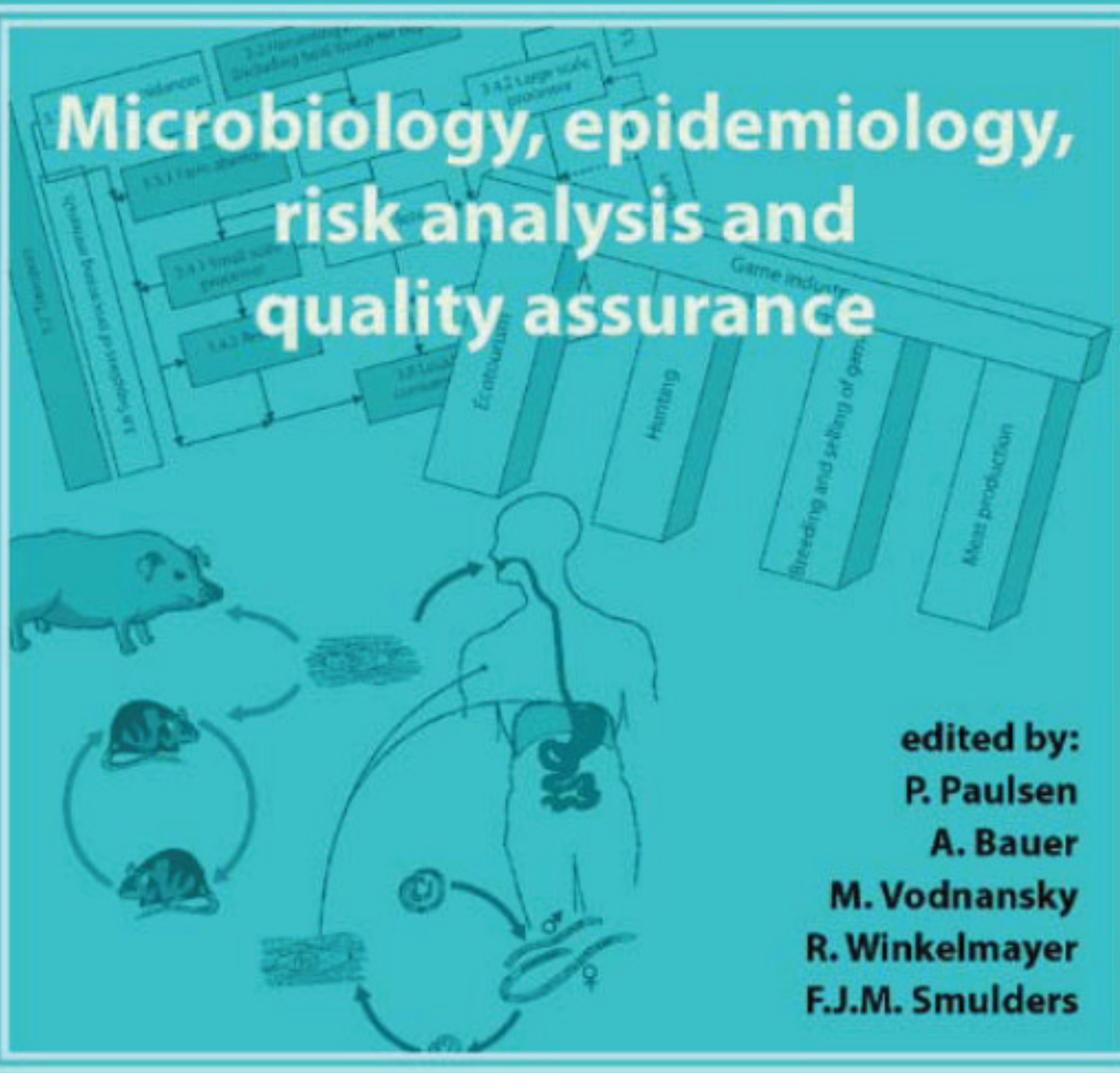
Game meat hygiene in focus

Microbiology, epidemiology, risk analysis and quality assurance



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Preface

Game meat is consumed world-wide. In most regions, it contributes only a small part to the overall meat and food supply, but for reasons of animal welfare and sustainability it is sometimes considered an alternative for meat from farmed animals. Despite differences in game species, *ante mortem* conditions (free-range or fenced; wild or semi-domesticated), hunting or harvesting procedures and further handling of the carcass, there are common requirements as regards meat safety and quality. Whereas meat safety and shelf life have been an issue in game meat for export/import for a long time, primary production, domestic supply and direct supply to the consumer have recently been addressed by legislation and these sectors still present unresolved questions and challenges.

'Hygiene' is commonly defined as all measures that promote and preserve health. It is often tacitly assumed that hygiene deals with the management of biological, chemical and physical hazards. However, considering that 'health' is 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity' (WHO, 1948), or, in a more recent definition '... a resource for everyday life ...' and ' ... a positive concept emphasizing social and personal resources, as well as physical capacities' (WHO, 1986), it seems entirely justified to include under 'hygiene' also aspects of sensory meat quality, ethics and sustainable production, as these contribute to 'social well-being'.

Having regard of the above-mentioned considerations, a consortium of food hygienists has established a forum that provides a platform for all questions of game meat hygiene, safety and quality, consequently termed 'International Research Forum on Game Meat Hygiene', IRFGMH. A major task of this forum is to promote research in all fields of game meat hygiene, quality and safety, and allow exchange of information in the form of biannual international conferences and by means of electronic communication.

The first conference of the IRFGMH was held at Brno, Czech Republic, in June 2009. The hospitality and professionalism of the local organizers, the University of Veterinary and Pharmaceutical Sciences in Brno, and, in particular, the Central European Institute for Wildlife Ecology, substantially contributed to the success of this conference.

This book includes chapters authored by key contributors and synopses of other contributions by participants. Based on their conference presentations, contributors were invited to update their contributions where necessary and their papers have gone through an extensive review process. The content is grouped into 4 main sections, viz. 'hygiene and microbiology', 'epidemiology', 'risk assessment and management' and 'muscle biology and meat quality'. The contributions represent research outputs, opinions and experiences of experts from 8 European countries, as well as from South Africa, a major game meat exporter.

This volume is the first of a book series on safety and quality assurance along the game meat chain, following an approach 'from forest to fork' and is targeted at scientists in academia and industry, graduate students as well as at governmental officials in veterinary public health and food safety.

Last but not least, we would like to acknowledge the 'Verein Grünes Kreuz', Austria for their financial support which made this publication possible.

Vienna and Brno, February 2011

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Hygiene and microbiology of meat from wild game: an Austrian view

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Summary

The food chain for meat from wild game in Central Europe differs from that of farm animals in the mode of killing (large game: free bullet in head, neck or anterior chest; small game: multiple shot pellets), conditions of evisceration, storage of carcasses (skin-on) and *post* and *ante mortem* inspection. Poor placement of shots, efflux of ingesta or feces during evisceration, delayed or insufficient cooling are known to compromise the microbiological condition of meat. Conditions at primary production level are poorly standardised which may explain the wide range: Total Aerobic Counts (TAC) on meat cuts from wild game. Counts in the order of 3-4 log cfu/cm^2 can be achieved when Good Hygiene Practice is strictly adhered to, but values of >8 log cfu/cm^2 are also reported. A number of studies report prevalences of pathogenic bacteria in intestines, tonsils or on skin, but relevant literature on the presence of a pathogen in the life animal and to what extent it will result in contamination of meat cuts is scarce. When meat from wild game is placed on the market the same way as that from farm animals, it is reasonable to demand that similar bacterial limits are set as performance objectives. This could be, for instance, for large game, that TACs must not exceed 6 log, and *E. coli* not 2 log cfu, per cm^2 on exposed muscle surfaces of skin-on carcasses arriving at game handling establishments and per g for meat cuts at retail. It is concluded that optimising the primary production level is the key to improving safety and shelf life of wild game meat.

Keywords: wild game meat, pathogenic bacteria, spoilage, good hygiene practice

1. Introduction

1.1 Hygiene and the game meat chain: how things developed

Hunting has been the very first way to provide meat for human nutrition, but its relative contribution to meat supply has – with few local exceptions – decreased. In the Middle Ages still regarded as 'substantial' for overall meat supply (Lerche, 1957; Kerschagl, 1964), meat from hunted wild game constitutes only ca. 1% of today´s average consumption of meat and meat products in Austria (Anonymous, 2010).

Practical hunting can be quite different between countries and continents, with respect to game species and hunting techniques. This contribution will thus focus on the situation in Austria and Germany, where hunting has a long tradition and a large number of hunters is

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more or less actively involved in meat production. The main differences of the game meat chain as compared to meat production from farm animals are:

- wild game is hunted either in the wild or in large fenced areas;
- for large game, hunters take either fixed positions and wait for the animal ('still hunting') or they stalk animals; another option are drive hunts/battues, where animals are disturbed and are awaited by hunters;
- for small game, drive hunts are most common;
- animals are killed by free-flying bullets/shot pellets;
- in case of single bullets the shot wound can be located in the anterior chest, head or neck;
- in case of multiple shot pellets, the wounding pattern is highly variable;
- *via* the shot wound, bacteria can be introduced in the blood stream;
- in some instances, evisceration can be done only with some hours delay, and for small game, even later;
- evisceration often done 'on the spot' with restricted access to clean water;
- traditions that may interfere with modern food hygiene standards;
- cooling of skin-on carcasses;
- *ante* and parts of *post mortem* inspection assigned to hunters/'trained persons';
- for most species, the meat production is seasonal, with peak-periods.

The implications for meat inspection are discussed elsewhere in this volume (Winkelmayer *et al.,* 2011) as are the ramifications with respect to local trade (Fettinger *et al.,* 2010a, 2011). The following subchapters will focus on the microbiology of game at the different steps in the food chain in the light of food safety and quality.

Although game meat has served as food for such a long time, it has not been a main concern of food hygienists for quite a long time. Admittedly, the need for a meat inspection system for game has been an issue before 1910, e.g. Kniewallner (1969) cites one reference from 1907, and the rationale for and feasibility of such inspection systems have been discussed in the following decades (e.g. Olt, 1943). It can be argued that processes as performed in game handling establishments (skinning, deboning, etc.) are basically the same as in slaughter/cutting facilities for farm animals, but the primary production area (including killing, evisceration, transport, cooling), which is different from that of slaughter animals received little attention.

Reviewing food hygiene literature, the Austrian food codex in its first edition (Anomymous, 1912) merely describes briefly the various species of meat game, differentiates 'haut-gout' (defined as a sort of 'acidic fermentation' with minor H_2S formation only) from spoilage and mentions that the current practice of shipping unviscerated, still warm carcasses is not acceptable. A food inspector´s textbook of 1930 essentially repeats these statements (Messner, 1930). In these times, the main Austrian textbook for veterinary meat hygienists did not address game meat at all (Postolka, 1903), or, in its last edition (Postolka, 1922) only reviewed selected aspects (e.g. identification of species, age and gender, and spoilage), but – in contrast to slaughter of farm animals – provided no specific information on the preferability of certain handling practices during evisceration, cooling, and transport. In German meat inspection textbooks by Ostertag (e.g. Ostertag, 1910), game meat was dealt with similarly. Lerche (1957), from a food hygienist´s view, provided more information on game meat and

also suggested cooling temperatures for game carcasses of 0 °C. He also questioned the term 'haut-gout': originating from an era with a lack of cooling facilities, 'haut-gout' was often used to present the onset of spoilage in an appealing light. Interestingly, a number of authors mention that meat from wild game would be more resistant to microbial spoilage, which they in part attributed to low pH and antibacterial activity of residual blood in muscles (Postolka, 1922; Ostertag and Schönberg, 1955; Lerche, 1957).

From the 1960´s onwards, there was a continuous discussion about inspection and hygiene of wild game meat (e.g. Englert *et al*., 1965; Prändl, 1976; Raschke, 1976), in part driven by the need for inspection of venison imported from e.g. New Zealand or hares from Argentina, and later by the discrepancy between imported venison (which was subject to inspection) and domestic venison (for which only *Trichinella* inspection was mandatory) (e.g. Kniewallner, 1969; Greuel, 1979; Riemer and Reuter, 1979). Later, the tendency to prepare meat from wild game 'rare' or 'medium' prompted food hygienists for intensified studies (Ring *et al*., 1988; Deutz *et al*., 2000).

In other words, scientific studies and food control focused on the final steps of the game meat chain for quite a long period, although it was principally agreed that meat hygiene principles have to be followed by hunters (Kniewallner, 1969).

In the area of primary production, some, although slow, developments could be observed. A brief view on major training textbooks published over the last 50 years can substantiate this. Originally, emphasis was put on the description of evisceration methods (carcass lying on the ground; facultative removal of thoracic organs) and it was stressed that evisceration should be done as soon as possible for large game (Voß, 1962) or all game species (Anomymous, 1956), but that carcasses should be opened only to an extent which would minimise contamination during further transport. It was pointed out that carcasses should be allowed to cool, i.e. to reach ambient temperature (within 12-24 h) before being transported. Cleaning of the body cavities should be done with a cloth or even blood (*sic*!), but not with water. To keep away or repel insects, storage under good ventilation was recommended, also swabbing of the body cavities with vinegar. For long-term storage, the skin-on carcass (for small game also un-eviscerated) could be stored deep-frozen (Voß, 1962).

In the 1950´s and early 1960´s, these recommendations were oriented towards the possibilities in practice: transportation of game from forest/field to game larders, etc. was generally considered difficult, and refrigeration facilities were scarce and not easily accessible or affordable for individuals. Recommendations also included traditional elements. It is interesting to observe that these were repeated in the major Austrian training books for hunters ('Der Jagdprüfungsbehelf') for more than 25 years and 7 editions with only minor modifications (Voß, 1962; Anomymous, 1989). The 12th edition of the textbook of 1992 (Anonymous, 1992) was the first to explicitly state that for game put on the market, even in the absence of formal inspection procedures, the responsibility of the hunter as food producer was applicable as in any other sector of food production. It also adopted parts of the EU wild game directive (EC, 1992) and stressed – *inter alia* – the necessity of proper cooling and the maintainance of low temperatures with specified maxima, and *Trichinella*-inspection of wild boar carcasses. The following editions up to the current one were then based on

the Austrian implementation of the wild game directive and, from 2004 onwards, on EU legislation ('hygiene package'). Education of hunters and 'trained persons' in order to comply with the 'hygiene package' and the role of hunters as 'food business operators' are discussed in detail elsewhere in this volume (Fettinger *et al.*, 2011 and Winkelmayer *et al.*, 2011).

In summary, improvements in hygiene of the slaughter of farm animals and in cooling facilities as established from the end of the 19th and first half of the 20th century onwards have found their way into game handling establishments, but there was some delay in adoption by primary producers of game meat. The next section will discuss this issue.

1.2 Separating science from tradition: best, good and common practice today and yesterday

Tradition plays a strong role in hunting, and this also holds true for handling of hunted game. Critical remarks on hunting traditions having negative consequences for meat hygiene have been made by Kniewallner (1969) and Riemer and Reuter (1979) and it has been emphasised that cooperation and compliance of hunters are needed to improve game meat hygiene.

For example, tradition required that during evisceration ('red work') sleeves of shirts should not be rolled up and protective gloves should not be used (e.g. Anonymous, 1956; Voß, 1962), which was abandoned in the 1970´s (e.g. Anonymous, 1977). The still widely practised ritual of post-hunt presentation of large and small game ('Streckenlegung') is not without hygienic complications, in particular at ambient temperatures >12 °C and when already eviscerated carcasses are arranged on the ground (Winkelmayer, 2009).

Current EU legislation suggests that 'guides to good practice' should be developed by the food sectors, and with respect to meat there are a number of textbooks in Germany and Austria which more or less explicitly deal with 'good practice'. Among some of the more recent are, e.g. Kujawski (2007), Martini (2008), and Winkelmayer *et al.* (2007).

Today's definition of good or best practice starts before the actual hunting event, and even before the *ante mortem* examination immediately before killing (Bandick and Ring, 1996). It is not only necessary to provide an adequate infrastructure for hygienic evisceration and refrigeration, but also to better organise drive hunts and battues to facilitate collecting and evisceration of large numbers hunted game in due time (e.g. Deutz, 2000; Deutz *et al.*, 2006).

Correct placement of shots and immediate death of the animal is relevant not only for animal welfare (Winkelmayer, 2009), but will also facilitate collecting of the game and allow early evisceration (Winkelmayer *et al.*, 2005). For large game and under conditions prevailing in Austria (i.e. numerous semi- or non-professional hunters, the need for short flight distances due to small hunting areas, the need to preserve head and antlers of male specimens as trophies), the correct aiming point was defined as an area delineated by the triangle: (1) caudal contour of shoulder blade; (2) humerus; (3) line from olecranon to the caudoventral edge of the scapula. This aiming point is slightly more cranial than traditional aiming points. By this, at the expense of damaging shoulder muscles, the risk of abdominal lesions (Winkelmayer *et al.*, 2005) and subsequent microbial contamination (Paulsen *et al.*, 2003) was minimised.

For large game, evisceration must be done without 'undue delay', e.g. Winkelmayer *et al.* (2008) specify a maximum of 3 h, a time span which is entirely realistic (see Table 1). The rationale is not only limiting the risk of microbial spread, but also to prevent hot, large carcasses (wild boar, red deer) from undergoing non-bacterial spoilage ('stickige Reifung', 'stifling maturation'). Recommendations to open the abdominal cavity only to a small extent and to make incisions in the axillary region, so as to improve cooling thus allowing delayed evisceration ('Lüften' as mentioned in textbooks from the 1970´s) may have been useful in times where cooling facilities were either not accessible within due time/distance or simply did not exist. However, nowadays it is an issue of hygiene management to have operational refrigeration rooms in suitable distance. Consequently, this practice has been completely abandoned in favour of evisceration and cooling at the earliest possible occasion. At still hunts, the majority of carcasses was eviscerated with 30 min. (Brodowski and Beutling, 1998; Deutz *et al.*, 2000), whereas at drive hunts, there will always be some delay between killing and evisceration (Deutz *et al.*, 2006), and it a logistic issue to keep this time span <1 h.

A variety of evisceration techniques for large game, with carcasses either lying on the ground or hanging, is suggested in literature and they all have their advantages as long as the contamination of the body cavity and muscle tissues with soil and feces is avoided or minimised and meat inspection is not impaired (see for example Bandick and Ring 1996). Also, for abdominal shots, different approaches are described (i.e. removal of the diaphragm and peritoneum or washing out the body cavity without opening the thoracic cavity). To date, there seem to be no systematic studies on which techniques actually result in a minimised microbial contamination of meat. For small game, the situation is somewhat complicated as current practice is to eviscerate small game with some days delay. Admittedly, in large drive hunts (which are then also societal events) with some hundred or thousand hunted pieces of wild game, it is a logistic challenge to ensure proper cooling, and timely and hygienic evisceration immediately after the event. In these instances, 2-3 smaller consecutive drive hunts with smaller hunting bags could allow a more effective game meat hygiene management.

EU legislation specifies maximum meat temperatures of +7 °C for large and +4 °C for small game (EC, 2004). However, to keep microbial numbers under control, best practice requires cooling room temperatures of ca. 0 °C, as already suggested by Lerche (1957), the rationale of which has more recently been confirmed in studies on carcasses as well as vacuum-packaged meat cuts (e.g. Paulsen *et al.*, 2005b; El-Ghareeb *et al.*, 2009; Fettinger *et al.*, 2010b). It is not exactly specified how long the period between killing and onset of refrigeration should be; national guides specify e.g. 12 h (Winkelmayer *et al.*, 2008). In the 1970´s, it was realistic

Table 1. Time from killing to evisceration, large game (% of carcasses).

<1 h	1-3 h	>3 h	n=	Reference
17.5	75.7	5.8	103	Riemer and Reuter, 1979
82.9	15.8	1.3	234	Brodowski and Beutling, 1998
96.5	2.0	1.5	195	Deutz *et al.*, 2000 (still hunt)
0.0	72.9	27.1	37	Deutz *et al.*, 2006 (drive hunt)

to expect that the majority of carcasses would be cooled to 0-2 °C within 24 h after killing (Riemer and Reuter, 1979). More recent studies demonstrate that the 12 h requirement is easy to meet (see Brodowski and Beutling, 1998; Deutz *et al*., 2000).

A 'pre-cooling' phase, as generally recommended for carcasses from slaughter animals in earlier literature (Postolka, 1922; Ostertag-Schönberg, 1955; Lerche *et al*., 1957), can be useful for preserving meat quality in slow-glycolysing muscles, and will allow the evaporation of moisture and subsequent drying of body cavities (Prändl *et al*., 1988). This is particularly useful when the carcasses are subsequently hung in refrigeration rooms with poor ventilation (as is the case for most small-scale units). This can, however, have negative consequences with respect to microbial surface contamination (Paulsen and Winkelmayer, 2004). Abandoning the pre-cooling phase requires professional cooling facilities with strong ventilation and the ability to remove moisture effectively. The necessity of an easy access to cooling facilities has been emphasised before (Riemer and Reuter, 1979; Brodowski and Beutling, 1989).

1.3 Panta rhei

'Good practice' as defined today can be obsolete within a few years, and the subchapter above has presented some already historical examples. Quite recent examples are studies conducted on the shelf-life of unviscerated carcasses of pheasants (Paulsen *et al*., 2008) and hares (Paulsen *et al*., 2005a) stored at +4 °C. The authors concluded that a storage for 3 days would result in acceptable total aerobic counts, with *E. coli* number remaining <10 cfu/g muscle tissue. Consequently, a recommendation to eviscerate refrigerated pheasants and hares not later than at day 3 *post mortem* was included in a textbook on game meat hygiene (Winkelmayer *et al*., 2007). However, practical experience with marketing of hares in the year 2009 showed that meat cuts from such carcasses, although not objectionable from a microbiological point of view, were already tainted, that shelf-life was impaired and thus that this meat is not easily accepted by supermarkets. Also, fermented sausages made of such meat were sensorily deterioriated. In this case, it proved to be best practice to eviscerate carcasses within 24 h *post mortem*.

2. Microbiology of meat from wild game

This section will – in conformity with EU microbiological food hygiene criteria (EC, 2005) – focus on total aerobic counts, Enterobacteriaceae and *E. coli*. Other bacteria, as micrococci, enterococci and clostridia, which can be found in large numbers on game carcasses (Riemer and Reuter, 1979; Bandick and Ring, 1995; Bandick and Ring, 1996; Schiefer, 1996) or meat cuts (Kniewallner *et al*., 1969; Kobe and Ring, 1992) and also pathogenic and zoonotic bacteria (Dedek and Steineck, 1994; Schiefer, 1996) will not be dealt with in detail. Also, the reader should not expect a comprehensive overview on all literature reports published in the last decades; this book chapter is rather somewhat like a round trip through a meat hygienist´s book shelf, section 'hygiene and microbiology', subsection 'wild game'.

Large game and small game, differing in the mode they are killed and handled *post mortem*, will be discussed separately, where appropriate.

2.1 Microflora of live game/uneviscerated carcass

Muscle tissue of healthy slaughter animals is generally considered as virtually sterile (Nottingham, 1982), and the same can be expected for game (Ring *et al.*, 1988; Scherling and Ring, 1989; Schiefer, 1996; Paulsen *et al.*, 2003). Exposed surfaces, as skin, hair/fleece or feathers and hooves, and also intestinal content will harbour a number of microorganisms, and for slaughter animals, the significance of the sources of microbial contamination has been quantified quite early, e.g. Lawrie and Ledward (2006) cite references from the period 1939-1941 on that subject. Skin/hide of slaughter animals can harbour from 3.5 to >10 log cfu/cm^2 (Ayres, 1955; Bell, 1997; Small *et al.*, 2005; Antic *et al.*, 2010), which has always been a matter of concern for food hygienists. Some studies demonstrate that for cattle, microbial transfer skin/hair to meat will be only 1% or less (see Antic *et al.*, 2010), but considering the high microbial concentration on hides and the potential presence of pathogens, microbial transfer is regarded as a significant problem.

Visually 'clean' cattle resulted in lower microbial numbers on the carcass (e.g. Reid *et al.*, 2002). With the emergence of enterohemorrhagic *E. coli* and the finding that cattle is the main reservoir, the role of cattle skin was re-examined thoroughly (Reid *et al.*, 2002; Nastasijevic *et al.*, 2008; Antic *et al.*, 2010) and the efficacy of different decontamination techniques assessed (Small *et al.*, 2005). These findings should be applicable to wild game also.

Interestingly, there are few data on the microbial load of skin and hair or feathers of wild game. For example, El-Ghareeb *et al.* (2009) examined various game bird species (partridge, pheasant, pigeon, quail) and reported total aerobic counts in the range of 2-6.5 log cfu/cm^2 skin/feathers, and median numbers of Enterobacteriaceae, *E. coli*, and *Staph. aureus* in the range of 2-3 log cfu/cm^2. It seems that, to-date, data on enterohemorrhagic *E. coli* on hides of wild ungulates are lacking, despite the evidence that such species constitute a natural reservoir (Bartels and Bülte, 2011). Admittedly, more data have been published on the presence of pathogens in the digestive tract, e.g. in tonsils and feces (e.g. for wild boar: Wacheck *et al.*, 2010).

2.2 Contamination at primary production level (shooting, evisceration and cooling)

2.2.1 Killing

It is well established for farmed game at slaughter, that captive-bolt stunning and sticking introduce bacteria in the bloodstream (see Lawrie and Ledward, 2006). However, there seems to be some antibacterial activity in *post mortem* tissues, as demonstrated by Gill and Penney (1979): bacteria experimentally inoculated in the bloodstream of animals before slaughter were disseminated in the body, but their numbers decreased in the following hour, or it took several hours before microorganisms started to multiply. Recently, and in the light of BSE, hematogenic dissemination of bacteria inoculated *via* the captive-bolt wound (Buncic *et al.*, 2002; Daly *et al.*, 2002; Prendergast *et al.*, 2004) has been studied as a model for CNS spread. It has become clear that the failure to recover artificially inoculated bacteria may to some extent be a problem of the concentration of the inoculum. With respect to large game killed

by a shot in the anterior chest, it may be argued that the massive loss of blood through severed major arteriae will wash away contaminants rather than transport them into deep vessels.

The mode of killing of large game and location of the shot wound have a distinct influence on the microbial numbers in deep muscle tissues (Lenze, 1977; Ring *et al.*, 1988; Deutz *et al.*, 2000). Adominal shot lesions are significantly associated with visible contamination of the abdominal cavity and exposed muscles (Deutz *et al.*, 2000; Paulsen *et al.*, 2003). For carcasses sampled 12-24 h after killing, Deutz *et al.* (2006) reported median and maximum TACs of 4.6 and 5.6 log cfu/cm^2 for thoracic shot wounds compared to 5.0 and 6.5 log cfu/cm^2 for abdominal lesions, respectively. Also, Paulsen and Winkelmayer (2004) and Langrange and Schmidt (2005) found higher surface counts for TAC, Enterobacteriaceae and *E. coli* in game with abdominal shot wounds, approximately in the same order of magnitude.

Drive hunts for large game are associated with a higher frequency of abdominal shot lesions and longer time to evisceration. However, it depends on the logistics of evisceration and cooling whether numbers of indicator bacteria are higher on such carcasses compared to those obtained from still hunts (Deutz *et al.*, 2006).

2.2.2 Evisceration

Evisceration of carcasses without undue delay is stipulated in legislation, but there is some evidence that, if intestines are not ruptured or otherwise damaged, bacteria will colonise muscle tissues quite slowly and that sensory deterioriation ('greening') will occur before microbial spoilage. Gill *et al.* (1976, 1978) have presented evidence for this hypothesis. In the case of small game, it was a traditional procedure to hang uneviscerated carcasses for days to weeks at near ambient temperature, and earlier studies (Barnes *et al.*, 1973; Mead *et al.* 1973, 1974) demonstrated that storage of uneviscerated pheasants at temperatures of 10 °C or less for several days does not necessarily result in sensory deterioriation or bacterial spoilage, unless the intestines are perforated and fecal material is released into the body cavity (similarly as observed for lambs, Gill *et al.*, 1978). Also, Paulsen *et al.* (2008) reported that during the storage of uneviscerated, hunted pheasants at 0 °C and 3-4 °C, total aerobic counts increased significantly during storage, but the absolute numbers remained below 6 log cfu/g. Whereas *E. coli* were <1 log cfu/g in muscles of hunted pheasants on day 3 at 4 °C, counts of up to 3.7 log cfu/g on day 7 at 4 °C indicate a loss of hygienic quality. Therefore, the authors recommended that hunted uneviscerated pheasants could be stored 3 days at 4 °C, but not 7 days or more after the hunt.

For hares, Meyer-Ravenstein *et al.* (1976) reported that eviscerated carcasses stored at 4 °C are more susceptible to spoilage than uneviscerated ones. From results reported by Heinz *et al.* (1977), who compared eviscerated and uneviscerated hares stored at 4 °C and at ambient temperatures (10-20 °C), it can be concluded that cold storage of uneviscerated carcasses tends to yield lowest bacterial counts on muscle surfaces, but sample numbers per group are low (n=5) and results cover a wide range (>2 log for most groups).

2.2.3 Surface microflora on wild game carcasses in the first 24 h p.m.

Atanassova *et al.* (2008) sampled 289 freshly shot large game specimens, comprising 127 wild boar, 95 roe deer and 67 red deer, and reported average TACs of 3.2, 2.9 and 2.6 log cfu/cm^2, respectively. Average Enterobacteriaceae concentrations were 2.2 log cfu/cm^2.

Under well-defined conditions (average time to evisceration: 42 min, average time to cooling: 3.1 h), median TAC values for 62 carcasses were 5.4 and 5.6 log cfu/cm^2 for abdominal cavity and thighs (Deutz *et al.*, 2000). The latter report also mentions higher values on 61 carcasses sampled at a game handling establishment (5.8 and 6.3, respectively). For Enterobacteriaceae, *E. coli*, and Staphylococci, a similar tendency was found.

Riemer and Reuter (1979) sampled 89 large game carcasses and reported TAC <2 log cfu/g deep muscle tissue in 53% of samples, and for the majority of the remaining samples counts <4 log cfu/g. Enterobacteriaceae (median <3 log cfu/g) and *E. coli* (median <2 log cfu/g) were detected in 11 and 12 of 89 carcasses, respectively.

2.2.4 Microbial contamination and visual assessment of 'cleanliness' of carcasses

The relation of visual assessment and microbial contamination of carcass surfaces has been studied by Deutz *et al.* (2003) at the level of 'trained persons' and by Paulsen *et al.* (2003) for veterinary meat inspectors. Deutz *et al.* (2003) classified carcasses according to contamination of body cavities and other alterations (such as emaciation, fractures, discolouration) in four categories, with the two best categories (i.e. not requiring veterinary intervention) being characterised by TACs <6 log cfu/cm^2 and *E. coli* counts <1 log cfu/cm^2. Interestingly, this correlated rather well with the finding of Paulsen *et al.* (2003), who examined roe deer carcasses (n=100) at the entry point of a cutting plant. The results were compared to approval/condemnation based on visual and olfactory examination performed by the meat inspecting veterinarian: 88% of the heavily contaminated carcasses (6 or more log cfu TAC/cm^2 abdominal muscle) were condemned or conditionally approved. Despite of microbial surface contamination, microbial growth was detected in only one core of 100 *extensor carpi radialis* muscles. Average TACs of abdominal muscles of 7.6 log cfu/cm^2 for visually contaminated abdominal cavities were found as compared to 5.3 log cfu/cm^2 for visually clean abdominal cavities. Respective numbers for Enterobacteriaceae were 5.1 and 3.5 log cfu/cm^2. From swabs of the thoracic and abdominal cavity, *E. coli* were isolated from 76 carcasses.

In addition to the previously described factors influencing the exent of microbial contamination, the time/temperature profile from killing to cooling is often underestimated. Paulsen and Winkelmayer (2004) found that 'pre-cooling' phases (ca. 12 h) at ambient temperatures of ca. 10 °C and 18 °C were associated with median TAC values of 4.1 as compared to 5.7 log cfu/cm^2. Respective numbers for Enterobacteriaceae were 2.5 and 3.5 log cfu/cm^2. Subsequent cooling at 0.4 °C allowed no microbial growth for 96 h, but differences in microbial numbers remained. This also demonstrates the need for a continuous cool chain.

2.2.5 Microbial limits for carcasses of wild game?

The data presented above raise the question, whether or not microbiological limits for game carcasses should be defined and if they should be in the range as given for slaughter animals or higher. To date, there are no standardised sampling methods for wild game (swabbing-destructive; surface-deep tissue, sampling site, step of the food chain). From various literature sources, recommendations have been collated in Table 2. Notably, these values are in the upper range or maximally 1 log unit higher than those for slaughter carcasses.

