein, 2005). In 2003, the wildlife ranching sector of South Africa was recognized as a fast growing sector in the agricultural industry. At the same time it was also estimated that South Africa had 9,000 commercial game ranches covering more than 17 million hectares of land (Reilly *et al.*, 2003). During 2014, South Africa produced 23,700 tonnes of game meat (Taylor *et al.*, 2015).

The increased demand for game meat directly correlates to the need for microbiologically safe meat. Health and safety regulations thus have to be in place to ensure that game meat intended for consumption is safe. The microbial safety of meat can be compromised in a number of

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ways. After an animal is killed in the field it is eviscerated. The carcass then has to be skinned and butchered as soon as possible but this is not always the case. Skinning and butchering may sometimes be delayed with a few hours to a few days (Gill, 2007; Klein, 2005). When this happens the probability of the gut being damaged during removal increases greatly as swelling of the intestines increases (Gill, 2007). The time the carcass spends at atmospheric temperature provides the perfect environment for microbial flora to be deposited onto damaged tissue and increase rapidly as the temperature increases (Klein, 2005). It is thus during this time that the carcass is most susceptible to microbial spoilage from its environment.

Further contamination can occur during the skinning of the animal. The hide of the animal naturally contains microbial loads due to their surrounding as well as contamination from faecal matter present in the field (Blagojevic *et al.*, 2011). If skinning of the carcass is thus not done properly, cross contamination can occur where microbial loads are transferred from the hide of the animal to the meat (Nørrung and Buncic, 2008). This study was done to investigate the microbial population and their prevalence on blesbok (*Damaliscus pygargus phillipsi*) meat after slaughter and dressing of the carcasses.

16.2 Materials and methods

16.2.1 Harvesting

Eight blesbok (*D. pygargus phillipsi*) were harvested from the University of Stellenbosch's experimental farm in the Western Cape, South Africa. After the animals were shot and bled out in the field they were transported to the slaughter facility on the farm. After dressing of the carcasses, two meat samples were taken and placed in sterilised sample bags. These samples were kept at 4 °C until they could be frozen (-20 °C) at the laboratory.

16.2.2 Microbial analysis

16.2.2.1 Sample preparation

Before analysis of the samples they were defrosted at 4 °C where after surface swabs (25 cm^2) of the meat samples were taken and placed in 10 ml buffered peptone water (Biolab, Merck, Johannesburg, South Africa). These samples were then used for analysis on the TEMPO system (BioMérieux, Johannesburg, South Africa) and Petrifilms (3M Company, St. Paul, MN, USA).

16.2.2.2 TEMPO system

Aliquots of the samples were transferred to the TEMPO vials (BioMérieux) containing the reconstituted media. This was prepared by adding specific volumes of autoclaved distilled water to the media powder in the vials. *Escherichia coli* and *Staphylococcus aureus* tests were performed using one millilitre of the sample thus making a 1/40 TEMPO dilution, whereas total coliforms test was performed using 0.1 ml of sample thus making a 1/400 TEMPO dilution. The contents of the vials were transferred to the TEMPO cards using the TEMPO

Filler. Each card consists of 48 wells, 16 of each of the three volumes (225, 22.5, 2.25 µl). *E. coli* and *S. aureus* cards were incubated at 37 °C while total coliform cards were incubated at 30 °C. All cards were incubated for 24 h where after the results were analysed on the TEMPO reader. This system makes use of software that detects which of the wells tested positive. The software uses the volumes of the positive wells as well as the dilution of the sample to mathematically calculate the cfu/cm^2 of the sample based on the most probable number tables.

16.2.2.3 Petrifilm

Aerobic count enumeration was done using the Petrifilm system (3M Company). Using the samples used for the TEMPO analyses a dilution series (10^{-2}-10^{-7}) was prepared. One-millilitre aliquots of each dilution were inoculated onto AC (Aerobic count) Petrifilms in duplicate. Using the spreader, the inoculum was spread over the 20 cm^2 surface. The Petrifilms were incubated at 30 °C for 48 hours. All red colonies were counted as per the manufacturer's instructions.

16.3 Results and discussion

During the study the microbial population and its prevalence was investigated to determine whether the blesbok meat was compliant in terms of food safety and food spoilage organisms. The following results indicate the prevalence of total coliforms, aerobic count, *E. coli* and *S. aureus* on the meat of eight blesbok carcasses.

The contents of Table 16.1 describe the acceptable microbial ranges for game meat that is destined for export (DAFF, 2010). All further results will be discussed with reference to this table. The prevalence of aerobic counts (Figure 16.1) for the eight animals ranged between 1.6-5.1 log cfu/cm^2. This clearly falls within the acceptable range according to the microbial

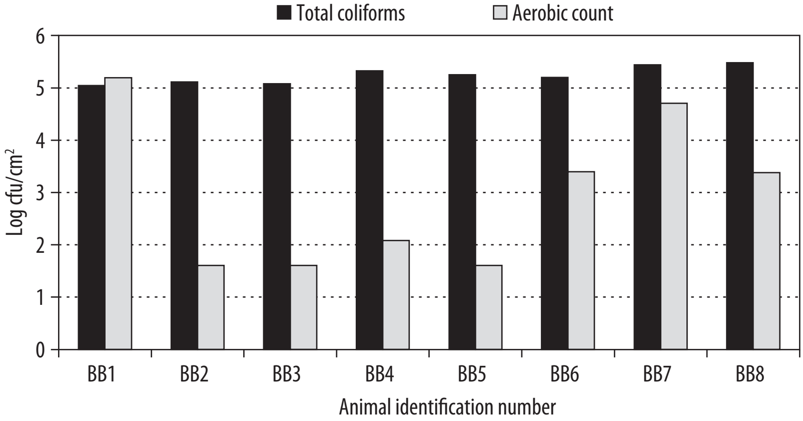

Figure 16.1. Bacterial contamination (log cfu/cm^2) of blesbok (BB) meat from eight carcasses (total coliforms in black; aerobic count in grey).

standards. Total coliforms on the other hand had a much higher prevalence with all the animals having log cfu/cm^2 count of more than five. Although there are no regulations pertaining to total coliforms as such, one could interpret it in terms of *Enterobacteriaceae*. No tests were done for *Enterobacteriaceae* so the following statement will be based on speculation. As described by Baylis (2006), total coliforms fall under the family of *Enterobacteriaceae* including other pathogens such as *Salmonella* and *Shigella*. One could thus speculate that the *Enterobacteriaceae* count would not be within specification as the total coliforms count already greatly exceeds the acceptable range. The prevalence of *E. coli* (Figure 16.2) was within the range of 0-2.19 log cfu/cm^2 which falls into the acceptable range. *Staphylococcus aureus* was present on all blesbok carcasses with a count of 1 log cfu/25 cm^2. Blesbok seven was the only animal that showed excessive contamination with a count of just above 3 log cfu/25 cm^2.

Gill (2007) stated that it is generally accepted that healthy animals' deep tissue meat can be regarded as sterile. Comparing the findings of this study to the before mentioned statement it is evident that cross contamination had to have taken place during the slaughtering process. The most significant factors contributing to the contamination of carcasses during the slaughter process is cross contamination from the hide and the hygiene of the personal responsible for the slaughtering (Barco *et al.*, 2015). Another important factor is the killing conditions. Depending on whether the animal was shot in the head or if the stomach was punctured it could affect the microbial load on the carcass (Atanassova *et al.*, 2008); Blagojevic *et al.*

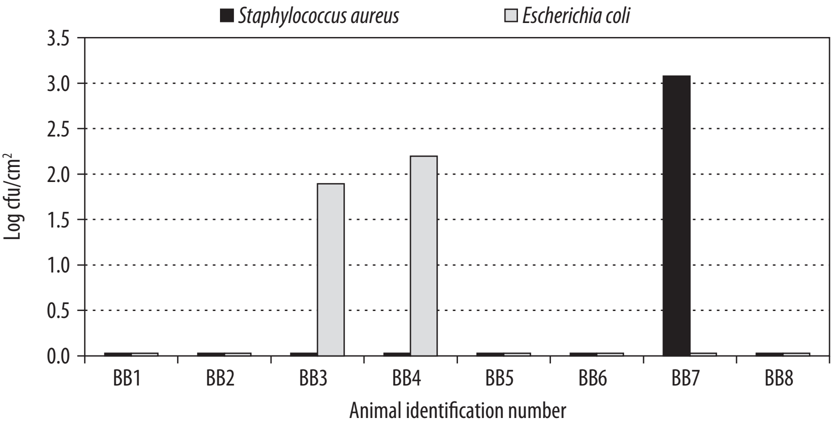

*Figure 16.2. Bacterial contamination (log cfu/cm^2) of blesbok (BB) meat from eight carcasses (*Staphylococcus aureus *in black;* Escherichia coli *in grey).*

Table 16.1. Acceptable microbial ranges according to the microbial standards for export meat (DAFF, 2010).

	Aerobic count	*Escherichia coli*	*Enterobacteriaceae*
Acceptable range (log cfu/cm^2)	5.0-5.7	1.7-2.7	2.0-2.5

(2011) showed a significant carryover of microbial load from the hide to the carcass during dressing of the carcass. This could be one of the major reasons for the contamination found on the carcasses. Another possible explanation is that the personal were touching the carcass after their hands had already been contaminated from being in contact with the stomach/intestines as well as the hide.

16.4 Conclusions

16.4.1 What has been achieved?

This study concluded that there was definitely cross contamination from the hide to the carcass suggesting that the dressing process was not executed properly. With regard to the microbial regulations in terms of food safety it can be concluded that the meat is safe for consumption. In terms of food spoilage the meat might be susceptible to spoilage due to the high total coliforms and aerobic counts; this indicates that the shelf life of the meat might be compromised.

16.4.2 What has been neglected?

This study only focused on blesbok from one farm in the Western Cape. Both number of tested animals and the array of screened indicator bacteria were limited.

16.4.3 What needs to be done?

It is recommended that this study be expanded to include more farms in South Africa as well as to a variety of different species. Further research should also include testing for *Enterobacteriaceae* and other indicator organisms, ad include larger sample sizes. A last aspect that could possibly be evaluated is if the level of contamination would change if the personnel underwent specific training on slaughtering hygienically.

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17. Evaluation of pH in game meat of red deer hunted in autumn in the Western Italian Alps

Roberto Viganò[1*], Jacopo Aprico[2], Martina Besozzi[1], Nicoletta Formenti[2,4], Tiziana Trogu[2], Chiara Donazzolo[3], Federica Obber[1,3], Nicola Ferrari[2,5] and Paolo Lanfranchi[2]

[1]Studio Associato AlpVet, Piazza Venzaghi 2, 21052 Busto Arsizio (VA), Italy; r.vigano@alpvet.it

[2]Università degli Studi di Milano – Dipartimento di Medicina Veterinaria (DIMEVET), Via Celoria 10, 20133 Milano, Italy

[3]Istituto Zooprofilattico Sperimentale delle Venezie, SCT2 Belluno, Via Cappellari 44/a, 32100 Belluno, Italy

[4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna 'Bruno Ubertini' (IZSLER), Via Rovelli 53, 24100 Bergamo, Italy (current address)

[5]Centro di Ricerca Coordinata EpiSoMI, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy

Summary

The need for data on the organoleptic and safety qualities of wild game meat derives from the will to promote this food as a valid nutritional resource. Besides the microbiological parameters, the pH measurement represents a valid indicator of meat quality. Therefore, the aim of the present work was to investigate the effect of animal age and sex and time after shooting on pH values in 262 free ranging red deer (126 females and 136 males) hunted in 2013-2015 in the hunting district VCO 2 – Ossola Nord (Verbania province, Central Western Italian Alps), during post-mating season. Our results show that in young and female individuals pH values decrease to ultimate values lower than 5.8; this leads to the proper maturation of game meat.

Keywords: pH, red deer, free ranging, game meat, quality

17.1 Introduction

The need for data on the safety and organoleptic qualities of wild game meat derives from the will to promote this food as a valid healthy protein source and as an alternative to red meat from domesticated animals. In addition to the microbiological control, and respecting proper hygienic practices to ensure food safety, evaluating physical-chemical characteristics of the final product is relevant to predict the ultimate sensory quality. Monitoring the decrease of pH values after shooting – a variable reflecting the intrinsic process involved in both the maturation of meat and the microbial growth – is a straight-forward and simple approach to achieve this.

Immediately after death, anaerobic metabolism depletes carbohydrate stocks, in particular glycogen in muscles, and the consequent conversion of glycogen into lactic acid leads to a

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reduction of the pH from 7.0 to 5.4-5.7 (Lonergan *et al.*, 2010). Depending on the degree of refrigeration, this process can be faster or slower. As the onset of *rigor mortis* occurs, muscles become stiff because the lack of ATP leads actin and myosin (contractile proteins of muscle) to bind to each other leading to muscle shortening. For this to happen, the animal should have a good body condition, i.e. without weight loss caused by any pathology or particular physiological status. Moreover, stress, such as injury or chase with dogs should be avoided during the hunt. After the shooting, the animal should be exsanguinated, evisceration of the animal must be carried out and the carcass cooled down as soon as possible (Pollard *et al.*, 1999; Winkelmayer and Paulsen, 2008). For these reasons, to ensure optimal organoleptic quality and microbiological safety of game meat (provided compatible with local needs) it would be preferable to hunt animals in a period of their annual lifecycle in which they are not particularly stressed.

In the Italian Alps, according to national guidelines (ISPRA, 2013), red deer (*Cervus elaphus*) is hunted from the 15th October onwards, during the post-mating season: this could affect the quality of meat. This might be of particular importance for breeding adult males, which experience a marked weight loss (even more than 40 kg) during the rut (Gaspar-Lopez *et al.*, 2011).

The aim of this study was to evaluate the most influential factors promoting *post mortem* pH decline in alpine hunted deer.

17.2 Materials and methods

A total of 262 red deer (126 females and 136 males) were sampled in the hunting district VCO 2 – Ossola Nord (Verbania province, Central Western Italian Alps), during hunting seasons 2013 (3rd November – 4th December; n=96), 2014 (15th October – 23rd November; n=103) and 2015 (18th October – 29th November; n=63). Red deer found wounded by track dogs were not sampled.

Sampled animals were classified into 4 age classes as follows:

- calves: born during the current year (31 females and 36 males);
- yearlings: 1-year-old individuals (25 females and 27 males);
- sub-adults: 2 and 3-year-old individuals (22 females and 38 males);
- adults: more than 4-year-old individuals (48 females and 35 males).