2.3 Microflora of meat cuts

2.3.1 Microflora of meat cuts: surveys and experimental findings

A number of studies reported that market samples from meat cuts from wild game have higher microbial numbers than those from farmed animals (Kniewallner, 1969; Baur and Reiff, 1976; Kobe and Ring, 1992; Bandick and Ring, 1996). As concluded by Schiefer (1996), surface counts of 7 log cfu/cm^2 are not uncommon, but meat cuts from farm animals can harbour similar numbers of bacteria on their surface (Kobe and Ring, 1992). In deep muscle tissue from wild game, total aerobic counts have been reported to be quite variable, i.e. from <2 to >5 log cfu/g (Kniewallner, 1969). A later study reports numbers to be significantly higher than in those from farm animals (Kobe and Ring, 1992; see also Table 3). In particular, the numbers of Enterobacteriaceae and *E. coli* (Kobe and Ring, 1992) or coliforms (Kniewallner, 1969) can be quite high. It is sometimes not easy to compare data, particularly those from older studies, which used semi-quantitative methods for assessment of bacterial contamination (e.g. Baur and Reiff, 1976).

Recent studies, conducted at different steps of producing and placing of the market of meat from wild game, still demonstrate a wide variation of results, but allow the conclusion that average TACs, Enterobacteriaceae and *E. coli* numbers of 3-4, 2-3 and 1-2 log cfu/g, respectively, can be achieved under GHP conditions.

In frozen meat cuts sampled in German game handling establishments, average TACs from 4.0-6.4, and Enterobacteriaceae and *E. coli* counts of 2.0-6.0 and 1.0-3.1 log cfu/g, respectively, were reported (Türck, 2008; Wacheck, 2008; Table 4).

Table 2. Suggested microbiological limits for wild game carcasses (log cfu/g), derived from various literature sources.

TAC	Enterobacteriaceae	*E. coli*	Based on
6.0	-	2.0	Deutz *et al.*, 2003 (non destructive sampling)
6.0	-	-	Paulsen *et al.*, 2003 (destructive sampling)
5.0	3.0	2.0	Lagrange and Schmidt, 2005 (destructive sampling)

Table 3. Microbial counts (total aerobic counts and Enterobacteriaceae) in game meat cuts; studies conducted in Germany and Austria (data are mean ± standard deviation or median plus range).

Sampling	n	TAC (log cfu/g)	EB (log cfu/g or cm^2) or coliforms*	Sensory assessment	Reference
Surface	62	7.33 (4-8.68)	5.48 (2-6.6)*	Not objectionable	Kniewallner, 1969
Surface	19	6.68±1.01	4.32±1.13	Not objectionable	Kobe and Ring, 1992
Surface	35	7.39±1.03	4.67±0.88	Objectionable	Kobe and Ring, 1992
Mix	103	6.29 (4.48-7.56)	5.1 (2.3-6.36)	Not objectionable	Paulsen *et al.*, 2005
Deep tissue	24	4.28 (<2-5.4)	4 (<2-5.38)*	Not objectionable	Kniewallner, 1969
Deep tissue	19	4.25±1.44	0.71±1.18	Not objectionable	Kobe and Ring, 1992
Deep tissue	35	5.72±0.98	1.93±1.49	Objectionable	Kobe and Ring, 1992

Table 4. Microbial counts in game meat cuts; recent studies conducted in Germany and Austria (data are mean or median plus range).*

Samples	Condition	n	Obtained at[1]	TAC (log cfu/g)	EB (log cfu/g)	*E. coli* (log cfu/g)	Reference
Hare	frozen	79	GHE	4.0 (2.2-6.2)	2.0 (1.0-3.8)	-	Türck, 2008
Hare	frozen, larded	85	GHE	4.0 (3.4-8.3)	2.7 (1.0-6.0)	-	Türck, 2008
Roe deer	frozen	44	GHE	5.7 (3.5-7.3)	3.6 (1.0-6.3)	2.3 (1.0-3.8)	Türck, 2008
Red deer	frozen	49	GHE	4.3 (1.1-6.6)	2.2 (1.0-4.2)	1.7 (1.0-3.7)	Türck, 2008
Wild boar	frozen	224	GHE	6.0 (2.6-8.6)	3.5 (1.0-5.8)	1.0 (1.0-4.9)	Türck, 2008
Various	frozen	63	GHE	6.4* (4.3-7.3)	6.0* (4.3-7.0)	3.1* (2.0-4.4)	Wacheck, 2008
Various	chilled, vac.	30	LSH-sc	4.4 (2.0-7.7)	2.3 (1.0-5.7)	1.0 (1.0-3.5)	Fettinger, 2011
Various	chilled	43	LSH-training	5.1 (3.0-7.7)	2.7 (2.0-5.7)	1.0 (1.0-3.0)	Fettinger, 2011
Roe deer	fresh after cutting	49	Experimental from visually contaminated carcasses	5.7	3.9	2.0	Irschik, unpublished data

[1] GHE: sampled at game handling establishments; LSH-sc: microbiological self control samples from hunters supplying directly to consumers; LSH-training: samples taken at training courses for supplying directly to consumers.

For some species, Türck (2008) observed significant differences in microbial contamination of different meat cuts. Similar observations have been reported by Kniewallner (1969) for venison and Heinz *et al.* (1977) for hares.

In 30 vacuum-packed fresh meat cuts obtained from hunters supplying directly to the consumer, Fettinger (2011) reported median counts of 4.4, 2.3 and 1.0 log cfu/g for TAC,

Enterobacteriaceae and *E. coli*, respectively. Similar results have been reported from training courses where hunters were cutting carcasses and packaging meat (Fettinger, 2011).

Meat cuts (n=103) from roe deer stored at 3.5 °C were characterised by 5.8 log cfu/g TAC and 4.0 log cfu/g Enterobacteriaceae (median values, all 132 h *post mortem*) (Paulsen *et al.*, 2005). This study included freshly prepared meat cuts as well as vacuum-packed stored meat cuts.

Studies as those mentioned above are based on carcasses where the initial microbial contamination of the carcass and contributing factors (e.g. time-temperature history, time to evisceration, visible contamination and location of the shot wound, see above) are not well-defined. Few studies have been published with the aim of explaining microbial numbers to be expected under 'good' or even 'best' practice options.

For roe deer (n=6), Paulsen (2005) reported average TACs of 4.3 and 5.1 log cfu/g for meat cuts and comminuted meats, when visibly clean carcasses were kept at 3 °C for 3-7 days and processed under hygienic conditions, whereas hygiene deficiencies resulted in log 2.5-3.5 higher microbial numbers.

For various game bird species, El-Ghareeb *et al.* (2009) studied the implementation of Good Hygiene Practice in the cutting process and concluded that median counts of 3-4, <2-2.3 and <1-1.5 log cfu/g are to be expected for TACs, Enterobacteriaceae and *E.coli*, respectively for a number of species. Somewhat higher numbers (and subsequent reduced shelf-life) were reported for pigeon (see Table 5). Fettinger *et al.* (2010b) studied GHP procedures for meat cuts of hare and reported similar results. Interestingly, these data are in the same range as reported previously for frozen hare meat imported from Argentina (Heinz *et al.*, 1977). Pecularities for small game, as frozen storage of uneviscerated carcasses (Heinz *et al.*, 1977; El-Ghareeb *et al.*, 2009) and freezing-thawing-freezing cycles during processing of hare (Türck, 2008) are not necessarily associated with higher microbial contamination of meat, but seem somewhat archaic in the light of modern food hygiene.

Table 5. Microbial counts in meat cuts from small game, obtained under GHP conditions (data are median plus range).

Samples	n	TAC (log cfu/g)	EB (log cfu/g)	*E.coli* (log cfu/g)	Reference
Hare thighs	20	3.4 (3.0-5.0)	2.6 (2.0-2.9)	-	Fettinger *et al.*, 2010b
Hare longissimus	6	3.0 (3.0-4.4)	2.9 (2.0-4.3)	-	Fettinger, unpublished data
Partridge breast	8	2.9 (2.0-3.9)	2.0 (2.0-4.0)	<1 (<1-4.1)	El-Ghareeb *et al.*, 2009
Partridge thighs	8	3.1 (2.0-5.3)	2.0 (2.0-4.5)	<1 (<1-4.5)	El-Ghareeb *et al.*, 2009
Pigeon breast	9	4.1 (3.0-6.0)	2.3 (2.0-5.3)	1.7 (<1.0-3.5)	El-Ghareeb *et al.*, 2009
Pigeon thigh	9	4.0 (3.3-5.7)	3.3 (<2-5.0)	3.0 (<1-4.0)	El-Ghareeb *et al.*, 2009
Quail muscles	25	4.0 (2.0-6.5)	<2 (<2-4.2)	<1 (<1-4.1)	El-Ghareeb *et al.*, 2009
Pheasant breast	32	3.7 (2.0-5.5)	2.0 (2.0-5.3)	1.0 (1.0-4.0)	El-Ghareeb *et al.*, 2009
Pheasant thigh	32	3.8 (2.3-5.5)	2.3 (2.0-5.3)	1.5 (1.0-4.0)	El-Ghareeb *et al.*, 2009

Generally, storage temperatures of ca. 0 °C afford keeping microbial numbers on vacuum-packed meat cuts nearly constant for at least one week (e.g. El-Ghareeb *et al.*, 2009 for wild birds; Fettinger *et al.*, 2010b, for hares; Irschik, unpublished data, for roe deer). This is of course not unexpected, but at higher temperatures multiplication of bacteria may be significant, e.g. as reported by Fettinger *et al.* (2010b) for hare meat stored at +4 °C (i.e. the maximum legally allowed temperature).

2.3.2 Sensory assessment and extent of microbial contamination

The relation of microbial numbers to sensory characteristics of wild game meat has been under study for quite a long time. Kniewallner (1969) and Türck (2008) could not establish a clear relationship, whereas Kobe and Ring (1992) found that average TACs of >7 log cfu / cm^2 surface or >5 log cfu/g deep tissue were associated with sensory deterioriation.

The relation to other parameters indicating protein degradation and thus ageing or the onset of spoilage was not convincing (Paulsen *et al.*, 2008; Türck, 2008).

Similarly, only a weak (Kniewallner, 1969) or no correlation (Baur and Reiff, 1976; Heinz *et al.*, 1977; Kobe and Ring, 1992) of pH values to microbial numbers has been found.

2.3.3 Microbial limits for carcasses of wild game?

To date, there are no explicit microbial limits for fresh meat from game, with the exception of generally applicable food safety criteria as laid down in EU legislation on microbiological criteria on foodstuffs (EC, 2005). Considering that meat from game is placed on the market as any other meat from farmed animals, it seems reasonable to adopt limits for fresh meat from farm animals. These may be included in national legislation or expertises (compiled by Eisgruber and Bülte, 2006), or part of quality meat programmes, e.g. the 'Agrarmarkt Austria' program (AMA, 2010). This approach was followed by two recent studies conducted at game handling establishments (Wacheck, 2008) and 'local supply' level (Fettinger *et al.*, 2010a). Basically, the evaluation scheme required that food safety criteria (pathogenic bacteria) had to be fulfilled, whereas for other microbial indicators pertaining to quality and shelf life, the limits would serve as guidance values only. Exceeding these guidance values would indicate that improvements in the game meat chain are necessary (example see Table 6 and Fettinger *et al.*, 2010a). Some recommendations are compiled in Table 7.

The pronounced seasonality in game meat production, and the small-scale of the operation plants and resulting poor standardisation of processes may contribute to the large variation of microbial numbers reported for game meat cuts. This seasonality and discontinuous production has become untypical for most meat species in Austria, maybe with exception of geese and sheep. However, this mode of production always bears some risk of high microbial numbers, e.g. Loncaric *et al.* (2009) reported 7.1±0.9, 3.3±2.2 and 1.3±1.2 log cfu/g for mutton cuts sampled at retail level in Austria.

Table 6. Microbial limits for game meat and products as used in a pilot trial for hunters supplying to the consumer (Fettinger, 2010a). Example: vacuum-packed meat (AMA, 2010; Eisgruber and Bülte, 2006).

Category 2 (fresh, vac-packed meat)	Tolerance level	Absolute limit
Listeria monocytogenes (cfu/g)	-	<100
Listeria monocytogenes in 25g	-	n.d.
Salmonella sp. in 25g	-	n.d.
Sulphite reducing anaerobes (kbE/g)	-	-
Staphylococcus aureus (cfu/g)	-	-
Bacillus cereus (cfu/g)	-	-
TAC (cfu/g)	5,000,000	-
Escherichia coli (cfu/g)	50	500
Enterobacteriaceae (cfu/g)	1000	-
Lactobacillus (cfu/g)	5,000,000	-

Table 7. Suggested microbiological limits for meat cuts from wild game (log cfu/g), derived from various literature sources.

TAC	Lactic acid bacteria	*Pseudomonas*	*E. coli*	Enterobacteriaceae	*C. perfringens*	*S. aureus*	Reference
6.7	-	-	-	-	-	-	Bandick and Ring, 1996
6	6	6	2.7		4	3	Wacheck, 2008
6.7	6.7	-	1.7	3	-	-	Fettinger, 2011

4. Conclusions

What has been achieved?

In the last 50 years, principles of slaughter hygiene have been adopted for wild game meat production, with respect to evisceration, cooling and the implementation of *ante* and *post mortem* meat inspection. Under these principles, it has been proven that it is entirely possible to produce skin-on large game carcasses with TACs not exceeding 6 log, and *E. coli* <2 log cfu per cm^2 on exposed muscle surfaces. Similarly, it can be expected that the resultant meat cuts should not contain >6 log TAC and >2 log *E. coli*/g meat tissue. For small game, traditional *post mortem* handling techniques (i.e. hanging of uneviscerated carcasses for several days; insufficient cooling) are increasingly replaced by rapid cooling and evisceration, and skinning within 24 h after killing.

What has been neglected?

Conditions at primary production level are still poorly standardised which accounts for a wide range of bacterial contamination on carcasses. This may be due to a lack of manual skills, organisation, infrastructure or simply ignorance. Continuing education of primary producers, currently on a voluntary basis in Austria, should be emphasised. Likewise, sampling strategies and techniques along the game meat chain are very diverse, which complicates comparisons of results.

What needs to be done?

Guides to good practice have been developed according to EU food hygiene legislation, but the application in practice should be enforced. Despite the variety of hygiene improvements suggested, stipulated and – in part – adopted in the last decades, average microbial counts on carcasses and meat have not declined in the order of magnitude expected. Although data exist on the prevalence of pathogens in the digestive tract, not much is known about the extent of transfer of these pathogens to the carcass and finally, to meat cuts. This is a crucial issue, as animals may be symptomless carriers and go undetected during *ante* as well as *post mortem* inspection.

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Essential food safety management points in the supply chain of game meat in South Africa

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Summary

Although the game industry is expanding in all areas, the most essential food safety management points in the supply chain of South African game meat has not yet been described. In order to determine the management points it was necessary to determine the supply chain and then to identify the most essential food safety management points. Information to better understand the supply chain and relevant food safety management aspects was obtained through a desk top study, observation of processes from farm to consumer in the local market as well as during export activities and analysis of questionnaire responses from game farmers, hunters, butcheries and municipalities. The description of essential food safety management points in the supply chain can assist policy-makers; law enforcers and the industry to establish and implement management programmes that will ensure a safe game meat product to the consumer.

Keywords: supply chain, traceability, sustainable utilisation, hunting, harvesting

1. Introduction

In South Africa, domestic stock has always been a major social, economic and livelihood support factor to the farmers and the people of the country. However, the process of desertification in Africa has produced an environment that can no longer support the increased numbers of domestic cattle economically (Van Schalkwyk, 2004). As a result, South African farmers were faced in the 1980s with finding alternative economic solutions for their increasingly marginalised land (Bothma, 2002a). However, from an economic perspective, extensive cattle farming is more efficient in terms of meat production than game farming and the total biomass of bovine culled per year is higher than that of game (Dekker and Van Wyk, 2002). Skinner (1970) also indicated that game animals are unlikely to compete directly with domestic animals as meat producers as they are not as efficient in converting feed into live weight and at that time doubts remained concerning disease and their management. In addition, wild animals were usually seen negatively, e.g. as crop raiders/pests or undesirable competitors for grazing that could be best used to produce domestic livestock (Bothma, 2002b; Bothma, unpublished data). On the other hand, Cloete *et al.* (2007) pointed out that game ranching can be more profitable, i.e. generate a higher gross margin per hectare than cattle.

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Africa has a high density and diversity of large wild herbivores, of which South Africa has some 300 different species of mammals (ABSA, 2003). In South Africa, as is in other countries with abundant wildlife, the national economic value of wildlife has traditionally been seen in terms of its potential to generate revenue through tourism, i.e. photographic tourism by overseas and local visitor's and hunting (Campbell *et al*., 2001). It has since been discovered that wildlife ranching is more profitable than domestic livestock farming in many parts of the country, mainly because it produces not only meat and other consumptive and non-consumptive products (ABSA, 2003) such as accommodation and service fees with a higher return potential (Dekker and Van Wyk, 2002), but it is starting to become a vehicle for conservation based rural community development (Bothma, unpublished data). Whilst conservation and development may be seen as conflicting agenda, attempts are nevertheless made to bring wildlife conservation closer under the general umbrella of sustainable development (Meadows *et al*., 1992; Traffic, 2008). Game farmers in South Africa now play a key role in the conservation of many game species (Ebedes, 2002). As the economic potential became clear, many South African farmers moved away from conventional agriculture towards wildlife production units and as a result of this, wildlife ranching has developed rapidly to become a multifaceted multi-million Rand industry with tremendous conservation benefits (Bothma, 2005). South Africa has now become a world leader in the sustainable extensive conservation and utilisation of game species (Ebedes, 2002). Many cattle farmers are changing over to game farming (Cloete *et al*., 2007; Carruthers, 2008) and it is now widely recognised that game farming is the fastest growing agricultural industry in South Africa. As a result game numbers seem to have increased and many livestock ranches carry enough wildlife to allow their commercial exploitation (Carruthers, 2008). The game industry as it is today provides for a variety of opportunities of utilisation of game that the farmers in the game industry can benefit from. Figure 1 illustrates the main sectors in the game industry.

Although these different sectors exist, this chapter will mostly discuss the food safety management points for meat production through harvesting and hunting of wild game. The term 'venison' refers to the meat of cervids, of which the red deer (*Cervus elapphus*) is the only true indigenous one in Africa (specifically North Africa). However, since the term 'game meat' is used most frequently in South Africa, it will be used for the purpose of this chapter.

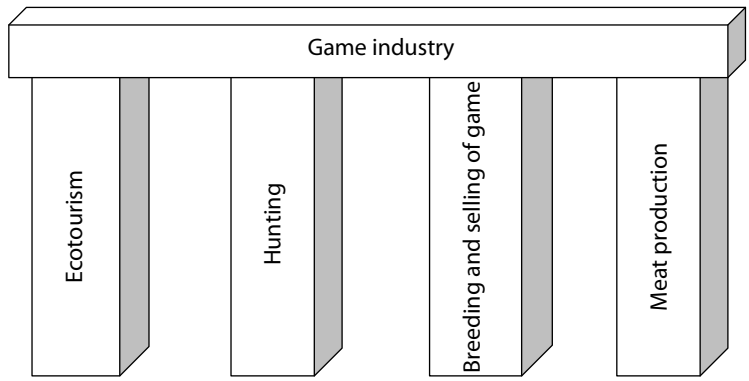

Figure 1. Sectors in the game industry.

Game meat has been recognised as a source for meat because:

- Farmers realised that wild ungulates in Africa, when managed properly could out-produce domestic animals in producing red meat per hectare (Skinner, 1984) and that game farming could supplement other forms of meat (Skinner, 1970).
- Game meat does not have a high fat content, because there is little marbling in the muscle (Aidoo and Haworth, 1995; Hoffman, 2008). It is also low in cholesterol, which forms an essential constituent of all cells of animal origin (Higgs, 2000). Game meat complies with the nutritional guidelines that place an increasing emphasis on reducing the ratio of n-6/n-3 polyunsaturated fatty acids in the diet (Hoffman *et al*., 2005). This was shown to be favourable with respect to decreasing the risk of coronary heart disease.
- African game meat is considered to be an organic product since the animals are wild and free-roaming (Hoffman and Wiklund, 2006). The meat is free of growth hormones and other artificial or chemical substances since no dipping takes place and no fertilisers or growth stimulants are used in the production system (Radder, 2002; Hoffman and Wiklund, 2006).

Consequently, alternative sources of meat, such as wildlife are increasing in popularity (Hoffman, 2003; Webb, 2003). The meat derived from game is consumed by the immediate family, sold locally in order to provide cash income, or exported as a sought-after delicacy, especially in Western Europe (Skinner, 1984). A study by Hoffman (2003) indicated that the majority (73%) of consumers in the Western Cape Province of South Africa had eaten game meat before. Most of these consumers had purchased the game meat in butcheries and then prepared it at home. Another study by Hoffman *et al*. (2003) indicated that game meat is very popular with tourists (Belgium's, Germans and Americans), not only as an African product but as a healthy product. The majority (82%) of tourists were aware of the health benefits of wildlife meat. Further, 92% of them indicated that they eat game meat when on tour and 88% when they are at home. Tourists also regard game meat as part of an African experience (Du Buisson, 2006). In the 2005 season it was estimated that South Africa exported the deboned meat from 160,000 game carcasses (Hoffman and Wiklund, 2006). However, due to the global economic climate, this number had decreased due to a lower international market demand to slightly over 76,000 in 2009 which was lower than the 85,500 of 2008.

The term 'food supply chain' is usually reserved for the total supply process from agricultural production, harvest/slaughter, through primary production and/or manufacturing, to storage and distribution to retailers or for use in catering and consumer practice (Stringer and Hall, 2007).

Game meat from game parks, big hunting farms and approved game abattoirs is finding its way to the consumer's table through the formal processes or structures. However, informally hunted game meat has also found a place in the local supply chain since 60-65% of the total income from game farming is generated from trophy and meat hunting (Van der Merwe, 2005). In identifying essential food safety management points and developing food safety interventions for game meat control purposes it is critical to understand the dynamics of the supply chain from the farm to the fork that also include the export of game meat to relevant countries. The purpose of this chapter is to use the South African supply chain for game meat as a model to describe essential food safety management points in the supply chain. A clear

understanding of the food safety management points can assist policy makers, law enforcers and the game meat industry to establish and implement management programmes that will ensure a safe game meat product to the consumer.

2. Methodology

Information to better understand the supply chain of game meat in South Africa was obtained through a study that consisted of (1) a desktop review of relevant subject material; (2) observations made during harvesting and hunting events and (3) the design of separate questionnaires for the respective target groups. The questionnaires, which were distributed to local game farmers, hunters, butcheries and municipalities (local authorities) were designed to obtain information regarding their involvement and related activities in preventative and legislative measures to ensure safe game meat. In designing the questionnaires international and local legislation and standards was taken into consideration.

The questionnaires were distributed to game farmers and hunters through their respective associations which form the official mouthpieces of game farmers and hunters in South Africa. Questionnaires to butcheries were distributed by senior students registered for the National Diploma: Environmental Health at the seven universities in South Africa that offer the course. After clear instructions were provided to students, the questionnaires were distributed to butcheries during their compulsory in-service training with municipalities. The questionnaire to municipalities was distributed electronically to the heads of the Environmental Health departments of all the metropolitan municipalities (n=6) and district municipalities (n=41) in South Africa.

A total of 139 game farmers, 290 hunters and 361 butcheries completed the questionnaires. Although two of the six metropolitan authorities submitted their responses per region in their metropolitan jurisdiction (metro 1 = 8 regions and metro 2 = 7 regions), all six metropolitan authorities responded. This resulted in a total of 19 questionnaire responses being received from the metropolitan areas. Twenty eight (68%) district municipalities completed the questionnaires. Consequently, the total n-value for the municipalities was 47 and a distinction between the metropolitan and district municipalities will not be made. The quantitative questionnaire responses were coded and analysed using the SPSS statistical software. Qualitative data were analysed using content analysis. This involved identifying specific *a priori* and emergent issues on South Africa that focus on the supply chain of game meat. Descriptive statistics were calculated to summarise the results in tables by means of frequencies and summary proportions.

3. Game meat supply chain

The game meat supply chain differs from the supply chain of meat from domesticated animals in that game animals are killed and partially dressed on the game farm by removing the viscera of the thorax and abdomen, the head, feet, reproductive organs and lactating udders.

Based on this difference and observations made during the study, Figure 2 was compiled to depict the supply chain for game meat in South Africa.

Figure 2 clearly illustrates that the supply chain for game meat in South Africa involves several role-players (including relevant authorities). The essential food safety management points for the different steps of the supply chain in Figure 2 are systematically described in the following sections with numbers corresponding to those in Figure 2.

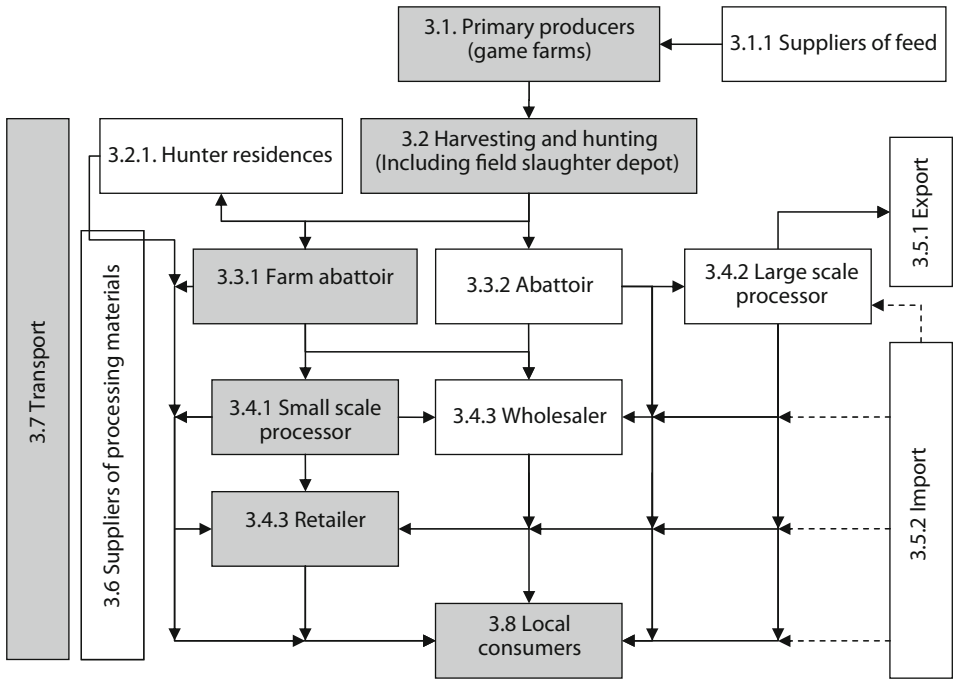

Figure 2. Game meat supply chain in South Africa.
Legend:
***Abattoir** refers to an abattoirs approved for local and/or export purposes and where the slaughtering of partial dressed carcasses is completed.*
***Farm abattoir** refers to an abattoir not necessarily approved by the relevant authorities and where the slaughtering of game carcasses is done for own consumption or the local market.*
***Large-scale processor** refers to a processor approved by the relevant authorities for local and/or export purposes and where value adding is done.*
***Small-scale processor** refers to a processor not yet approved by the relevant authorities and where value adding is done on a small scale for own consumption or the local market.*
***Wholesaler** refers to an enterprise that trades in the local market with approved carcasses and/or value-added products procured from approved abattoirs as well as large- and/or small-scale processors.*
***Retailer** refers to an enterprise such as a local or supermarket butchery, biltong shop, meat selling point (no processing), restaurant (including hotel), home industry, arts and crafts market, flea market, farmer market, or any other locality where the raw and/or processed and/or cooked product is made available directly to the consumer.*

3.1 Primary producers (game farmers)

From a financial perspective it is widely recognised that game farming is the fastest growing agricultural industry in South Africa (Patterson and Khosa, 2005) and increases by about 23% per annum in areas where there are no large predators (ABSA, 2003). Today there is more wildlife in South Africa than 40 years ago (Bothma, 2004). Wild ungulates that were objects of extermination prior to 1960 are now husbanded for a variety of purposes (Carruthers, 2008). All of the sectors indicated in Figure 1 help the farmer ensure that wildlife is a lucrative resource and a feasible land use option (Scriven and Eloff, 2003; Patterson and Khosa, 2005). The South African wildlife industry is well established, with a range of specialists including capture operators, wild-life transporting companies, wildlife marketers or brokers, wild animal auctioneers, wildlife insurance brokers and wildlife management consultants (Higginbottom and King, 2006).

Although the 65.7% of the game farmer respondents (n=134) indicated that they farmed with game only, the remaining 34.3% indicated that they farmed with domestic animals and game. In addition, 85.7% of the game farmer respondents indicated that their game was free roaming and wild while the remaining 14.3% indicated that they farmed game intensively. Regarding procurement strategies, 73.3% and 66.4% of the respondents (n=131) indicated that they buy game at auctions and from other game farmers respectively for breeding purposes. In addition, 24.4% of the respondents (n=131) indicated that they buy game at auctions and from other farmers respectively for hunting purposes.

Kriek (2002) warns that the prevalence of internal and external parasites, hoofs and leg problems due to malnutrition which in turn lead to fractures and nutritional deficiencies, e.g. copper deficiency, may be of importance for game production when farmed intensively. In addition, Bengis (2002) expressed concern regarding the dangers that game pose to domestic livestock by acting as parasitic hosts or as a source of epizootic disease and also that foreign animal diseases cycling in livestock may cross the interface and infect wildlife. From a public health point of view, Paulsen and Smulders (2004) highlight that: (1) some diseases are transmissible from animals to humans during handling (occupational hazard); (2) some diseases are transmissible to consumers of meat and meat products; and (3) medical therapy of meat animals could result in drug residues in meat and meat products. The control of infectious pathogens that originate from wild animals have therefore become increasingly important due to it having substantial impacts on human health, agricultural production, wildlife based economies and wildlife conservation (Bengis *et al*., 2004). Table 1 is a summary of diseases known to occur in game in South Africa.

Although certain diseases are identified by South Africa as notifiable, GlobalGAP which is an international standard for 'Good Agricultural Practices' requires that a written Veterinary Health Plan that supports optimal health of animals must be in place. This plan provides for aspects such as the availability of the services of a veterinarian, disease prevention strategies, vaccination protocols, endo and ecto-parasite control, identification and control of sick animals, isolation facilities of sick or injured animals, control over storage and administering of medication, maintenance of veterinary equipment and the keeping of records of animal health interventions (GlobalGAP, 2010). In South Africa, 75.4% of the game farmer respondents

Table 1. Summary of diseases occurring in game in South Africa (Bengis, 2002; Bengis et al.*, 2001, 2004; Godfroid, 2002; Keet* et al.*, 2001; Michel* et al.*, 2006; OIE, 2008, 2010).*

Disease	Species affected
African swine fever (OIE listed)	Warthog (*Phacochoerus africanus*) associated with bites from the eyeless tampan (*Ornithodorus porcinus)*; domestic and free ranging rustic pigs; and illegal translocated European wild boar in contact with warthogs
African horse sickness (OIE listed)	Zebra (*Equus burchelli*) associated with biting midge (*Cullicoides imicola*)
Anthrax (OIE listed)	Kudu (*Tragelaphus strepsiceros*)
Avian Influenza	Farmed and wild ostriches (*Struthio camelus*)
Babesia	White rhinoceros (*Ceratotherium simum*)
Bovine Malignant Catarrhal Fever (OIE listed disease for wildebeest only)	Wildebeast (*Connochaetes spp.*) as maintenance host which infects cattle
Bovine tuberculosis (*Mycobacterium bovis*) (OIE listed)	Baboon (*Papio ursinus*), buffalo (*Syncerus caffer)*, cheetah (*Acinonyx jubatus*), hyena, impala (*Aepyceros melampus*), kudu, leopard (*Panthera pardus*), lions (*Panthera leo*), meerkat (*Suricate suricate)* and warthog
Brucellosis (OIE listed)	Buffalo, eland (*Taurotragus oryx*), hippopotamus (*Hippopotamus amphibious*), impala, sable antelope (*Hippotragus niger niger*), waterbuck (*Kobus ellipsiprymnus*), zebra
Classical Swine Fever (OIE listed)	Feral pigs and indigenous bush pigs (*Potamochoerus larvatus*)
Cyanobacterial intoxication	Wildebeast, zebra, white rhino, buffalo, hippopotamus, giraffe (*Giraffa camelopardalis*), warthog, lion and cheetahs
Feline Immunodeficiency virus infection	Lions Kruger National Park (KNP)
Foot and Mouth Disease (OIE listed)	African buffalo, bushbuck (*Tragelaphus scriptus)*, giraffe, impala, kudu, nyala, warthog and cattle
Hydatid disease *(E. granulosis)* OIE listed	Necropsied lions
Newcastle disease (OIE listed)	Farmed ostriches
Rabies (OIE listed)	The viverid biotype were confirmed in common genets (*Genetta genetta),* selous mongoose (*Paracynictus selousi*), slender mongoose (H*erpestes sanguinea),* small spotted cats (*Felis nigripes)* and yellow mongoose (*Cynictus penicillata).* The canid biotype were confirmed in aardwolfs *(Protoles cristata)*, African civet (*Civettictus civetta*), bat-eared foxes (*Otocyon megalotis*), black backed jackals (*Canis mesomelas*) and side striped jackals (*Canis adustus*)
Rift Valley ferver (OIE listed)	Buffalo and gemsbok (*Oryx gazella*)
Theileriosis (Corridor disease) (OIE listed)	Buffalo as carrier which infects cattle
Trypanosomiasis (OIE listed)	Antelope, buffalo, elephant (*Loxodonta africana*), hippopotamus, rhinoceros, wild porcine
Tuberculosis (OIE listed) (*Mycobacterium tuberculosis*)	Meerkat

(n=138) indicated that they do not have a written Veterinary Health Plan in place. In addition, 89.4% also indicated that they do not have a screening and improvement programme in place for the control of zoonotic diseases. The absence of a written Veterinary Health Plan with the necessary controls and records of disease control may result in the accidental spreading of diseases to other animals, animal handlers and consumers of game meat.