Age classes, sex, biometric measurement, day time of shot and of pH measurement were recorded for each sampled animal. Value of pH was detected by means of inserting a pH-probe into the semimembranosus muscle with a resolution of ±0.001 pH units (HD2105.2; Delta OHM®, Soest, the Netherlands).

According to the protocol of Wiklund *et al.* (2004), pH values measured from 2 hours (120 minutes) and 4 hours (240 minutes) after culling were classified and used as predictors for possible alteration of the meat, i.e. the prevalence of dark, firm and dry (DFD) meat condition, as follows:

- pH>6.2: DFD;
- 5.8<pH<6.2: 'borderline' DFD;
- 5.2<pH<5.8: proper maturation.

The data were analysed through Generalised Linear Models in order to assess the effects of deer sex and age classes and time interval between shot and pH measurement as the response variable. Statistical analyses were undertaken with the software R 3.2.2 (R Development Core Team, 2015; https://www.r-project.org), setting *P*<0.05 for significance.

17.3 Results

In our study, mean pH value of the total observed sample was 5.58±0.25. Mean pH values in red deer males and females were 5.60±0.23 and 5.56±0.27, respectively (Table 17.1).

The more time elapsed between shot and pH measurement obviously resulted in lower pH values (Table 17.2). Statistical analysis showed a significant difference between genders and age classes, with younger animals showing lower pH values (Figure 17.1). Moreover, female red deer showed a faster pH decreases than males (Figure 17.2).

Table 17.1. Mean pH values measured in each age classes of red deer males and females.

Sex	Age	No.	Mean pH	Std. dev.
Male	calves (0 years)	36	5.51	0.18
	yearlings (1 year)	27	5.57	0.23
	sub-adults (2-3 years)	38	5.62	0.22
	adults (>4 years)	35	5.67	0.27
Female	calves (0 years)	31	5.55	0.25
	yearlings (1 year)	25	5.55	0.26
	sub-adults (2-3 years)	22	5.52	0.23
	adults (>4 years)	48	5.58	0.30

Table 17.2. Results of generalised linear models analyses on the effects of animal factor and time interval on pH variability.

	Degree of freedom	Deviance	*F*	*P*-value (>*F*)
Age	3	0.484	3.335	0.020
Sex	1	0.287	5.943	0.015
Time interval	1	3.113	64.298	<0.001
Sex interval	1	0.325	6.722	0.010

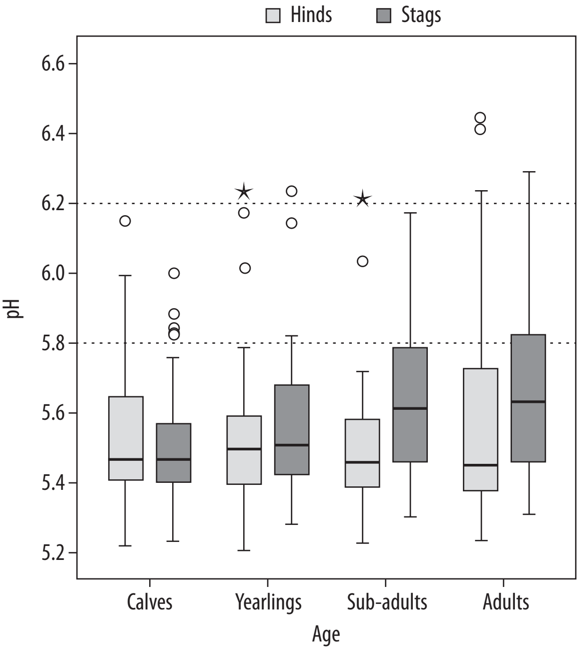

Figure 17.1. pH values measured in each classes of red deer males and females.

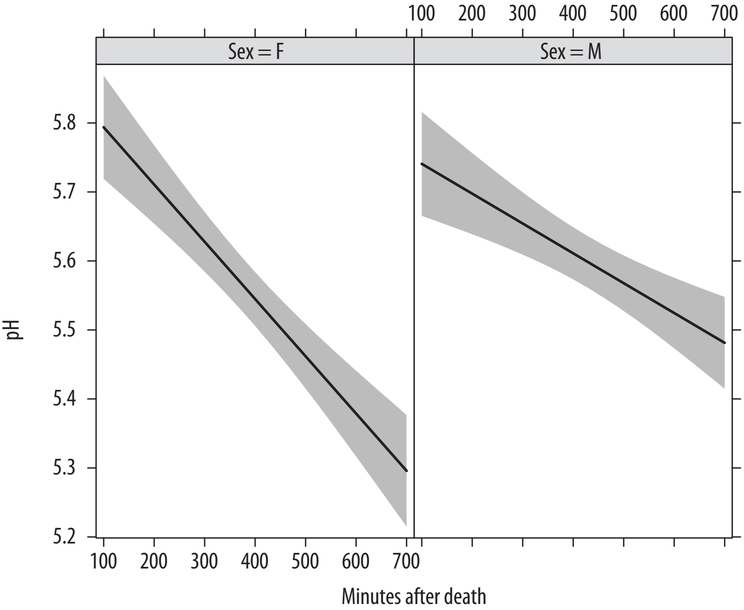

Figure 17.2. Effects of red deer sex and time interval on pH decrease (numbers on x-axis denote minutes post mortem).

At 120 minutes after shooting, pH values ranged from 5.8 to 6.2 in 19/126 males (4 calves, 1 yearling, 7 sub-adults, 7 adults) and 10/127 females (2 calves, 2 yearlings, 1 sub-adult, 5 adults). Moreover, 3 males (1 yearling and 2 adults) and 4 females (1 yearling, 1 sub-adult, 2 adults) showed pH values higher than 6.2. For the other animals, pH values were below 5.8.

At 240 minutes from culling, 11/94 males (1 calf, 1 yearling, 4 sub-adults, 5 adults) and 3/117 females (1 calve, 2 adults) showed pH values between 5.8 and 6.2; one adult male and one adult female had pH values higher than 6.2. For the other animals, pH values were below 5.8.

However, low pH values (<5.8) were observed in 83.2 and 92.0% of samples after 120 and 240 minutes after culling respectively (Table 17.3).

Table 17.3. Distribution of pH values recorded in different sex and age classes at 120 and 240 minutes after culling.

Sex	Age	Post 120 minutes			Post 240 minutes		
		pH<5.8	5.8<pH<6.2	pH>6.2	pH<5.8	5.8<pH<6.2	pH>6.2
Male	calves (0 years)	88.2%	11.8%	0.0%	96.6%	3.4%	0.0%
	yearlings (1 year)	92.0%	4.0%	4.0%	95.0%	5.0%	0.0%
	sub-adults (2-3 years)	80.0%	20.0%	0.0%	87.1%	12.9%	0.0%
	adults (>4 years)	71.9%	21.9%	6.3%	76.9%	19.2%	3.8%
Female	calves (0 years)	92.9%	7.1%	0.0%	95.2%	4.8%	0.0%
	yearlings (1 year)	87.5%	8.3%	4.2%	100.0%	0.0%	0.0%
	sub-adults (2-3 years)	90.9%	4.5%	4.5%	95.0%	0.0%	5.0%
	adults (>4 years)	83.7%	11.6%	4.7%	94.1%	5.9%	0.0%

17.4 Conclusions

17.4.1 What has been achieved?

In addition to microbiological control, pH measurement is a useful indicator for the ultimate safety and quality of game meat and derived products. Our data show a significant variation in ultimate pH dependent of sex and age classes. The number of sub-adult and adult males with pH values >5.8 was higher than in other sex and age classes. This may be due to the poorer body condition of adult males in the post-rut period, when hunting season takes place in our study area. However, the degree of *post mortem* muscle acidification in most of the hunted deer carcasses was acceptable, <5.8. Both genders and different age classes were represented in the DFD/'borderline' DFD class, i.e. no specific 'at risk' classes were observed.

17.4.2 What has been neglected?

In this work (although wounded animals found by means of track dog were not sampled) the hunting activity bias was not considered because the time of death and the time interval between shooting and picking up of hunted animals was not known. Actually, not every shot may have resulted in instant death of the animal. For this reason, at the moment of the check each hunter had to declare the death mode. Notwithstanding, data collected until now on the time interval between shooting and picking up of the red deer do not allow any evaluation on this variable. Finally, the presence of blood clots in muscles, affecting muscle metabolism, could explain the lack or delayed decrease of pH lower than 5.8.

17.4.3 What needs to be done?

For obtaining optimal 'conditioning and ageing' conditions in *post mortem* deer meat, particular attention should be paid to achieving an instant death and subsequent rapid exsanguination and refrigeration. This is particularly the case when hunting adult males and when the hunt takes place in autumn.

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Section 5

Game meat production and quality issues

18. A new agricultural industry in Scotland and the first new domesticated livestock species for 5,000 years

John Fletcher
Venison Advisory Services Ltd., Harthill, Reediehill Deer Farm, Auchtermuchty, Fife KY14 7HS, United Kingdom; johnfletcher@deervet.com

Summary

Although farming deer for meat began in Scotland during the 1980's, a large scale British taste for venison as demonstrated by retail sales has only become apparent in the 21st century. Now all UK supermarkets sell venison and are reporting yearly sales growth of around 25%. Most of this is farmed venison imported from New Zealand. Notwithstanding that, the largest source of UK venison remains wild deer with the majority by weight from Scottish red deer (*Cervus elaphus*) however this supply is declining. New Zealand farmed venison production is also declining. The Scottish government has therefore taken measures to increase the farming of red deer. A newly constructed privately financed abattoir for farmed deer, represents an entirely new initiative within Scotland with benefits for game meat hygiene. In addition, many new deer farms have been established including some projected to run 1000 breeding females. The addition of venison to the repertoire of mainstream red meats confers significant health benefits arising from the composition of this meat. However, whilst farmed venison can be treated in the same way as conventional abattoir killed meats, the provision of hygienically produced wild venison from wild deer killed in the field is challenging.

Keywords: deer, venison, abattoir, deer farming, hygiene

18.1 Introduction

The English word venison, from the Latin *venari* – to hunt, originally described all hunted meat but it has become restricted in modern English parlance to the meat from deer even when the deer has not been hunted, and it is with that meaning that it is used in this chapter.

Venison consumption must have been important historically within the UK. In the medieval period the overwhelming proportion of the human population was rural and it seems undeniable that the countryside in which most people lived must have provided ideal habitat for both red (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). The fallow deer (*Dama dama*) introduced by the Normans in the 12th century did not disperse widely from the enclosed deer parks into which they were placed. These were the only three deer species in Britain until the 19th century.

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Yet, despite the very low human populations of medieval England and the good breeding conditions for the deer, their numbers declined. By the end of the period, late in the 15th century, the Tudor monarchs were introducing measures to preserve wild deer. This decline is unlikely to be due in large part to the hunting pressures of royalty or even nobility as we have historical accounts of the likely numbers of deer they killed and it is clear that the numbers killed by these people were relatively few (Rackham, 2006). Nor were wolves a factor since they are thought to have become extinct in England by the beginning of the fifteenth century (Pluskowski, 2010). The only explanation is that the illegal killing or poaching of deer was widespread and this is confirmed in the proceedings of the forest courts of the 13th and 14th centuries (Birrell, 1996). Of course we cannot deduce the scale of medieval poaching because by its very nature it was clandestine. Thus excavations of the middens of medieval villages do not normally reveal large proportions of deer bones but poaching must have been sufficiently worrying for the landowners to have succeeded in having parliament make the sale of deer meat illegal from 1603-1831 (Fletcher, 2011).

Despite the legislation, the numbers of deer killed continued to reduce their numbers so that by the end of the eighteenth century wild deer numbers were approaching extinction throughout Britain. Red deer were restricted to small populations in South West England and Cumbria as well as larger numbers in the Scottish Highlands. Roe were virtually extinct and fallow were almost entirely restricted to enclosed deer parks whose numbers were declining. Since, in the 21st century, hunters with sophisticated modern rifles have difficulty keeping deer numbers within check we can only wonder at the skills and field craft of those covert medieval poachers.

This pattern of declining wild game numbers is not restricted to Britain. Typically wherever the human population in rural areas grows, as has normally been the case in most regions of the world, then large game species decline. However, as urbanisation subsequently reduces the rural population then those game animals, often including introduced species, become much more numerous, with populations soon exceeding their historical levels. This model holds most obviously for North America but also throughout most of Europe (Fletcher, 2013).

During the 18th and 19th century the human population in Britain became increasingly urban with an accelerating decline in the numbers of people living in the countryside. The proportion of those accustomed to eating venison became vanishingly small and during the 20th century deer numbers began to grow albeit from a very low base.

Given the rarity of deer, it is not surprising that by the beginning of the 20th century the consumption of venison in Britain had become restricted to the landowning classes and those who worked closely with deer in parks or the Scottish Highlands. The tradition of eating venison virtually disappeared and by the middle of the 20th century almost all the venison was exported, principally to Germany where venison had retained its appeal as a meat with strong cultural links to the traditions of the forest and hunting.

By the mid-twentieth century Scotland was annually producing about 25,000 wild red deer carcasses and a lower number of the much smaller roe. Of these, 1,500 tonnes were being exported (Blaxter *et al*., 1974). In other words virtually all the home produced venison was being exported, most of it to Germany.

18.2 The development of modern deer farming in Scotland

When the author began to farm red deer in 1973, initially as a part time occupation, he found that there was a domestic demand but that it was small. He believed that this sector of the market could be increased and made no effort to export venison – although he exported substantial numbers of live deer throughout Europe and also to Asia and the USA (Fletcher, 2003).

In an effort to stimulate interest in the product, he and his wife, Nichola Fletcher, developed a venison burger, the Veniburger™, which could be cooked and sold at agricultural shows and at country fairs. There was insufficient supply of farmed venison to meet demand and it became necessary to purchase wild venison to manufacture Veniburgers. The quality of this purchased venison was very poor with contamination from hair, bone, rifle bullets, and even larvae of the deer warble (*Hypoderma diana*).

This strengthened a resolve to build up the numbers of farmed deer as the difficulties of creating quality venison from wild deer became immediately obvious. This had been one of the key motives behind the experimental deer farm created in 1970 at Glensaugh in Kincardineshire by the Hill Farming Research Organisation and the Rowett Research Institute. These bodies published a progress report on their experimental deer farm in 1974 in which they stated (Blaxter *et al*., 1974):

> ...any meat industry of any scale based on red deer would have little chance of survival if the hygienic standards adopted in handling the carcasses were not impeccable, and similar to those applying to domesticated farm livestock. It did not seem likely that an industry could be based on the stalking and shooting of a managed stock; animals killed long distances from a road, disembowelled where they fell, transported by pony to a collecting point and only later transferred to a meat processing plant could hardly be said to be handled in an hygienic way. For this reason it was decided that if any attempt was to be made to exploit the red deer as a meat animal this exploitation should be based on a partial domestication so that suitable animals could be brought to a central point for slaughter and subsequent processing. An exploitation based on hunting or stalking seemed out of the question.