3.1.1 Suppliers of feed

In nature, wild animals will move to better pasture, sometimes over long distances when the availability of food is decreasing, usually during the dry seasons or drought (an example of such migrations are that of the wildebeest in East Africa). However, with modern game farming techniques, animals are fenced in which prevent them from leaving the fenced area. Ninety percent of the game farmer respondents who indicated that game were free roaming and wild also indicated that natural grazing was supplemented with feed during the dry winter months in order to get optimum production. South Africa has a Fertilisers, Farm feeds, Agricultural remedies and Stock remedies Act (Act 36 of 1974) that controls production and import of farm feeds (Anonymous, 1974). Although not specifically designed for wild game, GlobalGAP identify the following measures that game farmers may use as a guideline for the control of animal feed (GlobalGAP, 2010):

- Feeds must only be procured from feed manufacturers or suppliers that are registered as such by the relevant authorities.
- Labels of feeds must be checked and kept by the farmer as evidence of feed origin and ingredient composition.
- Feed must be traceable to the manufacturer.
- Mixing protocols must be available when feed is mixed by the farmers themselves.
- A procedure should be in place to deal with residues of medicated feed.
- Records should be kept of the suppliers and the batch numbers received from the suppliers.
- Adequate measures should be taken to protect the feed against contamination, damage and deterioration during storage. This includes pest and vermin control.
- Separate storage of medicated feeds in order to prevent cross contamination between medicated and non-medicated feeds.

3.2 Harvesting and hunting

The majority (65.7%) of the hunter respondents (n=290) in the survey indicated that hunting was part of the tradition of South Africans and was here to stay. Besides the estimated 200,000 local hunters, South Africa was visited by between 5,000 and 6,000 foreign hunters during the 2003/2004 season (Patterson and Khosa, 2005). In South Africa, hunters can be divided into professional hunters (PHs), sports or trophy hunters (SHs) and meat (biltong[1]) hunters (MHs). In terms of the South African Firearms Control Amendment Act, 2006 (Act 28 of 2006), a professional hunter is 'any person who supervises, escorts, offers to, or agrees to supervise or escort a client, for reward in connection with the hunting of a wild or exotic animal and who is authorised to do so in terms of any applicable provincial law'. The same legislation also refers to an occasional hunter as 'any person who, from time to

[1] Biltong is a salted dried raw meat product widely eaten in South Africa and prepared from the muscle of the antelope or cattle (Prior, 2008).

time, participates in hunting activities but who is not a member of an accredited hunting association' (Anonymous, 2006). Professional hunters are registered with the Professional Hunters Association of South Africa (PHASA) after the completion of a comprehensive training course at a professional hunting school.

3.2.1 Harvesting

Harvesting is a controlled process where compliance to specific legislation and standards is compulsory (Van der Merwe, 2005). Such legislation includes the Animal Protection Act, 1962 (Act 71 of 1962) (Anonymous, 1962), the Meat Safety Act, 2000 (Act 40 of 2000) (Anonymous, 2000) and Veterinary Procedural Notices (VPNs) of the Department of Agriculture, Forestry and Fisheries (Anonymous, 2008e). Harvesting can either take place during the day time by making use of feeding point, boma (closure) or helicopter methods or during night time, by using a spotlight from open vehicles in areas where less dense vegetation occurs (Hoffman, 2007). In accordance with South African rules the following matters should be in place before and during the harvesting process of the game:

- Registration of a game farm for export status: an application is made to the relevant national and provincial authorities to register the farm where harvesting is envisaged for export status. This registration provides for official inspection and approval and registration of farms; Requirements for residue monitoring as well as movement of partially dressed game carcasses from a registered farm to an abattoir. Prior to harvesting, officials from the relevant authorities must obtain a duly signed health attestation regarding the animal health status on the farm from the regional State Veterinarian (Anonymous, 2008a). This ensures that there is or was no recent disease outbreak on the farm or the direct vicinity of the farm.
- Harvesting teams: the team leader must ensure that the hunters and other staff have adequate competencies in hunting and slaughter techniques, that they are registered as such by the authorities and that they have adequate knowledge of legislative requirement (Anonymous, 2008b). At least one of the team members must be qualified as a Game Meat Examiner (GME) or a Game Meat Inspector (GMI). In South Africa a person can qualify as a GME after the successful completion of a six month competency based course while a GMI must be in possession of at least an appropriate three year tertiary training qualification.
- Temporary slaughter depots: the harvesting team must provide temporary slaughter depots where field slaughter is conducted (Figure 2, item 3.2). The depots must provide for (1) hard rolled floor surfaces covered with canvas or plastic to control dust; (2) hanging facilities for the dressing of carcasses and handling of viscera and high enough to prevent the head or neck of the carcass coming in contact with the ground; (3) meat inspection facilities for carcasses and viscera; (4) separate closable and washable containers for the storage of red offal, rough offal, inedible material and condemned material; (5) sterilizers for knives and other slaughter equipment; (6) sufficient potable water with hot running water at 40 °C or with an acceptable food grade approved disinfectant added; (7) adequate illumination for night slaughter (220 Lux for dressing and 540 Lux at the inspection point); (8) hand wash and disposable hand drying facilities with a supply of food grade approved liquid bactericidal soap; and (9) mobile thermograph equipped chilling facilities (preferably refrigerated trucks) with the capacity to reduce the carcass temperature to

below 7 °C within 24 hours for storage and transport of slaughtered carcasses and process. The accumulation of blood, waste, dust and mud below the frame and surrounding areas must be prevented throughout the slaughter process (Anonymous, 2008b). Samples of the water are taken at least one week before the harvest in order to determine the quality of the water.

- Hygiene management system (HMS): harvesting teams must have in place a HMS that provides for the control of *ante mortem* inspection, slaughter and dressing, personal hygiene of workers, medical fitness of workers, maintenance of sterilizers (chemical or high temperature sterilisers), availability of liquid soap and soap dispensers, toilet paper, and disposable towels, sanitation and continuous cleaning, availability and safety of water, waste disposal, including condemned material and continuous temperature control of the chiller vehicle (Anonymous, 2008b).
- Pre-harvesting inspection: the leader must ensure that the game to be harvested was not treated with veterinary medicinal drugs before harvesting or that all withdrawal periods for such veterinary medicines were adhered to. Prior to the start of harvesting, the slaughter depots and harvesting vehicles must be inspected by the GME or GMI to determine compliance with the hygiene requirements. In addition the hunting team must also have available for inspection by the GME or GMI the registration certificate(s) of the farms and for all hunters on the team, health attestation of the farm issued by the relevant authorities, harvesting programme, health certificates of hunter(s) as well as of assistants including copies of their identification documents, checklist for harvesting inspection, certificate of origin and proof of qualification the hunter is qualified to do *post mortem* inspections (Anonymous, 2008b,c).
- Shooting/killing: it is the responsibility of the hunter to ensure that the animals harvested has a normal healthy active appearance and that no animal with visible signs of injury or disease are hunted. If killed, hunters must mark all suspect animals and provide relevant information to the meat inspectors at the slaughter depot. The use of Professional Hunters is preferred as the meat derived from harvested game is mostly exported and only head and neck shots are used. Game killed with thoracic shots must be subjected to veterinary approval while carcasses with abdominal shots must be condemned for export purposes. Carcasses not approved for export purposes may however be considered for domestic use when it is fit for human consumption (Anonymous, 2008b,c).
- Bleeding: is done by means of severing the jugular vein and carotid artery on either side of the neck (throat slitting). As with domesticated animals, bleeding of game animals should be done within as short of time as possible after the kill. Considering that bleeding in the hunting field is not always that easy because of the terrain and the time lapse to get to the hunted animal, South African standards allows a maximum of 10 minutes after shooting (Anonymous, 2008b). Observations during harvesting however showed that bleeding takes place within 2-5 minutes after shooting, which is shorter than the prescribed time. South African standards require that small animals be bled in a hanging position; medium animals on a ramp at 20-30°, or in a hanging (elevated) position and large animals in a lying position. The bleeding knife used must be cleaned and sterilised by using water at 82 °C or an approved food grade chemical method. The provision of an adequate number of knives and the use of a two knife system is recommended in order to ensure the effective sterilisation of the knife not in use (Anonymous, 2008b). It is also important to ensure that the contact time is adequate for effective sanitation of the knives.

- Transport of harvested game: South African standards require that harvested game must be transported to a game depot within two hours after being bled. To shorten the time it is suggested that carcasses are off-loaded at the slaughter depot(s) as soon as they are in close vicinity thereof, even if the load is not full. Observations made during harvesting showed that on average, carcasses are transported to the depot within 45 minutes *post mortem*, which is shorter than the allowed two hours (Anonymous, 2008b). Care must be taken not to contaminate the neck slit area when transporting the carcasses. The vehicles used to transport harvested game carcasses from the point of kill to the farm abattoir (field slaughter depot) must be equipped with a hanging frame and/or a hoist and a ramp frame with a 20° to 30° slope to bleed carcasses. In addition vehicles must be equipped with facilities where bleeding knives can be cleaned and sterilised with water at 82 °C or by means of food grade chemical sterilisation; hand wash facility with potable running water and soap, and artificial light where game is bled at night with a minimum light intensity of 220 Lux. The structure of the frames, sterilisation equipment and had wash facilities must be constructed of a non-toxic material that is smooth surfaced, non-absorbent, resistant to impact and durable and easy to clean (Anonymous, 2008b).
- Field slaughter: is done at the temporary slaughter depot by transferring the bled animals from the harvesting vehicle onto a hanging frame where the heads, feet, lactating udders, scrotum and testicles are removed (leaving the *Lnn. inguinalis superficialis*) and carcasses are eviscerated in a hanging position. The hide or skin is however not removed and acts as protection against environmental contamination during the slaughter process. Evisceration is normally done at the slaughter depot, but as harvesting can take place any time of the year, bloating may occur in the hotter months of the year. If bloating occurs, the carcass must be brought to the depot sooner or the removal of the green offal (paunch and intestines) can as an emergency measure be removed by the hunter in the field within 30 minutes of being bled (Anonymous, 2008b). In such cases the hunter who is also a qualified GME or GMI must inspect the green offal during evisceration in the field. As with slaughter of domesticated animals in commercial abattoirs, the carcasses and the corresponding viscera must be identifiable for meat inspection purposes at the depot and abattoir. Throughout the field slaughter extreme measures must be taken to prevent contact of the exposed meat with soiled equipment, platforms, slaughter frames, ground or floor as well as the outer surface of the skin or hide. No cutting of the Rectum, small intestines, oesophagus, bladder and uterus must be allowed.
- Preliminary *post mortem* meat inspection: carcasses and the corresponding viscera must be inspected by qualified GMEs or GMIs. A comprehensive *post mortem* meat inspection must be done on the partially dressed carcass as well as all heads and feet, oesophagus, trachea, lungs, spleen, heart, kidneys (if removed), diaphragm, liver, mediastinum, abdominal organs. The partially dressed carcasses are inspected for thoracic shots resulting in gross contamination of the thoracic cavity with blood and/or bone splinters or gut shots resulting in contamination of abdominal cavity with ingesta; signs that an animal was wounded or required more than one shot to kill; and excessive contamination of the abdominal cavity with ingesta, soil, grass, mud or any other contaminant where game animals were eviscerated in the field employing poor evisceration techniques. Although such animals are not used for export it was found during observations that depending on the reason for the rejection of the carcass and, where possible, some carcasses such as heavily bruised carcasses remained on the farm for consumption by farm staff or further

processing of the parts of the carcasses still fit for human consumption (Anonymous, 2008c).

- Washing of partially dressed carcasses: in order to minimise contamination and to keep the carcass as dry as possible, no partially dressed carcass may be washed and accidental soiling/ contamination must be cut off (Anonymous, 2008c).
- Traceability: in order to ensure traceability to the abattoir, carcasses and corresponding pluck (heart, lungs and liver) must be tagged, each set with its own unique identification code. A list of the codes is attached to a Certificate of Origin that accompanies the refrigerated transport vehicle (Anonymous, 2008c).
- Chilling: after primary meat inspection the carcasses must be left to air dry. South African rules require however that inspected carcasses must be loaded into refrigerator trucks within 4 hours of inspection during summer months, which can be extended to 12 hours with an ambient temperature of 12 °C or less (Anonymous, 2008c). It was observed that on average the carcasses were loading into the refrigerated vehicles within three hours after inspection. Refrigerator vehicles must be able to chill the partially dressed carcasses to a deep bone temperature of below 7 °C within 24 hours (Anonymous, 2008b).
- Offal handling: the red offal must accompany the partially dressed carcasses in separate containers to the abattoir where final slaughter and inspection is conducted. As already indicated these must be suitably identified and must correlate with the carcass. The remaining offal after approval may be used by local consumers (Anonymous, 2008c).
- Transport: vehicles transporting partially dressed game carcasses and red offal must comply with regulations pertaining to vehicles transporting meat in terms of the design of the walls, floor and roof, i.e. it must be smooth, non-absorbent and washable. When all partially dressed carcasses are loaded, the GME or GMI must seal the truck with an official seal and record the unique seal number on the Certificate of Origin. The transport vehicle must be able to maintain a deep bone temperature of between -1 °C and 7 °C until offloading. A thermograph recording the temperature continuously must be available and the recording must provide for accurate actual time and temperature analysis, covering all phases of loading during the slaughter process and transport. Carcasses must be hung away from the floor and from each other in such a way as to ensure optimal airflow within the chiller space. The load must be accompanied by the health attestation, the Certificate of origin, the checklist for harvesting inspection and the inspection report of the GME or GMI (Anonymous, 2008c).
- Waste handling: lactating udders, reproductive organs and any organ not utilised commercially must be handled as condemned material and placed in appropriate containers. Condemned material must also be placed in the same containers and disposed of in an appropriate manner (Anonymous, 2008b,c).
- Monitoring for pharmaceutical substance residues: sampling of liver, fat, kidney, muscle tissue, blood serum and urine must be carried over a period of time and spreaded over the whole hunting season. The sampling programme must make provision for harvesting that may take place throughout the year in some areas, specific substances that are administered only in particular, the use of currently unknown substances and the presence of diseases in particular regions (Anonymous, 2009).

3.2.2 Hunting

Hunting refers to the act of chasing and killing wild animals for sport or food and is mostly an uncontrolled process, not necessarily following prescribed legislation. It sometimes takes place under the guidance of a professional hunter and is conducted by hunters that are not professional hunters (Van der Merwe, 2005). Hunting normally takes place in areas where wildlife viewing or photographic safaris are not possible (Barnett and Patterson, 2006). The following types of hunting are identified in South Africa (Humavindu and Barnes, 2003; Patterson and Khosa, 2005; Carruthers, 2008):

Trophy hunting: the hunter (national and foreign) is a client to a hunting concession owner and hunts purely for sport reasons with the objective of keeping some part of the animal as proof of the hunt. Once the hunter has taken the trophy, usually the horns, skull and/or skin, the left over meat is either used as bait for a subsequent hunt, or is given to local staff or local communities, although the hunters may request some for his/her own meals. Trophy hunting has became more and more popular and was the initial stimulus to develop wildlife ranching as a major economic force which generated an estimated income of R417 million for game ranchers in 2005 (PHASA, 2006). The trophy hunter's main objective is an undamaged trophy and not the health or hygiene of the meat (Van der Merwe, 2005). Game farmer respondents (n=114) indicated that on average 34.2% of meat obtained from trophy hunted animals was taken by the hunter, while 32.5% indicated that the meat remained on the farm, 17.5% said that it was taken by the professional hunter that accompanied the hunter during the hunt and 15.8% stated that the meat was sold to interested persons.

Biltong/meat hunting: the hunters are almost exclusively South African nationals who combine the experience if hunting with the desire for wildlife meat. The skin, horns or any other animal parts are seldom kept by the hunter as a trophy and sometimes hunting concession owners generate an income from these parts. The biltong hunter's objective is the thrill of the hunt and the biltong is a bonus (Van der Merwe, 2005). Van der Merwe and Saayman (2008) indicated that in 2007 the top five species hunted for biltong were springbok (*Antidorcus marsupialis*), impala (*Aepyceros melampus*), blesbok (*Damaliscus dorcas philipsi*), kudu (*Tragelaphus stepsiceros*), warthog (*Phachochoerus africanus*) and blue wildebeast (*Connochaetes gnou*).

Traditional hunting: traditional hunting refers to those whose hunting activity is aimed at provision of food, the satisfaction of cultural practices and/or the source of medicinal material and to test the speed of hunting dogs.

Subsistence hunting: this is where wildlife is hunted for its meat using a wide variety of methods and does not take place within any legal framework. This method is also frequently referred to as bush meat; although the latter may also include the illegal hunting of wild animals with the intention of selling the meat in an informal (and thus illegal) manner.

Very little of the rules that apply to harvesting are applied during hunting. Hunters may or not have some of the basic equipment that will assist them with the hunt such as a vehicle equipped with a hanging frame and a spotlight, a container with water and slaughter knife

to do the bleeding and the removal of the green offal (paunch and intestines). Bleeding and removal of the green offal mostly takes place on the hunting ground and is normally done by the hunter or an assistant that accompanies the hunter during the hunt. From there the carcass is transported on an open vehicle to the farm abattoir or transported to the hunter's residence (Figure 2, Item 3.2.1) where the slaughter is completed and sometimes the carcass is further processed into various products. Normally no meat inspection is conducted on the carcass or the viscera. However, the survey conducted indicated that meat and meat products derived from wild game animals is highest in demand by butcheries (43.3%) and consumers (90%) during winter months, which traditionally is the hunting season of the year. Although the majority of the hunter respondents in the survey indicated that they do not have adequate knowledge of legislation controlling meat hygiene and safety, 81.1% of the hunters (n=280) indicated their willingness to attend information sessions in order to obtain more knowledge. It is therefore important to ensure that all hunters receive relevant training to ensure that the meat and meat products derived from hunted wild animals are safe for consumption.

3.3 Slaughter

In South Africa, the Sub-directorate for Veterinary Public Health of the Department of Agriculture, Forestry and Fisheries (DAFF), is responsible for the slaughter of animals within approved abattoirs while the Sub-directorate Import/Export are responsible for the import and export of game meat. General Law enforcement is conducted by the veterinary public health units of the provincial government in their areas of jurisdiction.

3.3.1 Farm abattoirs

A farm abattoir is a slaughter facility that is not necessarily approved by the relevant authorities and where slaughter of game carcasses is done for own consumption or for the local market. Although the majority (91.6%) of the game farmer respondents in the survey indicated that they offered a slaughter service to hunters, the locations and the number of these abattoirs are unknown and therefore none or little control can be executed. No inspection of the hunted carcasses took place in the hunting field. In addition the carcasses of those game species wrongly shot during harvesting as well as carcasses with excessive damage or bruising and therefore not suitable for export purposes may end up in the farm abattoir for final slaughter. Normally no final meat inspection will take place at these farm abattoirs in order to determine the fitness thereof for human consumption. In cases where a farm abattoir is not available on the farm where the hunting take place, the carcasses of the hunted game is normally transported to another nearby farm abattoir or another abattoir registered for game slaughter (Figure 2, item 3.3.2) for final slaughter or the hunters residence (Figure 2, item 3.2.1) for final slaughter and further processing. The majority (80%) of hunter respondents indicated that they conduct the final slaughter and processing of the hunted game on the farm where the game was hunted. Meat for own consumption is exempted from inspection, but if meat is sold to an outlet or directly to the public, hunters have to meet the requirements of the Meat Safety Act, 2000 (Act 40 of 2000) (Anonymous, 2000; Patterson and Khosa, 2005) as well as the 'General hygiene requirements for food premises and the transport of food' promulgated under the National Health Act (Act 61 of 2003). It was evident from the survey that game meat reaches the consumer through the formal supply chain. Hunters (n=279) indicated that

they already market their hunted game meat prior to the hunt to game farmers for own use or further processing (7.9%), schools or other charity organisations (6.5%), family or friends (39.4%), wholesalers (3.4%) and retailers (24.8%) such as local and supermarket butcheries, biltong shops and restaurants. Forty percent of the game farmer respondents indicated that they procured game carcasses from hunters to process game meat products on the farm with the specific purpose of selling the meat or processed meat products to the retail (Figure 2, item 3.4.3) and wholesale markets (Figure 2, item 3.4.3) or directly to the consumer (Figure 2, item 3.8). However, in terms of fitness for consumption, 75% of hunter respondents indicated that the meat is not inspected by anyone, while the others indicated that the carcasses and offal are inspected by qualified game meat examiners (5%), hunters themselves (10%), the farmer (12%), or another hunter (3%). However, none of the latter three parties have received any training in game meat examination. This situation clearly states a gap in control in the meat supply chain.

3.3.2 Abattoir (game meat establishment)

The abattoir or as it is also referred to, the game meat establishment is defined in terms of the Meat Safety Act, 2000 (Act 40 of 2000) (Anonymous, 2000) as 'a slaughter facility in respect of which a registration certificate has been issued and in respect of which a grading has been determined'. In addition, separate regulations to manage the registration process, slaughter processes and hygiene management have been promulgated in terms of the said Act for red meat (bovine, ovine, porcine and equine), poultry meat and ostrich meat. Regarding game meat, draft Game Meat Regulations were published for comments during 2004 under the same Act but has still not been promulgated since an amendment needs to be made to the said Act, which currently prohibits the receiving and slaughter of dead animals in an abattoir. However, due to the administrative and prolonged nature of the amendment process, 27 abattoirs are already approved for the slaughter of game in South Africa (Anonymous, 2008d). Some of these abattoirs also slaughter other domesticated red meat animal species such as bovines, ovine, porcine and ostrich. It is the responsibility of the abattoir owners or managers to ensure that the abattoir is maintained and the slaughter process is conducted in accordance with the prescribed standards. In order to achieve this it is expected of abattoir owners or managers to implement a documented Hygiene Management System consisting of (Anonymous, 2004):

- schematic plan of the abattoir;
- flow diagram of the slaughter process;
- identification of potential biological, chemical or physical hazards and the prevention thereof;
- hygiene management programmes relevant to game slaughter including slaughter and dressing, meat inspection, personal hygiene and medical fitness of workers, temperature of water in sterilizers and maintenance of sterilizers, availability of liquid soap and soap dispensers, toilet paper, and disposable towels, sanitation and continuous cleaning, availability and quality of water, vermin control, waste disposal, contact wrapping and packing materials, maintenance, thermo control and visitor control.

Although it is also expected of abattoir owners or managers to conduct a regular self assessment based on a prescribed Hygiene Assessment System (HAS), veterinary public

health officials employed by the respective provinces also evaluate the abattoirs on a regular basis against the same HAS (Anonymous, 2000, 2004).

Partially dressed carcasses and their corresponding plucks are delivered to abattoirs. If the carcasses originate from a harvest, the seal is checked against the Certificate of Origin and ensured that it is still intact and the number of carcasses concurs with the prescribed register that accompanied the vehicle. The carcasses are offloaded and stored in holding chillers before slaughter is completed by removal of the hides and skins and comprehensive final primary and secondary meat inspections are conducted on the carcasses and the accompanying viscera. The approved carcasses are normally deboned or processed into primal cuts, chilled or frozen and stored until dispatched for export (Figure 2, item 3.5.1) or to the local markets, i.e. large-scale processors (Figure 2, item 3.4.2), retailer and wholesalers (Figure 2, item 3.4.3) or directly to the local consumer (Figure 2, item 3.8). The results of the *ante mortem* inspection in the field, primary meat inspection and secondary meat inspection must be recorded and where zoonotic and notifiable diseases are diagnosed, the local state veterinarian must be notified.

3.4 Processing, wholesale and retail markets and export

Something that causes some difficulty in control of game meat (and for that matter all other meats) in South Africa is that the control of the meat after the gate of the farm abattoir (Figure 2, item 3.3.1) or the abattoir premises (Figure 2, item 3.3.2) falls under the metropolitan and district municipal health authorities jurisdiction and no longer with the relevant veterinary public health authorities. There is very little liaison and cooperation between the veterinary public health authorities, the municipal health authorities and the abattoirs. The mandate and role of municipalities in food control lies within the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) (Anonymous, 1972) and the National Health Act, 2003 (Act 61 of 2003) (Anonymous, 2003). The Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) deals with matters regarding the sale, manufacture and importation of foodstuffs while the latter Act deals with hygiene requirements for food premises and the transport of food. Typical hygiene requirements include aspects such as the registration of the premises and the issuing of Certificates of Acceptability; handling and transportation of food; structural and design requirements for food premises; facilities, containers and equipment; display, storage and temperature control of food as well as food handlers and protective clothing. Although it is the primary responsibility of the enterprise owners or managers to ensure compliance with the legislation, the control over the implementation and maintenance of the requirements are done by authorised Environmental Health Practitioners (EHPs) and in selected cases by veterinarians (Basson, 2006). Visiting EHPs are also responsible for sampling and analysis of foodstuffs; health education of food processors, handlers and consumers and advising on legal requirements (Basson, 2006). The survey has however shown that 7% of local and supermarket butcheries, meat processing plants and restaurants, 14% of wholesalers and 16% of biltong shops are not in possession of Certificates of Acceptability. By not issuing the required Certificates of Acceptability, municipalities are not aware of food premises or they are ignoring their responsibilities regarding this matter.

In addition to hygiene and processing requirements stated by legislation it is imperative for all processors to establish, implement and maintain a food safety management system i.e. a

Hazard Analysis Critical Control Point (HACCP) system or The Food Safety Management System of the International Standards Organization (ISO, 2005). The purpose of the mentioned system is to establish policies and procedures whereby food hygiene, safety and quality can be managed. In terms of the requirements of such as system a hazard analysis of all processes and products are conducted and the necessary controls are implemented to prevent and control identified hazards.

3.4.1 Small-scale processors

The term 'small-scale processors' refers to processors not yet approved by the relevant authorities and where value adding is done on a small scale for own consumption or the local market. Small-scale processing is often done on game farms in a separate room adjacent to the slaughter area. Seventy percent of the game farmer respondents indicated that they processed game meat products on the farm themselves and that the top five products processed were biltong (50%), dried sausage[2] (30%), salami (10%), fresh cuts (5%) and fresh sausage (5%). This confirms the finding of Hoffman *et al.* (2004) that there is still a demand for the processing of biltong (including dried sausage) as a delicacy amongst South African consumers. Other products include mince, hamburger patties, and cabanossi sausages. Although game meat is not new to the South African consumer, it is no surprise that processors experiment with alternative processed products in order to satisfy the growing need for lean meat (Hoffman and Wiklund, 2006). According to the survey conducted most (49.4%) of the game meat processed on the farm remains on the farm for own home use, use in game lodges; use as rations for farm workers; and selling to hunters and interested persons or the public. Approximately 17% is donated to family, friends or charity and 31.9% of the processed meat processed on the farm reaches the consumer (Figure 2, item 3.8) through local retail and wholesale markets (Figure 2, item 3.4.3).

Currently, neither veterinary public health nor municipal health authorities know the location and number of farm processing facilities and these are therefore not yet approved in terms of the applicable legislation referred to above. Another complicating factor is that the amount of game meat received, processed and sold in municipal areas of jurisdiction is unknown to the municipal health authorities.

3.4.2 Large-scale processors and exporting

Large-scale processors are normally located in urban areas and this definition refers to processors approved by the authorities for local and/or export purposes and where value adding is done and the final products are distributed to the wholesale (Figure 2, item 3.4.3) and retail markets (Figure 2, item 3.4.3) from where the local consumers purchase the products.

[2] South African dried sausage (droëwors) is a ready-to-eat dried seasoned and intermediary moisture meat product which can be distinguished from European types of dried sausages in that it is not cured nor fermented, but preservation is obtained by an artificial drop in pH and subsequent drop in water activity (Burnham *et al.*, 2008; Osthoff *et al.*, 2002).

3.4.3 Wholesalers and retailers

As previously indicated, game meat and game meat products also enter the supply chain at the wholesale and retail points. This was confirmed when butchery respondents in the survey indicated 64.5% of the game meat processed and sold by butcheries is obtained from hunters (including own hunting). In addition, 52.2% confirmed that that hunters offer game meat for sale to them prior to hunting, while 36.2% indicated that they deliberately order or buy game meat from hunters for the purpose of processing and sale in their butchery. Butchery respondents also indicated that only 63.6% of the game meat received by their butcheries is processed on behalf of leisure hunters for their own use while the remaining 36.4% of the meat is processed and sold directly to the consumer from the butchery. Carcasses are mainly (63.4%) received without the hide or skin which complicates species identification. On the other hand, the remaining 36.6% that are received with the hide on requires separate chilled storage facilities to prevent cross-contamination of other carcasses. It must also be remembered that the carcasses obtained from hunters are not inspected by a qualified PME or PMI. The absence of the head, feet and viscera at this point also prevent complete inspection of the carcass and its entrails as required by the legislation. Despite of all these complicating factors, only two of the municipality respondents indicated that they have policies specifically aimed at the control of game meat entering their area of jurisdiction.

3.5 Export and Import of game meat

Import and export of game meat is an important contributor to the economy, but also forms part of the larger goal of sustainable food security in South Africa.

3.5.1 Export

Approximately 450 tons of game meat are exported annually (mainly to Europe) with a value of about R15 million (Du Toit, 2007). In South Africa there are five game handing establishments approved for export purposes by the European Union (EC, 2008). Two of them are also approved as cutting plants. These facilities as well as the export of the game meat are controlled by the Sub-directorate: Import/Export within the Directorate: Veterinary Health of the Department of Agriculture, Forestry and Fisheries. It is expected of these facilities to comply with the requirements of the Meat Safety Act, 2000 (Act 40 of 2000) as well as a series of Veterinary Procedural Notices (VPNs) prescribed by the same sub-directorate. The VPNs prescribe procedures in line with European Union standards and deal with controlling of animal diseases on the farm; hunters used for harvesting; *ante* and *post mortem* meat inspection and hygiene control at point of harvest and game meat establishments; control of meat; residue monitoring; and law enforcement (Anonymous, 2008e). These establishments also have full time veterinarians and meat inspectors on site that oversee the compliance with the requirements.

3.5.2 Import

Game meat is imported from approved cutting plants in countries such as Australia, Namibia and New Zealand, mainly for commercial purposes, but also by hunters who have hunted

in neighboring and other countries. The meat derived from the latter may be for the hunters own use but also for commercial purposes. Although policies regarding the import of meat are determined by the Import/Export unit within the Directorate: Veterinary Services of the Department of Agriculture, Forestry and Fisheries, the Sub-directorate: Port of Entry Point Control of the same department administers these policies. Control policies consider the following:

- Restriction of the mass of meat for own consumption. South Africa allows a maximum of 250 kg of meat per individual for own consumption. The import of meat exceeding 250 kg per importer is regarded as import for commercial purposes (Anonymous, 2008f).
- Issuing of veterinary import permits and veterinary health certificates by the relevant veterinary authorities. Specific conditions regarding the import may also be stipulated in the permit i.e. temperature requirements during transport and proof that the meat is coming from a Foot and Mouth free area (Anonymous, 2008f).
- Animal health status at the point of origin of the meat (Anonymous, 2008f). In South Africa this matter is evaluated and approved by the Directorate: Veterinary Services of the Department of Agriculture, Forestry and Fisheries.
- Approval of game meat processing plants in various countries: these are visited and when found to be complying with the relevant requirements they are registered as approved suppliers. Currently, South Africa has plants approved as suppliers in Australia, Namibia and New Zealand (Anonymous, 2008g).
- Placement of governmental officials with relevant knowledge and experience at air, land and sea ports of entry to ensure that the meat is imported in accordance with requirements of the import permit (Anonymous, 2008f). In South Africa, inspectorate of the Sub-directorate: Port of Entry Point Control is responsible for this function. On approval of meat for own consumption, the meat is released into the hands of the owner who is the consumer (Figure 2, item 3.8).
- Final inspection and approval of consignments meant for the commercial market. In South Africa, the meat is finally inspected and approved by a state veterinarian at a designated end-point before it is released (Anonymous, 2008g). Only thereafter, the meat may be distributed to the large-scale processor (Figure 2, item 3.4.2), the wholesaler (Figure 2, item 3.4.3) and the retailer (Figure 2, item 3.4.3).