18.3 Current status in the venison chain in the UK

18.3.1 Deer farming systems

The farming of red deer in Britain by private individuals has followed the patterns developed by the experimental deer farm at Glensaugh. Well-designed handling systems and paddocks allow the deer to be gathered and calves to be weaned in the autumn and either sold to finishing units or housed through the winter. Deer are rotationally grazed on grass paddocks until ready for slaughter when they can be transported to purpose built abattoirs. Forage crops such as turnips or other brassicas are sown for winter feeding. Single sire mating is often employed to permit rapid genetic improvement. The only routine veterinary intervention is usually administration of anthelmintics and, where necessary flukicides, to young stock although copper boluses may also be indicated on some farms where copper status is low.

18.3.2 The procurement of venison from Scottish wild red deer

Since the 1970's the wild venison industry has made huge progress in attempting to prove those authors, Blaxter *et al.* (1974), wrong. Extraction of carcasses is less often by pony and more often by All Terrain Vehicles, movement into refrigerated premises within a few hours of shooting and the introduction of quality assurance for wild venison (Scottish quality wild venison scheme) have all permitted wild venison to enter supermarkets and also to make big inroads into the restaurant trade.

Nevertheless, wild red deer carcasses are inescapably eviscerated where they are shot in the field and dragged across the hill until they can be loaded on to a vehicle or pony. This is probably incompatible with the levels of hygiene that modern food chains require. Because arguably the chief objective of the Scottish stalking industry is to reduce deer numbers so as to protect commercial forestry and to increase botanical biodiversity, deer of all species are shot at all ages. This is further compounded by the secondary objective, namely the sporting interest in trophies which require male deer to be shot only when they are mature.

Consequently, deer entering the game dealers' premises may be of any age and within Britain may be any one of five species varying in live weight from the muntjac (*Muntiacus reevesii*) or Chinese water deer (*Hydropotes inermis*) at 15 kg to the red deer at 150 kg (Fletcher, 2007). It is this variability which makes the marketing of wild venison to the supermarkets very difficult and explains the preference of retailers for farmed red deer venison. This preference is reflected in the price premium which farmed venison carries over the wild product. At the time of writing deer farmers are receiving around £5.40/kg of carcass whilst Scottish producers of wild venison are probably receiving about half that price from the game dealers. Certainly the price paid for wild venison carcasses normally includes the hide but the high costs of collecting small numbers of carcasses from remote Highland glens as well as the higher levels of wastage make it difficult for game dealers to pay prices comparable to those paid by retailers for farmed deer.

Wild venison producers are perilously close to an economic predicament that would make it more cost effective to leave the carcasses of wild deer where they are shot on the hill. This has been accepted by some environmentalists who have emphasised the benefits to carrion eating birds and insects. Such a move would however have deleterious consequences for employment amongst those who work in the Highlands of Scotland and it would be damaging to the culture associated with hunting including the public perception of game meat. It would probably also make it more difficult to achieve the increased cull levels demanded by environmentalists to increase biodiversity.

18.3.3 The regulations controlling wild, farmed and park venison within the UK

During the 1980's and 1990's British deer farmers considered that their product was so different from wild venison that they wished their farmed meat to be treated in the same way as beef, lamb and pig meat. Domestic legislation was drafted to permit farmed deer to be killed in licensed abattoirs although an optional route was provided for those deer farmers who wished to shoot their deer in the field with a rifle. Deer farmers are therefore in the privileged position of being able to kill their free ranging deer with a free bullet or restrain them in their handling yards and use a captive bolt, yet at the same time they can also now opt to send their deer to an abattoir. Whichever route they choose the venison is treated as a red meat with requirements for veterinary *ante mortem* inspection and inspection of carcasses by professional meat inspectors. In addition carcasses are bled within seconds of shooting in conformation with the welfare of animals at time of killing regulations introduced to enforce the requirements of Council Regulation (EC) no. 1099/2009 (EC, 2009) on the protection of animals at the time of killing. These procedures as well as the premises in which farmed venison is handled are under the control of Food Standards Scotland (FSS) or the Food Standards Agency (FSA) in England. By contrast wild deer producers must register as food business operators and their collection facilities are supervised by the local environmental health officers administered by the local authorities; however, unless exempted as direct sales the wild deer carcasses must then be transported to an approved game handling establishment (AGHE) (see below).

There is a further category of deer within Britain recognised as 'park deer'. These are deer bred and killed in deer parks and are defined by the British Deer Farms and Parks Association, as follows:

> Deer raised in a park type setting where they are able to roam freely with minimal input. They may be provided with supplemental feed, calves are unlikely to be weaned, nor antlers removed, stock is not housed and management is generally with a rifle. When contact with deer is minimal, they may be classified as being wild and not farmed and as a consequence the slaughter and carcass handling procedures are those applicable to wild deer.

The criteria that decide whether a group of enclosed deer are wild or farmed have been delegated to Member States by the European Regulation (EC) no. 853/2004 (EC, 2004b) which defines wild game as:

> wild ungulates … that are hunted for human consumption and are considered to be wild under the applicable law in the Member State concerned, including mammals living in enclosed territory under conditions of freedom similar to those of wild game.

In an attempt to interpret this European Regulation the UK FSA in 2013 distinguished wild (park) deer from those on farms using the following criteria:

> The conclusion as to whether animals are wild or farmed should be reached with reference to the conditions and circumstances in which the deer live. Each operation should be judged on its own merits with consideration of local issues including the following:
>
> - There should be sufficient room for the herd to roam naturally.
> - The enclosed area should provide sufficient natural foodstuffs for the herd to survive. However, the provision of additional foodstuffs is acceptable as long as the animals are not fed continuously throughout the year.
> - Numbers of deer should be kept up through natural reproduction from animals within the herd. However, culled deer may be replaced with deer brought in from outside subject to animal health rules.

In November 2015, this definition was revised and FSA (2015) issued the following guidance:

> Some animals, such as deer, may live in an enclosure such as a large estate or park. If they are hunted, the fact that they are enclosed does not prevent them from being classified as wild game. Game animals with sufficient grazing to enable them to live throughout the year without supplemental feeding are also considered to be wild. All these animals must be killed by hunting to be eligible for human consumption. Wild game animals killed in other ways, e.g. by road traffic, cannot be supplied for human consumption.

This guidance in its November 2015 revision is unworkable in its definition of deer parks as it excludes any park which feeds its deer during the winter. In practice, all park managers feed their deer during the winter and if they did not, they could be expected to be prosecuted as compromising the welfare of the animals in their charge. If enforced, the 2015 guidance would class all park deer as farmed and there are around 40,000 deer in over 250 British parks predominantly in England (The Deer Initiative, 2015). Within these parks keepers can generally only kill small numbers in a day so that active culling takes place over many days of the year; *ante mortem* inspection on each occasion would not therefore be feasible without the FSA employing many more veterinarians.

The distinction between parks and farms is extremely important from the game meat hygiene point of view in that farmed venison is classified as 'red meat'. Farmed deer must be subjected to a veterinary *ante mortem* inspection and must be killed in compliance with the welfare at time of killing regulations and processed through an approved farmed game handling establishment subject to the control of the FSA in England or FSS in Scotland. In practice, the November 2015 guidance is not enforced and venison derived from park deer is classified as wild. As such it must be killed only during the hunting season, requires no veterinary *ante mortem* inspection, and can be processed through an AGHE. AGHE are premises which have been approved by FSA or FSS as being in compliance with Regulations (EC) no. 852/2004 and 853/2004 (EC, 2004a,b). Park venison is also, as wild, able to benefit from the same 'hunting' and 'small scale' exemptions as free ranging deer. Wild or park deer carcasses can therefore be sold into an AGHE game dealer but may be previously collected and held in premises whose hygiene controls are enforced by the environmental health officers of the local authority rather than the FSA and FSS.

18.3.4 The development of a market and a route to market for farmed venison

In Britain, where the tradition of eating venison had almost disappeared by the mid-20th century, almost all consumers were unfamiliar with deer meat during the early development of deer farming. Since most wild venison was being exported the chances of the naïve venison consumer having his first experience with farmed venison gradually increased. And since farmed venison was from young deer processed hygienically it was more easily marketed. It was consistent and ideally suited to the modern taste for low fat meat, cooked quickly and eaten 'rare'.

Nichola Fletcher and others worked hard to promote farmed venison by writing books, articles in newspapers, and giving cookery demonstrations on television and at shows (Fletcher, 2007). Gradually the market grew and it is this from which we benefit now. Celebrity chefs followed, and venison became a 'must have' for supermarkets. The price paid for farmed venison rapidly outgrew that of wild venison.

However, deer farming in Britain did not reflect this. Handicapped by agricultural policies which restricted agricultural support within the pastoral industries to cattle and sheep, deer farming did not develop in Britain whereas in New Zealand, where all support was removed in 1984, deer farming grew strongly. Deer slaughter premises (DSPs) were constructed and initially staffed and managed by deer farmers which avoided the necessity for farmed deer to enter the massive and heavily unionised sheep abattoirs. All farmed venison had to be killed within those DSP's. By 2000, New Zealand's national farmed deer herd had reached two million breeding females with exports of farmed venison returning well over one hundred million pounds sterling *per annum*. These venison exports were predominantly to Europe. The promotional investments of the New Zealand deer industry assisted our UK markets and the British Deer Farmers' Association even ran generic promotional campaigns for farmed venison in collaboration with New Zealand in an effort to boost UK sales.

Due to the proximity of markets and consumers many small British deer farms killed their deer on farm and processed and marketed the venison locally. This contrasted with the situation in New Zealand where local markets were almost non-existent compelling deer farmers to export. As a consequence, New Zealand deer farmers were more co-operatively minded and willing to pay levies on the carcasses they sold which created substantial funds for the promotion of farmed venison.

Small British deer farms, especially those wishing to market their own venison continued to kill deer on the farm or occasionally within conventional livestock abattoirs. Shooting farmed deer with a free bullet as they fed in the field in a group was the most common means of killing farmed deer during the early years. It was found that provided the deer were part of a large group and were being fed, the noise of a rifle shot, and the sight of their colleague falling to the ground, did not cause the surviving deer to panic and they would return to feed close to the dead deer lying nearby. If shot in the head using a large calibre rifle at less than fifty metres, there is instantaneous destruction of the brain and immediate death. Several deer can be killed in this way in a few minutes. In order to satisfy the welfare at time of killing regulations each shot needs to be followed within a few seconds by severing the carotid arteries to exsanguinate the deer. This was considered to be the most humane possible system for slaughtering farmed deer and became a significant selling point for many consumers wishing to buy welfare friendly meat.

However, it was soon recognised that an abattoir dedicated to deer and in which deer could be humanely killed was needed if larger deer farms were to be developed. This gap was filled by Round Green Farm in South Yorkshire which during the 1980's created a dedicated abattoir killing only deer. They developed systems which provided the high levels of hygiene and humane handling demanded by the supermarkets and provided the levels of assurance which supermarkets required. An official visit by the Farm Animal Welfare Committee in 2013 could find no fault with the ways in which farmed red deer were processed through this abattoir (FAWC, 2013).

Nevertheless, UK deer farming did not develop significantly in Britain during the 20th century while agricultural subsidies continued to favour cattle and sheep and, by excluding farmed deer, effectively penalise them. This gradually changed as the common agricultural policy moved towards 'decoupling' by which support in the UK was paid from 2005 on either a historical or an area-based basis to all *bona fide* agricultural enterprises regardless of the nature of the agricultural business. Thus a 'level playing field' was created in which deer farming could compete on equal terms with traditional livestock enterprises.

By about 2010 all the principal UK supermarket chains were selling venison, predominantly sourced from farms. In 2015, most of these supermarket chains were reporting annual growth in venison sales of around 25% and were expressing concern as to future supplies.

Retail sales of venison across the whole UK rose from £32 million in 2006 to £43 million in 2009, an increase of over 34%. Figures published in 2012 indicate that that growth continues. Marks & Spencer sold three times as much venison in 2011 as it did in 2010, Sainsbury's reported sales of its 'own brand' venison had almost doubled year on year, and some other retailers reported sales up by 50%. Asda said its sales were up by a third, and in 2012 the Co-op stocked venison for the first time. Waitrose reported an increase in sales of 92% in 2013 Sainsbury reported its venison sales up 115% in December 2015 over the previous year. In summer 2014, analyst Kantar Worldpanel reported an increase in UK venison sales of more than 400% over the previous year (Anonymous, 2016).

In 2015, venison production was listed as one of the 50 most promising commercial sectors (Mintel, 2015):

> Soaring venison sales have put game meat in the spotlight. While the size of the game meat market is dwarfed by that of poultry (with sales of £97 million in 2014 versus £1.7 billion for poultry) the game meat market has enjoyed strong growth in 2014, increasing about 9% from 2013. This rise in sales has been largely thanks to the popularity enjoyed by venison. The fact that many more UK consumers have expressed an interest in trying game than have eaten this type of meat before highlights the significant growth potential in this sector.

> Leveraging the health credentials of game such as venison will help to position it as a better-for-you alternative to red meat, thus boosting sales further.

The Waitrose supermarket chain within the John Lewis group which has been buying British farmed venison since the 1980's is in the forefront of this sector and has established a very active producer group of deer farmers under the name *First Venison*.

In Scotland, Richard Lochhead, cabinet secretary for rural affairs and the environment, aware of the health benefits of venison in comparison to conventional meats said in May 2013:

> We need to increase our venison production by a third to keep pace with demand. Our venison is another high quality and delicious product that is increasingly desired by customers at home and abroad.

18.3.5 The profitability of UK deer farms

In Britain figures for venison production on deer farms show very positively against other pastoral enterprises (Table 18.1). Gross margin figures for a hundred red deer hind unit carrying the progeny to finishing are estimated at £17,470.00 in comparison to a sheep farm carrying 200 ewes at £9,250.00 and beef production at around £242.00/cow. Gross margin figures make no allowance for labour but the sheep and cattle enterprises will have a very much higher labour requirement than the deer. However, by contrast the deer farm will have higher capital costs in terms of fencing and construction of handling systems. Nevertheless, despite its proven profitability UK farmed venison remains a small source of venison in comparison to wild venison production.

18.3.6 The competitor: New Zealand deer farming

Meanwhile in New Zealand venison production has been falling for several years. Virtually all their venison is produced on farms and the remarkable growth in the dairy industry producing milk solids for the Chinese market caused the sale of many deer farms. This has now reversed following a rapid decline in dairy income during 2015 and deer farming is probably at present the most profitable agricultural sector in New Zealand. This remains subject to currency exchange rate fluctuations and the decline in the strength of the Euro (€) creates pressure for New Zealand to develop new markets outside Europe. More than 80% of NZ venison production is now licensed for export into the Chinese market although few sales have yet been made (Deer Industry New Zealand: http://tinyurl.com/h8l25v7).

The success of the New Zealand deer farming industry is often attributed to sales of growing antler, 'velvet antler', which is sold to China and Korea as a valued constituent in the traditional Chinese medicine trade. The amputation of the growing antler which is necessary to provide this product is illegal in most if not all European member states as being deemed the product of an inhumane mutilation, thus placing European deer farmers at an economic disadvantage. Nevertheless it seems unlikely that the European public would accept the amputation of live antlers to supply a Chinese market for a product whose efficacy is largely unproven.

Table 18.1. Financial data for venison production from farmed red deer produced by Venison Advisory Services (www.venisonadvisory.co.uk).[1,2]

	Total (£)
Potential output from established 100 red deer hinds (breeding and finishing)	
100 hinds @ 90% calving yield 45 finished hinds less 8 retained as replacements = 37 of which 26 (70%) sold as breeding @ £350/hind	9,100.00
Balance of hinds (10) as venison (45 kg DCW @ £5/kg = £225/hd	2,250.00
Cull hinds (8 @ 55 kg DCW @ £3.80/kg)	1,672.00
Stags for venison (45 @ 53 kg DCW @ £5/kg)	11,925.00
Total sales	24,947.00
Costs per 100 hinds (red deer breeding and finishing)	
Feed – hinds (silage 10.5 ME/6 kg/hd/165 days, 990 kg/hd @ £26/t)	2,573.00
Feed – hinds (concentrate 0.5 kg/hd/90 days), 45 kg/hd @ £240t	1,080.00
Feed – calves 80 (silage 10.5 ME/3.5 kg hd/180 days) 630 kg @ £26t plus concentrate (0.5 kg/hd/180 days) @ £220t	2,894.00
Feed – stags (4 breeding and 2 replacements) £30/hd	180.00
Fence maintenance	500.00
Tags, drench, sundries £2.50/hd	250.00
Total costs	7,477.00
Gross margin for 100 hinds	17,470.00

[1] All UK deer farms are now eligible for farm subsidies but these are not included in this calculation. Some supermarket buyers are offering significantly more that £5.00/kg dressed carcass weight (DCW) but no account has been taken of costs of transport to abattoir and therefore the lower figure of £5.00/kg has been used. No suggested stocking density figures are given as this depends on the quality of ground but as a rough indication red deer hinds will need about twice the acreage of ewes. All feed costs are based on purchased prices.

[2] Compare: gross margin for 200 lowland ewes 2013/2014: £9,250.00 (Aberystwyth University, 2014); gross margin for lowland suckler beef (average): £242.00/cow (Quality Meat Scotland, 2013).

The amputation of growing antlers is closely controlled in New Zealand with those wishing to carry out the procedure trained and certified. Local anaesthetic is used as a ring block around the coronet and the cut antler is treated hygienically as a food. However over the last decade sales of antler velvet have only contributed around 10% of net receipts to the New Zealand deer industry which is based firmly on venison production (Deer Industry New Zealand: http://tinyurl.com/h8l25v7).

Despite the encouraging growth of the venison market favouring British and Scottish deer farmers, progress in the development of deer farming remains slow. Scotland produces an estimated 3,500 tonnes of venison *per annum*, of which just 50 tonnes come from farmed deer. In addition the production of wild venison in Scotland is stable or declining over the long term (Figure 18.1). Since 2013 cull figures have increased slightly but this is believed to reflect efforts to reduce the population and are unlikely to be sustainable. Consequently, to meet growing demand, imports from New Zealand, Poland, Spain and elsewhere in Europe

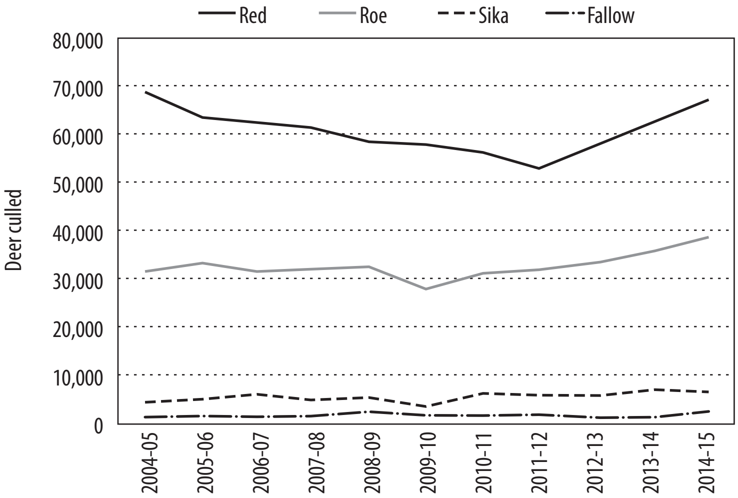

Figure 18.1. Annual cull figures from Scottish wild populations (data presented by Scottish Natural Heritage: http://tinyurl.com/gtmyree).

are increasing and are sustaining a year-round market. Scotland's two major game dealers reported that they imported 25,000 carcasses (over 1000 tonnes of venison) in 2012 to meet market demand, and in 2013 the UK imported the equivalent of 29,000 carcasses from New Zealand alone.

The Scottish Venison Partnership aims to encourage increased production of Scottish farmed venison and reduce the UK's reliance on imports. That group considers that an additional 1,200 tonnes of venison *per annum* is required to keep step with demand.

18.4 Ensuring food safety: venison hygiene control

In Britain red deer are almost unique in that the same species exists both in the wild and also as farmed deer. The ways in which venison from the two classes of deer are processed are very different.

18.4.1 Venison from wild deer

A recent outbreak of *Escherichia coli* O157 arising from Scottish wild venison has brought the issue of wild venison hygiene to the forefront although the presence of verocytotoxin producing *E. coli* O157 has been recognised in deer for many years: in wild white-tailed deer in North America and in farmed red deer in Britain since 1997 (Chapman and Ackroyd, 1997). A further unpublished description of the recovery of four positive samples from 15 wild red deer faecal samples collected at one remote Scottish site, including one high shedding individual with 7.5×10^4 cfu/g, was made by Synge (2006). However Synge examined 740 more samples from Scottish deer without finding any positives and concluded that infection rates in wild deer in Scotland were low (Synge, 2006).

In Britain all wild deer are killed by rifle. This entails a careful, concealed approach to the animal taking account of the direction of wind to avoid disturbing the deer. When sufficiently close and depending on the light and weather, the stalker will take a shot. The preferred area of impact is the thorax ideally striking the heart but if the stalker is confident and able to approach closely enough a neck shot may be taken which causes less damage to the carcass.

The marksman may be a professional stalker employed by the landowner to kill deer to meet cull targets or they may be an amateur shooting deer as a recreation. Within Britain as in many other developed countries deer numbers are often increasing and there may be difficulties achieving cull targets designed to minimise damage to agricultural and forestry crops, the environment or even injuries to people arising from collisions between deer and cars. There is some evidence that the numbers of stalkers (hunters) may be falling and if this becomes a well-established trend then it may become necessary for government, whether national or local, to recruit deer hunters.

Once the stalker has approached the deer and confirmed that it is not conscious it is exsanguinated and a ventral incision is made with the deer lying on its back. The oesophagus is cut and knotted and the rectum cut and the abdominal viscera removed. This is essential for the larger species, the red and fallow deer, so as to lighten the carcass to enable it to be handled and also to assist in cooling it. Extraction of red deer carcasses from remote Scottish Highland deer forests depends on rapid evisceration to reduce the weight.

Once eviscerated the removal of the carcass to the larder begins. On steep remote Scottish hillsides it may not be feasible to reach the carcass even with a four wheel drive all-terrain vehicle or a pony. In those circumstances the carcass will need to be dragged downhill to a more accessible point where it may be loaded on to a vehicle or pony.

Dragging of an open carcass makes contamination almost inevitable. The use of ‘drag bags’ into which the deer can be placed after evisceration, is costly, and few materials can withstand the impact of dragging a carcass that might weigh 80 kg over rocky terrain.

The carcass is then taken to a game larder where the sternum is split, the lungs, heart and liver – ‘the pluck’ – are removed and the head and feet taken off. The carcass is then left until it can be collected and removed to an AGHE.

For the smaller species including roe deer which are often killed close to the game larder it may be considered feasible to take the carcass, viscera intact, to the game larder. For the smaller species a drag bag becomes a more realistic proposition where the location demands evisceration in the field.

In some circumstances, by use of the ‘hunter exemption’ or the ‘small volume exemption’, the carcass may be sold or given directly to the consumer. This is most likely for the smaller species but also occurs commonly with red and fallow deer. In that case the carcass is skinned and may even be butchered by the stalker and the venison distributed with no hygiene controls. In addition to the venison passing through legitimate exemptions sanctioned by EU regulations an unknown quantity of wild venison is shot illegally.

It can be seen from the above that hygiene control of venison from wild deer is entirely dependent on how competent and conscientious the stalker is. Operating in extremely difficult conditions of cold, wet and windy weather and often at the close of a physically demanding day, the work can be challenging and short day length during the autumn and winter mean that many carcasses will be recovered in poor light. Preparation and publication of best practice guides were initiated by the Deer Commission for Scotland (DCS) and published from 2008. A series of best practice days further encouraged stalkers to process deer carcasses to a high standard. DCS was incorporated into Scottish Natural Heritage in 2010 and funding for these training days became unavailable.

In the wake of the *E. coli* O157 outbreak in wild venison in 2015 efforts are being made to reinforce the Best Practice guidance and encourage stalkers to adopt these measures. Where wild deer producers are registered as quality assured under the scottish quality wild venison scheme compliance can be monitored but where stalkers are operating on a small scale or as recreational hunters it is virtually impossible to control levels of hygiene.

Ante mortem inspection of wild deer other than by the trained hunter is obviously impractical as also is the examination of the viscera. In addition, delays in transporting carcasses of wild deer from remote regions to AGHE are inevitable.

18.4.2 Venison from farmed deer

The hygiene problems associated with wild venison are minimised when deer are farmed. Conversely, however, it is generally assumed that species which are domesticated are more likely to carry pathogens that are more virulent, more novel and more communicable. This was discussed by e.g. Vågsholm (2014). Briefly, Vågsholm suggests that the increased risk factors include the likelihood of higher population densities in a farm situation, possible higher stress levels in newly domesticated species, controlled breeding programmes increasing risks of vertical transmission of diseases, and the increased risk of zoonoses due to close contact between humans and domesticated animals.

As consumption of farmed venison increases, more farms are developed, and farmed deer become genetically distinct from the wild animal, some of these risks may diminish. Farmed deer may become much less susceptible to stress. And, in any case it could be argued, that for example, wild red deer in Scotland are frequently subject to nutritional stresses leading to high mortality so that in some respects farmed deer are likely to be less highly stressed.

By passing farmed deer through dedicated abattoirs where inspectors can develop extensive experience of the normal in both live and dead animals, the risks of disease escaping detection must be reduced.

Farmed game including farmed venison must receive *ante mortem* inspection by a veterinarian within 72 hours of the deer being killed. For those deer farms which kill their deer by free bullet then the FSA considers that *ante mortem* inspection is best conducted without isolating the animals. This permits the deer to behave in an undisturbed manner and is thus considered to be most likely to reveal abnormal behaviour indicative of disease. If isolated then deer will behave abnormally and disguise signs of disease.

18.5 The composition of venison

When considering the risks posed to modern human populations by food products, infectious agents are important and together with contamination comprise the risks which are generally assumed to fall within food safety and hygiene. However, food safety in developed societies should also encompass a consideration of nutrition. In such societies very many more people will experience early deaths as a result of consuming excess sugar or fats than through ingesting pathogens from contaminated meat.

An analysis of game meat, even farmed game meat, shows that it has a composition hugely more favourable than conventional beef, lamb, pig or even poultry meat. For example, game meats have about twice the iron of red meats from domestic livestock (Chan *et al.*, 1995). In Britain more than 5% of children aged 1.5-2.5 years, women aged 15-18 years and 35-49 years and men aged more than 65 have iron deficiency anaemia (Scientific Advisory Committee on Nutrition, 2010). In addition, game meats including red deer venison have been shown to have low fat content and high poly unsaturated fatty acids compared to conventional livestock (Hoffman and Wiklund, 2006).

Indeed, the composition of game meat is so close to the dietary requirements of humans that many scientists have considered that the human physiology is adapted to the game meat which we have been eating for millennia and has not yet evolved to meet the challenges of eating livestock to which we have only been exposed for a few centuries. This view has been held for many decades (e.g. Crawford and Marsh, 1989).

In considering food quality, regulatory bodies in each nation must consider not only hygiene but also the nutritional quality. In procuring wild game, meat hygiene may not always reach the levels expected of abattoir killed livestock but their composition may constitute higher nutritional quality. Farmed deer should be able to achieve the highest levels of both measures.

18.6 Conclusions

18.6.1 What has been achieved?

The development of new red deer farms in Scotland together with associated abattoirs and routes to market is still in its infancy but already systems have been established which make red deer farming more profitable than conventional agriculture. Markets have been created which show strong and growing demand sustained over many years for a game meat that is nutritionally of the very highest standards. Veterinary controls of farmed venison are in place and appear cost effective.

18.6.2 What has been neglected?

Less encouraging are the controls for wild venison the enforcement of which is extremely challenging due to the nature of hunting wild deer in difficult terrain and recovering the carcasses. Nevertheless, there have been very few cases of contamination leading to clinical disease.

18.6.3 What needs to be done?

In the future there is a need for many more deer farms to displace imported venison. Systems need to be put in place to do everything possible to improve the hygiene of wild venison. Refinements of farming systems have to ensure that deer farming improves productivity and the welfare of the deer. Finally, continuing public education on the healthy composition of venison and the means of cooking it will be needed in order to assure that the demand from the consumers for venison will not decline in future.