3.6 Suppliers of processing materials

Suppliers of any processing materials such as ingredients, additives and packaging material are primarily responsible for supplying materials that are safe and of a high quality. The import of any processing materials is regulated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) (Anonymous, 1972). Law enforcement at establishments where manufacturing take place is done by Environmental Health Practitioners employed by the municipal health departments. Composite products such as pre-mixed spice mixes must indicate the ingredients on the label in order for the user to identify ingredients such allergens, stabilisers and colorants. This allows the processor to determine compliance with the relevant and local legislation.

3.7 Transport

Game meat is transported from the point of kill throughout the supply chain to the customer. Even though standards and regulations exist, the control of hygiene and safety practices through this part of the supply chain is often neglected and may have a negative impact on the safety and quality of the meat. Table 2 provides information on controls that can to be taken during transport through the supply chain.

Table 2. Description of transport methods in the supply chain as observed during the study.

Transport	Controls
Point of kill to field slaughter depot (Figure 2, item 3.2) or farm abattoir (Figure 2, item 3.3.1) on an open pick-up vehicle.	Protection of exposed neck bleeding slit and abdomen in cases where the green offal is removed in the field. Prevent neck or carcasses coming in direct contact with the ground. Keep the time from kill to delivery at slaughter depot or farm abattoir as short as possible.
Field slaughter depot (Figure 2, item 3.2) to farm abattoir (Figure 2, item 3.3.1) on an open pick-up vehicle	The thoracic cavity is open and its viscera, the head and feet are removed and it is therefore necessary to protect the exposed parts against contamination during transport.
Field slaughter depot (Figure 2, item 3.2) to abattoir (Figure 2, item 3.3.2) in refrigerated vehicles.	Proper design of the internal structure of the refrigerated vehicles to enhance cleaning and prevent contamination. Installation of a refrigeration unit capable of maintaining the temperature below 7 °C. Equip vehicle with thermometer couplings linked to a thermo control device that will monitor the temperature continuously. Sealing of refrigerating unit doors to prevent unnecessary opening of the doors during transport. Non-edible or rejected products may not be transported in the same compartment as carcasses. Measure and record carcass temperature during loading and off-loading.
Farm abattoir (Figure 2, item 3.3.1) to local consumer (Figure 2, item 3.8) for own use in the consumer's (including hunter's) own vehicle(s).	If the carcass is transported with the hide or skin on, the carcass must be protected against contamination. If the hide or skin is removed, the meat must be protected against contamination by packing in suitable clean containers. If the meat was chilled and is transported over a short time (maximum one hour), the meat can be transported in cooler boxes or something similar that will maintain the temperature. Transport over longer times must be refrigerated.

Table 2. Continued.

Transport	Controls
Farm abattoir (Figure 2, item 3.3.1) to small scale processors (Figure 2, item 3.4.1) or wholesaler and retailer markets (Figure 2, item 3.4.3) in refrigerated vehicles.	Proper design of the internal structure of the refrigerated vehicles to enhance cleaning and prevent contamination. Installation of a refrigeration unit capable of maintaining the temperature below 7 °C when fresh or below -18 °C when frozen. Equip vehicle with thermometer couplings linked to a thermo control device that will monitor the temperature continuously. Sealing of refrigerating unit doors to prevent unnecessary opening of the doors during transport. Non-edible or rejected products may not be transported in the same compartment as carcasses. Measure and record carcass temperature during loading and off-loading.
Abattoir (Figure 2, item 3.3.2) to large scale processor (Figure 2, item 3.4.2) or wholesaler and retailer (Figure 2, item 3.4.3) in refrigerated vehicles.	The same precautions as with the previous item will apply.
Large scale processor (Figure 2, item 3.4.2) to point of export (Figure 2, item 3.5.1) in refrigerated vehicles.	The same precautions as with the previous item will apply.
Point of import (Figure 2, item 3.5.2) to large scale processor (Figure 2, item 3.4.2) or wholesaler and retailer (Figure 2, item 3.4.3).	The same precautions as with the previous item will apply.
Point of import (Figure 2, item 3.5.2) to consumer (Figure 2, item 3.8) for own use in the consumer's (including hunter's) own vehicle(s) and is normally without the hide or skin.	The meat must be packed in suitable clean containers to protect it against contamination. Meat must be chilled or frozen and maintained at temperatures below 7 °C when fresh or below -18 °C when frozen.
Point of purchase by consumer to consumer's home mostly in consumer's own vehicle with no refrigeration or facility to maintain the temperature.	Consumer and retailer education regarding the maintenance of the cold chain and how it can be maintained when shopping. Encouraging of retailers to make cooler bags available for sale to customers.

3.8 Consumers

Consumer confidence in the quality (including safety) of their food supply depends in part on their perception as to the effectiveness of food control measures (CAC, 1995). Although consumers consider game meat consumption as a healthier alternative to red meat (Hoffman and Wiklund, 2006), they also consider aspects such as quality, i.e. tenderness, leanness, juiciness; meat safety i.e. the presence of antibiotics, chemical residues and growth hormones;

and animal welfare, i.e. health status and stress that the animals were subjected to (Krystallis *et al*., 2006; Blokhuis *et al*., 2008).

It is however often the customers themselves that cause undesired changes in the game meat through aspects such as temperature abuse, unhygienic handling and poor preparation practices. Although consumer education is an enormous challenge, stakeholders in the game meat supply chain should take responsibility to educate consumers on proper handling practices. The offering of programmes such as the 'Five Keys to safer food' of the World Health Organization (WHO, 2006) at schools, universities and other public forums may assist to overcome this problem. The five keys relate to (1) Keeping clean; (2) separation of raw and cooked food; (3) Cooking food thoroughly; (4) Keeping of food at safe temperatures and (5) the use of safe water and raw materials.

4. Conclusions

What has been achieved?

South African game meat and game meat products obtained from harvesting or hunting as well as imports and exports are reaching both the local and the international consumer through the game meat supply chain described in this chapter. In order to assist policy makers, law enforcers and the game meat industry, essential foods safety control points were described and are summarised in Table 3.

The establishment and implementation of proper and appropriate policies, legislation and programmes by the respective stakeholders in the supply chain as appropriate to what they are responsible for will prevent potential food safety breakdowns in the supply chain for game meat.

What has been neglected?

The essential food safety management points described applies only to the hunting of larger game (antelope) such as impala, springbok, kudu, zebra, eland, etc. and does not include the supply chain for (1) bird hunting, i.e. guinea fowls, partridge, etc. or (2) bushmeat that is obtained through informal and frequently illegal hunting of other wildlife such ascertain rodents, reptiles, porcupine and birds.

What needs to be done?

More research is needed on the supply chain and the appropriate essential food safety management points for the harvesting and hunting of birds and bushmeat. Bush meat is often also associated with traditional practices of certain communities and a better understanding of these traditional practices may assist with the establishment of food safety management points. Although bird hunting is not done on large scale in South Africa and is mostly used by the hunters and direct communities, it has the potential to also reach the formal supply chain. Aspects that may impact on the hygiene and safety as well as the general quality of

Table 3. Essential food safety management points for game meat.

On the game farm	After the game farm
Compliance with national and international legislation.	Compliance with national and international legislation.
Implementation of an animal health plan.	Transport control throughout the supply chain.
Control programme for animal feeds.	Approval and registration of abattoirs and other meat processing establishments, including large and small meat processors, wholesale and retail facilities.
Hunting and hunter control.	Establishment of procedures and policies for game meat control by the relevant health authorities.
Field slaughter depot and farm abattoir approval and maintenance.	Establishment of programmes for Good Hygiene Practices (GHP's) as well as Good Manufacturing Practices (GMPs).
Small processor identification and approval of the establishments.	Training of slaughter and processing staff.
Establishment of programmes for Good Hygiene Practices (GHP's) and conducting regular Hygiene Assessments.	Training of health authority officials and meat examiners.
Implementation of traceability procedures.	Conducting of a Hazard analysis to determine possible food safety hazards and the control thereof.
Water control.	Implementation of traceability procedures.
Training of slaughter and processing staff.	Programmes for correct packaging and labelling.
Training of meat examiners.	
Conducting of a Hazard analysis to determine possible food safety hazards and the control thereof.	

the product include (1) the harvesting or hunting methods (shot guns are used frequently) utilised for bird hunting as well as (2) the trend not to eviscerate the carcasses after hunting (often with perforated intestines).

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Game harvesting procedures and their effect on meat quality: the Africa experience

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Summary

This paper discusses the major methods employed to harvest game on a commercial basis in South Africa and Namibia. These two countries are presently the major exporters of game meat from southern Africa. The methods employed are determined by the specific species and the terrain where these species are found. The wild behaviour and extensive nature of game species mean that inevitably the mechanics of game meat production are infinitely more complex than those of domestic production systems where stock can be driven to a central meat production facility. Unlike with domestic animals, good management practices that minimise stress during pre-slaughter handling are difficult to employ with wild ungulates since factors such as terrain, time limitations, weather and the behaviour of specific species will hinder the efficiency of the harvesting process. As the export and local consumption of game meat in Africa increases, it is becoming increasingly important to maximise its quality in order for it to compete with that of domestic species. One of the major quality aspects that can be controlled through proper management is the use of cropping methods suited to the specific species being cropped and efficient in minimising *ante mortem* stress. Relating this *ante mortem* stress to the meat quality of wild ungulates is also essential in understanding the importance of the harvesting process when it comes to the quality of the product being produced.

Keywords: harvesting, meat quality, venison, Africa

1. Introduction

South Africa and Namibia are well-known for their high quality game meat and game meat products. Tourists often praise this attribute of game meat (Van Schalkwyk and Hoffman, 2010) as it is regularly offered on the menu in restaurants, guest houses and lodges – tourists wish to consume game meat as part of their African experience (Hoffman *et al*., 2003). In both countries, game numbers increased when land owners were granted ownership of the wild animals found on their property. Namibia's freehold farmers have had ownership rights over land and livestock since the early 1900's, although the commercial rights over wildlife and indigenous plants had only been given to freehold farmers in 1967. Farmers in communal areas received the same rights over wildlife much later (1996) when policies were adopted to promote community-based natural resource management (Barnett and Patterson, 2006). The implementation of these policies resulted in wildlife being utilised and valued by the private sector, driving the wildlife sector into a rapid growth phase (Mendelsohn, 2006).

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Although South Africa has had no nation-wide census as pertaining to game numbers, it is estimated that there are more than 5,000 game farms, 4,000 mixed livestock-game farms which amount to approximately 13% of the total surface area of the country. Springbok (*Antidorcis marsupialis*) is the major species harvested and exported (Table 1) from South Africa (70% of game animals harvested in 2008) and Namibia (80% of game animals harvested in 2008). In 2000 it was estimated that the gross income generated by game meat sales in South Africa alone was R20 million (Eloff, 2002). Kudu and blesbok were respectively the second and third most utilised species for game meat production in South Africa between 2002 and 2004 (Table 1), even though the amount of meat produced from these two species were far less than that produced from springbok (Patterson and Khosa, 2005). In 2005, it was estimated that South Africa exported de-boned meat from 160,000 carcasses, predominantly from springbok (*Antidorcus marsupialis*), blesbok (*Damaliscus pygargus phillipsi*) and kudu (*Tragelaphus strepsiceros*). Other species such as zebra (*Equus burchelli*), blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*) and gemsbok (*Oryx gazella*) were also exported in smaller numbers (Hoffman and Wiklund, 2006). However, due to the global economic climate, this number had decreased due to a lower international market demand to slightly over 76,000 in 2009 which was lower than the 85,500 of 2008.

In Namibia there are at least two million head of game (Table 2), a figure roughly similar to those for cattle, for sheep and for goats (Barnes *et al*., 2009). Approximately 90% of the wildlife is located outside formally proclaimed conservation areas. More than 80% of the larger game species are found on privately owned farms which comprise about 44% of the surface area of Namibia (Brown, 2008). Currently at least 41% of Namibia is under wildlife management. Some 60 communal conservancies are now registered bringing the area under communal conservancy management to about 15.3% of the area of Namibia. State protection comprises

Table 1. Species and numbers of game harvested commercially for meat production in South Africa for the period 2002 to 2004 (Patterson and Khosa, 2005).

Species	2002		2003		2004	
	Number	Weight (tons)	Number	Weight (tons)	Number	Weight (tons)
Springbok	19,252	287,956	25,133	322,030	20,664	307,374
Kudu	733	64,572	256	21,155	646	51,869
Blesbok	811	29,755	31	1,002	1,379	49,241
Black wildebeest	285	22,460	0	0	222	18,744
Zebra	84	14,240	337	64,914	88	16,633
Eland	14	3,282	0	0	82	16,343
Gemsbok	29	2,491	7	820	139	13,537
Impala	117	3,616	28	794	169	4,296
Deer, fallow	51	1,519	1	33	65	1,733
Bushbuck	6	190	1	32	0	0
Blue wildebeest	29	3,005	1	72	0	0
Total	21,457	433,771	25,816	411,080	23,455	479,783

16.5%, freehold conservancies 6.1%, private protected land 2.1% and community forests and concessions 1.3% (Brown, 2009).

Table 2. Wildlife numbers in Namibia.

Species	Protected areas NVCF[a]	Protected areas SVCF[b]	Communal land NVCF[a]	Communal land SVCF[b]	Private land	Total
Springbok	33,811[c]	1771	37,150	37,270	621,561	731,563
Kudu	2,063[c]	1484	1,545	1,000	345,801	351,893
Gemsbok	11,450[c]	3115	18,670	5,084	350,092	388,411
Red hartebeest	1,468[c]	115	700	0	122,805	125,088
Eland	1,704[c]	524	245	0	34,743	37,216
Plains zebra	18,098[c]	0	20	0	7,303	25,421
Mountain zebra	8,564[c]	4347	2,130	2,175	55,520	72,736
Ostrich	3,947[c]	530	2,840	2,020	36,336	45,673
Blue Wildebeest	4,975[c]	224	470	0	16,623	22,292
Black faced impala	1,500[c]	0	0	0	1,870	3,370
Common impala	77[c]	0	385	0	14,980	15,442
Roan	440[c]	120	95	0	435	1,090
Sable	256[c]	60	15	0	902	1,233
Lechwe	0	0	250	0	284	534
Tsessebe	0	15	0	0	162	177
Waterbuck	0	0	0	0	4,475	4,475
Buffalo	1,025[c]	250	90	0	0	1,365
Giraffe	3,683[c]	229	666	68	5,769	10,415
Warthog	148[c]	61	40	0	173,866	174,115
Cheetah	706[c]	149	405	270	2,970	4,500
Leopard	1,970[c]	430	960	640	4,000	8,000
Lion	574[c]	23	109	22	0	728
Elephant	9,043[c]	24	735	155	0	9957
Hippo-potamus	1,262[c]	0	300	0	0	1,562
Black rhino	816[c]	43	45	75	134	1,113
White rhino	54[c]	62	0	0	75	191
Total	107,634	13,576	67,865	48,779	1,800,706	2,038,560

[a] NVCF = North of the Veterinary Cordon Fence.

[b] SVCF = South of the Veterinary Cordon Fence.

[c] Game counts are not representative of the current numbers of wildlife in protected areas.

The major wildlife species in Namibia under consideration for commercial game meat export are gemsbok, springbok, kudu (*Tragelaphus strepsiceros)*, mountain zebra (*Equus hartmannae)*, red hartebeest (*Alcelaphus buchelaphus)* and eland (*Taurotragus oryx*). The suitability of these species is not only based on their population numbers, but also on other factors such as their reproductive performance, the fact that they occur in large herds in easily accessible regions, their suitability for commercial harvesting and proximity to de-skinning, de-boning and processing facilities. In Namibia the impact of wildlife use on the economy is estimated to be some N$ 1.3 billion when the indirect contributions are included as a result of a multiplier effect of 1.86 (Barnes *et al*., 2009).

In both South Africa and Namibia tourism is a growing industry. In Namibia tourism is the strongest driving force behind the growth of the wildlife industry. This sector is envisaged to grow at 6.9% per annum between 2008 and 2017. Namibia's Tourism Satellite accounts show that in 2006 tourism contributed directly and indirectly (through support industries to the tourism sector) about 71,777 jobs and N$ 6.8 billion to the Gross Domestic Product (GDP). These tourists expect to see unspoiled habitat, or at least what they perceive to be unspoiled. At the same time they expect to see an abundance of game animals. However, surplus animals need to be removed to maintain populations in equilibrium. There are a number of options available for the game farmer or owner to control or remove surplus game animals such as:

- making use of predators;
- live auctions;
- recreational hunting: trophy hunting and fresh meat (own use, biltong hunting);
- harvesting or harvesting for commercial meat production.

Although predators are high on the non-consumptive tourists' list to see and photograph, this form of control is seldom suitable as the fenced game farms are often small and predators then consume large numbers of game animals which are frequently scarce and expensive animals (Power, 2002, 2003; Lehman *et al*., 2008). Until recently live sales were a feasible option for managing wildlife populations, but auction prices reached a peak (Eloff, 2002) and are about half of the price obtained for commercial meat sales (Brown, 2008). Trophy hunting earns more foreign currency for Namibia than it does for South Africa, which makes Namibia one of the preferred hunting destinations in Africa. Humavindu and Barnes (2003) suggested that trophy hunting is about five times more important as a contributor to the national economy in Namibia as South Africa. It is only Tanzania that earns more foreign currency from trophy hunting than Namibia (Agriforum, 2007).

Harvesting game for commercial meat sales has huge potential (Von la Chevallerie, 1970; Van Schalkwyk and Hoffman, 2010). Consumers expect the meat products on the market to have the required nutritional value, be wholesome, fresh, lean and have adequate juiciness, flavour and tenderness (Dransfield, 2001, 2003; Ngapo and Dransfield, 2006). The export of game meat from South Africa and Namibia to the European Union is on the increase (Hoffman, 2003; Van Schalkwyk and Hoffman, 2010). Most of the game harvested on large scale is destined for export. The standards set for processing of game meat are high and enforced stringently (Hoffman, 2003). Game harvesters hunting game for the commercial meat trade and meat processing are however food business operators in their own right and responsible

for the safety of the food they deliver to the game meat value chain (Atanassova *et al*., 2008). Von La Chevallerie (1970) listed the requirements for successful harvesting of game as follows:

- humanity;
- economy;
- efficiency;
- low wounding percentages;
- low disturbance and scattering;
- selectivity of correct ages and sexes;
- minimal damage to meat;
- ability to bleed carcasses;
- no association with humans.

It is imperative that game animals are handled correctly prior to harvesting and dressing, as incorrect handling can result in meat that is unwanted (Hoffman, 2001). If a game animal is killed with minimal stress then there is a normal pH decline and the quality of the meat is good. However, when the animal is killed after a running period, little glycogen is left in the muscles and the pH stays high. No lactic acid is formed and the meat is dark, firm and dry (DFD meat). Meat with a high pH is also prone to bacterial spoilage. If the game animal runs for a very long period the liver cannot break down the lactic acid fast enough and lactic acid builds up in the muscle. This results in the denaturation of the muscle protein and the meat is pale, soft and exudative/watery (PSE meat) (Van Schalkwyk and Hoffman, 2010). The traditional season for harvesting is usually in winter when animals are normally better fed and more water is available. Ambient temperatures are cold enough during winter to prevent carcasses from spoiling before being dressed and cooled. However, if the necessary cooling facilities are nearby, the season or time of the year does not have to hamper the harvesting of game animals (Hoffman, 2003).

2. Harvesting techniques

With the onset of fencing and zoning of large areas of land for use as game ranches and wildlife conservancies, harvesting of wildlife populations has invariably become an integral component of the management of game ranches and wildlife conservancies. According to Bothma (1996) there are three broad types of management options for wildlife populations on a game ranch:

- Conservation: the manipulation of a small or reduced population to increase its density.
- Sustained harvest: the utilisation of a population through sustained yield over a long time.
- Control: the manipulation of a population whose numbers are too high or which has an unacceptably high growth rate, to stabilise the population or to reduce its numbers.

The type of management option followed will depend on a number of factors, including the definitive objectives for the use of the ranch as well as the population dynamics of the species being considered (Bothma, 1996). Sustainable yield of game for meat production could be accomplished by regular harvesting of game. Maximum sustained yield assumes a certain constancy of environmental conditions seldom realised in Africa, particularly in arid regions (Skinner, 1989). For this reason, harvesting programmes should follow the feedback principles

of Stocker and Walters (1984), taking into account vegetation and also ungulate population numbers as well as age and sex ratios. The harvesting technique applied depends on the species, its habitat and the vegetation of the area. The harvesting techniques are continuously being adjusted so as to harvest the most animals in the least amount of time (Mostert, 2007).

Harvesting of ungulates can either be contracted, in which case professional harvesting teams are brought in, or a farmer may choose to harvest the animals himself. In the former case, abattoir facilities are customarily provided by the contractor and there is little flexibility in the scheduling of such activities. If, for example, an opportunity for harvesting is missed, it may be some time before a contractor is available again (Skinner, 1989). The use of contractors is also a necessity if the meat is to be exported or sold on the local market, whereas meat for own use is exempt from such regulations. In this case, the ranch owner is most likely to do the harvesting himself. For meat to be of export quality, only head and upper neck shots are acceptable (Hoffman, 2003).

The use of a specific harvesting technique is also important with regard to the stress placed on the animal prior to death since *ante mortem* stress has been shown to have deleterious effects on the meat quality of domestic livestock as well as on that of game (Smith and Dobson, 1990; Veary, 1991; Wiklund *et al*., 1995; Hoffman, 2000a; Kritzinger *et al*., 2002; Hoffman and Wiklund, 2006). Inefficient harvesting techniques also lead to higher labour costs while inaccurate shooting wastes ammunition and may result in excessively damaged carcasses. Inefficient harvesting may also lead to lower game productivity as a result of injured and stressed animals (Ruggiero and Ansley, 1992). The cost of harvesting has been shown to affect the price of game meat directly (Van Rensburg, 1992). According to Tinley (1972), there are four general requirements for successful harvesting:

- Instantaneous death: from humanitarian considerations and also because *ante mortem* stress causes inferior meat quality.
- Minimum disturbance to the population, e.g. if a number of animals have been harvested from one herd, it is unwise to continue chasing the same herd as they become excitable and later unapproachable.
- Throughout the year, daily human activity on foot, frequent passage of motor vehicles and spotlighting at night without hunting will accustom animals to the rancher's presence and activities and ultimately facilitate harvesting.
- If carcasses are required for consumption of fresh meat, they are required to be in an unspoilt condition, obtainable only with head and upper neck shots.

2.1 Night harvesting

Night harvesting in the field is undoubtedly the most popular method employed in large-scale game harvesting operations (Veary, 1991; Lewis *et al*., 1997; Hoffman, 2000a; Kritzinger *et al*., 2002; Hoffman an Wiklund, 2006; Le Grange, 2006; Van Schalkwyk and Hoffman, 2010). As early as 1964, Dassman reported that this method had the greatest success rate (Dassman, 1964). Hunting commences after dark and usually on moonless nights since animals are difficult to approach in moonlight and because the moonlight reduces the efficiency of the spotlights used to blind the animals (Le Grange, 2006). Strong spotlights are used to detect and blind the animals and hunting may continue until dawn the following day to take maximum

advantage of the moonless conditions (Kritzinger *et al*., 2002). The spotlights immobilise the animals so that they can be shot from distances ranging from 25 to 100 m. Ruggeiro and Ansley (1992) found that shots in excess of 150 m, on average, resulted in unacceptable accuracy. The latter is especially true when light calibre rifles are used and there is a strong prevailing wind; conditions frequently found out on the South African plains (Karoo) when plains game species are harvested. During large-scale operations, several marksmen may be employed at one time, each with a quota of animals to shoot. The marksmen each proceed in open chase vehicles and often the shooter may also be the driver so as to avoid delayed reactions from communication errors between drivers and shooters. One or two people are usually equipped with spotlights. They stand on the back of the vehicles, sweeping the light over the terrain to detect the animals. Animals are spotted by the reflection from their retinas and firing should only commence if clear shots are possible (Kritzinger *et al*., 2002). Head and upper neck shots are preferred, using high-velocity, small calibre rifles, since these reduce carcass loss to a minimum (Bothma, 1996; Le Grange, 2006). Von La Chevallerie and Van Zyl (1971) found that these shots resulted in the least amount of carcass damage in springbok and impala, while shots in the shoulder and buttocks can contribute up to 20% and 50% of carcass weight loss, respectively. According to Le Grange (2006), good-quality telescopic sights are also a necessity, since open sights do not provide the accuracy required, especially at ranges as far as 100 m.

A study by Lewis *et al.* (1997) noted that, with a single marksman, the average time that lapsed between the culling of impala in a herd was 28 seconds, with the maximum time being 3 minutes and 18 seconds and the minimum time being 2 seconds. It must be noted that in this study there was a high density of animals and the animals had been, as suggested by Tinley (1972), habituated to humans. Animals need to be collected as soon as possible after being shot since the darkness may impede the finding of the animals and in areas that harbour large populations of predators, these animals may compete with the harvesting team in recovering the carcasses as they become aware of the hunting routine (Le Grange, 2006).

Studies conducted by Hoffman and Ferreira (2000), Kritzinger *et al*., (2002), Veary (1991) and Von La Chevallerie and Van Zyl (1971) indicated that the least amount of *ante mortem* stress is experienced during night harvesting so that as a result, the use of night harvesting holds beneficial effects on certain meat quality parameters. Other advantages of night harvesting include the fact that animals in a herd are less disturbed by the harvesting (Ledger *et al*., 1967; Bothma, 1996) and lower night temperatures may allow for better meat quality as well while it also results in the absence of flies (Bothma, 1996). Disadvantages, on the other hand, are that wounded animals are more difficult to recover and in the absence of moonlight, the method is unsuitable in areas of dense bushveld where the vegetation makes it difficult to see the animals. Night harvesting may also be more expensive than harvesting methods employed during the day, since labour costs at night tend to be one and a half times those of day costs (Van Rensburg, 1992). Another disadvantage is that the culling is limited to moonless nights of two weeks per month and with the growth in the export market, insufficient numbers are taken off resulting in alternatives such as day shooting having to be implemented (Le Grange, 2006). In addition, night harvesting can prove disadvantageous if shooters and drivers are unfamiliar with the terrain since navigation is more difficult at night and this in turn may hinder the predictability of animal movement.

Night harvesting is also limited in its suitability to certain species. In the case of species that are not sexually dimorphic like the red hartebeest for example, the sexes are difficult to tell apart (Ruggeiro and Ansley, 1992; Bothma, 1996). Animals such as the kudu are also not suitable for this method as they are predisposed to look away from the spotlight or to close their eyes (Joubert, 1983). This is in contrast to springbok and impala, which are ideal since they have a tendency to remain still once they have been caught in the spotlight (Lewis *et al*., 1997; Conroy, 2005). According to Ruggeiro and Ansley (1992), the territorial nature and relative tameness of gazelles may reduce their flight distance, whereas the more skittish temperament of wildebeest and zebra, as well as their habit of running in tight groups, makes them more difficult targets when using this method. These authors also found that the dark grey colour of wildebeest made finding its head in the telescopic sight more difficult and the zebra's stripes made distinguishing one individual from another more difficult, particularly when moving in a tight group. During night harvesting, harvesting teams also tend to get tired so that accuracy tends to decline after 23:00 (Ruggeiro and Ansley, 1992) and more errors are made the longer the harvesting sessions proceed.

2.2 Day harvesting

There are a number of different harvesting methods employed during the day time. A method commonly applied in commercial harvesting operations is that of hunting from a vehicle in a method similar as described under night harvesting. Unlike night harvesting, this method can be used at any time of the month and animals are spotted by two or more people on the back of the vehicle. A definitive advantage of harvesting during the day is that marksmen are able to distinguish sexes (even when species are not sexually dimorphic) as well as age classes and social groupings so that selective harvesting is possible (Bothma, 1996; Hoffman and Laubscher, 2009a, 2010). This method is easily used with most species of game since sighting of the animals is less complicated during the daylight hours. Shots can also be fired at distances in excess of 150 m since animals are more easily distinguishable from each other and their surroundings than at night time, although it then becomes increasingly important for marksmen to be well trained in shooting at such distances. These distances also inevitably increase the chances of prevailing winds blowing the bullet off course and animals being wounded although during the day, wounded animals as well as carcasses are more easily located than at night thereby decreasing the risk of shooting losses.

Another method employed during the day although not commonly used, involves the herding of animals towards shooting lines using scrambler motorcycles, pick-up vehicles or horsemen. Marksmen position themselves either in camouflaged bunkers or behind bushes where they are unseen by the approaching animals (Kritzinger *et al*., 2002). Veary (1991) found that this method caused the largest amount of stress to the animals and he reported final pH values for springbok, harvested using this method, that were similar to a value reported by Hoffman (2000a) for a single severely stressed impala ram. According to Kritzinger *et al.* (2002), this method also causes severe sub-dermal abrasions and bruising as a result of the animals bumping into each other and falling. The effect of the *ante mortem* stress on the *post mortem* pH of the muscle also causes several deleterious meat quality attributes. Field observations also indicated that this method is also not suited to small, agile species such as warthog, since they tend to lie down and hide in the thicket when being chased, making it impossible for the

marksmen to spot them. Bothma (1996) observed similar behaviour in bushbuck and nyala, which tend to run in any direction and try to hide.

2.3 Hide harvesting

This method is employed during the day time and animals are lured to either a drinking hole or feeding point. Animals are then killed from a nearby hide, using a silenced rifle (Hoffman and Wiklund, 2006). The method may be used in areas with dense bush where animals are difficult to locate on foot or by vehicle. It works well with species such as kudu that are prone to approaching feeding points although it does not work well with other species such as impala (Hoffman and Wiklund, 2006). This method may be used by ranchers for meat for own consumption although it is not commonly used in commercial operations since the off-take rate is very slow.

2.4 Boma harvesting

The technique employed in this method is similar to that of the mass boma capture of animals for relocation. Game is herded by a helicopter into a large capture boma, where they are then shot with a light calibre rifle from the ground (Bothma, 1996). Bomas are usually constructed from dark coloured plastic since animals are reluctant to challenge an apparently solid wall and because the animals are unable to see through the plastic. This also ensures that as the animals proceed forward to 'escape', they can be moved into separate compartments (Le Grange, 2006). Wind direction plays a critical role in setting up the boma so that the position of the front gate must be downwind from the direction from which the animals will approach (Le Grange, 2006).

Once the animals are herded into the main area of the boma, it is recommended that they stand for a short period, usually less than 2 hours (Hoffman and Wiklund, 2006). After this, they are broken up into smaller groups (± 10 animals per group) and moved into smaller compartments. It is in these smaller compartments that they are shot and this activity usually takes approximately 60-90 seconds (Hoffman and Wiklund, 2006). According to Le Grange (2006), it is best if animals are moved into the smaller compartments during the day and left until night, when shooting commences. This allows them to settle down and accept the enclosure as well as the intrusion of the light and the marksman later on, and the animals can then be dispatched off relatively quickly. From there, the animals are removed from the compartment to a transport truck where they are hung (head hanging down) and after the vehicle has moved away a short distance, they are exsanguinated. This is done to minimise the blood spilt in the killing enclosure as this may stress the next batch of animals. The carcasses are then transported further to a field abattoir set up in the veld (Mostert, 2007).

This method of harvesting has a practical advantage for dense bushveld areas where the landscape is inaccessible to vehicles. In these areas, the animals can be driven to areas which are accessible to trucks and refrigeration vehicles (Bothma, 1996). It allows for a large number of animals to be harvested and processed within a very short time period and ensures that no wounded animals are left behind. It also allows for a certain level of selectivity in terms of which animals are harvested thus allowing animals of trophy status or specific breeding

animals or very young animals to be selected and set free. Animals are processed on the spot and this allows for easy maintenance of hygiene and easier inspection of the carcasses by the relevant authorities (Le Grange, 2006). The use of light calibre rifles also causes less damage to the carcass.

No research has been done with regard to the effect of this harvesting method on meat quality and according to Hoffman and Wiklund (2006), dominant males in a herd may start fighting with submissive males and may even kill submissive males. This is in agreement with Bothma (1996) who found that males of certain species, for example impala, kudu, waterbuck, gemsbok, blue and black wildebeest, red hartebeest and eland, often fight with one another or with the females soon after being captured and may need to be separated to prevent injury. Care should also be taken with those species that have horns since panicked animals may cause serious injuries to those around them. Fighting and pushing between animals may result in bruising which will negatively affect meat quality.

Capture myopathy may also become a problem if animals were herded over long distances and for prolonged periods of time. Certain species such as eland bulls, kudu and waterbuck may cause problems when held in the boma since they are excellent jumpers and may jump out if they become overly nervous. This is normally overcome by placing of shade net over the specific boma area. Buffalo do not challenge the plastic at all unless they can see out or through it (Le Grange, 2006). Certain species are also more amenable to being driven, for example eland and blesbok, while others, such as the kudu, easily become nervous and difficult to herd (Ledger *et al.*, 1967; Bothma and Van Rooyen, 2005). Because species such as impala are naturally large-herd animals, they are also easily herded and captured in a boma (Furstenburg, 2005). Other methods of harvesting may be preferred over this method since its set-up may be more costly and labour needs to be trained in an assembly line-type approach to process the carcasses as quickly as possible (Bothma, 1996; Le Grange, 2006).