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19. Fatty acid composition of game meat: implications for human health and variability between free-ranging and farmed game

Teresa G. Valencak[1], Sarah A. Ohrnberger[1], Katharina Brugger[2] and Matthias Schreiner[3]*
[1]Institute of Physiology, Pathophysiology and Biophysics, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria; teresa.valencak@vetmeduni.ac.at
[2]Institute for Veterinary Public Health, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria
[3]Institute of Food Science, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria

Summary

Whilst game meat increasingly gains attractiveness among consumers for its healthy nutrient composition, it cannot be obtained as easily from the supermarket as other meat products. In countries like the US, it is even illegal to harvest wild game meat for commercial use. Thus, game meat purchased from specialty suppliers in the USA originates from a 'ranch' or a farm where the animals are raised in free-range conditions. Like its feral counterpart, farmed game meat has been reported to contain little fat. Yet, it needs to be determined whether or not the ratio omega-6:omega-3 fatty acids is comparable. Precursor omega-6 and omega-3 polyunsaturated fatty acids have to be ingested through the diet and can subsequently be elongated enzymatically in the mammalian body. According to dietary recommendations, human uptake of omega-6 to omega-3 fatty acids should not exceed 5:1 but in western societies this ratio is generally higher. We assessed total and phospholipid fatty acid composition from free-ranging game in Austria as well as from 5 local farms in lower Austria producing meat from red (*Cervus elaphus*) and sika deer (*Cervus nippon*). Interestingly, we found that fatty acid composition was well comparable and not significantly different between free-ranging and farmed game. Species-specific profiles in the content of monounsaturated fatty acids were assessed for *Cervus dama*, which contained ca. 5% more (15%) monounsaturated fatty acids than did red deer (10%). In all samples, the ratio between omega-6 and omega-3 fatty acids was lower than 5:1. This suggests that, independent of rearing regimen, fatty acid composition of game meat is beneficial from a human nutrition point of view. A balanced diet in omega-6 and omega-3 fatty acids reportedly: (1) lowers the risk of contracting coronary heart disease; (2) improves eyesight and memory; and (3) even helps to reduce inflammatory processes in the body.

Keywords: human nutrition, farmed game, fatty acids, n-3 polyunsaturated fatty acids, n-3 and n-6 fatty acid balance

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19.1 Introduction

As hunters and gatherers, humans did not interfere with the cycle of nature. Rather, their lives were dependent on the availability of both plant and animal food sources so they did not impact on the ecological balance on a large scale. However, with the first forest clearances in order to cultivate plants, humans began farming and agriculture was born. These events took place ca. 12,000 years ago. Over the centuries, this endeavour was most labour intensive. Crop rotation, the use of mineral fertilisers and the increase in production due to mechanisation changed agriculture as one knows it today, but these developments had a very profound impact on livestock farming and human meat consumption. While reportedly in Austria the annual meat consumption has tripled from 1950 to 2010 (Willersdorfer, 2013), the current intake of game meat is as low as 0.7 kg per person and year (Anonymous, 2015) and does not seem to change much over the years, in spite of game meat having an excellent reputation in terms of nutritive value. Previously, we already emphasised the favourable fatty acid composition in game meat total lipids (TL) and phospholipids (PL) (Valencak and Gamsjäger, 2014; Valencak *et al.*, 2006). While the composition of TL refers to all lipids present in the tissue (triglycerides, free fatty acids and PL), the fatty acid content of the membrane constituents i.e. the polar PL refers to the species-specific and biologically relevant proportion of the different lipid classes. TL fatty acid composition is largely influenced by factors such as individual age and constitution as well as diet. We therefore suggest that – in identifying the nutritive value of game meat samples for humans – both types of profiles need to be considered.

Apart from its importance as a healthy food, game meat is also known for its excellent taste and is considered a delicacy. The game meat recipes in Austria's biggest hunting magazine (Österreichs Weidwerk) are among the most accessed and sought after articles of the magazine. Even people who are vegan or strictly vegetarian agree that game meat indeed may represent an ethical meat source today (Winkelmayer, 2014).

This contribution aims at assessing fatty acid profiles of farmed game meat originating from the three common species red, sika and fallow deer and at determining whether or not a particular profile is possibly associated with housing conditions and/or feed preferences of these three species.

19.2 Fatty acid chemistry

Fats are chemically defined as acylglyerols, i.e. compounds in which carbohydrate chains containing an acyl group are linked to a glycerol molecule by an ester bond. Fat tissue (in terms of visible fat layers) are triacylglycerols containing 3 fatty acid molecules bound to a single glycerol backbone. According to their saturation degree, fatty acids can be assigned to three major categories: (1) saturated fatty acids (SFA); (2) monounsaturated fatty acids (MUFA); and (3) polyunsaturated fatty acids (PUFA). The third category, the PUFAs get most attention from nutritionists as they occur in 2 families, the n-3 and the n-6 (or synonymous omega-3 and omega-6). PUFA are essential to mammals and need to be obtained from the diet. The sources for n-3 and n-6 PUFAs are very different: while n-3 PUFAs are found in fish, sea food and the green parts of plants, n-6 PUFAs dominate everyday industrial vegetable oils (sunflower oil, peanut oil, soy oils, etc.). Recent evidence shows that MUFAs and some PUFAs are health-promoting 'good fats' while most SFAs and the so-called trans fatty acids (Cordain *et al.*, 2005; Smit *et al.*, 2010) are detrimental to human health and their nutritional uptake should be avoided or at least kept at low levels (Cordain *et al.*, 2005). Thus, for a healthy diet it is more important to select the type of fat than to avoid fat in general. Our western diet is characterised by large amounts of SFA and trans fatty acids while it contains too few n-3 PUFAs in relation to n-6. It is well-known that, n-6 and n-3 PUFAs should be ingested in a defined relationship of about 5:1 (Kris-Etherton *et al.*, 2001). Yet, the relationship between ingested PUFAs in daily meals in industrials countries is mostly 20:1 (Kris-Etherton *et al.*, 2001). Not surprisingly, many authors have suggested that very common nutritional disorders such as overweight and obesity may have their cause in long-term malnutrition with the 'wrong' fats. Proponents of the so-called 'Palaeo diet' are bringing forward the idea that mimicking the Neolithic food rich in fats and meat (a diet that may have been approved by 'natural selection and evolution') may help reducing the negative consequences of today's refined food products such as refined sugars, vegetable oils and cereals (Cordain *et al.*, 2005). It has been suggested that not only do people ingest too many PUFAs in general but also that the 'wrong' n-6 PUFAs are consumed. Apart from impaired eyesight and memory, an unbalanced n-3 to n-6 ratio may give rise to cardiovascular disease, overweight and obesity and even some sorts of cancer (Kris-Etherton *et al.*, 2001; Ruxton *et al.*, 2007). The n-3 or omega-3 PUFA in tissues are the 'good' fats as they have an anti-inflammatory and cardioprotective function (Ruxton *et al.*, 2007). In contrast, n-6 PUFA originating from C 18:2 n-6 (α-linoleic acid) and most abundant in our western diet in vegetable oils (Cordain *et al.*, 2005) have very negative properties such as triggering inflammatory processes. Populations relying on fish as the major part of their diet, e.g. those inhabiting the Mediterranean area, are known for a better healthspan throughout their lives than those living in an area characterised by less intake of n-3 PUFAs. Game meat represents a high-value nutrient in terms of healthy and balanced fat supply, which may even compensate a diet with little n-3 PUFAs.

19.3 Regulation of game meat fatty acid composition

Game meats in general and certain lagomorph and ungulate species in particular represent a very healthy food source for humans (Cordain *et al.*, 2005; Valencak and Gamsjäger, 2014; Valencak *et al.*, 2015). Meat from European hare (*Lepus europaeus*) may even contain more than 20% n-3 PUFA in their membrane constituents, i.e. PL (Valencak and Gamsjäger, 2014; Valencak *et al.*, 2003, 2006). The fatty acid composition of game meat is highly dependent on the species. While ungulates such as red deer and roe deer possess very high contents of n-3 PUFAs (>10%) and a beneficial n-3 to n-6 ratio for human health, wild boar is among the species that are less advantageous from a nutritional viewpoint (Valencak *et al.*, 2015). The data from ungulates were somewhat surprising in the first place as the rumen digestive system clearly gives rise to biohydrogenation, i.e. both the shortening of chain lengths of PUFAs and also reducing the number of double bonds through microbial processes by turning PUFAs into SFAs. Therefore, less PUFAs may be available to ungulates than to mammals with a different digestive system. Wild fowl has a similarly diverse, species-specific fatty acid composition (Valencak and Gamsjäger, 2014) which may be reflected in different demands of certain wild fowl for special berries and fruit. From the view point of comparative biology, however, (even given the described differences in fatty acids composition) membranes are built up from SFAs, MUFAs and PUFAs in a straightforward manner: while the content of SFAs is relatively constant, the exact proportion of MUFAs and PUFAs may vary between species. That raises the question why wild animals have over 60% PUFAs in their membrane PL (Valencak *et al*., 2006). Three hypotheses come to mind (Figure 19.1):

1. Homeoviscous adaptation (Cossins and Prosser, 1978; Cossins *et al*., 1977). This hypothesis suggests that long-chained PUFAs may act in a similar manner as anti-freezing compounds in the tissues and therefore may lower the melting points of fats in the tissues when exposed to cold. In support of this, gradients in tissue fatty acid composition with more PUFA in the more peripheral body parts were found in a variety of species including European hares (Valencak *et al*., 2003) and Svalbard reindeer (Irving *et al*., 1957). Also from whale blubber, differentially saturated or polyunsaturated parts were reported that were attributed to cold adaptation (Budge *et al*., 2008). Conceivably, the function of PUFAs as anti-freezing compounds is more evident for the structural lipids (i.e. the PL in the membranes) than for energy lipids such as triacylglycerides.
2. Membrane pacemaker hypothesis (Hulbert, 2007; see Valencak and Ruf, 2007). Smaller mammals such as rodents possess membranes with more n-3 PUFA whereas larger mammals such as deer both contain less TL and tissue PL. Hence, certain long chain n-3 PUFA may trigger metabolism in small mammals while the more abundant n-6 PUFAs may restrict metabolic rate in mammals with bigger masses.
3. Closely connected to the pacemaker function of certain fatty acid molecules is a third hypothesis that relates to muscle function (reviewed in Arnold *et al*., 2015). It starts with the observation that the highest values of C 22:6 n-3 (i.e. docosahexanoic acid) have been reported for the rattlesnake shaker and the hummingbird pectoralis muscle (Infante *et al*., 2001) amounting to over 30% of this long-chained and highly unsaturated fatty acid. Later, Valencak *et al.* (2003) and Ruf *et al.* (2006) suggested that certain PUFAs may impact on muscle physiology by triggering membrane-bound enzymes that regulate calcium homeostasis and muscle contraction. Thereby, rate-limiting processes such as the calcium uptake into the sarcoplasmatic reticulum in fast running mammals may drive maximum running speed in mammals (Ruf *et al*., 2006).

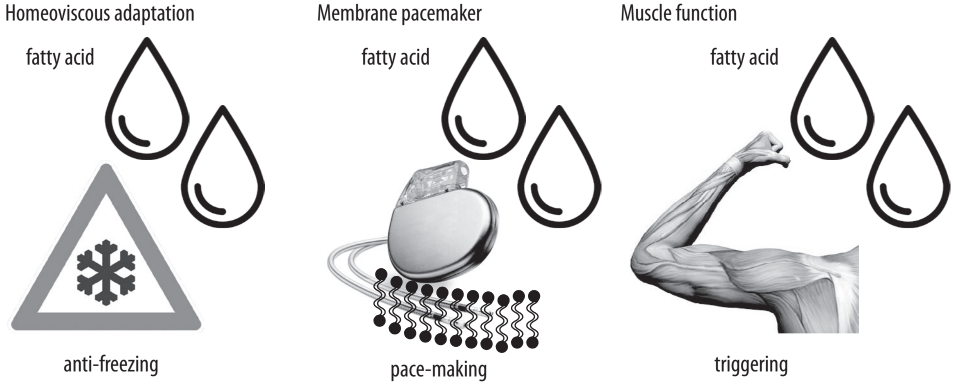

Figure 19.1. Relevance of fatty acid composition in wild animals for body and cell functions.

Please note that while it is very common in literature to assume that fatty acid composition in mammals is mainly a result of their diet, we observed in several studies that the prime determinant of an individual's tissue composition is its phylogenetic origin and we have suggested previously that in order to compare membrane lipid profiles one has to account both for body mass and phylogeny of the species (Valencak and Ruf, 2007). That said, sika deer and fallow deer may have a species-specific membrane composition while it is still possible to influence a deer's membrane composition through dietary supplementation. The effect size of this dietary intervention is estimated to be in the range of 5-8%, species-specific differences representing up to 20% (Valencak and Ruf, 2007).

19.4 Farmed game in Austria

Although criticised by animal rights groups and also by several other parts of the Austrian population, game farming does provide an emerging opportunity for diversification by Austrian farmers. In our alpine region, modern and sustainable game farming may represent a profitable animal production enterprise with a small but dedicated market. While most of the demand comes from gastronomy and gourmet-type customers, direct marketing from the farmyard also drives the attractiveness of farmed game.

Three species are mainly used for game farming in Austria: fallow deer (*Cervus dama*), sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*). The semi-domesticated, outside rearing mode all the year round together with the near-natural conditions result in a high-quality nutrient that meets high ethical standards too. Yet, an often overlooked aspect of game farming is the obvious trade-off between the animal density in the enclosure and the availability and uptake of natural pasture that is clearly affecting meat quality. Before we started our study on fatty acid profiles in farmed game in lower Austria, we set out to identify those farms that feed the animals few or even no commercially available pelleted food but rather have herds that can pick up their food themselves as free-ranging deer albeit within an enclosure. We did this to keep the influence of feeding as low as possible so membrane composition of the farmed game could be compared with that of free-ranging game.

The following main aspects may differ between farmed game and game meat from free-ranging individuals:

1. Weather and climate. Free-ranging deer species may be exposed to harsh climatic conditions more than farmed deer. Whilst their wild conspecifics might be exposed to harsh winter frosts, wet spring conditions and very arid and dry summers in lower Austria, farmed game individuals may benefit from wooden sheds or similar shelters. Potentially, they may be more protected and able to conserve energy and fat reserves under given constraints. Unfortunately, this environmental, (climatic) effect on meat quality has not yet been addressed systematically.
2. Nutrition and diet. Depending on the actual location of the enclosure (e.g. in a more alpine region, in the forest) in lower Austria, farmed game animals of the three species are exposed to different amounts and qualities of food. Also, the population size within the enclosure drives food availability as does the capability of the individuals to access nutrients.

3. Pathogens and disease. Due to space limitations and the effects of population size, pathogens and disease may spread easier within enclosures for farmed game. On the other hand, due to regular monitoring of the herds by veterinarians this difference may be absent or missing.
4. Genetic pollution. Game farming is restricted to native species only due to the potential risk to the genetic integrity of wildlife posed by the possibility of escape of game farm animals (Bunnage and Church, 1991). Yet, if indeed selection within the enclosure may make the animals maladapted to the natural habitat, this is not too relevant as it is very unlikely that farmed animals successfully escape and are not eliminated from the wild environment.

19.5 Methods applied when analysing fatty acid composition from 5 different enclosures in lower Austria

With all these differences between farmed and free-ranging game in mind, we set out to compare fatty acid composition from red, fallow and sika deer from 5 different enclosures in lower Austria and to compare their nutritive value for the consumer. We collected a sample of ca. 0.5 g of the Musculus vastus from all animals that were from both gender and aged between 15-30 months. Within the first 30 minutes after the death of the animal, samples were taken and immediately frozen at -20 °C until transported (on ice) to the laboratory where lipids were extracted and prepared for analysis. From each sample we obtained information on age, gender and body mass of the individual and the size of the enclosure. Also, information on the quantity and quality of the food provided to the animals was collected and we always chose those enclosures within the lowest food supply so as to avoid any potential bias due to feeding. Lipid extraction and separation into lipid classes was done according to Valencak *et al.* (2015). Briefly, muscle samples were homogenised and lipids were extracted by using chloroform and methanol (2:1, v/v), then either directly transesterified (by heating for 30 minutes at 100 °C in acidic atmosphere (H_2SO_4 in methanol) as for TL or separated on silica gel thin layer chromatography plates before being made visible under ultraviolet light with the polar, i.e. phospholipid fraction (PL) isolated. Fatty acid methyl esters of a set of 39 fatty acids were identified by using a gas chromatograph equipped with a flame ionization detector and a Rtx-225 column (crossbond, 50% cyanopropylmethyl 50% phenylmethyl polysiloxane; Restek, Bellefonte, PA, USA). Chromatograms were analysed by Chrom-Card (Brechbühler AG, Switzerland). After completion of the biochemical analysis, all data were statistically assessed using R (https://www.r-project.org). First we identified the 10 most influential fatty acids in both TL and PL on the basis of their abundance in the tissues (more than 5%) and by using the graphical approach with a heatmap (Figure 19.2).

We excluded the proportions of 6 determined saturated fatty acids, 6 MUFA, four dienoic fatty acids, three trienoic fatty acids and one tetraenoic, one pentaenoic and finally docosahexaenoic acid from our statistical analyses as the mentioned fatty acids each amounted to less than 5% in the sample and were therefore considered as nutritiously less relevant for our contribution. We used analyses of variance (ANOVA) for identifying significant differences between the species and we accounted for the influence of individual body mass on the proportion of respective fatty acid classes by adding masses into all models presented below.

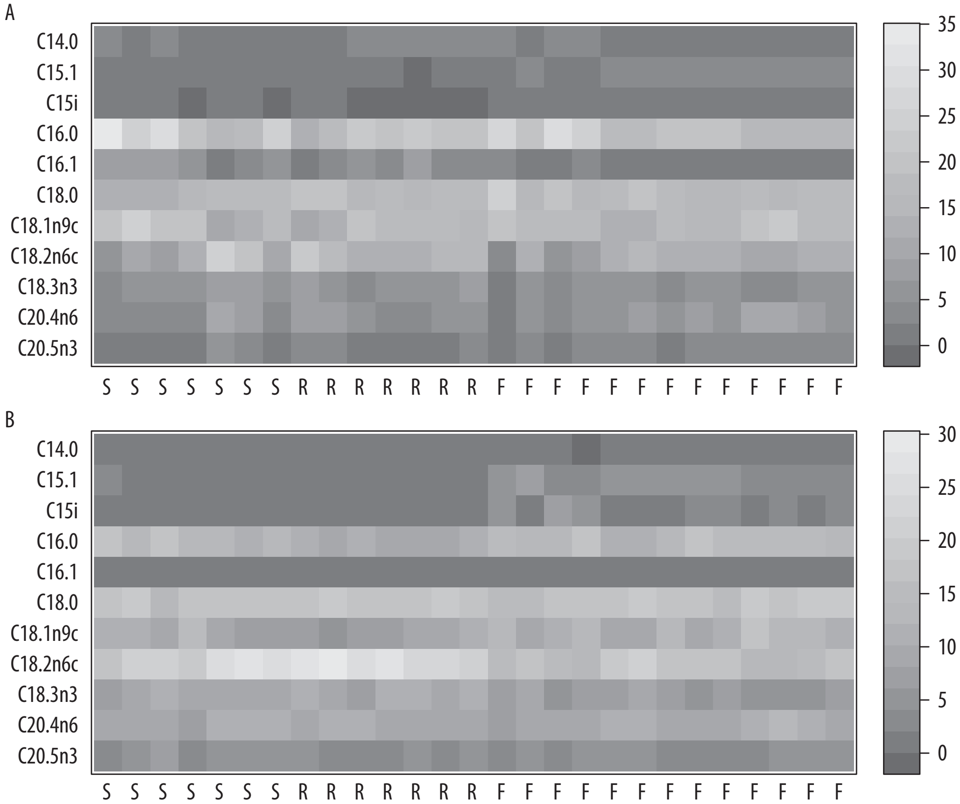

Figure 19.2. Heat map of the 11 most abundant fatty acids in Musculus vastus tissues of sika (S), red (R) and fallow (F) deer in (A) total lipids and (B) phospholipids.

19.6 Results in phospholipids and total lipids

We found significant differences between the three species (Table 19.1). Both single fatty acids and fatty acid classes that were grouped on the basis of their saturation degree or the location of their first double bond varied between PL and TL (Table 19.1).

The summed proportion of SFA was largest in TLs of sika deer where it amounted to 40.37±9.12% and did not differ from the closely related species ($F_{2,23}$=0.08, *P*=0.92). Similarly, the other fatty acid classes such as MUFA and PUFA were not significantly different between the three species in the TL (MUFA: $F_{2,23}$=0.6, *P*=0.56; PUFA: $F_{2,23}$=0.06, *P*=0.9; Table 19.1). From a nutritionist´s viewpoint, the very important n-3 and n-6 PUFAs were in an excellent ratio of n-3:n-6 of 1:2 to each other in all examined TL tissues (Table 19.1). Again, there was no significant difference between the species (n-3: $F_{2,23}$=0.54, *P*=0.6; n-6: $F_{2,23}$=0.09, *P*=0.91).

In the PLs, the amount of SFA was largest in fallow deer, showed fewer variation between individuals (31.98±2.86) and was similar between the three species ($F_{2,23}$=1.4, *P*=0.28).

Table 19.1. Fatty acid composition of polar, i.e. phospholipids and total lipids from fallow, red and sika deer; values are given in weight % with means ± standard deviation.[1]

	Phospholipids			Total lipids		
	Fallow	Red	Sika	Fallow	Red	Sika
Sample size	13	7	7	13	7	7
Body mass (kg)	28.29±3.7	77.29±10.7	37.79±3.6	28.29±3.7	77.29±10.7	37.79±3.6
C 14:0	0.21±0.11	0.25±0.05	0.26±0.11	1.94±1.02	2.86±0.96	1.91±0.83
C 15:1	4.13±1.07	0.65±0.15	0.81±0.78	3.04±1.03	0.34±0.15	0.43±0.38
C 15i	2.49±0.41	0.1±0.01	0.22±0.12	0.26±0.17	0.09±0.07	0.13±0.08
C 16:0	14.37±1.6	10.07±1.1	13.97±2.2	19.82±4.87	18.83±2.77	23.4±6.34
C 16:1	0.9±0.2	0.74±0.2	0.74±0.3	1.85±0.44	4.38±1.67	5.93±3.16
C 18:0	17.4±1.2	18.03±0.8	16.35±1.5	17.54±2.39	17.51±1.86	15.06±1.95
C 18:1 n9c	11.6±2.3	8.2±1.8	10.3±3.1	17.20±2.45	16.04±3.38	17.6±4.5
C 18:2 n6c	16.39±2.6	25.14±2.2	22.63±3.7	11.3±3.38	15.86±3.92	13.61±6.12
C 18:3 n3	6.74±1.3	9.97±1.5	9.08±1.4	4.88±1.34	6.77±1.49	5.63±1.5
C 20:4 n6	9.86±1.5	9.85±1.2	9.59±1.9	6.61±2.43	5.59±1.49	5.05±2.69
C 20:5 n3	4.16±0.4	3.97±0.5	4.88±0.99	2.86±0.82	2.37±0.73	2.6±1.26
C 22:5 n3	5.97±0.65	4.04±0.4	4.52±0.9	4.32±1.22	2.57±0.34	2.45±0.87
SFA	31.98±2.86	28.35±2.01	30.58±3.82	39.31±8.28	30.2±5.59	40.37±9.12
MUFA	16.62±3.49	9.58±2.18	11.82±4.2	22.09±3.92	20.76±5.2	23.97±8.05
PUFA	43.12±6.4	52.97±5.81	50.71±8.77	29.98±9.18	33.16±7.97	29.33±12.43
n-3	16.87±2.35	17.98±2.39	18.49±3.24	12.06±3.38	11.71±2.56	10.68±3.63
n-6	26.26±4.05	34.99±3.42	32.22±5.53	17.92±5.8	21.45±5.41	18.65±8.8
n-9	11.6±2.25	8.19±1.79	10.28±3.09	17.20±2.45	16.04±3.38	17.6±4.5

[1] MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

Interestingly, in the PL's we found a species specific compositional difference between fallow, red and sika deer with fallow and sika deer showing higher amounts of MUFAs in their PL's ($F_{2,23}=8.9$, $P=0.001$; Table 19.1). In both the MUFA and the PUFA class there was a significant impact of individual body mass on the respective fatty acid proportion (e.g. PUFA $F_{2,23}=26.7$, $P<0.0001$).

The amount of PUFAs varied accordingly between these species ($F_{2,23}=5.7$, $P=0.009$; Table 19.1). Finally, the amount of n-6 PUFAs was significantly different between the species and highest in red deer ($F_{2,23}=4.76$, $P=0.018$; Table 19.1). Interestingly, the proportions of n-6 PUFAs in muscle PLs were also influenced by individual body mass with the heavier animals having the higher n-6 proportions ($F_{2,23}=26.8$, $P<0.0001$; Table 19.1). Again in the PL class, the ratio between n-3 and n-6 PUFAs was ca. 1:2 and thus very beneficial for human consumption.

Expectedly, body masses significantly differed between the three species ($F_{2,24}$=18.4, P<0.0001; Table 19.1). This was mostly related to the different age when individuals were shot and samples were taken.

19.7 Implications of lipid research in game meat

19.7.1 Impact on for human health

Our study revealed that game meat from five nature-oriented enclosures in lower Austria possessed a very beneficial fatty acid composition in both TL and PL (Table 19.1). The three species showed some differences in the proportions of MUFAs with fallow and sika deer having higher MUFAs than red deer (Table 19.1). This observation appears to confirm the species-specificity of fatty acid composition in general (Valencak and Ruf, 2007). It also shows that these differences occur on top of body weight differences between the species and thus may indicate certain needs of a species for one or another fatty acid in order to establish or maintain tissue homeostasis. Previously, we stressed the importance of accounting for individual body mass when comparing lipid profiles between different species (Valencak and Ruf, 2007). Also, phylogenetic relationships between species need to be considered (Valencak and Ruf, 2007).

Our chosen species coming from very close taxonomic groups such as *C. elaphus*, *C. dama* and *C. nippon* did not differ in the content of n-3 PUFAs (Table 19.1) and thus all three had a ratio of n-3:n-6 amounting to 1:2. This is in accordance with all our previous work (Valencak and Gamsjäger, 2014; Valencak *et al.*, 2006, 2015) and again highlights the importance of game meat as healthy nutrient for human consumption. While we recently critically evaluated changes to fatty acid composition due to cooking (Valencak *et al.*, 2015) and found no change of the n-3:n-6 ratio, we can report here that the beneficial fatty acid profiles of game hunted from the wild do indeed resemble those of farmed game as well (Figure 19.3). We feel that this result is both interesting and important.

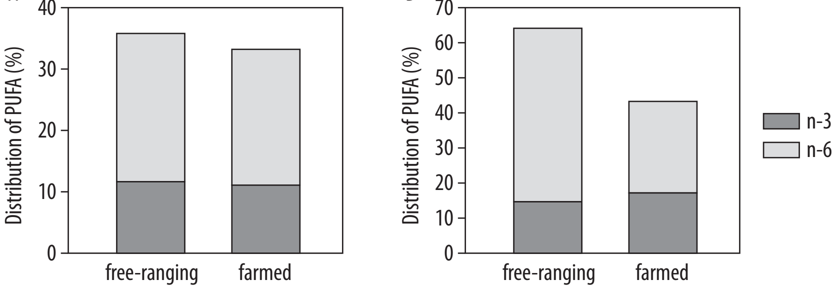

Figure 19.3. Proportion of n-3 divided by n-6 polyunsaturated fatty acids (PUFAs) in (A) total lipids and (B) phospholipids from tissues of free-ranging versus farmed red deer. Please note that the data from free-ranging animals were taken from our previous study (Valencak et al.*, 2006), whereas the data from farmed game originate from the present study.*

Since decades, nutritionists are warning about excessive intakes of n-6 fatty acids when the intakes of n-3 PUFAs are too low (Lands, 2014). Yet, it is clearly less obvious to them how to obtain food items composed of a ratio of 1:5 n-3:n-6 (or even better). This is particularly true for a landlocked country as Austria that relies on importing seafood when intending to supply the population with maritime n-3 PUFA sources.

We feel that game meat consumption could contribute to a healthy diet due to its high n-3 to n-6 fatty acid ratio and we suggest that the actual origin of the game (from a farm or from the wild) has less impact than has been claimed in the past. We had no difficulties in identifying five responsible game farms producing high-quality meat with an excellent n-3:n-6 ratio of 1:2. Our findings may contribute to an increased visibility and consumption of game meat in Austria, which is in a good position to locally supply people with meat of healthy fat composition. Currently, according to OECD data (http://www.oecd.org/els/health-systems/Briefing-Note-AUSTRIA-2014.pdf) Austria has slightly lower numbers of obese people than our neighbouring countries and it is tempting to speculate that this may be the result of a somewhat more healthy (maybe more nature-oriented and traditional) lifestyle. The consumption of locally raised and hunted game meat may have contributed to that.