2.5 Helicopter harvesting

This method consists of shooting animals from a helicopter during the day time (from an altitude of around 6 m) using a 12-bore shotgun while a ground team follows in a vehicle to collect the dead animals (Veary, 1991; Bothma, 1996). Hunting may be carried out with semi-automatic shotguns so that as many as six animals can be shot in succession as they run in a line (Le Grange, 2006). Shooting from a helicopter has the advantage of selective culling as well as being practical in dense bushveld areas where it is difficult to locate animals on the ground (Rudman, 1983). A quick estimate of the game population can also be made during the harvesting operation and harvesting can take place over a larger area than would be possible with boma harvesting (Bothma, 1996).

According to Van Rensburg (1992), this method is the most expensive when compared to boma harvesting and harvesting from a vehicle and is usually only employed in large scale commercial operations since it requires high capital investment. This method has been successfully used for impala, blesbok, springbok and buffalo in Africa as well as red deer in New Zealand (Le Grange, 2006). Veary (1991) noted similar muscle ultimate pH values as those obtained during night harvesting, although according to Le Grange (2006), experience

in Zimbabwe has shown that the high levels of adrenaline released in the animals during shooting and the excessive increase in body temperature result in extremely rapid meat decay (probably due to high ultimate pH values and the occurrence of dark, firm and dry meat), usually rendering the carcasses unfit for human consumption.

A high success rate is also dependent on the skill and accuracy of the shooter as well as the open nature of the terrain so that only head and neck shots can be utilised. According to Mostert (2007), this may not always be the case and broken legs can sometimes be observed in species such as springbok that tend to jump when fleeing. Good communication between the pilot and ground crew is also essential so that carcasses can be recovered quickly. In many cases, it may be necessary for the ground crew to use hand-help GPS navigational equipment for locating the carcasses. Carcasses also need to be eviscerated as soon as possible to cool the carcasses and reduce decay (Le Grange, 2006). Another disadvantage to this method is that it may inflict unnecessarily high stress (due to exercise) and bruising on the animals as well as damage to fences when larger animals attempt to escape the property (Rudman, 1983).

Capture myopathy may also become a problem if animals are chased for extended periods before being shot. This occurs because of over exertion since wild animals are generally not equipped to run fast over long distances or for long periods of time. Over exertion results in increased plasma glucose levels and the oxidative capacity of the mitochondria is exceeded with the intense simultaneous involvement of the majority of the animal's muscles. In the resultant hypoxia, anaerobic metabolism in increased, lactate levels raise dramatically, cell membrane permeability is increased and various intracellular enzymes are released with a simultaneous reduction in blood pH, i.e. blood acidification (Veary, 1991). Following a number of chemical changes in the blood, reactions take place in the damaged tissues and the kidneys become affected as well. The resulting physiological and chemical effects interfere with the normal functioning of several vital organs. Animals then die either as a result of kidney degeneration or heart failure (Bothma, 1996). The high acidification in the muscles of such animals will hold detrimental effects for the meat quality since a high ultimate pH in meat results in meat with a high spoilage potential and a resulting short shelf-life (Newton and Gill, 1981).

2.6 Conventional hunting

This method is suited to all game species and can be applied either on foot, from a hide or from a vehicle. Harvesting should be done during the day time, preferably in the early morning or late afternoon if there are no cooling facilities available (Tinley, 1972). It is advantageous as it causes little disturbance, specifically if done on foot which prevents the animals associating vehicles with hunting, and game do not run much and consequently yield a higher quality of game meat. Selective harvesting can also take place with regard to age, sex and social grouping (Bothma, 2006; Hoffman and Laubscher, 2009b). Although it is unethical to hunt game at waterholes, it is effective for game which occurs in small groups, for example warthogs, for timid game such as bushbuck and nyala and in dense bushveld areas where walking is virtually impossible (Bothma, 2006).

2.7 Harvesting losses

According to Von La Chevallerie and Van Zyl (1971), harvesting losses are usually the result of three factors:

- Loss of meat unfit for human consumption because of bullet damage.
- Animals shot and not recovered.
- A decline in meat quality because of *ante mortem* stress to which hunted and wounded animals are subjected.

Wastage of meat due to bullet damage may be prevented by the right placement of shots (Table 3) as well as by ensuring good marksmanship of the shooter beforehand since the more difficult shots such as head and upper neck shots are also the most preferred shots (Bothma, 1996). Studies by Hoffman (2000a,b) and Hoffman and Ferreira (2000) found that head shots resulted in no wastage of meat and high neck shots resulted in less than 2% wastage of meat. In the meat trade, the neck is also classified as a lower value joint (Hoffman, 2001) so that the damage done with regards to carcass value by shots through this region is almost negligible. On welfare grounds, head shots are preferred since they usually result in instantaneous death while neck shots may result in paralysis and may not render the animal immediately insensible (Lewis *et al*., 1997). However, Hoffman and Sales (2007) has shown that in animals such as the warthog (*Phacochoerus africanus*), placement of the bullet in a specific area in the brain is important as incorrect placement may cause excessive kicking movements (and thus muscle activity) that could result in pale soft exudative (PSE) meat developing.

Traditionally, hunters prefer to shoot animals through the shoulder rather than through the head or upper neck since it gives the shooter a larger and more stationary target area and less chance of missing. This type of shot is usually placed at the top of the crease at the back of the foreleg, in the position where large vital organs such as the heart and lungs can be found as well as major blood vessels and nerves. A bullet in this area will either hit the heart, resulting in massive haemorrhage or will hit the lungs and large blood vessels, resulting in lung collapse and a 'quick' death (Hoffman, 2001). Although this shot can result in up to 20% carcass damage (Table 3), it is the type of shot that is most likely to result in death since, even if the exact target area is not hit, most shots in this shoulder area will either result in death (even if

Table 3. Bullet damage from shots at various localities as a percentage of total carcass weight (adapted from Von La Chevallerie et al.*, 1971).*

Locality of the bullet wound	Percentage of carcass damaged
Neck	3.18
Neck and shoulder	15.66
Shoulders	20.58
Shoulder and ribs	22.22
Ribs	5.47
Back	12.47
Other (e.g. stomach, hind quarters)	15.61

not instantaneous) or at least severe wounding of the animal causing no or poor mobility. In the latter case, a second shot should be enough to kill the animal. According to Van Rooyen *et al.* (1996), gut shots should also be avoided since contamination of the carcass from the stomach and intestinal contents may occur, which is unacceptable. The behaviour of the animals may also affect harvesting losses and Lewis *et al.* (1997) found that males generally respond more actively to disturbances than females and show an increase in response when in breeding herds, leading to a higher percentage of animals being wounded.

Rifle calibre is also important and light calibres are preferred where possible since they cause the least amount of damage (Hoffman, 2001). An impala should, for example, preferably not be shot from a short distance with a 7 mm Remington Magnum but rather with a 7 × 57 mm or a .30-06 with a heavy bullet (Bothma, 1996).

3. Harvesting operations for meat export

3.1 Legislation

There is a difference in definition for large and small game between the South African legislation and the European Union legislation. The South African legislation (Anonymous, 2000) defines gemsbok, kudu, hartebeest and zebra as large game (Category B) and springbok as small game (Category C). The definition section in the European Union legislation (EC, 2004c, Annex I) defines small wild game as *wild game birds and lagomorphs living freely in the wild* (paragraph 1.7). A lagomorph is any plant eating mammal with two pairs of incisors in the upper jaw specialised for gnawing, i.e. rabbit and hare. Large wild game is defined as *wild land mammals living freely in the wild that do not fall within the definition of small wild game* (paragraph 1.8). All the species suitable for commercial harvesting in southern Africa would thus be classified as large wild game in the EU legislation. Certification of exports of animal and animal products in South Africa and Namibia is done by the competent authority of the exporting country. The legislations pertaining to the export of game meat to any other country are governed by the requirements of the particular importing country. Official veterinarians are required to make certain health attestations which cover both animal health and public health conditions (Kamwi and Magwedere, 2007).

The following legislations and guidelines are applicable to the export of game meat from Namibia to South Africa (Kamwi and Magwedere, 2007):

- South African requirements:
  - Codex Alimentarius: Recommended International Code of Hygiene Practice for Game (CAC, 1993).
  - Meat Safety Act No. 40 of 2000 (Anonymous, 2000) and draft game meat regulations.
  - Veterinary Procedural Notices.
- Namibian requirements:
  - Animal Diseases and Parasites Act No. 13 of 1956 and its regulations as amended (Anonymous, 2005a).
  - Prevention of Undesirable Residues in Meat Act No. 21 of 1991 as amended (Anonymous, 1991).

- Animal Protection Act No. 71 of 1962 as amended (Anonymous, 1962).
- Veterinary Circulars (VC).

The following legislations and guidelines are applicable to the harvesting, dressing and export of game meat from Namibia and South Africa to the European Union (Kamwi and Magwedere, 2007):

- Council Decision 79/542/EEC as regards certification requirements for imports into the community of certain live ungulate animals and their fresh meat (EC, 1979).
- Council Directive 92/45/EEC on public health and animal health problems relating to the killing of wild game and the placing on the market of wild-game meat (EC, 1992).
- Council Directive 93/119/EC on the protection of animals at the time of slaughter or killing (EC, 1993).
- Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products (EC, 1996).
- Council Directive 98/83/EC on the quality of water intended for human consumption (EC, 1998).
- Council Directive 2002/99/EC laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption (EC, 2002b).
- Commission Regulation No. 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (EC, 2002a).
- Commission Decision 2003/73/EC-amending Decision 97/468/EC as regards the inclusion of Estonia and Namibia establishments in provisional lists of third country establishments from which Member States authorise imports of wild game meat (EC, 2003a).
- Council Directive 2003/99/EC on the monitoring of zoonosis and zoonotic agents (EC, 2003b).
- Commission Regulation No. 1441/2007 amending parts of Regulation No. 2073/2005 on microbiological criteria for foodstuffs (EC, 2007).
- Commission Regulation No. 852/2004 on the hygiene of food stuffs (EC, 2004a).
- Commission Regulation No. 853/2004 laying down specific hygiene rules for food of animal origin (EC, 2004b).
- Commission Regulation No. 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption (EC, 2004c).
- Commission Regulation No. 2073/2005 on microbiological criteria for foodstuffs (EC, 2005a).
- Commission Regulation No. 2075/2005 laying down specific rules on official controls for *Trichinella* in meat (EC, 2005b).
- Commission Decision 2008/752/EC amending Annexes 1 and 11 to Council Decision 79/542/EEC as regards certification requirements for imports into the community of certain live ungulate animals and their fresh meat (EC, 2008).

3.2 Areas approved for harvesting

Game should not be harvested from areas which are subject to official prohibition of harvesting, whether the prohibition is for reasons of conservation, animal health, animal or plant chemical control, or any other reason (CAC, 1993). Animals may only be harvested from the OIE recognised Foot and Mouth Disease (FMD) free zone without vaccination. In Namibia this zone extends south of the Veterinary Cordon Fence which extends from Palmgrave Point in the west to Gam in the east of Namibia (for bovine, ovine, caprine, wild and farmed game). The meat should be obtained from animals originating in areas which are free of OIE (Office International des Épizooties/World Animal Health Organisation) notifiable diseases prior to slaughter (Kamwi and Magwedere, 2007).

3.3 Requirements for harvesters

Harvesters should note any abnormal condition they detect in the live game animal, or during the evisceration or bleeding of a game carcass, and such abnormal condition should be reported to an inspector if that game carcass is taken to a game establishment (CAC, 1993). Harvesters are required to be registered and must meet certain requirements understanding the normal disposition of the animal (Anonymous, 2000). For meat exports to the European Union harvesters must be trained in health and hygiene and must have sufficient knowledge of the pathology of wild game and of the production and handling of wild game and wild game meat after harvesting to be able to undertake an initial examination of wild game at the point of harvesting.

Training of harvesters should cover at least the following subjects (EC, 2004c: Section 4, Chapter 1, par 1-5):

- The normal anatomy, physiology and behaviour of wild game.
- Abnormal behaviour and pathological changes in wild game due to diseases, environmental contamination or other factors which may affect human health after consumption.
- The hygiene rules and proper techniques for the handling, transportation, evisceration, etc. of wild game animals after killing.
- Legislation and administrative provisions on the animal and public health and hygiene conditions governing the placing on the market of wild game.

Results from a study by Atanassova *et al*. (2008) concluded that freshly shot game has a very good hygienic status when all requirements are properly carried out. They detected a connection between the shooting methods of expertly and non-expertly shot animals and the occurrence of Enterobacteriaceae which can cause meat spoilage.

3.4 *Ante mortem* inspection

If an animal is under stress when harvested, the quality and shelf-life of the meat will be negatively affected. The meat of stressed animals may discolour. Blood supply to the muscles is increased and this can result in poor bleeding and meat that is not tender. Only game which passed the *ante mortem* inspection and that are seemingly alert may be shot (Van Schalkwyk and Hoffman, 2010). *Ante mortem* inspections must be carried out by the harvester prior to

the harvesting operation (CAC, 1993; Anonymous, 2000). If no abnormalities were observed amongst the animals during the examination, no abnormal behaviour was found before harvesting and there was no contamination to the environment, the trained person must attach a numbered declaration to the shot animals stating the condition of the animals as well as the date, time and place of the killing (EC, 2004c: Chapter II, par 4(a)). For meat exports to the European Union, harvesters must comply with the requirements imposed by the European Union to permit the monitoring of certain residues and substances in accordance with Council Directive 96/23/EC (EC, 1996).

3.5 Shooting

Game should be shot in the field in a humane manner (CAC, 1993). Shooting must be done by a competent marksman, ensuring immediate death. Only head shots are allowed for commercial harvesting. This is essential to limit decay and contamination of the meat (Van Rooyen *et al*., 1996). Game killed with thoracic and abdominal shots are subject to secondary inspection (Anonymous, 2000: Part V, Section 11.(1)(h), par 61). For meat exports to the European Union, shooting must be executed in accordance with Council Directive 93/119/EC (EC, 1993).

3.6 Bleeding

Game intended for commercial purposes must be bled without delay (CAC, 1993) and preferably within 10 minutes of being shot (Van Schalkwyk and Hoffman, 2010). Blood is an ideal growth medium for bacteria and when not well-bled, a carcass will deteriorate faster (Van Rooyen *et al*., 1996). Bleeding is done by means of severing the jugular vein and carotid artery on either side of the neck (throat slitting) with a clean sterilised knife. In any event, the bleeding must be carried out before the animal regains consciousness. All animals which have been stunned (shot) must be bled by incising at least one of the carotid arteries or the vessels from which they arise (EC, 2004c: Chapter III, par 1). The knife used for bleeding must be washed and sterilised before each cut. Large numbers of bacteria are found on the skin of any animal and contaminate knives when cutting through the skin. A system for sterilising the knives should be available. Ideal would be water at a minimum of 82 °C, but since this is sometimes not practical; an approved chemical steriliser in an enclosed holder which is fitted to the harvesting vehicle should be used instead. When large numbers of game are harvested several knives should be used to prevent cross-contamination between carcasses. A two-colour knife system is often recommended to ensure the effective sterilisation of the knife not in use. Workers bleeding the game must wash their hands between each carcass with bactericidal (food grade approved) soap and potable warm water (42-45 °C) (Van Schalkwyk and Hoffman, 2010).

The different categories must be bled in the following ways (Anonymous, 2000: Part V, Section 11.(1)(h), par 62):

- Category A (large animals): may be bled in a lying position.
- Category B (medium animals): on a ramp at a minimum of 20°.
- Category C (small animals): may be bled in a lying position.

Wounded animals requiring a second shot must be condemned if a time period of 10 minutes is exceeded after the first shooting. All suspect animals, including those that have been wounded, must be identified and clearly marked. Detained carcasses can either be totally condemned or passed conditionally. Suspect carcasses must be separated or fully covered in plastic when transported to the export abattoir for examination by the State Veterinarian. On the Suspect Carcass Form, which must accompany the consignment to the game handling facility, the following should be indicated by the Game Meat Examiner (Van Schalkwyk and Hoffman, 2010):

- species, age, gender and weight of game animal;
- part of carcass affected;
- possible cause of detention;
- additional observation made during hunting.

3.7 Evisceration in the field

If the shooting site is far from the field abattoir, the intestines and stomach should be removed from the carcass. Care must be taken to ensure that the abdominal cavity and the cut surfaces are not contaminated with rumen content or dust or dirt (Ebedes and Meyer, 1996). This should be done within fifteen to twenty minutes after the animal has been shot. A knife with a rounded cutting edge is needed for this purpose. Evisceration is easier when the carcass is in a hanging position with the head hanging downwards. It is advisable to staple the cut skin of the abdominal wall together for transporting (Van Rooyen *et al*., 1996).

3.8 Transport of harvested game to field abattoir

Game carcasses should be transported to a field abattoir within two hours of bleeding. The neck slit area must not be contaminated when transporting the carcass to the field abattoir/depot (Anonymous, 2000: Part V, Section 11.(1)(h), par 65). Vehicles used for harvesting Category C (small game: Namibian and South African category) game or springbok (considered as large game by the EU) must be (Van Schalkwyk and Hoffman, 2010):

- designed with a corrosion resistant hanging frame to bleed carcasses in a hanging position;
- designed to provide sufficient space (no heaping) between carcasses to allow effective air flow for cooling;
- corrosion resistant and free from holes and cracks;
- durable, non-toxic, smooth surfaced and impervious;
- resistant to impact;
- easily cleanable;
- free from equipment or loose objects, other than what is required for the harvesting of game;
- designed in such a manner that the animal's feet are not touching the ground while in transit.

Vehicles used for the harvesting of Category B (medium) game must:

- comply with the requirements above;
- have a hoist and a ramp manufactured at a minimum slope of 20° for hanging of game.

3.9 Partial dressing of the game carcasses at the field abattoir

Carcasses must be transferred from the collecting vehicle to a clean slaughter frame at the field abattoir in such a manner as to avoid contamination. Labels must be provided for the identification of each carcass and its organs (Ebedes and Meyer, 1996) since maintaining traceability is essential for export purposes. At the field abattoir the heads and feet may be removed provided that it can still be correlated with the carcasses when meat inspection is done. Horns may be removed with part of the cranium and stored separately (Anonymous, 2000: Part V, Section 11.(1)(h), par 64). In specific cases like the zebra, heads and feet are not cut off at the field abattoir/depot since the skin has more value when the skin of the head and feet is also preserved.

Partial evisceration, normally restricted to the removal of the intact gastrointestinal tract, serves to reduce the weight and bulk of the carcass and to speed cooling. Such removal should be restricted to those parts which will not increase exposure to contamination to an unacceptable level and which the controlling authority determines are not required for inspection. A game carcass should not be skinned or dressed beyond the extent required in the Codex guideline (CAC, 1993). Incision lines for opening the hide or skin must be spear cuts from the inside to the outside. A clean sterilised knife must be used. Lactating udders and reproductive organs are regarded as condemned material and must be removed with the skin on in such a way as to prevent contamination. Contact with outer surfaces and soiled equipment must be avoided at all times. Carcasses may not be washed and soiled or contaminated areas must be cut off (Van Schalkwyk and Hoffman, 2010).

The trachea and oesophagus are cut loose from the surrounding muscles, from the lower jaw to the breastbone and the diaphragm is cut away from the ribs. The trachea, oesophagus, lungs and heart (red offal) are hung in enclosed bags next to the carcass (Van Rooyen *et al*., 1996). It must be kept identifiable with the carcass of origin until inspection (Anonymous, 2000: Part V, Section 11.(1)(h), par 65). The game meat inspector at the field abattoir must inspect each carcass and matching viscera, head and feet and any abnormalities must be noted down in a report to be forwarded to the game meat abattoir. If a game meat inspector is not available at the field abattoir/depot, the viscera, heads and feet must be transported with the carcasses to the game abattoir while maintaining identification between the carcasses and the organs. Lockable fly-proof containers must be available during evisceration for the collection of condemned material (Anonymous, 2000: Part V, Section 11.(1)(h), par 66).

Continuous cleaning and sanitation should be practiced throughout the evisceration process at the field abattoir (Clean-as-you-go). Workers must continuously clean and sanitise with warm water (82-87 °C) or chemical steriliser (1-2 ppm free chlorine) all hooks, knives, tools and other equipment used during evisceration to prevent contamination. The temperature and/or chlorine level of the water must be tested throughout the harvesting process. The chemical data sheets of all detergents and sanitizers, as well as the dilutions and contact times thereof must be available on site. When the floor surface becomes covered in blood and dirt it should be swept clean (Van Schalkwyk and Hoffman, 2010).

3.10 Chilling of game carcasses

Partially dressed carcasses and offal must be chilled within 12 hours of culling to a temperature not exceeding 7 °C, but when the ambient temperature is more than 15 °C, it must be chilled within four hours of being killed (Anonymous, 2000: Part V, Section 11.(1)(h), par 67). Where the ambient temperature is sufficiently low to achieve the required temperature, carcasses should be placed under refrigeration soon after harvesting, either in a game depot, game establishment or other specifically approved facility (CAC, 1993).

Veterinary maturation of meat destined for the European market is necessary. This is a control process whereby the Foot and Mouth virus is deactivated. Carcasses must be submitted to maturation at a temperature above + 2 °C and below + 7° C for at least 24 hours before de-boning. All carcasses that have gone through the maturation period should have a pH of less than 6.0. The maturation period starts when the door of the chiller truck is closed after the last carcass have been placed in the chiller truck. This requirement is described in Commission Decision 2008/752/EC amending Annex I and II of Council Decision 79/542/EEC as regards certification requirements for imports into the Community of certain live ungulate animals and their fresh meat (EC, 2008).

Maturation of the meat is critical regarding quality. Game has a high metabolic rate and incomplete maturation may occur. Conditions before shooting may increase metabolism (Fink, 1992; Kappelhof, 1999). Cooling of carcasses is hampered due to aponeuroses which firmly surround the muscles and the often, thick hairy skin (Altemeier *et al*., 1998). If the glycogen reserves are reduced by stress, meat maturation and acidification can be impaired resulting in game meat which is tough, has a limited shelf life and a higher pH (Hofmann, 1987; Fink, 1992; Deutz *et al*., 2000).

3.11 Vehicles transporting harvested game

Vehicles used for the transport of partially dressed carcasses must comply with the standards for a meat transport truck according to the requirements for Food Premises under the South African Health Act No. 61 of 2003. If partially dressed carcasses and offal need to be held in a chiller truck for periods exceeding eight hours, the chiller unit must have the potential to chill such carcasses to a core temperature of less than 7 °C within 24 hours of being loaded. Carcasses must hang away from the floor in such a way as to ensure optimal air flow between carcasses and to avoid contact between skin surfaces and exposed meat of the body cavities. Edible rough and red offal transported in the same load space as the carcasses must be packed in closable leak proof containers (or bags) (Anonymous, 2000: Part V, Section 11.(1)(h), par 68). These organs must preferably hang together with the carcasses.

Game meat can only be marketed commercially if it was transported to a game handling establishment as soon as possible after the *ante mortem* inspection. The viscera must accompany the body and must be identifiable as belonging to a given animal (EC, 2004c: Chapter II, par 3). If no trained person is available to carry out examinations on the body, then the head (except for tusks, antlers and horns) and all the viscera except for the stomach and the intestines must accompany the body (EC, 2004c: Chapter II, par 4 (c)). The heaping

of carcasses should be avoided while travelling to the game handling establishment (EC, 2004c: Chapter II, par 6).

3.12 Hygiene control

An adequate supply of chlorinated, drinkable water (river or dam water is not acceptable) must be available at the field abattoir as well as on the vehicle that transport the carcasses. All washing should be preferably done with hot (42 °C) running water. No cloths may be used to dry meat, equipment or hands (Ebedes and Meyer, 1996). An approved Hygiene Management System must be implemented by the management of the game establishment which includes hygiene controls for harvesting. Control measures must be taken to ensure that no contamination of meat and edible products occur. All workers must be trained in correct harvesting techniques including principles of hygiene practices (Anonymous, 2005b: Schedule 2, par 4.7.2).

3.13 Receiving of game carcasses at the game processing plant

Flaying and final dressing of the partially dressed game meat carcasses may only be done in a game meat processing plant. Carcasses must be offloaded and removed to the holding chillers without delay. In the case where the chiller truck is used to hold carcasses before dressing, the doors must be closed when not loading out (Anonymous, 2000: Part V, Section 11.(1)(h), par 69).

3.14 Dressing and portioning of carcasses

Incision lines to a hide or skin must be made with a spear cut from the inside to the outside with a clean sterile knife. Separate knives must be used for cutting the skin and the rest of the carcass (Van Rooyen *et al*., 1996). Mechanical flying knives cannot be used for this purpose. Contact of the exposed meat with platforms, walls, floors, outer surfaces of the hide or skin and any soiled equipment must be avoided.

All organs received separately at the game processing plant must be available and identifiable for meat inspection. Final washing with water is allowed to remove bone chips and blood from the carcass. Substances intended to prevent spoilage by inhibiting the activities of insects, or the development of bacteria or moulds, may not be applied to the meat unless it applies with the requirements of the Foodstuffs, Cosmetics and Disinfectants Act No. 54 of 1972. Approved carcasses may be halved and quartered before or after chilling. Any further cutting must be done in a approved cutting plant (Anonymous, 2000: Part V, Section 11.(1)(h), par 71. 72, 73).

Un-skinned game carcasses may only be skinned and placed on the market if it was handled separately from other food before skinning and not frozen. The de-skinned carcass must undergo a final inspection in accordance with Regulation (EC) No. 854/2004 (EC, 2004c: Chapter II, par 8). Cutting and boning must be organized in such a way as to prevent or minimise contamination. Meat intended for cutting must be brought into the workrooms progressively and as needed. During cutting, boning, trimming, slicing, dicing, wrapping and packaging, the meat must be maintained at a temperature of not more than 7 °C with

an ambient temperature of not more than 12 °C. Where premises are approved for different species, cross-contamination must be avoided by separating the operations of different species in either space or time (EC, 2004c: Chapter V, par 2).

3.15 Controls at the game establishment

The official veterinarian at the game processing plant will verify that the seal of the truck off-loading the partially dressed carcasses is not broken and that the seal number corresponds with the seal number as indicated on the Game Harvesting Control Document and that the amount of partially dressed game carcasses and their tag numbers concur with information provided. The official veterinarian will also note the temperatures. Continuous thermo-control recording is however recommended from loading of the carcasses to arrival and unloading at the game meat handling facility. The recording must provide the accurate actual time and temperature analyses covering all phases of harvesting and transport (Van Schalkwyk and Hoffman, 2010).

The primary responsibility for food safety rests with the food business operator (EC, 2004a: Chapter I, Article I, par 1) and it is necessary to ensure food safety throughout the food chain, starting with primary production (par 2). Food business operators must therefore establish, implement and maintain hygiene control procedures based on HACCP (Hazard Analysis and Critical Control Points) principles (EC, 2004a: Article 5, par 1). This is applicable to the harvesting of game for meat exports to the European Union. Records must be available of observations, checks, results, laboratory analyses and corrective actions taken (Anonymous, 2000: Part III, Section 11.(1)(e), par 47). Personnel must be trained in hygiene procedures and personal hygiene and records thereof must be kept (Anonymous, 2000: Part V, Section 11.(1)(f)). In order to comply with EU regulations on the monitoring of specific residue in meat (EC, 1996), the testing for residue of Lead and Cadmium in kidneys and liver of game animals harvested for commercial purposes is compulsory.

4. Conclusions

What has been achieved?

As the export and local consumption of game meat from Africa increases, it is becoming increasingly important to maximise its quality in order for it to compete with that of domestic species. One of the major quality aspects that can be controlled through proper management is the use of harvesting techniques suited to the specific species being harvesting and the efficiency of minimising *ante mortem* stress. It has been shown that the terrain as well as the specific behaviour of the targeted species influences the harvesting technique(s) to be employed. Although very little scientific proof exists that the techniques used in the industry maximise the meat quality, the techniques are efficient and work.

The hygienic handling of the carcasses prior to skinning and de-boning is another crucial factor when it comes to the quality of the product being produced. The commercial harvesting teams in South Africa and Namibia are subject to stringent legislations, regulations and

monitoring to ensure a quality game meat product to the discerned consumer. Most of these were derived from the formal red meat industry and may require further refining.

What has been neglected?

The harvesting and role of inspection (or lack thereof) in the whole bushmeat trade has not been discussed at all in this section. Bushmeat is the informal (frequently illegal) harvesting of wildlife (not only limited to mammals but could include primates, reptiles, birds, etc.) and the informal trading of the 'fresh' meat on the market.

What needs to be done?

An aspect that warrants further research as pertaining to the different methods of harvesting the various game species is a more intensive quantification of the effect of the boma harvesting method on the meat quality of the animals. Aspects within this scenario that require analysis include the lairage duration (time from chasing to the start of the culling) as well as the aspects that are linked to this (such as duration of chasing by the helicopter into the boma). It is obvious that these requirements (and recommendations that are derived from the research) will differ from species to species. The effect of species and environmental conditions on the time period before removal of the gut and contents also needs elucidation. Personal observation have also indicated that the cooling regimes in the chiller trucks need to be investigated further as there are quality issues (colour stability and drip loss) with the meat further down the supply chain when the protocols and cold chain are not maintained. This is of particular importance with the larger species (zebra, wildebeest, eland, etc.) when the surface area to volume ratio is such that rate of chilling is slow.

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Zoonotic diseases and direct marketing of game meat: aspects of consumer safety in Germany

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Summary

Direct marketing is a traditional way of distributing game meat. If direct marketing of small quantities takes place within a radius of 100 km with delivery directly to the consumer or the local trade an official meat inspection is not required. However, a high level of responsibility lies with the hunter, which he can only take, provided he has received a sound education to be refreshed continuously. The risks caused by consumption of game meat are primarily associated with lack of hygiene during processing and with unrecognised zoonotic diseases which can be transferred to humans consuming the meat. Important zoonotic risks in Germany include Trichinellosis, Shiga toxin producing *Escherichia coli* (STEC), enterohaemorhagic *Escherichia coli* (EHEC), Hepatitis E and Tularaemia.

Keywords: distribution, sociological aspects, safety strategies, zoonoses

1. Introduction

According to Regulation (EC) No. 853/2004 (EC, 2004), persons who hunt wild game with a view to placing it on the market for human consumption must have sufficient knowledge of the pathology of wild game, of the production and handling of wild game and wild game after hunting, especially as regards anatomy, physiology, (abnormal) behaviour, of wild game, pathological changes in wild game which may affect human health after consumption, hygiene rules/legislation and administrative provisions on animal and public health, and hygiene conditions governing the placing on the market of wild game.

2. Importance of hunting and wild game consumption in the society

Hunting plays an important part in human culture and represents an ancestral strategy of survival. Hunting, especially in Europe and particularly in Germany can be seen as a significant part of the historical development, although – at present – hunting is only carried out by a small part of the population. Hunting itself and related gains – like the hunting tradition, language, and techniques, its economic importance and benefits, the actual prize (the trophy), the hide and meat of the hunted animal – have not only dramatically decreased in significance in the eyes of today´s society but also faces ostracism in wide circles of the population, due to the changes in the societal and moral values over the past few decades. The latter is without doubt geographically influenced and therefore varies markedly from

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region to region and is likely particularly valid for urban societies, whilst greater acceptance is observed among the rural population. This is entirely understandable because hunting originates from- and is widely carried out in rural areas, whose local population is more acquainted with this practice: customs which are familiar and experienced on a regular basis are rarely met with aversion.

The consumer still associates certain exclusivity with the eating of game meat, although nowadays it is available year round, due to import and storage logistics and can be bought at moderate prices. In comparison to the constantly increasing consumption of meat from farm animal species, game meat consumption is relatively insignificant. The *per capita* consumption lies at 1.3% (0.8 kg/yr) of the total meat consumption in Germany (NVS, 2008). Yet, the game meat market is economically relevant. The animal consumption in Germany is about 73,000 tons, whilst the domestic supply with meat from large feral game species approximates 35,000 tons, representing a monetary worth of about 150 million Euros. Exact data on marketing of game meat are not available, so these figures are based on approximations of the Federal Association of Game Meat (European Egg, Poultry and Game Association (EPEGA)), which estimates one half to be distributed through commercial game handling establishments, the other half by hunters directly supplying to consumers (oral communication EPEGA, 2009).

3. Safety strategies on game meat hygiene

Although the European Feed and Food law explicitly prohibits local direct marketing of game meat in small amounts to consumers or to retail dealers, this issue falls under the jurisdiction of national law. Since 2006, European food law regarding game has changed. Hunters (in their capacity as food business operators) are responsible for the safety of their products. National German legislation also considers aspects of responsibility and traceability. The intention is to provide a level of food safety as high as possible and consequently mandatory measures of hygienic game meat production and handling under proper conditions (e.g. boning in adequate rooms, chilling as soon as possible to +7 °C) are included.

Safety strategies for handling game meat include establishing efficient surveillance, providing (continuing) education for hunters as regards hygiene and zoonotic pathogens, assuring traceability of products by means of documentation and manageable markets, and the provision of well equipped facilities.

As result of these strategies, in 2007 11% of all shot large wild game was inspected by official veterinarians (Table 1). A total of 114,000 hunters in Germany participated in continuing education, which has allowed them to obtain the status of a 'trained person'. Of the approximately 350,000 persons in Germany holding a hunting licence, only about 33% have received such training, although one may assume that those individuals who have completed their hunting training after 1987 have satisfactory knowledge due to improved training quality from this year onwards.

The direct marketing of game meat is at present being forcefully propagated through marketing campaigns by the umbrella organisation for German hunters. The key strategy of

Table 1. Statistics of meat inspection of large wild game in Germany 2007 (Statistisches Bundesamt, 2007: Fachserie 3, Reihe 4.3).

Species	Number of meat inspection	Whole carcass unfit for consumption
Red deer	10,772	91
Fallow deer	9,732	88
Roe deer	75,286	619
Wild boar	92,129	1,222
Others	1,144	38
Total	189,036 (11%)[a]	2,058 (1%)
Testing on *Trichinella*	282,442	9
Samples taken by hunters	125,468	

[a] Hunting bag 2007 total of 1,664,824 animals.

the campaign is drawing attention to the unpolluted condition of game and the high quality of game meat. However, by adopting a direct marketing strategy the hunter takes on a very high responsibility for consumer safety. This responsibility must be taken seriously to be able to successfully fulfil expectations.

4. Food chain and traceability

Article 18 Regulation (EC) No. 178/2002 (EC, 2002) makes the traceability of food that is put on the market the obligation of the food business operators. This system is also well established in the local direct marketing of game meat. The attachment of an identification mark to each carcass is obligatory, as is further documentation by way of special forms, once game is brought to market in some states of the German Federation. However, the initiative to make this a nationwide uniform procedure in all states was recently overruled in a German Parliament voting, which outcome is explained by the fact that the handling of game meat takes place within close and local quarters, and the origin of the game is usually known as it is received directly from the hunter.

5. Pathogens and health risks

Outbreaks associated with game meat consumption or handling are rarely reported (except Trichinellosis and Tularaemia, see below). Yet, despite an increasing quality of hygiene standards, human cases of infections in which game meat is incriminated occur. Major examples of the transfer of zoonotic agents from wild game to humans include *Trichinella*, *Escherichia coli* (STEC, EHEC), *Salmonella*, Hepatitis E virus and *Francisella tularensis*.

5.1 *Trichinella* – situation in Germany

As in other European countries, this muscle parasite is also found in Germany and affects various wildlife animals, like martens, foxes and wild boars. Due to insufficient heating of the meat, the larvae can be transferred to humans and cause allergic reactions, which may lead to severe illness or death.

There have been reports on human *Trichinella* outbreaks after the consumption of meat of wild boars. For instance, in the year 1977 in Ebermannstadt (Bavaria), 69 persons fell ill after having eaten raw sausages made from wild boar´s meat. Due to the steadily growing populations of martens, foxes and boars, an increase in *Trichinella* occurrence in wildlife animals can be anticipated. According to figures from the meat inspection authorities, more than 3.697 million wild boars were examined for *Trichinella* between 1991 and 2006 (Table 2). A total of 186 *Trichinella*-positive animals were recorded, which represents a prevalence of 0.005%. In Germany, 1 to 10 registered human cases of Trichinellosis occur per year (mostly imported contamination). Occasionally *Trichinella* outbreaks occur through meat from wild boars or domestic pigs (last major outbreak in 2006 in Mecklenburg-Vorpommern affecting 16 persons). In contrast, of the 425.64 million domestic pigs slaughtered between 1997 and 2006 only 1 animal (in the year 2003) was tested positive for *Trichinella*.

This example for *Trichinella* indicates the importance of thorough meat inspections, as the only reliable way to detect a rare infestation of the muscle parasite in an early stage and thus prevent transfer to the consumer. However, due to its rare occurrence, the awareness of the necessity of game meat inspection for *Trichinella* is still very low among hunters. A *Trichinella* infestation

Table 2. Hunting bag and positive findings of Trichinella *(wild boar, 1991-2006).*

Year	Number	Tested	(%)	Positive	Prevalence (%)
1991	312,768	215,494	68.90%	6	0.0028
1992	248,898	160,901	64.65%	12	0.0075
1993	339,242	214,426	63.21%	20	0.0093
1994	313,214	201,442	64.31%	26	0.0129
1995	253,788	179,385	70.68%	13	0.0072
1996	362,214	251,656	69.48%	10	0.0040
1997	281,916	215,926	76.59%	14	0.0065
1998	251,431	192,764	76.67%	12	0.0062
1999	418,667	292,460	69.86%	9	0.0031
2000	350,976	265,417	75.62%	8	0.0030
2001	531,887	389,008	73.14%	4	0.0010
2002	512,050	397,425	77.61%	12	0.0030
2003	470,283	370,187	78.72%	10	0.0027
2004	476,042	390,570	82.05%	11	0.0028
2005	476,645	402,996	84.55%	11	0.0027
2006	287,080	272,258	94.80%	8	0.0029

is not macroscopically visible and the relevance of selecting specific sample locations is difficult to understand for the lay person. This was significantly better understood by hunters, once they had been given special training, enabling them to take samples themselves instead of having to take the whole animal to an inspection site. Unfortunately, the number of authorised inspection offices is constantly decreasing since the legal requirements for accreditation have been elevated, as it is not considered worthwhile to start a laboratory, only carrying out a small number of inspections. In addition, the current regulations stipulate that inspections must be done using the digestion method instead of the traditional compression method (which occasionally gives false negative results). Consequently, increasing findings of *T. pseudospiralis* are now reported, which subspecies remained undetected in using the compression method.

5.2 Shiga toxin producing *Escherichia coli* (STEC) in game meat

The degree of STEC contamination in meat from wildlife animals is equal to or higher than that in food from farm animals. The presence of STEC in food is frequently associated with its processing, e.g. animal slaughter practices and hygiene playing a major role in contamination of meat with STEC.

It is not clear if STEC is present in higher proportions in game than in domestic animals or if high contamination of wildlife meat with STEC is caused by improper slaughter practices. Few reports are available on wild life as carriers of STEC, and some of these animals serve as a food source for humans. Surveys show that game holds the 2nd rank of STEC contaminated meat samples in Germany (9.9%). Most STEC positive samples are found in mutton (11.1%), beef is far behind (5.2%) and pork samples are least STEC contaminated (0.7%).

5.3 Enterohaemorhagic *Escherichia coli* (EHEC)

Since 2001, when obligatory registration was instituted in Germany, approximately 1,100 EHEC-infections per year have been registered in humans, equalling 1.4 infections per 100,000 inhabitants, with beef being the primary source of infection.

Findings of the National Reference Laboratory for Epidemiology of Zoonoses at the BfR have presented another scenario: in year 2002, 3% of game samples showed contamination with EHEC, in 2005 the quota was 14.8%, i.e. during this time period, the percentage of contaminated samples of wild game meat was significantly higher than that of beef. According to present findings as well as information from other research organisations, it appears that wildlife (as a reservoir), and game meat as a source of possible EHEC infections in humans have been underestimated. Possibly, not adhering to proper hygienic methods during game meat processing explains these observations, similarly as is the case with contamination with STEC.

5.4 Hepatitis E

In 2009, 106 cases of hepatitis E were registered in Germany, and there has been a yearly increase in the number of cases recorded. It was originally assumed that the infections originated from travels to areas where the virus is endemic. However, it was later found that cases of infection included persons who had not travelled to risk areas. In recent years,

there have been increased reports of hepatitis E being found in pigs and wild boars in Europe and Japan, in the latter country being transmitted to humans through consumption of insufficiently heated wild boar meat. A recent study from Wichmann *et al.* (2008) has identified the consumption of meat and intestines of wild boars as an increased risk factor for hepatitis E infection in Germany.

Recent studies also show that the virus has widely spread among the wild boars population in Germany (Schielke *et al.*, 2009). The virus was found in 22 of a total of 148 examined animals (14.9%), albeit with significant regional differences. This was also confirmed by findings of other scientists.

To date it remains unclear if the detected strains can be directly transmitted to humans and how easily this occurs. One of the viruses detected in wild boars show a very close link to a virus from a human hepatitis E case in Germany. This would seem to suggest the possibility of virus transmission between both hosts in this particular case. However, the low number of human infections – in consideration to the widely spread hepatitis E virus in wild boars – indicates that additional factors are necessary for the transmission of the virus or that only high virus concentrations lead to infection of humans.

Studies are currently being carried out in various different research organisations in Germany, with the aim to gain further insight in the spreading of the virus in the wild boars and domestic pig populations, which would allow improving risk assessments of virus transmission between wild boars, domestic pigs and humans.

5.5 Tularaemia

Tularaemia is an infection, predominantly of hares, rabbits and other rodents (beaver, mice, rats). Infected hares and rabbits show symptoms of a haemorrhagic septicaemia (blood poisoning). In addition, deer, wild boars and domestic animals (e.g. sheep, cows, pigs, dogs and cats) are susceptible to this pathogen. In a recent retrospective study, antibodies for *Francisella* bacteria were found in the serum of 3.1% of a total of 763 examined wild boars in Mecklenburg-West Pomerania, proving that the tularaemia pathogen is present in some regions in Germany. According to various reports there is a relationship between the contact with hares and tularaemia infection in humans. For example, two persons were infected in Ortenaukreis (Baden-Württemberg) towards the end of 2001 after having eaten hare meat. In both patients the pathogen was detected in blood serum and by immune histological tests.

Following a report from Griesheim (Hessen) in November 2005, at least 6 persons of a hunting group fell ill after helping with the gutting of the hunted hares. After an incubation period of a few days, they developed high fever and lymph nodes swellings. Examination in the University Clinic in Heidelberg confirmed tularaemia infection.

6. Conclusions

What has been achieved?

Direct marketing of game meat gains importance in Germany even though its role in the transfer of zoonotic diseases cannot be excluded. In the recent past, many hunters have received continuing education by which both the level of their knowledge has been increased and their awareness that hygienic handling is important for the safety of game meat (cooling, etc.) has been consolidated.

What has been neglected?

The process of accreditation of laboratories for the testing of *Trichinella* has lead to a decrease in the number of laboratories for economic reasons. Consequently – also because of the often large distances to the still existing inspection laboratories – the willingness to subject wild boars to *Trichinella* examination has decreased.

What needs to be done?

A careful hygienic practice in dissecting and preparing game meat must be promoted, the thorough washing of hands being one of the most important preventive measures. As most zoonotic pathogens are sensitive to heat, the safe preparation of game meat (i.e. adequate heating) offers the best protection against infection.

A proper education of hunters to improve their consciousness for good hygiene practice and their knowledge about zoonotic diseases would appear to be the best assurance for consumers when game meat is acquired *via* direct marketing. Although this is the primary responsibility of the hunters associations, official food surveillance agencies are in a position to support such activities.

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Dog bites in hunted large game: a hygienic and economical problem for game meat production

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Summary

Main objectives of this study were to monitor dog bite occurrence in game meat and to evaluate the damage caused. For this purpose, a total of 526 animals were evaluated: 337 red deer (C*ervus elaphus*), 142 wild boar (*Sus scrofa*), 29 fallow deer (*Dama dama*), and 18 mouflon (*Ovis musimon*), in hunting zones located in the county Idanha-a-Nova (lat 39° 55'N: long 7° 14'W). A total of 100 (19.01%) of the analysed animals had suffered from dog bites. Of those, 64 were classified as level 1, 20 as level 2 (i.e. removal of affected tissues necessary) and 16 as level 3 (i.e. necessitating total rejection of the carcass). Apart from the animal welfare issue this study emphasises the hygienic, microbiological and economic relevance of this problem in the game meat production chain. The necessity of improving dog behaviour during drive hunting so as to avoid meat rejection, promote animal welfare and game meat hygiene and quality.

Keywords: drive hunting, dog bite

1. Introduction

Large game hunting campaigns, part of them related to ecotourism, are an important source of revenue and development for a considerable number of Portuguese regions. Among the large game species hunted in Portugal, red deer and wild boar are most relevant. Hunting activity and game management are regulated by national law (number 201/2005, 24th of November 2005). According to this document, large game species may be legally hunted using procedures such as stand, stalking, battue, drive hunting, and by spear. Of these approaches the most important method to hunt large game is drive hunting, where in a previously designated location, the hunter waits for the animal, which is driven by dog packs moved forward by beaters.

Although dogs are indeed important for large game hunting in Portugal, excessive dog bites are undesirable side-effects, as the resulting wounds have several negative consequences including:

- Impairment of animal welfare.
- Quantitative losses in game meat; resulting from rejection of the affected and surrounding areas, as recommended in the Codex Alimentarius (1994).

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- Hygiene defects and, generally, increase of microbiological contamination of game meat.
- Increased risk of dog infection by diseases transferred from wild game, such as tuberculosis, leptospirosis, Aujeszky's disease and parasitoses (echinococcosis, cysticercosis), some of which are also pathogenic to humans (Coye, 1992). The affected animal can also contribute to the dissemination of diseases to other geographical regions, and thus affect other wild or domestic animal species.

In addition, the microbiological condition of game meat can be compromised by poor placement of shots, and by poor carcass evisceration and dressing practices in the field, as indicated by Gill (2007). The long time period between the hunt and the beginning of carcass preparation, as well as improper carcass storage temperature, may also adversely affect the level of meat contamination (Vieira-Pinto *et al.*, 2005). Contributing to this is the infliction of carcass wounds resulting from dog bites.

The main objectives of this study where to monitor dog bite incidence in game meat and to evaluate the damage level.

2. Materials and methods

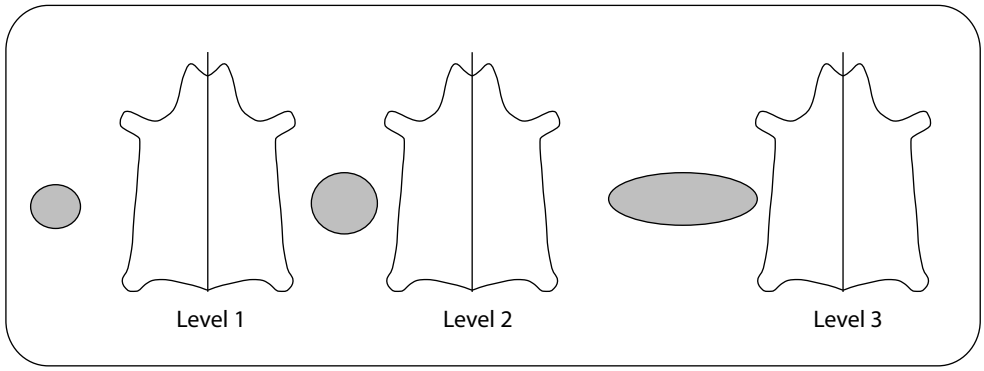
The study was conducted in the county Idanha-a-Nova in the central-eastern part of Portugal (lat 39° 55'N: long 7° 14'W) with 1,412.7 km^2 and 10,561 inhabitants. Located in a plateau region, it has a large border line with Spain, which is crossed by the river Erges in the east and the river Tagus in the south. Idanha-a-Nova is a typical Portuguese rural area, with approximately 50% of land dedicated to agriculture (43% dry agriculture, 9% watered agriculture, and 5% graze land); 30% are forested areas, mainly oaks, and 13% is shrub land with sparse vegetation. This region is considered to be one of the best hunting areas in Portugal with numerous game estates, many of which also breed cattle and sheep in an extensive free ranging system, grazing in pastures alongside wild artiodactyls.

From November 2008 to February 2009, 20 organised hunts rendering a total of 526 animals were evaluated, i.e. 337 red deer (*Cervus elaphus*), 142 wild boar (*Sus scrofa*), 29 fallow deer (*Dama dama*), and 18 mouflon (*Ovis musimon*).

On every hunting day, before sanitary inspection, the hunted animals were visually evaluated and classified, according to the extent and depth of dog bites, based on the scale presented in Figure 1. The grey area represents the damaged carcass site.

3. Results

From the 526 animals analysed, 100 (19.01%) were affected by dog bites. Of those, 64 were classified as level 1, 20 as level 2 and 16 as level 3 (Figure 1). Affected and surrounding areas of all animals with level 1 and 2 wounds were partially rejected. Level 3 carcasses were judged completely unfit for human consumption (Figure 2).



Bite level	Level 1	Level 2	Level 3	Total
%	64	20	16	100

Figure 1. Classification of carcass damages through dog bites. Grey areas indicate the size of damaged tissues related to carcass size. Intensity of alterations through dog bites in affected carcasses (n=100).

Figure 2. Bite level 2 (left) and level 3 (right).

4. Discussion and conclusions

This study emphasises the relevance of this problem in game meat production. According to Coye (1992) and Talan *et al.* (1996), dog teeth harbour a considerable quantity of bacteria. These authors observed that the majority of the microflora cultured from human wounds contained a mixture of aerobes and anaerobes, mainly derived from the oral flora of the biting animal. In these studies, both *Pasteurella* spp. and *Staphylococcus* spp. have been recognised as potentially important pathogens.

Meyers *et al.* (2008) conducted a study to identify the bacteria present in dogs wound as related to various grades of bites and reported that 16% of the cases were aerobes, 1% anaerobes and

67% a mixture of both (including *Clostridium*). *Pasteurella canis* and pyogenic streptococci were common in infected wounds, whilst *Bacillus* spp., *Actinomyces* spp. and the oral streptococci were usually found in contaminated wounds. Two other studies on dog bite wounds revealed *Staphylococcus intermedius* as the most common isolated bacteria, followed by *Streptococcus* spp. and coliforms (Kelly *et al*., 1992; Griffin and Holt, 2001).

Thus, one may assume that the same bacteria commonly found in wounds resulting from dog bites are present in affected game meat carcasses. Additionally, these wounds constitute a port of entry for the game skin flora. Coye (1992) and Moreno (2003) reported that the external layer of the skin has a rich microbial flora that can cross the skin when its integrity is compromised by any kind of mechanical action, including dog bites. When the bite occurs while the animal is already dead, bacteria will not spread from the local area. Yet, the microbial profile of the carcass can be compromised when the animal is bitten when blood circulation is intact, through which bacteria from the oral cavity can spread to the entire organism.

There is concern that the killing process or deficiencies during evisceration and chill storage can have a detrimental effect on hygienic quality (Gill, 2007). In this context, carcass wounds resulting from dog bites represent an additional negative factor that may influence the microbiological profile of game meat. It is important to draw attention to this problem as it has practical animal welfare relevance and should be considered in hygienic rules to improve the level of consumer protection. The latter is highlighted in Regulation (EC) No. 853/2004 (EC, 2004) that lays down specific hygienic rules for foodstuffs. As previously argued by El-Ghareeb *et al*. (2009), the introduction of 'Good Manufacturing Practice' (GMP) represents an important element in securing safe and wholesome game meat.

In consideration of our results, GMP should also include stricter rules on dog behaviour during drive hunting, so as to prevent jeopardising animal welfare and the safety of game meat obtained by drive hunting practices. The authors believe that the result of our study should prompt hunters to improve dog behaviour during game drives.

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Verocytotoxigenic *Escherichia coli* (VTEC) in wild ruminants in Germany

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Summary

This contribution addresses the role that feral game animals, hunting practices and the marketing and distribution of game meat may play in the transmission of verotoxinogenic *E. coli* (VTEC) to domesticated ruminants and to humans. Recent data generated in Germany indicate that the epidemiological role of game meat in the spread of VTEC is underestimated.

Keywords: faecal contamination, translocation of bacteria in game meat

1. Introduction

Escherichia coli is a gram negative, facultative anaerobic, non-spore-forming bacterium and a member of the Enterobacteriaceae (Rolle and Mayr, 1993; NCBI Taxonomy, 2009). *E. coli* strains belong to the physiological gut flora of humans and nearly all mammals, guinea pigs and chinchillas being notable exceptions.

Of a total of about 10^4-10^9 cfu/g gut content, *E. coli* represents a maximum of 1% of the eubiotic gut flora and is therefore characterised as belonging to the 'accompanying microflora' (Rolle and Mayr, 1993). However, some strains of these gut commensals can be facultative pathogenic. The importance of verocytotoxigenic foodborne infectious agents was first recognised in 1982, when the consumption of insufficiently heated hamburgers led to serious illnesses and deaths in the United States (Riley *et al*., 1983) and subsequently enterohaemorrhagic *Escherichia coli* (EHEC) were described as a new group of enteropathogenic bacteria for the first time (Karch *et al*., 2005). *E. coli* strains of the serotype O157:H7 are considered to represent the prototype of enterohaemorrhagic *E. coli*, causing haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic-thrombocytopenic purpura (TTP) in humans. The production of verotoxins (shigatoxins) is seen as a primary virulence-factor of this group of pathogens, so they are also termed verotoxin-producing (shigatoxin-producing) *E. coli* (VTEC/STEC). In contrast to this virulence-associated nomenclature, the term enterohaemorrhagic *E. coli* (EHEC) is based on the resulting clinical symptoms. The differentiation between EHEC and VTEC/STEC derives from the observation that not all *E. coli* strains, which can produce verotoxins, lead to illness in humans. Nevertheless, up to the present day, there is controversy concerning the distinct definitions of EHEC, VTEC and STEC.

Whilst VTEC infections of farm animals (e.g. cattle, sheep, goats) have been in the focus of scientific interest since the early 1980's, the possible epidemiological role of game animals,

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especially feral ruminants, has only more recently received increased attention. Particularly the reports of the Federal German Institute of Risk Assessment (BfR) have prompted the consideration of game as a primary reservoir of verotoxinogenic *E coli*, and it is now feared game meats might represent a source of infection for humans and that their role in the transfer of human colibacillosis has to date been underestimated (Lehmann *et al.*, 2006, BfR, 2007).

2. Occurrence of VTEC in wild ruminants in Germany

Bülte *et al.* (2002) tested faecal samples of healthy cattle and found up to ca. 70% animals within a herd to contain VTEC, which findings are corroborated by other studies (e.g. Naylor *et al.*, 2005). Consequently, one considers VTEC to belong to the normal intestinal flora, as they generally do not lead to illness in ruminants, with the possible exception of calves which occasionally develop clinical symptoms (Baljer *et al.*, 1990).

German studies conducted in the past few decades have shown that VTEC strains can also be isolated from samples of game. Bülte and Wrocklage (1992), observed that fallow deer were shedding VTEC at a rate of 10%, all of these *eae*-negative strains. From meat sampled from roe deer two serovars (incriminated in human infections) were isolated, which indicates that insufficiently cooked meats from these game animal species represent a potential VTEC source (Thoms, 1999; Trumpf *et al.*, 2000). In more recent studies by the reference laboratory for zoonoses at the German Federal Institute of Risk Assessment (conducted in the period 2002-2006), a large number of game meat samples have been investigated. In 2005, 14.8% of the samples analysed were found to be positive for verotoxin-producing *E. coli*, i.e. higher than the prevalence generally found in beef. Also, VTEC's such as O26:H11, O128:H2 and O103:H2 were detected, which serovars are known to possibly cause severe illness in humans (BfR, 2007). In another German study from 2006, 29 out of 56 faecal samples (51.8%) of wild ruminants were detected positive for VTEC; among the 13 different O-serogroups isolated were O21, O128 and O146 strains (pathogenic for humans), albeit none of these were *eae*-positive (Lehmann *et al.*, 2006). An overview of the detection of VTEC in wild ruminants in Germany is presented in Table 1.

Table 1 illustrates that game meat species may represent a potential reservoir for the transmission of *E. coli*-strains to domestic ruminants and humans in Germany. A horizontal transmission of the infection in farm- and wild ruminants is entirely conceivable as both wild and domesticated ruminants use the same pasture and the former animal category can contribute to a constant presence of the pathogens in the population. In addition, human infection may result from the consumption of VTEC contaminated foodstuffs, e.g. insufficiently cooked game meat (Busch *et al.*, 2007) or herbal foodstuffs, which have been in contact with droppings of wild animals (Akashi *et al.*, 1994; Thoms, 1999).

Table 1. Proof of verocytotoxigenic E. coli *(VTEC) of wild ruminants in Germany.*

Animal species	Number of examined faecal samples	Number of examined game meat samples	Number of positive samples (%)		Reference
			VTEC in general	*E. coli* O157	
Fallow deer	100	0	10 (10.0)	0	Bülte and Wrocklage, 1992
Roe deer	0	53	4 (7.5)	no information	Thoms, 1999
Different wild animals species (general term 'game meat')	0	from 80 to 160 per year (not specified)	year 2002 (3.0) year 2005 (14.8) year 2006 (10.0)	no information	BfR, 2007
Red-, roe- and fallow deer	56	0	29 (51.8)	0	Lehmann *et al.*, 2006
Red- and fallow deer in deer park	20 (cumulative faecal samples)	0	3 (15.0)	0	Lehmann *et al.*, 2006

3. Ports of entry of VTEC in game meat

3.1 Contamination pathways related to hunting practices

It is of interest to consider the possible pathways by which the more common VTEC strains may be transferred. Relevant factors may include the following: type of hunting, the condition of the animal before the shot, the location of the shot, the behaviour of the animals after the shot and the subsequent supply of game.

3.1.1 Type of hunting and the condition of the animal before the shot

The hunter plays an important role in assuring the quality of game meat, not only in the supplying of game according to best practices, but even in the choice of method of hunting (Krug, 1998, Deutz *et al.*, 2006). Favouring the entry of VTEC are the so-called movement hunts (e.g. driven hunt and battue, particularly with the employment of dog packs). In this type of hunting, game animals often escape (Krug, 1998) and are therefore exposed to increased stress. Physical activities, such as chasing animals, lead to an increase in the level of endotoxins (Zucker and Krüger, 1998; Seidler *et al.*, 1999), which in turn, are responsible for increased permeability of the intestinal tract for bacteria. This could lead to a premortal endogenous contamination of the organism, even with serious harmful bacteria (Zucker and Krüger, 1998; Seidler *et al.*, 1999). This mechanism has been reported for slaughter pigs, which are known to be particularly vulnerable in view of their genetic make-up, the currently applied husbandry systems and transportation stress. Thus, premortal stress situations may lead to the translocation of microorganism from the colonised body regions, such as the gut or locally infected regions, into generally sterile organs and muscles (Zucker and Krüger, 1998; Fehlhaber and Alter, 1999; Seidler *et al.*, 1999). In addition, it has been shown that such takes

place more frequently, the more pronounced the premortal stress, as the serum bactericidal activity is adversely affected. Consequently, even *post mortem* antibacterial activities remain reduced (Fehlhaber and Alter, 1999).

The physiological dormancy of deer-like animals in winter represents an additional stress factor. This may trigger an accelerated glycogen breakdown in game animals especially during the winter months, when their metabolism is lower. The reduced energy turnover, the scarcity of food and the simultaneous release of endogenous reserves, have to be considered in choosing the type and the date of hunting (Balfanz, 2007). Hence, in late winter premortal VTEC contamination is more likely to occur and consequently culling programmes for these wildlife species should be completed by the end of December (Balfanz, 2007).

3.1.2 Location of the shot and the condition of the animal after the shot

Krug (1998) reported that, whereas stalk hunting of a single animal allows 90% effective chest hits, this figure decreases to 25% in hunting drives, which conforms to more recent findings of Deutz *et al.* (2006). According to the latter study the time interval between killing and evisceration is generally considerably longer in drive hunts, whilst a major proportion (i.e. one-third of the animals) are killed by 'soft shots' (i.e. injuring the gastrointestinal tract). Such undesirable shots do not only jeopardise carcass hygiene by the spread of endogenous gut microflora, but as they are not necessarily instantaneously fatal, such shots may result in bacteriemia, by which potentially pathogenic organisms (such as prevailing VTEC's) are distributed to muscle tissues (Hadlok, 1993; Kappelhoff, 1999; Deutz, 2000).

3.2 Cross-contamination during game meat portioning

Shot game is usually eviscerated on the spot, sometimes under adverse light and weather conditions. Unless the hunter possesses the necessary skills and has a basic knowledge of game evisceration and dressing hygiene, this often results in significant carcass contamination. In addition, special care must be taken during processing of roe deer, which have a looser connective tissue structure, increasing the risk of bacterial penetration in surrounding tissue during breaking up and portioning of the carcass (Deutz, 2000). Therefore, especially in this species, a careful treatment must be warranted.

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Microbial quality of venison meat at retail in the UK in relation to production practices and processes

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Summary

Venison is a popular game meat in the UK with steadily increasing sales. Deer can be wild, kept in parks or farmed and this affects whether deer are shot in the open or slaughtered indoors. The distance the carcasses need to be transported affects whether evisceration is outside or inside and the time before the carcasses can be chilled. Our work aims to identify best hygienic practice from the different methods used to produce venison meat in the UK. We have visited five major UK producers of venison and recorded the production practices and processes used. We have also examined the microbiological quality of retail venison meat from these producers with the aim of relating food hygiene status to the production practices employed.

Keywords: Approved Game Handling Establishment (AGHE), venison, *Salmonella*, enterobacteriaceae

1. Introduction

Sales of venison (deer meat) in the UK are steadily increasing as consumers consider that deer are free from the concerns that exist over the intensive production and harvesting systems associated with many other meats (Mintel, 2008). Within the EU, producers that intend to retail large quantities of venison, either within the EU or for export, must process deer carcasses in an approved game handling establishment (AGHE) approved by the competent authority which in the UK is the Food Standards Agency (FSA). The FSA is an independent government department established to protect the public's health in relation to food. There is a large degree of variation in the practices employed in the production of venison prior to it being despatched from the AGHE and placed on the market. This study aims to identify hygienic best practice from the many different methods used to produce venison. We have visited five major UK producers, whose combined output represents a significant proportion of the venison retailed (*via* multiple retail outlets) to the consumer, where we observed the range of production practices and processes employed. In addition, we have also examined the

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microbiological quality of the venison (steak and diced meat) from these producers at retail and determined any correlation of product hygiene with the production practices employed.

2. Material and methods

2.1 Observational studies

This study made confidential observational studies of five UK AGHEs that processed deer for venison.

2.2 Microbial analysis

2.2.1 Sample preparation

Samples of venison were obtained from retail sources where attribution to known AGHEs was possible, or obtained as a retail pack directly from the AGHE. Samples were attributed as 'wild', 'farmed' or 'farmed & park' according to the known source of deer for each AGHE as given in Table 1. It was not possible to source product from AGHE C. Samples for AGHE E were purchased directly through a farm shop on site (Ei) and through a supermarket (Eii). Products were limited to those types known to be produced solely from UK venison. Retail meat (diced meat and steaks) from wild (n=25), farmed (n=16) and farmed & park (n=30) deer were examined. A test portion of twenty five grams of meat was weighed and homogenised with 1:10 volume buffered peptone water (Oxoid Ltd., Hamps., UK).

Table 1. Production practices of UK approved game handling establishments (AGHE).

AGHE	Source			Alive	Slaughter			Condition of carcass on receipt		Venison origin		
					Shot							
	Wild	Farm	Park		Head/Neck	Thorax	Stunned/ Exsanguinated	Skin on	Eviscerated	Culled	Sport shoot	Farmed
A	✓			×	✓	✓		✓	✓	✓	✓	
B	✓			×		✓		✓	✓		✓	
C	✓			×		✓		✓	✓		✓	
D		✓		✓			✓	✓				✓
E		✓	✓	✓	✓		✓	✓		✓		✓

2.2.2 Direct microbial culture methods

The levels of *Escherichia coli*, Enterobacteriaceae and *Staphylococcus aureus* in addition to the total aerobic count were determined according to the appropriate ISO protocol (Anonymous, 2009). Ten-fold serial dilutions of samples were made using Maximum Recovery Diluent (Oxoid Ltd.) and appropriate dilutions were plated in duplicate. One-way ANOVA and student's T-test was performed in order to evaluate possible differences between counts from samples produced by the differing processes. Where no organisms were recovered on count plates a value of half the minimum level of detection for the method was used to allow statistical analysis.

2.2.3 Detection of Salmonella *spp.*

The remaining buffered peptone water was pre-enriched overnight at 37 °C and sub-cultured onto MSRV semi-solid agar (Oxoid Ltd.) for a further 24 h at 42 °C. Where growth characteristic of *Salmonella* was observed, the culture was inoculated onto XLD agar (LabM Ltd., Lancs., UK) and incubated for 24 h at 37 °C. Colonies with typical *Salmonella* morphology were confirmed as *Salmonella* by agglutination with Poly-O A-S antiserum.

3. Results

3.1 Current practices of the UK venison industry

Venison is obtained from three distinct sources of deer (Table 1): (1) wild, unmanaged, free-ranging herds; (2) domesticated, farmed herds that are reared and husbanded under controlled conditions; (3) park herds that are fenced-in, may be subject to some limited husbandry practices and are commonly less cautious of man than wild deer and held in a more controlled environment so shot placement is generally easier.