19.7.2 Significance of lipid analysis in the discussion on free-ranging vs farmed game

As commonly known, it is very difficult to identify the origin of game meat offered in a restaurant. The common opinion is that game meat offered in restaurants often originates from game farms in New Zealand or elsewhere rather than from forests in Austria or from local places that practise game farming. By showing that locally farmed game meat has a very healthy fatty acid distribution, we aim to increase consumer's demand for game in general and maybe reduce the mental reservation towards game farming in general. This may also improve the public perception of sustainable hunting and modern views on healthy and contemporary nutrition.

Unfortunately, but largely influenced by the recent food scandals, the public very often is negatively biased towards meat production albeit they trust governmental measures to promote hygienic meat production that are in accordance with animal welfare and care. Having spoken to people who are critical about game meat (irrespective of whether free-ranging or wild game is addressed), it became apparent that game meat may require a kind of official 'trademark'. Game meat with such a seal of quality should meet specified hygiene criteria and may convince the broad public that its consumption does not have to be the privilege of hunters or reserved for the clientele of premium restaurants.

Undoubtedly, a lot has been done and can still be done to improve game meat hygiene (Paulsen *et al.*, 2015). We feel that these relatively recent achievements had a tremendous impact on the quality of game meat in general and that they have received too little attention from the public. We propose that both through improved marketing and by maintaining high standards aimed at improving game meat as a 'premium' and delicacy food item, its attractiveness for the consumer could be raised. While this would have economic advantages for the hunters and owners of game farming units, it would also: (1) contribute to maintaining high quality standards of the meat as only premium quality meat achieves a high price on the market; and (2) would contribute to a healthier diet.

19.7.3 Trans fatty acids in lipids from game?

Trans fatty acids are those unsaturated fatty acids with at least one double bond in the trans (hydrogen on opposite sides) position. They are found in foods produced commercially via hydrogenation of unsaturated fatty acids found in vegetable oils. As they have been blamed for causing systemic inflammation, deteriorating heart health and affecting high and low density lipoproteins, their consumption should be monitored and kept to a minimum. While the majority of dietary trans fatty acids originate from industrially produced hydrogenated unsaturated fatty acids (Smit *et al.*, 2010), there are 'natural' trans fatty acids that arise from biohydrogenation of linoleic acid by fermentative bacteria in ruminants and by endogenous synthesis from vaccenic acid by delta-9 desaturase (Smit *et al.*, 2010). Thus, we feel that the amount of trans fatty acids needs to be addressed in game meat particularly in view of what happens during hydrogenation in the rumen of red deer, sika and fallow deer. However, it is rather unlikely that this will change the general view that game meat represents a high-quality nutrient for humans. Rather, it may allow interesting additional comparisons of fatty acid composition between livestock ruminant animals and game meat.

19.8 Conclusions

19.8.1 What has been achieved?

Game meat from five nature-oriented enclosures in lower Austria possessed a very beneficial fatty acid composition in both TL and PL. The three species showed some differences in the proportions of MUFAs, but were similar in their ratio of n-3:n-6 amounting to 1:2. Since this favourable ratio is preserved during culinary preparation of venison, game meat consumption could contribute to a healthy diet. The actual origin of the game – whether from a farm or from the wild – has less impact on nutritional quality than is often thought.

19.8.2 What has been neglected?

The demand for game meat produced in Austria is less than the actual offer and therefore prices for game meat remain moderate especially for species such as European hare, wild boar and roe deer. We feel that the role of game meat as healthy nutrient has received not enough attention and thus one must continue to analyse the quality of farmed game in Austria and its contribution to a healthy diet.

19.8.3 What needs to be done?

Improvements in methodology need to be identified, such as gaschromatographic profiling which has been upgraded by the usage of modern mass spectrometers for which no external standards are required and by which even unknown compounds can be determined. In order to fully characterize the dietary significance of lipids in game meat, research on natural trans fatty acids in wild ruminants needs to be conducted.

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Biographies of keynote contributors

Lenie A. Algra-Verkerk is senior inspector involved in the development of supervision at the Netherlands Food and Consumer Product Safety Authority. She studied veterinary medicine in Belgium, Gent. After working several years as official veterinarian in slaughterhouses for domestic ungulates and later in poultry slaughterhouses and wild game establishments, she obtained her present position in 2006.

Johanna Baily qualified as a vet in 2002 from the École Nationale Vétérinaire de Toulouse in France before completing a masters in fish pathology at the Institute of Aquaculture in Stirling. She went on to spend a year in mixed practice and the following two in a private practice dedicated to disease investigation in fish, in both farmed and wild species, in Inverness. She was awarded the Official Veterinary Surgeon qualification by the University of Glasgow in 2005 and in 2010 she passed the European College of Veterinary Pathology (ECVP) board exams having completed a 3 year residency in veterinary anatomical pathology at Edinburgh University. She then went on to complete a wildlife PhD at the Moredun Research Institute, investigating the pathology and epidemiology of disease in juvenile grey seals, *Halichoerus grypus*, around the Scottish coastline with particular emphasis on *Campylobacter* and *Salmonella*. She has since moved back to the Dick Vet as a part-time lecturer in veterinary pathology. Her main areas of interest are comparative pathology and wildlife disease, especially within the aquatic environment. In addition she works as a health consultant for a Europharma, a Glasgow-based fish diagnostic company servicing the Scottish salmon industry.

Niels Bandick is head of the working group Food Technology at the Federal Institute for Risk Assessment (BfR), Department for Biological Safety and Deputy Head of Unit Food Technologies, Supply Chains and Food Defense. Wissenschaftlicher Oberrat Dr med. vet. Bandick started his career 1989 with employments as veterinary surgeon. His first scientific activities were 1991-1993 research on psycho-neuro-immunology in rats, Hannover Medical School. From 1993 until 1995 he focused on research of game meat hygiene at Hannover Veterinary School. Subsequently, until 2000, he was involved in studies on risk based visual meat inspection at Bonn University. From 2000 on he was, for eight years, senior lecturer and scientist at Freie Universität Berlin, Institute of Meat Hygiene and Technology. Since 2008 he is involved in risk assessment and research on meat hygiene and food technology at BfR. He is hunter since 1992.

Katharina Brugger is a senior, research driven lecturer at the Department of Farm Animals and Veterinary Public Health at the University of Veterinary Medicine in Vienna. Dr Brugger has a background in epidemiology of arthropod-borne diseases transmitted to livestock and wild animals. Apart from her research in this area, she is a widely recognised expert in climatic changes impacting veterinary sciences.

Cosmin Chintoan-Uta graduated from the Veterinary School of the University of Bristol in 2010 with a BVSc degree. The following year, he studied at the same University for an MSc by research, under a Welcome Trust Veterinary Entry Research Fellowship, investigating the potential of wild deer to transmit anthelmintic resistant nematodes to livestock farms. At the same time he worked in the First Opinion Small Animal Practice of the University of Bristol. In 2012, he moved to Edinburgh where he studied for a PhD at The Roslin Institute, University of Edinburgh. His PhD focused on development of vaccines against Campylobacter in poultry. Since October 2015 he has been working as a postdoctoral research fellow at The Roslin Institute. His research focuses on studying host-pathogen interactions with a view to develop control strategies, particularly as regards vaccines. He also teaches the food microbiology course on the veterinary public health module at the Royal (Dick) School of Veterinary Studies and he is a member of the steering committee of the Early Career Vaccinologists division of the Veterinary Vaccinology Network.

Hans Dannenberg is a senior policy officer of the Netherlands Food and Consumer Product Safety Authority (NVWA), directorate of inspection (now he has retired). He worked since 1992 in the field of veterinary public health (training of inspectors, approval of establishments, export, developments and new EU legislation) and was specialised in wild and farmed game meat, poultry meat and fishery products. He was involved in several TAIEX missions regarding the evaluation of veterinary services concerning progresses in upgrading of the establishments processing meat, milk, and fish.

J. Paul Duff joined Animal Health and Veterinary Laboratories Agency (VLA) in 1987 and has worked as a Veterinary Investigation Officer at several regional laboratories and is currently based at APHA, Penrith in the Lake District, UK. Paul has always tried to combine being a veterinarian with a lifelong interest in natural history and since 1997 he has been the project leader for DEFRA funded Disease of Wildlife Scheme. APHA chairs the GB Wildlife Disease Surveillance Partnership which with the Disease of Wildlife Scheme delivers wildlife disease surveillance to government.

John Fletcher is a vet who graduated from Glasgow and then completed a PhD from Cambridge on the breeding physiology of wild red deer on the Isle of Rum, West Scotland. He then established Britain's first red deer farm at Auchtermuchty, Fife in 1973 together with his wife, Nichola. The farm has exported deer breeding stock throughout Europe, to the USA, New Zealand, Taiwan and Japan and received the Queen's Award for Export in 1990. With Nichola he developed a business killing and processing deer on the farm for sale through the farm shop, at farmers' markets and by mail order. He conceived and established the Scottish Deer Centre, Cupar, Fife. He was the first vice-chairman of the British Deer Farmers' Association and later its chairman, and was founder and later president of the Federation of European Deer Farmers' Associations and of the Veterinary Deer Society. He is now president of the British Deer Farms and Parks Association. He published 'Fletcher's game', an account of his work with Scotland's deer in 2003; 'Gardens of earthly delight – the history of deer parks' in 2011 and 'Deer' in 2013. He was elected an honorary fellow of the Royal College of Veterinary Surgeons in 2006 and a fellow of the Royal Agricultural Society in 2014. Having sold the venison business John continues to keep a very special herd of red deer using the Inshewan bloodline as a source of breeding stock. He is a partner in Venison Advisory Services Ltd providing advice to those interested in establishing commercial deer enterprises and is hugely rewarded by the number of new entrants to Scottish deer farming. Above all his priority is to maintain the image of deer as beautiful and magical wild animals and to avoid them being treated as vermin.

Antje Gerofke is a biologist and has joint the German Federal Institute for Risk Assessment (BfR) in 2007. She is working in the unit Feed and Feed Additives at the Department Safety in the Food Chain. Her main area of work is risk assessment of contaminants in food.

Andrea Gröne is professor of pathology at the Faculty of Veterinary Medicine, Utrecht University, the Netherlands. She studied veterinary medicine in Germany and, after working as veterinarian in small animal practice in England, went to the Ohio State University in Columbus, OH, USA, to obtain her PhD and train as a veterinary pathologist. Following a year working as a post-doc in Giessen, Germany, she went to Berne, Switzerland, to obtain the habilitation. Before obtaining the present position she worked for a year in Hannover, Germany. Andrea Gröne is diplomate of both, the American and European Colleges of Veterinary Pathologists. Since 2008 she is also director of the Dutch Wildlife Health Centre, located at the university.

Martin Hennecken is senior veterinary officer for meat hygiene involved in the development of supervision at the Netherlands Food and Consumer Product Safety Authority. After his study of veterinary medicine at Utrecht, the Netherlands, he gained experience as a private veterinarian with small and farm animals in the Netherlands. In 1997 he joined public service as official veterinarian in the slaughterhouse and was involved in animal disease control of the CSF-outbreak in the Netherlands. From 1999 onward, he worked at the Dutch Ministry of Agriculture/Economic Affairs as a policy officer on different subjects, including from 2006 onwards on food safety of the meat supply chain. Since 2015 he is working for the Netherlands Food and Consumer Product Safety Authority on the same subject.

Andreas Hensel is the president of the Federal Institute for Risk Assessment in Berlin since 2003 and Germany's representative in the scientific council (Advisory Forum) of the EFSA. Before accepting the position as president of the Federal Institute for Risk Assessment, Professor Dr Dr Hensel was senior scientist at the University of Veterinary Medicine and the University of Vienna (1990-1997). After that he became full professor for 'animal hygiene and federal animal pest control' and took over the position of director and chair at the 'Institute for Animal Hygiene and Veterinary Public Health', University of Leipzig (1997-2003).

Eric Hoberg is former chief curator and zoologist at the US National Parasite Collection, among the largest archives of specimens and information documenting global parasite biodiversity. A field biologist and biogeographer, he has traversed regions of Alaska, Canada, Siberia, and Antarctica and since 1999 he has been a principal of the Beringian Coevolution Project an interdisciplinary exploration of historical processes, biodiversity and structure of host-parasite systems among northern mammals. He has authored or co-authored ca. 300 publications in the global literature where parasites serve as a portal to historical/ ecological insights about the biosphere, emphasizing the role of episodic events, climate, environmental perturbation and patterns of geographic colonization as determinants of diversity and emergent diseases in evolutionary and ecological time. Given the opportunity he chases large salmon with small flies.

Louwrens C. Hoffman received his academic education (BSc in animal science 1987; MSc in meat science 1985) at Stellenbosch University, South Africa. His PhD (1995) titled 'Factors influencing the meat quality of catfish' was awarded from the same university. Since 1997, he has been researching various factors influencing the meat quality of animals 'from stable to table'. Dr Hoffman has published over 225 peer reviewed meat related papers and has attended and read papers at numerous international and national conferences. His major research focus is on the more exotic meat types produced from low input agricultural systems such as ostriches and wild ungulates found in Africa. Dr Hoffman is the recipient of several research awards. His expertise in game meat was emphasised further when he was invited in 2013 to write two chapters on this topic for the encyclopaedia of meat sciences. Prof. Hoffman is regularly invited to read plenary papers at international conferences and is a regular guest speaker at farmer's days in South Africa and Namibia. As from 2013 he is the incumbent of the highly competitive SARChI (South African Research Chair Initiative) chair in meat science: genomics to nutriomics. This chair allows him to focus primarily on research.

Anniina Holma-Suutari (PhD in animal physiology) has studied persistent organic pollutants on reindeer and moose and has also published articles on harmful environmental contaminants in different animal species in the Arctic food chain. Her interests are reaching other areas including parasitic studies and wellbeing of northern ungulates.

Pikka Jokelainen holds a DVM and a PhD degree and is adjunct professor of zoonotic parasitology in the University of Helsinki, Finland. She has published over 20 scientific articles. Her research work in Finland, Estonia and Denmark focuses on zoonotic parasites.