Wild and park deer are slaughtered in the open by rifle bullet after a stalked hunt. Wild deer are shot by both sport and professional hunters, whereas park deer are generally only culled by expert marksmen. Farmed deer are slaughtered in AGHEs specifically approved to use a method similar to other red meat species (captive bolt stunning and exsanguination). Apart from deer slaughtered in an AGHE, a common problem is the lack of an integral cold chain throughout the whole process. For example, park deer processed in AGHE E were shot in the open and transported to a well equipped game larder where they were eviscerated, had head/hooves removed and were placed in a chiller (7 °C) <2 h after being shot. Carcasses were then detained in the chiller for ~72 h prior to being transported (<3 h) under ambient temperatures to the AGHE for final dressing.

3.2 Hygienic status of venison products

Salmonella was not isolated from any of the samples examined. *E. coli* was isolated from 100% of the samples from wild deer (Figure 1; AGHEs A & B) and from 32% of the samples from farmed/park deer (AGHE E), but was not isolated from any of the samples from farmed deer

(AGHE D). Levels of *E. coli* and Enterobacteriaceae isolated from samples of wild deer meat were significantly higher than those from farmed/park (diced, $P<0.001$; steak $P<0.01$ Figure 1) and farmed venison (steak, $P<0.0001$). Enterobacterial contamination on farmed/park steaks was significantly higher than on farmed steaks ($P<0.0001$). There was no significant difference between the levels of *S. aureus* and aerobic colony counts recorded in each of the groups ($P>0.05$).

4. Conclusions

Slaughter and/or evisceration of deer within a specialised environment (slaughterhouse or AGHE) permit a level of control on factors that influence food hygiene. In the field the increased likelihood of sub-optimal slaughter through poor shot placement and/or evisceration in non-ideal conditions may lead to carcass contamination which is further complicated by increased time between slaughter and chilling and/or breaks in the cold chain prior to further dressing (Sumner *et al*., 1977). Each of these factors may account for the greater levels of *E. coli* and enterobacterial contamination found on venison from wild deer (Atanassova *et al*., 2008; Paulsen and Winkelmayer, 2004). However, unlike *E. coli* and Enterobacteriaceae, aerobic colony counts and the presence of *S. aureus* are not correlated with faecal or environmental contamination but more so with the degree of handling carcasses undergo during dressing procedures. The similar levels of such contamination found on all venison regardless of origin may therefore be due to the comparable way that all deer carcasses are skinned, trimmed, boned-out and prepared for retail when processed at an AGHE. Cross-contamination, for instance by the use of common butchery boards and manual handling, during the preparation of venison for retail and the growth of cold tolerant spoilage bacteria may obscure any differences in the aerobic colony count that existed on as-dressed carcasses.

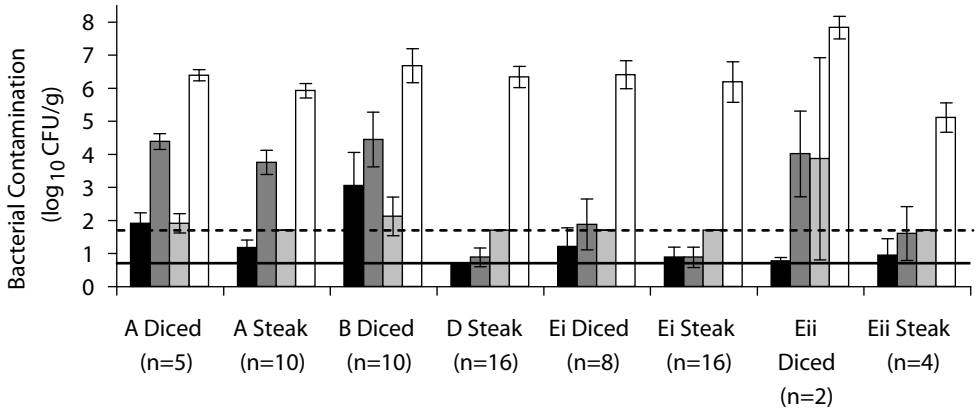

Figure 1. Bacterial contamination (log_{10} cfu/g) of venison meat. Escherichia coli *(black); Enterobacteriaceae (dark grey);* Staphylococcus aureus *(light grey); Aerobic Colony Counts (white). Solid line = 1/2 minimum level of detection of* E. coli*/Enterobacteriaceae; dashed line = 1/2 minimum level of detection for* S. aureus *and total aerobic count. n = sample size.*

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Detection of *Alaria* spp. mesocercariae in game meat in Germany

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Summary

A steadily increasing number of incidental findings of *Alaria alata* mesocercariae in meat of wild boars during official *Trichinella* inspection necessitates the development of a specific detection method for this parasite. A reliable verification of infested paratenic hosts seems to be one of the key factors for both understanding the biology of *Alaria* spp. and determining a sound risk assessment within the meaning of the consumer's health protection. Consequently, our studies concentrate on (1) the verification of suitability of the official digestion methods for *Trichinella* spp. for *Alaria alata* mesocercariae detection in wild boars, (2) development, optimisation and validation of methods, and, (3) the distribution of the parasites within their paratenic hosts.

Keywords: *Alaria alata* mesocercariae, *Distomum muscularis suis* (DMS), detection method, predilection sites, magnetic stirrer method for pooled sample digestion, *Trichinella*

1. Introduction

Distomum musculorum suis (DMS, Duncker, 1896; syn. *Agamodistomum suis*, Stiles, 1898) is the mesocercarial stage of the trematode *Alaria alata*, a small fluke (0.5-1.5 mm) usually found in the small intestine of various carnivores in the western hemisphere. The life cycle of this parasite includes freshwater snails (e.g. *Helisoma* and *Planorbis* spp.) as first intermediate hosts. Cercariae emerge from the snails, penetrate tadpoles, and develop into mesocercariae. A wide range of paratenic hosts can acquire infection by ingesting tadpoles or other infected paratenic hosts (Figure 1). Dogs, cats, foxes, mink, and other carnivores become infected by feeding on these animals. The young flukes migrate through various organs of the definitive host, including the diaphragm and lungs, before reaching the small intestine. Although the flukes are generally considered to be non-pathogenic for the definitive host, large numbers may cause pulmonary haemorrhages during migration or enteritis when they mature in the small intestine.

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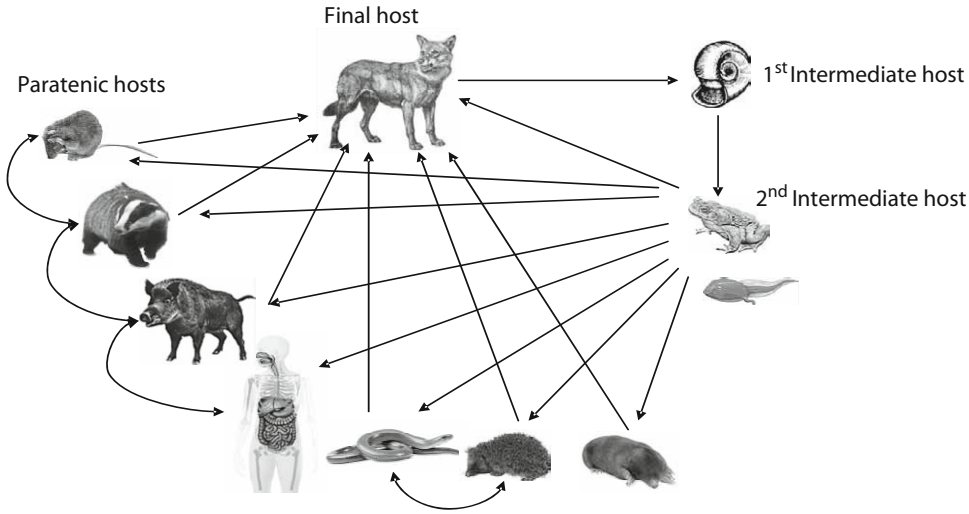

Figure 1. Life cycle of Alaria alata.

General information about biology, prevalence and pathogenicity of this parasite has been given in a previous review (Möhl *et al.*, 2009).

In mid-20th century scientists took notice of the potential human health risk posed by this parasite. Experimental infection of a primate demonstrated that DMS can cause severe damages within a paratenic host closely related to humans. Since 1973 several reports about human larval alariosis in North America have been published (Shea *et al.*, 1973; Byers and Kimura, 1974; Fernandez *et al.*, 1976; Freeman *et al.*, 1976; Beaver *et al.*, 1977; McDonald *et al.*, 1994; Kramer *et al.*, 1996). Nearly all cases of human alariosis could be linked to the consumption or handling of game meat (paratenic host) and/or frog legs (second intermediate host). Nevertheless, the risk for humans was generally ignored or at least postulated to be negligible until this issue re-emerged in Europe: Jakšić *et al.* (2002) and Große and Wüste (2004; 2006) published results on repeated incidental findings of DMS in meat of wild boars during routine *Trichinella* inspection in certain areas of Croatia and Germany respectively. Figure 2 shows *Alaria* spp. mesocercariae isolated from a wild boar.

In view of deficiencies in methodology, lack of data on prevalence, and the human DMS cases which were reported in the meantime, an increased scientific attention should be paid to this subject. Consequently, the German Federal Institute of Risk Assessment concluded in its opinion (BfR, 2007) that meat which contains *Alaria alata* mesocercariae should be regarded as unfit for human consumption. A final statement concerning the health risks for consumers could not be given due to the lack of information about both the prevalence of DMS and the suitability of *Trichinella* inspection methods to detect this parasite in wild boar meat. Therefore an appropriate diagnostic method for detection of DMS should be developed in order to acquire further information about the occurrence and the parasite´s importance in Germany (BfR, 2007).

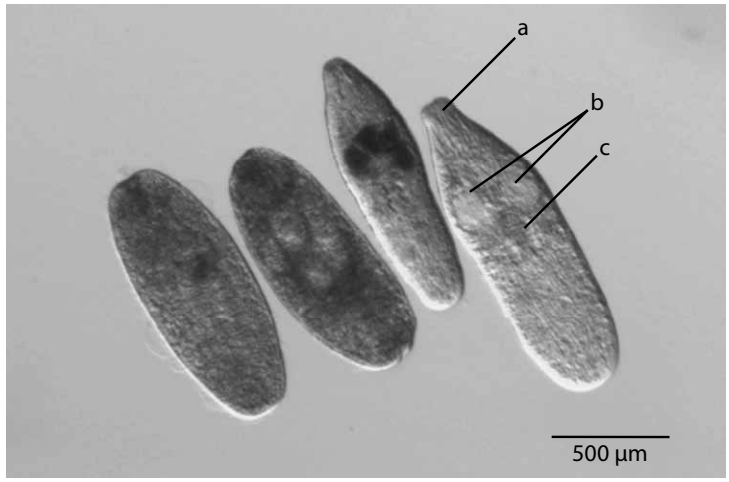

Figure 2. Living Alaria *spp. mesocercarial stages with intact viscera (a = oral sucker, b = penetration glands, c = acetabulum) alongside dead and partly dissoluted DMS after digestion with TIM.*

Our studies concentrate on the most pressing questions of (1) the development, optimisation and validation of methods for reliable DMS detection, (2) the distribution of the mesocerariae within their paratenic hosts, i.e. identification of potential predilection sites, particularly in wild boars, and (3) their prevalence in sylvatic populations of animals with respect to their introduction into the human food chain. Here we present our first results on predilection sites we obtained by use of the reference method for *Trichinella* detection in meat samples as stipulated in specified in Annex I, Chapter I No. 3 of Regulation (EC) No. 2075/2005 (EC, 2005; in the following abbreviated as *Trichinella* identification method 'TIM') and a modification of this method up to the presentation at IRFGMH conference, Brno, 2009.

2. Material and methods

In our studies on DMS in wild boars we first applied the reference method for *Trichinella* detection in meat samples according to Regulation (EC) No. 2075/2005 (EC, 2005). We digested the sample material from the carcasses of 20 wild boars in a total of 218 digestions. The carcasses were dissected and the available muscle tissue totally sampled for detection of DMS according to 12 anatomically defined sampling sites (pillar of the diaphragm, tongue, masticatory muscles (*Mm. masseter, temporalis, pterygoideus lat.*), 'cheek' (i.e. various tissues in the caudoventral region of the head containing among others muscle, connective, adipose, glandular and lymphatic tissue), neck, foreleg, shoulder, intercostal muscle, loin, back, abdominal muscles (*Mm. rectus abdominis, obliquus externus abdominis, transversus abdominis*), hind leg, adipose tissue) and completely digested. All carcasses and sample materials were stored at +2 °C until dissection and preparation and examined within 24 hours. In addition to the original protocol for the magnetic stirrer method for pooled sample digestion as specified in Annex I, Chapter I No. 3 of the Regulation (EC) No. 2075/2005 (EC, 2005) we modified the TIM in order to optimise its efficiency to detect DMS. Numerous

authors (e.g. Pearson, 1956; Shoop and Corkum, 1981; Shoop *et al*., 1990; Kimber and Kollias, 2000) report, that DMS prefers locations rich in adipose tissue. Since adipose tissues is undigestable in the HCl/pepsin digestion we tested a method in which Pankreatin© and bile acid in a magnetic stirrer apparatus are used for the digestion of adipose tissue. For a 50 g pool sample 5 g Pankreatin© and 1 g bile acid is added to a 2 litre cylinder containing 1.0 litre of tap water, preheated to 37 °C; a stirring rod is placed in the beaker, the beaker is placed on the preheated plate, the stirring is started and $NaHCO_3$ was added until pH 8 (± 0.5) is reached. 50 g of sample material is chopped in the blender and afterwards transferred to the 2 litre cylinder. After 60 min the stirrer is switched off and the digestion fluid is poured through a sieve (mesh size 180 µm). The digestion fluid is allowed to stand for 30 minutes and subsequently a 40 ml sample of digestion fluid is quickly run off into a small measuring cylinder. The 40 ml sample is allowed to stand for 10 minutes. A portion of 30 ml supernatant is then carefully withdrawn by suction to remove the upper layers and leave a volume of not more than 10 ml. The remaining 10 ml sample of sediment is poured into a larval counting basin or petri dish. The cylinder is rinsed with not more than 10 ml of tap water, which has to be added to the sample in the larval counting basin or petri dish. Afterwards, the sample is examined by a trichinoscope or stereo-microscope at a 15-20× magnification. To date, 89 single samples have been analysed by this lipid digestion method. In parallel, distribution patterns of DMS in 35 positive wild boars were analysed.

3. Results and discussion

The carcases of 20 wild boars were examined by use of TIM. In 14 cases (70%) DMS was demonstrated in one ore more of the analysed tissues. The number of DMS isolated from the different tissue samples varied considerably, ranging from 1 to 48 larvae in samples from 11.6 to 125 g. The distribution within the carcasses was heterogenic in all cases and a clear distribution pattern as known in *Trichinella* spp. could not be determined. Table 1 shows the number of DMS as retrieved from the tissue samples by TIM. It is noticeable that in some highly infested animals, tissues like 'cheek' and masticatory muscles show high larval burdens, whereas the same tissues are completely negative in other DMS-positive animals. Although nearly all tested body tissues were infested by the parasites, our results show in accordance with literature that DMS seems to prefer muscular tissue which contains high amounts of adipose-, connective- and/or glandular tissues. Cheek, neck, intercostal muscle and shoulder were infested in more than 40% of all positive animals. The larval burden decreases with smaller amounts of adipose and connective tissues in the sample material. Tongue, masticatory muscles, and back are infested in 38.5 resp. 28.6% of all positive cases, abdominal muscle, hind leg and loin in only 7.1%. The foreleg was the only tissue in which no DMS were detected at all. Furthermore, it became evident, that a large percentage of mesocercariae die during the pepsin/HCl-digestion, and they did not demonstrate the typical structure/shape of DMS during stereo-microscopical examination (see Figure 2). The use of Pankreatin© and bile acid digestion revealed 2 positive carcasses (No. 10 and 12) that had remained unidentified by conventional *Trichinella* digestion. Moreover, an increased number of DMS was isolated in some cases, even though a maximum of only 10-15% of the sample material was actually digested. In one case DMS could be exclusively detected in the

Table 1. Number of DMS as retrieved from muscle tissue of samples from different locations of the carcass (n=20) by application of magnetic stirrer method for pooled sample digestion acc. to Regulation (EC) No. 2075/2005 (results of analyses carried out until 11.09.2009).

Carcass	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Pos/all[1]
Tongue	2	0	n.a.[2]	0	0	6	0	0	0	0	0	0	0	0	0	5	1	1	0	0	15/19
Masticatory muscle	6	2	n.a.	0	0	2	48	0	0	0	0	0	0	0	1	0	0	0	0	0	59/19
Cheek	2	9	0	1	0	12	30	30	0	0	0	0	0	0	0	3	11	2	0	0	120/20
Neck	2	1	5	0	0	11	1	0	0	0	0	0	3	5	0	0	0	0	0	2	30/20
Shoulder	1	11	0	1	0	16	0	0	0	0	0	0	0	28	0	0	0	2	0	0	59/20
Foreleg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/20
Intercostal	1	0	2	0	0	7	6	0	0	0	0	0	0	39	0	0	5	0	0	4	64/20
Back	1	9	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	0	0	0	16/20
Abdominal	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3/20
Loin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1/20
Hind leg	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4/20
Total	15	32	10	2	0	61	85	30	0	0	0	0	3	75	1	9	17	5	0	6	351/218

[1] Pos/all = number of DMS/number of tested samples.

[2] n.a. = not analysed.

abdominal adipose tissue. Table 2 shows the number of DMS as retrieved from adipose tissue by lipid digestion of wild boars' carcasses.

After the first experiments with this new method it turned out, that the parasites showed increased survival rates and a distinctly higher vitality after lipid digestion compared to TIM. On the basis of these promising approaches we also applied modifications of the Pankreatin© and bile acid digestion in order to optimise its digestion efficiency.

Our results show that DMS distributes heterogeneous throughout the whole body of its paratenic host. Preferences are mainly identifiable for tissue composition; they further indicate, in accordance with literature, that DMS prefers muscular tissue which contains high amounts of adipose-, connective tissues and/or glandular tissues. Sampling as stipulated

Table 2. Number of DMS as retrieved from adipose tissue by application of Pankreatin© and bile acid digestion (n=20).

Carcass	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Pos/all[1]
Adipose tissue	n.a.[2]	n.a.	n.a.	n.a.	n.a.	21	2	6	n.a.	4	0	12	29	5	1	4	n.a.	n.a.	n.a.	0	84/11

[1] Pos/all = number of DMS/number of tested samples.

[2] n.a. = not analysed.

in Annex I, Chapter I, No. 2 and Annex III (a) of Regulation (EC) No. 2075/2005 (EC, 2005) is therefore non-applicable for detection of DMS. Especially the muscles of the distal foreleg were not infested with DMS.

The vastly differing infestation mode and resulting differences in the predilection sites of *Trichinella* and *Alaria* spp. also suggests, that the official digestion method for *Trichinella* spp. as laid down by regulation (EC) No. 2075/2005 (EC, 2005) is inadequate for DMS detection as this method was optimised for the detection of *Trichinella* in muscular tissue free of all fat and connective tissues. At the same time our studies have shown that a large percentage of mesocercariae die during the pepsin/HCl-digestion and their motility, as a major diagnostic feature, gets lost. In addition, the use of a sieve harbours a certain danger that mesocercariae could be retained in the mesh or that vital parasites attach to it with their oral suckers. The promising approaches with the Pankreatin© and bile acid digestion indicate that this method, possibly in combination with TIM might be more applicable than the original digestion methods for *Trichinella* spp.

4. Conclusions

Our results show that the official digestion methods for *Trichinella* spp. as stipulated in Regulation (EC) No. 2075/2005 (EC, 2005) are inadequate for the detection of DMS due to differing infestation modes and tissue preferences of both parasites. In conclusion, we can state that further studies are imperative with respect to the high potential pathogenicity of DMS and their presence in the German wild boar population. The development of a reliable method for detection of the mesocercariae in meat seems to be one of the key factors in this context. Simultaneously the distribution patterns of the mesocerariae within their paratenic hosts need to be investigated thoroughly with the view to determine potential predilection sites in wild boars. Finally the parasites' prevalence in sylvatic populations of animals needs to be elucidated (update: Riehn *et al.*, 2010).

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Hygiene management systems for commercial game harvesting teams in Namibia

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Summary

There are more than two million game animals in Namibia, with numbers increasing at a rate of 20-40% per annum. Around 90% of the country's wildlife is located outside formally proclaimed conservation areas whilst more than 80% of the larger game species are found on privately owned farms comprising 44% of the surface area of the country. The Namibian wildlife sector offers a commercially viable alternative for generating farm income. The quality of the meat harvested from game meat depends on several factors such as the skill and attitude of the hunter, the health of the game animal before being shot, the position of the shot and the hygienic handling after shooting. Quality aspects further rely on the time before cooling and the transport and treatment of game carcasses as well as the period prior to cooling. Food business operators must therefore establish, implement and maintain hygiene control procedures based on HACCP (Hazard Analysis and Critical Control Points) principles (EC, 2004a) before exports of game meat to international countries are approved.

Keywords: microbiological spoilage, HACCP, Africa

1. Introduction

Namibia is well-known for its high quality game meat and game meat products. Tourists often praise this attribute of Namibian game meat, as it is often offered on the menu in restaurants, guest houses and lodges. Namibia has a number of regulations that apply to the sustainable use of game animals which are applicable when the harvesting of game animals for commercial game meat production is used to remove excess animals (Nature Conservation Ordinance, 1975). Countries importing game meat, such as South Africa and the European Union, also lay down specific rules and regulations whereby countries willing to export game meat, must abide.

Only harvesting teams registered with the Namibian Directorate of Veterinary Services and the Ministry of Environment and Tourism are allowed to harvest for the commercial export of game meat. Each of the harvesting teams should have a well documented and implemented hygiene management system, as required by the importing country, in place, before the meat harvested will be allowed to be exported by the competent authority, which is the Directorate of Veterinary Services in Namibia. Game may only be harvested from the Office International des Épizooties (OIE) – the world organisation for animal health – recognised foot-and-mouth disease (FMD) free zone without vaccination. The fresh meat should be obtained from areas free of FMD and Rinderpest (Kamwi, 2007).

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2. Hygiene management systems

The primary responsibility for food safety rests with the food business operator (EC, 2004a) and it is necessary to ensure food safety throughout the food chain, starting with primary production. Food business operators must therefore establish, implement and maintain hygiene control procedures based on Hazard Analysis and Critical Control Points (HACCP) principles (EC, 2004a). This is applicable to the harvesting of game for meat exports to the European Union and other countries such as South Africa (Anonymous, 2000, 2004).

2.1 Standard Operating Procedures (SOP'S)

Hunters must be trained in health and hygiene and must have sufficient knowledge of the pathology of wild game, and of the production and handling of wild game and wild game meat after hunting, to undertake an initial examination of wild game on the spot (EC, 2004b).

Ante mortem inspections must be carried out by the hunter prior to hunting (CAC, 1993). Only head shots are allowed for commercial harvesting. This is essential to limit decay and contamination of the meat (Van Rooyen *et al*., 1996). Game killed with thoracic and abdominal shots are subject to secondary inspection (Anonymous, 2000, 2004). A farmer may only employ a night harvesting team for commercial purposes (Nature Conservation Ordinance, 1975).

Game intended for commercial purposes must be bled within 10 minutes of being shot. Blood is an ideal growth medium for bacteria and when not well-bled, a carcass will deteriorate faster (Van Rooyen *et al*., 1996). Carcasses must be transferred from the collecting vehicle to a clean slaughter frame in such a manner as to avoid contamination. Labels must be provided for the identification of each carcass and its organs (Ebedes and Meyer, 1996). Animals should be partially eviscerated within 20-30 minutes of harvesting. Partial evisceration, normally restricted to removal of the intact gastrointestinal tract, serves to reduce the weight and bulk of the carcass and to speed cooling. Chilling must begin within a reasonable period of time after killing, preferably within 12 hours after the harvest. When the ambient temperature is more than 12 °C, carcasses must be chilled within 4 hours (Anonymous, 2000, 2004). Veterinary maturation of meat destined for the European market is necessary. This is a control process whereby the foot-and-mouth disease virus is deactivated. Carcasses must be submitted to maturation at a temperature above +2 °C and below +7 °C for at least 24 h before de-boning (EC, 2008).

Game meat can only be marketed commercially if it was transported in a refrigerated truck to a registered game handling establishment as soon as possible after harvesting. The red offal must accompany the body and must be identifiable as belonging to a given animal (EC, 2004b).

2.2 Sanitation Standard Operating Procedure (SSOP's)

Hygiene management systems for game harvesting teams comprise Sanitation Standard Operating Procedures for pre-, during and post-operational cleaning and sanitation. Sterilizers used to sanitise knives contain 10 ppm free chlorine derived from a chemical

sterilizer. Drinking water is adjusted to a free chlorine level of 1-2 ppm. All equipment, including the trucks used for harvesting, are cleaned and sanitised.

2.3 Good Hygiene Practices (GHP's)

Employees handling the game carcasses wear outer garments suitable for hunting and in such a manner that the clothing protects against contamination. These include jackets, aprons, rubber boots and hair nets. Employees undergo medical check-ups regularly and abide by a strict hygiene code. At least one team member is trained as a game meat inspector and also maintains the records of the hygiene management system.

2.4 Critical Control Points (CCP's)

A hygiene risk assessment is used to determine Critical Control Points for the game harvesting process. Typical Critical Control Points defined are:

- Checks on the potability of water from farms where the harvesting take place.
- Checks on faecal contamination of the partially dressed carcasses.
- Checks on temperatures of the carcasses after being loaded into the refrigerator trucks.

The treatment of water to an acceptable chlorine level is essential since most of the time untreated water from the farms is used during the harvesting of game. Faecal contamination can result in unacceptable pathogenic bacterial growth. Maturation of the meat (between +2 °C and +7 °C in 24 h) is critical regarding safety (EC, 2008). The detection of metal fragments from bullets is not considered as a Critical Control Point. It is controlled by the Standard Operating Procedure where only head shots are accepted for commercial harvesting. A study undertaken by Haldimann *et al.* (2002) concluded that frequent consumption of game meat has no significant effect on blood lead levels.

3. Conclusions

Hygiene management systems assist game harvesters and processors in ensuring that all harvesting, transporting, dressing and processing procedures are done under hygienic conditions. Micro-organisms are mostly responsible for causing severe food poisoning in humans who eat contaminated meat. Game meat however, has an inherent resistance to contamination by micro-organisms and this gives it a competitive edge over other types of meat (Ebedes and Meyer, 1996).

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Salmonella spp. in wild boar (*Sus scrofa*): a public and animal health concern

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Summary

Wild boars constitute a potential reservoir and may spread zoonotic agents, including *Salmonella* sp. and thus they represent a source of infection for (wild and domestic) animals and humans. During the 2006 hunting season, 77 rectal faecal samples from animals shot by hunters in Northern Portugal were collected and analysed to determine the prevalence and serovars of *Salmonella* sp. in wild boars (*Sus scrofa*). The results showed that 17 (22.1%) were positive for *Salmonella* sp. In these positive samples, the most prevalent serovar was *Salmonella* Typhimurium, identified in 11 (64.7%) isolates, followed by *Salmonella* Rissen in 6 (35.3%). These results confirm the importance of wild boar as a reservoir of pathogenic serovars of *Salmonella* and as a potential risk for humans and livestock and emphasise the importance of intervention procedures for improving surveillance.

Keywords: wild boar, *Salmonella*, zoonoses, wildlife, food safety

1. Introduction

Salmonella is defined, both at the European and national level, as being the main responsible pathogen for foodborne diseases, with approximately 170,000 annually notified human salmonellosis cases within the European Union (EFSA, 2007). Because of its importance as a zoonotic agent, extensive surveillance programmes exist in all Member States. However, in spite of sanitary campaigns, and according to recent literature reviews (Bengis, 2002; Gortázar *et al*., 2007), the possibility of persistent cycling of infection in wildlife is real and this could limit the success of domestic animals disease control programmes. In this context *Salmonella*

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may serve as a model for studying the role that wildlife plays as zoonotic agent reservoir and how this could compromise control programmes in use by veterinary authorities.

Previous studies on the occurrence of *Salmonella* sp. in wildlife highlight the significance of game animals as carriers contributing to animal and human contamination, e.g. hedgehogs (Handeland *et al*., 2002), wild birds (Refsum *et al*., 2002), gulls (Wahlström *et al*., 2003), wild birds and mammals (Millan *et al*., 2004) and white-tailed deer (Renter *et al*., 2006). However, to date, data on the epidemiological distribution of *Salmonella* sp. in wild boars are very limited. In particular, bibliographic references for Portugal are lacking.

Wild boar infected with *Salmonella* sp. may play an important role in the epidemiology of this zoonotic pathogen. *Salmonella* sp. shed in their faeces may be ingested by other wild animals or by domestic livestock animals, either through direct contact or when these resources are shared by food animals (specially pastured livestock) or through water cross contamination (Bengis *et al*., 2002; Vicente *et al*., 2002; Renter *et al*., 2006). This wildlife/domestic animal interface is observed in rural areas in Northern Portugal, where pasturage of domestic animals and backyard pig production is a tradition.

Human health risks from wild boar infected with *Salmonella* sp. arise indirectly from agricultural areas and vegetable products contamination, through direct animal contact, during the hunting process and carcass manipulation, or directly from ingestion of contaminated meat or meat products, such as sausage (Renter *et al*., 2006).

Considering the importance of wild boars as a major game species in Northern Portugal, as well as their potential role in transmission of *Salmonella* sp. to domestic and wild animal populations with the attendant risk for human health, assessing the prevalence and serotypes of *Salmonella* sp. in free-ranging wild boars harvested by hunters was the main objective of this study.

2. Material and methods

2.1 Study area

The study area is located in Northern Portugal, where rural and hunting areas comprise numerous rural settlements (poorly fenced) that are used by local people to produce vegetables for sale in local markets. The domestic animal production system is characterised by outdoor production of ruminants and backyard pig production. Within this area, hunting activity (wild birds, rabbits and hares, wild boars) represents an important contribution to the development of the local economy, not only in terms of hunting fees but also from the viewpoint of complementary expenses spent by hunters for lodging, meals and purchases.

2.2 Sampling procedure

During the hunting season of 2005/2006 (December 2005 to February 2006) several hunting associations from Northern Portugal were contacted with the request to indicate the calendar

of their hunting activities so as to allow collecting a representative number of wild boar samples. After the hunting events, animals were transported to a site where the research team directly sampled rectal faeces from harvested wild boars (aseptically, i.e. using latex gloves). From a total of 253 wild boars (*Sus scrofa*), 77 hunted animals were sampled, whereupon samples were coded and transported under refrigerated conditions to the laboratory. All tested animals were subsequently marketed for human consumption.

2.3 Cultural microbiology

All samples were analysed by means of standard culture methods, according to annex D of ISO standard 6579:2002 applied to *Salmonella* detection in animal faeces. Isolates of presumptive *Salmonella* (1 to 2 colonies from each sample) were confirmed by means of biochemical tests (Oxidase reaction, Triple Sugar Iron Agar (Oxoid® – CM277), Urea broth (Merck® – 1.08483), L-Lysine decarboxylation medium (Oxoid® – CM308S)) and serological agglutination with Poly A-I & Vi antiserum (Difco® – 222641). *Salmonella* isolates were serotyped from each positive sample according to the Kauffmann-White scheme (Popoff, 2001) in the LNIV – National Reference Laboratory for *Salmonella*.

3. Results and discussion

This study is the first report of *Salmonella* sp. identification in wild boars´ faecal samples in Portugal, and demonstrates the presence of this microorganism in 17 (22.1%) faecal samples from 77 harvested wild boars. This prevalence highlights the importance of the wild boar as reservoir and as a faecal shedder of *Salmonella* sp., and indicates that faeces from apparently healthy wild boars can be a source of this pathogen and be potentially transferred, to livestock, other animals and humans.

Vicente *et al.* (2002) reported a small seroprevalence of *Salmonella* in Spain, as based on antibodies against *Salmonella* serovar B in 4% and *Salmonella* serovar C in 3% of the cases. In contrast, Vengust *et al.* (2006) reported a seroprevalence of 47% of *Salmonella* sp. in wild boars in Slovenia. So far, no data on the prevalence of *Salmonella* sp. in faecal samples of wild boars have been published.

In our study, only two serovars were identified: *Salmonella* Typhimurium (64.7%) and *Salmonella* Rissen (35.3%). This is partially explained by the small number (one or two) of *Salmonella* sp. colonies isolated and identified per sample, limiting identification of other possible serovars. Nevertheless, there is a clear dominance of *Salmonella* Typhimurium (identified in 64.7% of the positive samples), which suggests this is a predominant serovar in fecal sample of wild boars, as established in several national (Vieira-Pinto *et al.*, 2005) and international studies (Davies *et al.*, 2000; Giovannacci *et al.*, 2001; Swanenburg *et al.*, 2001; Botteldoorn *et al.*, 2004; Castagna *et al.*, 2004), and confirmed by the report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006 (EFSA, 2007).