Arja Helena Kautto studied subarctic ecology and genetics in University of Oulu, Finland (1980-1986). She graduated with a MSc thesis in grazing ecology of reindeer in Finnish reindeer herding area. After participating in different research projects at the Natural Research Institute in Finland she studied veterinary medicine at the Swedish University of Agricultural Sciences (SLU), Uppsala. She got the Diploma in Veterinary Medicine in 1993 and her final thesis was chosen as 'the best of the year'. Her Swedish veterinary specialisation in food safety (2006) was on game meat hygiene. From 1993-2001 she combined mixed practice with working as a veterinary officer in the slaughterhouse and being head of a 'state veterinarian clinic'. During 2001-2006 she was employed by the National Food Agency (NFA), focusing on the approval of food business establishments and implementing the new EU food hygiene legislation in Sweden. During 2006-2012 she was in charge of implementing the new NFA organization as a head of the department in the north of Sweden. Currently she is working as a veterinary expert in quality assurance and modernization of meat inspection at NFA which work is a relevant part of her residency in European College of Veterinary Public Health. Since 2012 she has been a member of the governmental national game board and of the board of veterinary program at the SLU. She is a (mainly moose) hunter in Sweden.

Susan Kutz is a Professor of ecosystem and public health at the Faculty of Veterinary Medicine, University of Calgary, Canada. Passionate about Arctic and Sub-Arctic ecosystems and peoples, she has worked in partnership with communities in northern Canada for over 20 years studying the impacts of climate and anthropogenic change on the health and sustainability of northern wildlife. She also established the 'Northern Community Health' program, delivering veterinary services and animal health education to remote communities in the NWT since 2008. Her areas of expertise include wildlife parasitology, disease ecology, ecosystem health, climate change, and community-based disease surveillance.

Monika Lahrssen-Wiederholt is veterinarian, since September 2009 she is head of the department 'Safety in the Food Chain' at the German Federal Institute for Risk Assessment (BfR). From 1999 to 2002 she worked as a scientific officer in the Directorate General for Agriculture and the Directorate General for Health and Consumer Protection in Brussels at the EU Commission in the unit 'Animal Nutrition – feedingstuffs'. Afterwards she was head of the unit 'Feed additives and animal nutrition' of the Federal Institute for Consumer Health Protection and Veterinary Medicine in Germany and later became head of the unit 'Contaminants in the food chain and feed safety' at the Federal Institute for Risk Assessment.

Sauli Laaksonen is adjunct professor of cervid diseases in the University of Helsinki. He is the creator of Finnish hunters' hygiene training. He has published ca. 60 articles on game animal health in international scientific series. His textbook on game meat hygiene, originally published in Finnish, has been made available as English version by Wageningen Academic Publishers. He has also worked for over 30 years in game and reindeer meat inspection and the hygiene control of primary production and food industry facilities. Currently he is working as a leading researcher in the project 'Reindeer health in a changing environment'.

Alison J. Leslie is a senior lecturer and wildlife ecologist in the Department of Conservation Ecology and Entomology at Stellenbosch University in South Africa, earned her MSc and PhD degrees at Drexel University in the United States working on sea turtles and crocodiles. Dr. Leslie currently studies many aspects of a broad range of wildlife species; a common goal of her projects is to develop management plans for governments and various wildlife organisations. She has conducted wildlife research in South Africa, Botswana, Namibia, Zambia, Malawi and India. She is also a well-known TV personality, having worked with National Geographic television, the BBC, and a number of other wildlife documentary producers.

Annett Martin is member of the team of Epidemiology, Statistics and Mathematical Modelling unit at the Federal Institute for Risk Assessment in Berlin. She holds an engineering degree in biomedical technology/electronics and a MSc in epidemiology. Her work involves the statistical analyses of data from scientific studies at the institute – often in the area of microbiology – as well as modelling risk related questions.

Margriet G.E. Montizaan has been professionally occupied with wildlife for almost thirty years. For more than twenty years she worked at the Royal Dutch Hunters Association. There she worked as a wildlife biologist, her main topics being bag statistics, mammals, diseases, the Dutch an EU regulation concerning the food safety of game and the development of a course to become a Trained person. Nowadays she works at the Dutch Wildlife Health Centre, where her main activity is the communication of information with respect to wildlife diseases to both professionals and the broader public. She is still involved in the actualisation of the course to become a trained person.

Christine Müller-Graf graduated from the University of Freiburg and took a DPhil in zoology (specializing in parasitology) at Oxford University in 1994. Postdocs at Oxford University, the University of Warwick, Université de Perpignan and Université Pierre-et-Marie Curie (UPMC, Paris) followed. She held a lecturer position at Université Pierre-et-Marie Curie in the ecology laboratory. In Paris she obtained her habilitation in 2002. In 2006 she joined the Federal Institute for Risk Assessment (BfR) in Berlin, Germany as head of the Epidemiology, Statistics and Mathematical Modelling Unit. The focus of her present work is the statistical analysis in food and feed related projects, as well as the quantitative risk assessment of microbial pathogens and the application and development of methods in qualitative and quantitative risk assessment. She was member of the AHAW panel of EFSA (2006-2009) as well as member of the BIOHAZ panel (2009-2012). She is furthermore teaching at the Humboldt-University in Berlin.

Rauni Niskanen is currently head of the Department of Microbiology at National Veterinary Institute (SVA) in Uppsala, Sweden. SVA is an expert authority under the Ministry of Enterprise and Innovation, and is nation’s leading knowledge centre for infectious diseases in veterinary medicine. Rauni Niskanen graduated in veterinary medicine at the Swedish Agricultural University in Uppsala in 1982, and then obtained a PhD degree at the same university in 1995 based on the studies on diagnostic methods and epidemiological investigations of bovine virus diarrhoea virus infection in cattle Swedish dairy herds. Subsequently, she worked as lecturer and as senior lecturer in ruminant medicine. In 2002, she was appointed associated professor in ruminant medicine. Rauni Niskanen was also appointed head of the department of Ruminant Medicine and of the department of Clinical Science, in total about 6 years. Between 2007 and 2011 she acted as head of Food Control department and two years as head of Food Control division (2014-2015) at the Swedish National Food Agency in Uppsala. The leadership for food control in Sweden was included in her duties as the head of Food Control division. During the years at Swedish National Food Agency she also acted as deputy head of the agency (2012-2014).

Delphine H. Nourisson received a master degree in environmental biology at Florence University in 2009, with a thesis on the ecosystem quality of coastal water bodies. In 2014 she got an European PhD in ethology and ecology, studying the arthropod community responses to human-induced environmental changes on sandy beaches. During the following two years she was in charge of monitoring the arthropod community of the Maremma Natural Park. Meanwhile she got a 20-months post-doctoral fellowship, allowing her to spend six months at DWHC, at Utrecht University, the Netherlands. Here she developed a project proposal under Horizon 2020, aimed at improving the hygiene and food safety of game meat at the European level. She is now teaching biology and environmental sciences in Florence high schools.

Sarah A. Ohrnberger is a PhD student with T.G. Valencak at the Department of Biomedical Sciences at University of Veterinary Medicine in Vienna working on the role of overheating on milk production in golden hamsters. She has a background in wildlife medicine and requirements of maintaining wild animal herds in captivity.

Antti Oksanen is a parasitologist, professor and head of Production Animal and Wildlife Health Research Unit of the Finnish Food Safety Authority EVIRA.

Thomas W. Pennycott graduated from the Royal (Dick) School of Veterinary Studies in 1978, and has spent over 30 years investigating diseases of gamebirds and providing advice on treatment and control measures. After five years at Worcester Veterinary Investigation Centre in England and 25 years at Ayr Disease Surveillance Centre in Scotland, he joined St David's Poultry Team (Gamebird Services) in 2014, where he is currently developing site-specific gamebird health plans.

Jolianne M. Rijks works as epidemiologist at the Dutch Wildlife Health Centre (DWHC), Utrecht University, the Netherlands. After studying veterinary medicine in Utrecht, she first worked in primary animal health care and other projects in Africa for fourteen years, using participative approaches. In the last twelve years, she has worked on wildlife health in the Netherlands, including on food safety in the wild game value chain since 2013. She obtained her PhD in 2008 at the Erasmus MC, Rotterdam, the Netherlands, and is a European veterinary specialist in wildlife population health (ECZM).

Helmut Schafft is a senior staff scientist of the Federal Institute for Risk Assessment (BfR) in Berlin, Germany, head of the unit Feed/Feed Additives/Contaminants in the Food Chain, and senior lecturer at the Faculty of Life Sciences at the Humboldt-University of Berlin. Before joining BfR, Dr Schafft was on the Faculty of Agriculture at the Humboldt-University of Berlin. He is an expert in risk assessment in feed and food. His focus includes methodology for consumer health risk assessment, including approaches to weight of toxicological evidence for human hazard and cross-species extrapolation. Dr Schafft received a PhD in animal sciences from the University of Göttingen and a Dr habilitation from Humboldt-University of Berlin.

Matthias Schreiner, associate professor at the Institute of Food Science at the University of Natural Resources and Life Sciences, Vienna, Austria, is expert for lipid chemistry, in particular analyses of FAMEs from all sources (plant, animal, etc.). Apart from his role as lecturer for students in food safety, he conducts research in the area of food chemistry and readily shares his expertise on new, exciting and emerging areas in the field. Before his current role as associate professor he conducted postdoctoral studies in Vienna, at the Freie Universität Berlin, Germany and at the University of Sao Paulo, Brazil.

Alessandro Seguino graduated from the Veterinary School of the University of Naples 'Federico II' in 1997 with a DVM degree. He worked for three years in a mixed practice and, at the same time as veterinary officer in the Animal Health State Veterinary Service in Italy. During this time he undertook postgraduate study in the 'Inspection and control of foodstuff of animal origin', also in Naples qualifying as recognised specialist in 2001. In 2002, he moved to the UK where he worked in England for one year as official veterinarian for the Meat Hygiene Service, and was then promoted to veterinary manager. Then he moved to Scotland supervising vets working in a wide range of food processing plants. In 2009, he joined the Royal (Dick) School of Veterinary Studies as head of Veterinary Public Health. Currently, he is a senior lecturer in Veterinary Public Health and director for the 4th year Veterinary Public Health (Food Safety) course. Alessandro is a diplomate of the ECVPH and a senior fellow of the Higher Education Academy.

Thomas Selhorst received a doctorate in agriculture (1990) from the University of Bonn, Germany, with a specialisation in plant pest epidemiology and biological control and a habilitation (1996) in theoretical ecology also from the University of Bonn. From 1997 until 2014 he worked at the Friedrich Loeffler Institute (FLI), Department of Epidemiology and during this time his research interests were: ecology of terrestrial rabies and rabies control, epidemiology of BSE and BSE surveillance, and the spread of infectious diseases on livestock trade networks. Since 2014 he works at the BfR, in the unit of Epidemiology, Statistics and Mathematical Modelling. His research focuses on studies of the social contact structure between livestock in a barn, and the dynamics of local, regional and global food and feed chains as well as collaborating on several projects in the BfR and advising on statistical analyses. He continues to lecture at the University of Bonn.

Cristina Soare obtained her veterinary degree at the University of Bucharest, Romania, in 2005. She worked for two years in small animal practice and subsequently undertook a position for the Romanian Civil Service as veterinary adviser. In 2010, she moved to the UK to work in the veterinary public health sector. Cristina's interest in veterinary public health covers all aspects of 'farm to fork' and zoonoses with an emphasis on emerging zoonotic diseases and their impact on global health. To pursue her interests Cristina enrolled in 2012 into a 3 years international Master's degree programme which covers veterinary public health and animal health aspects. Her MSc dissertation will present the current scenario related to zoonotic origin of hepatitis E virus. In 2014, Cristina was invited to work as a teaching fellow at Edinburgh University, a position that she fulfils enthusiastically at present.

Monlee Swanepoel obtained a BSc degree in conservation ecology from Stellenbosch University, South Africa, in 2010 and a PhD in conservation ecology from the same institution in 2015. The work for her dissertation focussed on the distribution, management and utilisation of the common warthog in South Africa. She has published four peer-reviewed papers which emanated from her dissertation before being awarded the degree, and is working on further publication from the research. She is currently conducting research as a post-doctoral fellow at Stellenbosch University on the development of processed game meat products from popular game ungulates hunted in South Africa to encourage game meat utilization and sustainable hunting practices.

Ellen Ulbig graduated in 1994 at the Berlin University of Technology, Faculty for Environment and Society; focus: ecological risk assessment. After her diploma in environmental planning (1983), she spent four years with the Federal Biological Research Centre for Agriculture and Forestry (BBA), Department for Ecological Chemistry, focusing on research for the health and productivity of plants. Between 1988-2004 she worked as scientific employee at the Federal Environment Agency in different fields of the environmental risk assessment (forest damage research, Antarctic environmental protection). Since 2004, she is working at the Federal Institute for Risk Assessment, currently in the Department for Safety in the Food Chain, focusing on the assessment of contaminants, residues and other undesirable substances from food or feed and the safety assessment of feed additive applications with regard to consumer and animal health.

Ivar Vågsholm is veterinarian and professor in food safety at SLU since 2009 and since 2015 head of the department of Biomedicine and Veterinary Public Health. Graduated as veterinarian in 1984, and obtained his PhD from the University of California in 1989. Over the past 30 years he has worked with the European Union, relating to animal health and food safety, starting with the EFTA Surveillance Authority in 1992-1995, and later as head of the Swedish zoonosis centre in 1997-2006. He has been a diplomate (population medicine) of ECVPH since 2001 and contributed as member of the ECVPH Education Committee since 2002. A key contribution to translating science into public benefits has been his membership of the EFSA scientific panels on animal health and welfare from 2012-2015, biological hazards from 2003-2012, and EU Commission's Scientific Committee of Veterinary Measures relating to Public Health from 1997-2003. Moreover, he is member of the editorial board of Preventive Veterinary Medicine, and member of the advisory group of the Swedish Medical Products Agency. He has participated in 2 WHO consultations during 2012 on Campylobacter control and environmental health. The revision of meat inspection and food safety legislation, and control of TSE have been major topics during the last years. Currently his main interests include circular food production systems and avoiding cycles of infectious agents becoming public or animal health risks, food safety of composite foods and improving food control having regard to public health, animal welfare and economics and political science.

Teresa G. Valencak, is assistant professor in wildlife biology working at the Department of Biomedical Sciences at University of Veterinary Medicine in Vienna, Austria. She is conducting experimental research on the role of fatty acids as signalling molecules, as important constituents of membranes and as potential pacemakers of metabolism. Her research concerns both wild animal tissues as well as commonly known laboratory animals such as mice and golden hamsters. She also lectures physiology and genomics in mammals with a special focus on wild animals. Before settling in Vienna, Dr Valencak conducted research on energy metabolism of wild and laboratory animals during lactation in the UK and studied processes of overnutrition, i.e. obesity in mammals and humans in the People's Republic of China.

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