The similarity of the *Salmonella* serovar prevalence pattern of pig and wild boar populations indicates a possible bidirectional circulation of *Salmonella* sp. through both animal ecosystems. This is not unlikely to occur in Northern Portuguese rural areas, as physical contact between wild boars and backyard raising pigs is frequently reported. Yet, at the present time, this hypothesis cannot be substantiated since the sanitary status of the northern Portuguese pig population with respect to *Salmonella* sp. is not known.

According to Vicente *et al*. (2002), *Salmonella* Typhimurium is pathogenic for animals, and could affect the dynamics of European wild boar populations as well as interfere with the health of other wild animals, such as birds. In addition, this serovar deserves special attention in view of its virulence in humans (Botteldoorn *et al*., 2004) and because it is highly resistant to antibiotics (Fedorka-Cray *et al*., 1999; Nielsen *et al*., 1999; Cruchaga *et al*., 2001; Botteldoorn *et al*., 2004). Currently, *Salmonella* Typhimurium, is the second most prevalent *Salmonella* identified in the European Human Salmonellosis cases in 2006 (EFSA, 2007). In respect to the second most prevalent serovar, *Salmonella* Rissen, no references were found on its prevalence in wildlife.

Wild boars may indirectly represent a reservoir and source of infection for humans, by uncooked contaminated vegetables (lettuce, cress), water (streams, stagnant pools) or domestic animals, or directly through contact with infected carcasses, by consuming meat or meat products (Everard *et al*., 1979; Kruse *et al*., 2004; Vengust *et al*., 2006). *Salmonella* sp. occurrence in wild boar meat and carcass was also demonstrated in studies by Decastelli *et al*. (1995) in Italy, Kanai *et al*. (1997) in Japan and Wisniewski (2001) in Poland. For example, Wisniewski (2001) reported the presence of *Salmonella* spp. in 11 animals (7%), in carcasses and internal organs of 156 hunted wild boars. Since hunted wild boars are marketed for human consumption, they are a potential source of infection to man, if they harbour *Salmonella* sp. in their edible tissues, or if meat is contaminated by intestinal content during evisceration or processing of the carcass (Lillehaug *et al.,* 2005). Faecal contamination of wild boar carcasses can be expected when hunted animals are poorly bled, eviscerated and skinned under precarious hygienic conditions (Decastelli *et al*., 1995; Lillehaug *et al.,* 2005). In Portugal, this scenario is of particular concern, since the majority of the hunted animals are used for human consumption, in almost all cases after a deficient transportation of the hunted animals, and technically and hygienically undesirable evisceration and dressing procedures.

4. Conclusions

The results presented in this study show that wild boar can represent a vehicle of pathogenic serovars of *Salmonella* to humans and animals, suggesting that more attention should be paid to game meat hygiene. Our study also indicates that systematic serological and bacteriological surveillance of wild boar populations should be improved with a view to better understand and minimise the impact of such diseases on wild and domestic animals as well as humans.

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Preliminary results indicating game meat is more resistant to microbiological spoilage

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Summary

The aim of this study was to investigate whether game meat has an inherent antimicrobial activity. To test this, game samples from impala, nyala, warthog, wildebeest, ostrich, zebra and as controls; beef, mutton and pork were challenged with *S. aureus* and the growth determined after overnight incubation at 37 °C. Concluded from the results of this study, meat from game animals repressed growth of *S. aureus* much stronger than that of domestic animals. Further studies to determine whether this phenomenon is applicable to other animals are in progress.

Keywords: microbiological spoilage, *Staphylococcus aureus*, Africa

1. Introduction

Most foodborne diseases are caused by pathogens such as *Escherichia coli, Salmonella, Campylobacter, Clostridium botulinum* and *Staphylococcus aureus* (Abee *et al*., 1995). Some of these microbes originate from soil, water, the intestinal tract of humans and animals. *S. aureus* is almost always present in the nose, mouth and skin (Gill and Newton, 1978).

The purpose of this study was to determine whether game meat, in comparison to that of domestic farm animals such as beef, mutton and pork, had an inherent antimicrobial activity.

There are a number of factors that could cause this apparent phenomenon. The first is the ultimate pH (pHult) of the meat (Gill and Newton, 1981), which is subsequently the result of the amount of lactic acid produced from glycogen during anaerobic glycolysis (Aidoo and Haworth, 1995). When glycogen levels of muscles are depleted due to *ante mortem* stress the meat has a high pHult and this increases the likelihood of meat spoilage (Gregory, 1996). Meat with a high ultimate pH is also classified as dark firm and dry (DFD) and has a high water binding capacity (Scanga *et al*., 1998). Game meat that has been stressed *ante mortem* show signs of DFD and a strong water binding capacity (Hoffman, 2000). The correlation between meat water content and microbiological spoilage is well-documented (Gill and Newton, 1981).

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2. Materials and methods

A naturally occurring Gram- and Catalase-positive isolate from pork, positively identified as *S. aureus* was used in the experiments. Approximately 1g grounded meat samples from nine different animals (sourced from a commercial game meat processor) were each inoculated with 105 cfu/g (200 µl) of the isolate and 400 µl of sterile distilled water. As controls, 1g of each meat sample (beef, mutton, pork, impala (*Aepyceros melampus*), nyala (*Tragelaphus angasii*), warthog (*Phacochoerus africanus*), wildebeest (*Connochaetes taurinus*), ostrich and zebra (*Equus burchelli*)) was inoculated with sterile distilled water (600 µl). The samples were then incubated at 8 °C for 24 h and tested for the proliferation of the isolate. After incubation every sample was vigorously mixed with 9 ml sterile distilled water and serial dilutions were made in duplicate. Dilutions were plated out onto Baird Parker agar, a selective medium for the identification of *S. aureus*. Cell counts were determined after overnight incubation at 37 °C.

3. Results and discussion

After determining the cfu/g in each sample on the appropriate medium, the data was plotted (Figure 1) to reveal the proliferation of the pathogen present in the meat.

In this study beef was taken as the comparative standard, although mutton or pork could also be chosen. This is done to compare the results obtained from game meat with that of domestic farm animals. The difference in cell numbers of the *S. aureus* inoculated beef before and after 24 hours was designated a value of 100. The resistance of the other meat samples was then compared with that of beef. Of the other two domestic animals the mutton had a value of 130 and pork 60. Wildebeest had a value (76) between that of beef and pork. Ostrich displayed a slightly better value (67). Nyala (44) was fairly better to that recorded for pork. The impala (31), zebra (17) and especially the warthog (1), showed surprisingly high resistance to the proliferation of this pathogen.

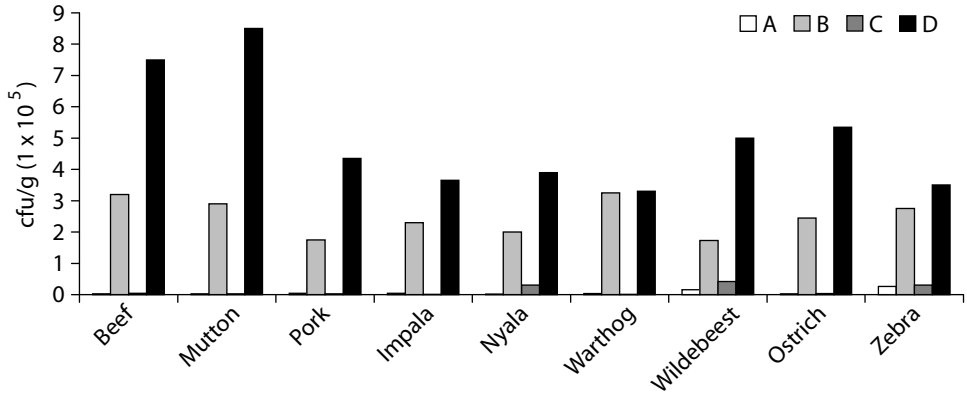

*Figure 1. Meat experiments done with Baird Parker Agar. The bars marked A represent the meat sample plus H_2O at 0 hours. B indicates the meat sample plus H_2O plus the isolate (*S. aureus*) at 0 hours. The meat sample plus H_2O after 24 hours is indicated by C, and D indicate the meat samples plus H_2O plus the isolate after 24 hours.*

As the meat samples did not contain high numbers of *S. aureus* before inoculation, the values recorded at time zero were thus considered negligible.

It would also seem from the results that game meat has a stronger ability to naturally preserve itself than that of domestic farm animals. It was thought that possible *ante mortem* stress might be an influencing factor, but MacDougall *et al*. (1979) could not find any difference in microbiological spoilage of farmed young red deer exposed to different levels of *ante mortem* stress.

It would appear that the reticulo-endothelial system in some species is more effective than in others, since venison can be hung for a considerable period without any submission to decreased temperature or other precaution methods (Lawrie, 1985). Gill *et al*. (1976) confirmed that the surviving action of the reticulo-endothelial system destroys bacteria entering the lymphatic system from the intestines up to 24 hours *post mortem*.

This phenomenon clearly requires further elucidation.

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Section 2
Epidemiology

Trichinellosis in wild and domestic pigs and public health: a Serbian perspective

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Summary

Among a large number of susceptible animal species, the domestic pig is the most important source of human *Trichinella* infection worldwide. Trichinellosis is commonly defined by two cycles; 'domestic' (in pigs on-farm) and 'sylvatic' (in wildlife). Under conditions on industrial farms (particularly indoor) with good hygienic practices and efficient management including biosecurity, combined with effective governmental/veterinary services, *Trichinella* transmission *via* the domestic cycle is unlikely. In countries effectively implementing these strategies and with officially recognised negligible risk of *Trichinella* in domestic pigs, testing for this parasite at meat inspection is no longer mandatory for slaughter pigs reared in integrated production. In other countries, testing for *Trichinella* of slaughtered pigs is a very important component of the control system. Under conditions on small farms with pigs having access to the outdoors and where control measures are poorly implemented, or are lacking, the domestic cycle can play a very important role in trichinellosis transmission. This possibility is further enhanced where socio-economic and political problems temporarily diminish the efficacy of the governmental/veterinary services. Additionally, the practice of making uncooked products from meats of uninspected domestic and/or wild pigs at home represents a major risk for human infection. The risk of further spreading trichinellosis is additionally exacerbated by increased globalisation in modern times, including increased movements of livestock, food and people. Traditional farming practices facilitating a mixture of domestic and sylvatic cycles of *Trichinella* need to be modified/improved so to ensure the separation of the cycles. Furthermore, hunters need to be educated to avoid leaving animal carcasses or their entrails in the field because this increases the probability of transmission to new hosts. Also, the farmers, the hunters and the consumers should be educated to freeze pork (including meat from wild boars) before its further home-processing into products, or to cook the product before consumption, or both, aimed at the larvae inactivation.

Keywords: *Trichinella*, wild boar, pig, meat, meat products

1. Introduction

Trichinellosis is an infectious parasitic disease transmissible from animals to man (zoonoses). Natural *Trichinella* infections in more than 100 species of mammals, seven avian species, and three reptile species have been reported (Pozio, 2005). Nevertheless, the domestic pig is the

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most important source of human infection worldwide. In addition, meats of wild boars and horses have played a significant role during outbreaks over the past few decades. Trichinellosis is commonly defined by two cycles; 'domestic' and 'sylvatic'.

With the 'domestic cycle', trichinellosis is considered in domestic pigs that can become infected through feeding on: (a) uncooked swill and other organic wastes that can contain pork; (b) carrion that are not removed/disposed; or (c) synanthropic animals from the surroundings (e.g. rodents). Under conditions on industrial farms (particularly indoor) with good hygienic practices and efficient management including biosecurity measures, *Trichinella* transmission *via* the domestic cycle is unlikely, as indicated by the lack of reports of infections on industrialised farms in developed, western countries (EFSA, 2005a). However, under conditions on small farms with pigs having access to the outdoors, and where control measures are poorly implemented or are lacking, the domestic cycle can play a very important role in trichinellosis transmission. This possibility is further enhanced where socio-economic and political problems temporarily diminish the efficacy of the governmental/veterinary services.

With the 'sylvatic cycle', trichinellosis is considered in wildlife hosts comprising a number of carnivorous and omnivorous wild animal species, between which the infection is transmitted through feeding on: (a) infected prey animals; (b) tissue from infected carrion of the same species ('cannibalism'); or (c) tissue from infected carrion of other species. The likelihood of the transmission is enhanced by the fact that *Trichinella* larvae can survive in decaying muscles of dead animals for extended periods of time.

The domestic and sylvatic cycles can function either independently from each other or interactively (Pozio, 2007). The interaction between the cycles, i.e. a switch from wild animals to domestic animals, can occur where there is an improper management in segregating husbandry and wildlife (Gottstein *et al.*, 2009). This usually leads to increases in incidence/prevalence in susceptible food animals and humans, which is accompanied by serious problems in international meat trade. The risk of spreading trichinellosis is further exacerbated by increased globalisation including increased movements of livestock, food and people that is evident in modern times.

2. Main characteristics of disease

2.1 Animal infection

Trichinellosis is a foodborne parasitic zoonotic disease caused by nematode worms of the genus *Trichinella* including *T. spiralis* T1, *T. nativa* T2 (and related genotype *Trichinella* T6), *T. britovi* T3 (and related genotypes *Trichinella* T8, T9), *T. pseudospiralis* T4, *T. nelsoni* T7, *T. murrelli* T5, *T. papuae* T10 and *T. zimbabwensis* T11 (EFSA, 2004). All the species of the genus are known to infect mammals (mostly carnivores), rodents and omnivores including pigs, and occasionally also herbivores (e.g. horses), but *T. pseudospiralis* also can infect birds and both *T. papuae* and *T. zimbabwensis* also can infect reptiles (EFSA, 2004). In pigs in Europe, *T. spiralis* is of the highest concern; followed by *T. britovi* and *T. pseudospiralis*. Only

the larval stage of the parasite is infectious; the infection occurs only *via* the ingestion of the muscle tissue containing the larvae (Figure 1).

However, the infectivity can vary with *Trichinella* species-animal species combinations (Table 1). Furthermore, both the infective dose and the total number of larvae in muscles can differ between pigs and wild animals. For example, some reports indicate that in pigs and foxes,

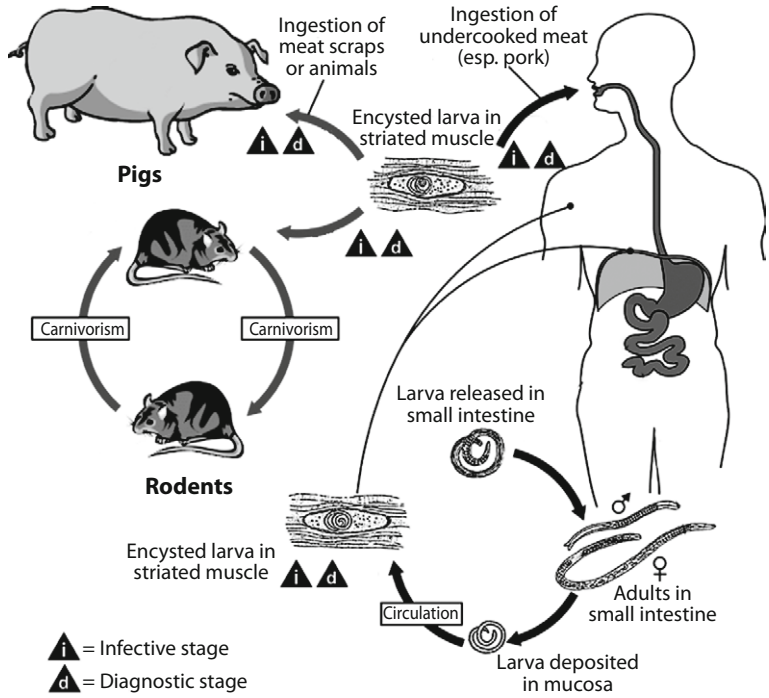

Figure 1. Life cycle of Trichinella *(source: http://www.dpd.cdc.gov/dpdx).*

Table 1. Trichinella *infectivity variations in animals (adapted from Kapel and Gamble, 2000; Trichiporse, 2005; EFSA, 2005b).*

Trichinella species	Domestic animals		Wildlife			Birds
	Pigs	Horses	Wild boars	Rats	Foxes	
T. spiralis	+++[1]	+++	+++	+++	+++	-
T. nativa	-	?	+	+	+++	-
T. britovi	++	+	++	++	+++	-
T. pseudospiralis	+	++	++	++	+++	+

[1] Infectivity: +++ highest; + lowest; - negligible.

the infective dose is around 50,000 and 500 larvae, respectively, and average larval burden in an infected host is around 950 and 20 per host, respectively (Olsen *et al*., 1964; Kapel *et al*., 1995). Normally, infected animals do not show clinical signs of the disease; therefore, in most EU member states and non-EU European countries, slaughter pigs, horses, wild boar and other wildlife intended for human consumption are tested for *Trichinella* at meat inspection.

2.2 Human infection

In contrast to animals, infected humans can develop serious and life-threatening disease. Historically, most cases of human trichinellosis worldwide have occurred following consumption of pork. *Trichinella* infection in humans is strongly associated with the consumption of raw or undercooked meat; thus, cultural factors such as traditional dishes based on raw or undercooked meat or meat-derived products play an important role in the epidemiology of the disease (Gottstein *et al*., 2009). However, in the last fifty years it has also become clear that meat from other species – wild boar, bears, foxes, walrus, cougar, dog and horse – can be a vehicle of trichinellae (EFSA, 2004). Hunters, their relatives and their friends are at risk of trichinellosis infection when raw meat from game animals is not tested for *Trichinella* before consumption. The migratory flow of humans with their own food practices including the consumption of raw meat, the illegal importation of non-controlled meat from endemic to non-endemic countries, and new food practices and dishes including raw meat has resulted in outbreaks in Denmark, Germany, Italy, Spain, and the United Kingdom (Gottstein *et al*., 2009).

Trichinella infection with low number of larvae can remain asymptomatic in humans, but in the case of ingestion of a higher number of larvae (e.g. few hundred), the disease exhibits two main phases: intestinal and muscular. The intestinal phase is manifested by gastroenteritis (diarrhea, abdominal pain) approximately two days *post*-infection, caused by the larvae penetrating intestinal mucosa and migrating *via* blood circulation throughout the body until reaching their final location: the striated skeletal muscles. Migrating *Trichinella* larvae and their metabolites provoke an immediate reaction, which causes immunological, pathological, and metabolic disturbances and the various clinical phenomena observed during the acute stage of the infection (Gottstein *et al*., 2009). Human infection data from outbreaks indicate that some differences in the disease pattern may exist between *Trichinella* species. For example, the incubation period was 5-20 days after ingestion of 5,000-18,000 *T. spiralis* larvae, but 12-40 days after ingestion of 300-30,000 *T. britovi* larvae (Pozio *et al*., 1993; Gari-Toussaint *et al*., 2004; EFSA, 2005b). In the case of the former species, not only the incubation was shorter, but the symptoms were also more intense.

The therapy in cases of human trichinellosis must be applied as early as possible, with application of antihelmintics at the intestinal invasion stage so to eliminate intestinal forms of *Trichinella* sp. from the lumen of the gastrointestinal tract. The drugs principally include preparations like albendazole and mebendazole. In the case of delayed start of the antihelmintic therapy, i.e. during advanced stage of disease, the effects on already encysted larvae are poorly elucidated to date. Despite therapy, lethality in cases with high infection intensity is up to 5%, whilst in milder cases most patients exhibit a disappearance of symptoms within 2 to 6 months (Gottstein *et al*., 2009).

3. Current trichinellosis status

3.1 Current European Union perspective

3.1.1 Wild pigs and other wildlife

In the EU in 2007 (EFSA, 2009), the reported *Trichinella*-positive wildlife animals included 0.1% of non-farmed wild boars (443,890 examined), 4.5% of bears (403 examined), 2.6% of foxes (6,680 examined), 19.6% of lynxes (224 examined), 19.4% of racoon dogs (222 examined) and 21.8% of wolves (55 examined).

3.1.2 Domestic pigs

In the EU in 2007 (EFSA, 2009), *Trichinella* was reported in <0.1% of 220,680,358 examined domestic pigs (EFSA, 2009) and in 0.4% of 6,615 examined farmed wild boar. The highest numbers of *Trichinella*-positive slaughter pigs were reported by Poland, Romania and Spain. In 2007, Denmark was assigned the status as a region where the risk of *Trichinella* in domestic pigs is officially recognised as negligible in accordance with Regulation (EC) No. 2075/2005 (EC, 2005). This was the first time this status was granted to any EU member state. Countries with this status are allowed to use a risk-based monitoring programme for *Trichinella*, and testing for this parasite at meat inspection is no longer mandatory for slaughter pigs reared under controlled housing conditions in integrated production.

3.1.3 Humans

In the EU in 2007 (EFSA, 2009), a total of 779 confirmed human cases of trichinellosis were reported. The highest numbers of cases were recorded in Bulgaria, Poland and Romania. Bulgaria and Romania became EU member states in 2007, thus their contribution has resulted in a higher number of recorded cases of trichinellosis compared to previous years. In 2007 in the EU, for 69.1% of confirmed human cases, the *Trichinella* species was not reported, but where reported, *Trichinella spiralis* was the most common species (28.2% of all cases). Apart from *T. spiralis*, *T. nativa* and *T. pseudospiralis* were detected in humans; but no cases due to *T. nativa* or *T. pseudospiralis* were reported.

3.2 Current Serbian perspective

3.2.1 Wild pigs

Trichinellosis is endemic and prevalent in the wildlife in Serbia. This includes the sizeable wild boar population (roughly 10,000-11,000), of which roughly 25% were hunted annually during the 2005-2008 period. Whilst it is not known whether every hunted boar was subjected to examination for *Trichinella* infection, among annually reported *Trichinella* tests in hunted wild boars, between 0.45% and 1.26% were *Trichinella*-positive (Table 2). On the other hand, published data for *Trichinella* in wildlife other than wild boar in the country is scarce.

Table 2. Trichinellosis in wild boars in Serbia.

Year	Total number of boars	Number of hunted wild boars	Positive wild boars: number (% of tested)
2005	10,781	2,631	14 (0.53)
2006	10,647	2,638	12 (0.45)
2007	10,583	2,531	32 (1.26)
2008	10,839	2,749	27 (0.98)

3.2.2 Domestic pigs

Total numbers of annually reported *Trichinella*-positive slaughtered domestic pigs in Serbia during the 1994-2007 period varied between 153 and 1,698 with the incidence ranging roughly between 0.004% and 0.04% (Table 3). Industrial pig farms usually have well-controlled farming practices, so have only extremely rarely been involved in trichinellosis. In contrast, very small farms, most often holding only a few pigs ('backyard'; often with access to the outdoors), to be slaughtered on-farm and for home consumption pose the main trichinellosis risk; this is much higher than that posed by industrial farms. This is supported by data for 2004 (Mirilovic, 2005), showing that the mean number of pigs on positive farms in Serbia was 13.2, with 40.4% of the positive farms having only 1-7 pigs. The Serbian experience is that the farm-level epidemiological situation within a given region could be more relevant

Table 3. Trichinellosis in domestic pigs in Serbia.

Year	Positive pigs	
	Number	%
1994	153	0.0041
1995	394	0.0094
1996	866	0.0195
1997	1,416	0.0336
1998	1,519	0.0366
1999	1,575	0.0360
2000	1,698	0.0415
2001	1,147	0.0317
2002	920	0.0256
2003	877	0.0241
2004	645	0.0188
2005	391	0.0124
2006	426	0.0113
2007	586	0.0147

for the prevalence of *Trichinella* in domestic pigs than the geographically defined region itself (EFSA, 2005b).

When considering the temporal trend of trichinellosis in domestic pigs in Serbia, it is clear that socio-economic factors can play a major role in the occurrence of trichinellosis in domestic pigs (Djordjevic *et al.*, 2003; Cuperlovic *et al.*, 2005; EFSA, 2005b). Before the political and military turmoil in the Balkans, i.e. in 1970s-1980s, pig farming in Serbia was based on very large state-owned industrial farms, with reasonable farming practices and under very well-organised veterinary and regulatory activities. At that time, the occurrence of trichinellosis in domestic pigs was roughly stable, ranging from 0.009% to 0.02% annually (data not shown). Subsequently, during the decade-long Balkan problems in the 1990s, a sharp decline in large industrial farms was associated with a sharp increase in very small farms and 'backyard' pig rearing. The latter brought about much worsened or sometimes 'improvised' farming practices that were often not subject to proper regulatory controls. This was accompanied with a correlated increase in trichinellosis occurrence in domestic pigs in the 1990s (Figure 2). However, since the political and military problems ended and democratic transition occurred in Serbia in 2000, gradual improvements in farming practices and regulatory activities took place over the following years, which resulted in a clear decrease of the trichinellosis occurrence in domestic pigs (Figure 2).

3.2.3 Humans

The total number of reported human cases of trichinellosis in Serbia during the 1994-2007 period varied between 117 and 791, with the incidence ranging roughly between 0.001% and 0.01%, respectively (Table 4). When considering the temporal trend of trichinellosis in humans in Serbia, it is clear that socio-economic factors can play a major role in occurrence of trichinellosis in humans, similar to the situation with domestic pigs (Djordjevic *et al.*, 2003; Cuperlovic *et al.*, 2005; EFSA, 2005b). In 1970s-1980s, the occurrence of human trichinellosis was mostly stable, at around 0.5 cases per 100,000 population, with two exceptional peaks

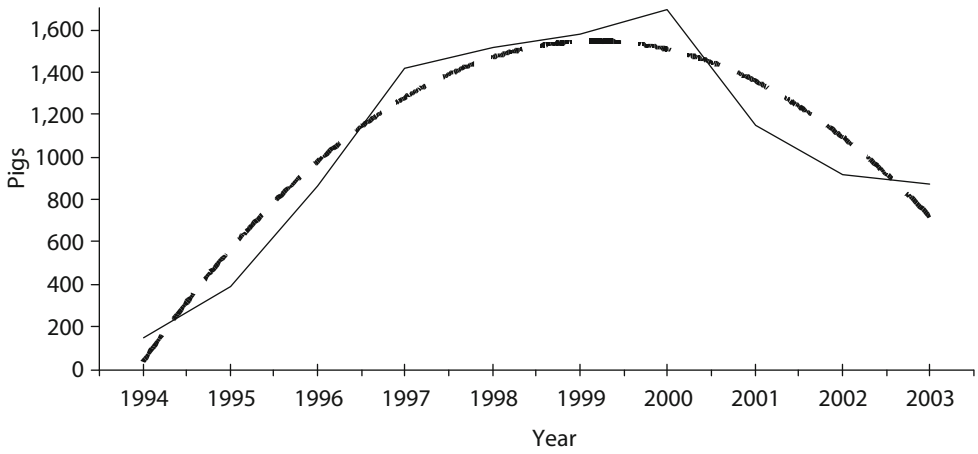

Figure 2. Temporal trend of trichinellosis in pigs in Serbia (Mirilovic, 2005).

Table 4. Trichinellosis in humans in Serbia.

Year	Human cases	
	Total number	Number per 100,000 population
1994	492	6.56
1995	791	10.55
1996	594	7.92
1997	766	10.22
1998	408	5.44
1999	559	7.46
2000	411	5.48
2001	383	5.11
2002	575	7.67
2003	178	2.37
2004	221	2.95
2005	339	4.52
2006	188	2.51
2007	117	1.56

of 1.0 due to two larger outbreaks (data not shown). In 1990s, due to the Balkan political and military problems, the occurrence increased to between 5.4 and 10.5 per 100,000; subsequently, starting in late 1990s and through 2000s, it significantly decreased following the democratic and economic transition in the country (Figure 3).

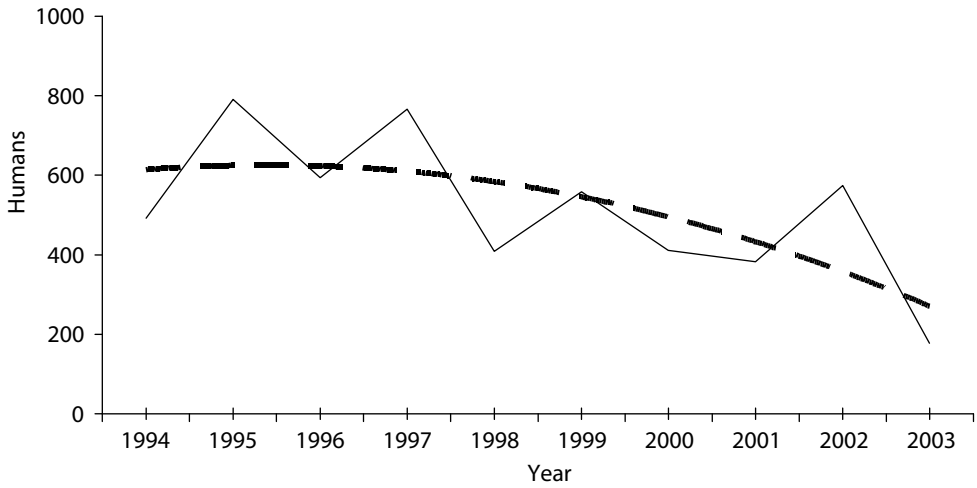

Figure 3. Temporal trend of trichinellosis in humans in Serbia (Mirilovic, 2005).

When considering some factors associated with infections in humans, no clear effect of gender on trichinellosis occurrence has been observed (Table 5). On the other hand, fewer cases of trichinellosis occurred in young children and older individuals (≤ 6 and ≥ 60 years, respectively) compared to other age groups (Table 5). The reasons behind this phenomenon are unclear, but it could be hypothesised that the diets of very young and older contain somewhat smaller amounts of uncooked meat (pork) products and/or that their digestive system is somewhat less efficient in digesting meat (so fewer larvae are freed) and/or that their age groups are smaller in proportion to the total population, compared with other age groups. In any case, it is clear that the large majority (approximately 70%) of all human cases occurred in individuals being between 20 and 60 years of age.

3.2.4 Source attribution for human infection

When considering source attribution for human trichinellosis, it is important to bear in mind that not all larvae in ingested meat are infective. The larvae infectivity, so human dose-response, are affected by duration and temperature (chilled, frozen) of meat storage before consumption, salt concentration i.e. water activity (a_w) of meat/product and cooking regime (temperature, time) of meat/product. These factors are highly variable and inter-related, and so are difficult to evaluate and/or quantify. From a practical perspective, the two most important meat-associated factors that can completely inactivate the most important species, *T. spiralis*, in meat are effective freezing and cooking regimes (Table 6).

With respect to the effects of freezing, *T. spiralis* and *T. pseudospiralis* can be considered as cold sensitive trichinellae when in the muscle of pigs and wild boar (EFSA, 2004). Concerning the cold resistant *Trichinella* species/genotypes that have been identified (e.g. *T. nativa*, *Trichinella* T6 and *T. britovi*), it has been noted that present cold treatment and time/temperature combinations targeting *T. spiralis* may not be effective in respect to the cold resistant strains should they be present in meat of pigs; however, there is a lack of scientific data on this issue (EFSA, 2004). With respect to the effects of cooking, no information is available regarding the heat tolerance in meat of species/genotypes of trichinellae other than *T. spiralis*. Furthermore, it should be noted that the effectiveness of heat inactivation of trichinellae can also vary with the meat heating method; a somewhat higher temperature for

Table 5. Age and gender of human trichinellosis cases in Serbia (1994-2003).

Age groups (years)	Males (number)	Females (number)	Total	
			Number	%
≤6	101	103	204	3.96
7-19	518	411	929	18.01
20-39	1,102	866	1,968	38.16
40-59	930	700	1,630	31.61
≥60	210	216	426	8.26
All age groups	2,861	2,296	5,157	100.00

Table 6. Inactivation of Trichinella spiralis *by freezing and cooking (adapted from EFSA, 2004).*

Meat freezing inactivation of *T. spiralis*		Meat cooking inactivation of *T. spiralis*	
Core temperature (°C)	Required duration	Core temperature (°C)	Required duration
-17.8	106 hours	49.0	21.0 hours
-20.6	82 hours	50.0	9.5 hours
-23.3	63 hours	52.2	2.0 hours
-26.1	48 hours	54.5	30 minutes
-28.9	35 hours	56.7	6 minutes
-31.7	22 hours	58.9	2 minutes
-34.5	8 hours	60.0	1 minute
-37.2	0.5 hours	62.2	instantaneous

complete inactivation may be required in the case of microwave-oven heating, compared to conventional-oven heating (EFSA, 2004).

Regarding source attribution for the human cases in Serbia, the published data is limited and poorly documented, which does not allow detailed related analysis. Nevertheless, it is considered that no confirmed human cases have been linked to meat/pork from industrial abattoirs to date. On the other hand, anecdotal evidence from most human trichinellosis cases points to home-made traditional meat products that are cured, cold smoked and dried but not subjected to any heat treatment either during the production or before the consumption, as the sources of infection. The two main examples of those meat products are country-style dried meats and fermented, dry sausages (salamis).

In home-making of cured, cold smoked and dried pork joints (ham, shoulder), the production parameters vary and are largely undocumented but, based on the epidemiological information, *Trichinella* larvae can survive in such products if they contain infected meat. For illustration purposes, typical parameters in industrial production of corresponding dried meats are: rapid chilling of pork (0 °C after 24 h; pH 5.8), salting at 5 °C (until $a_w \sim 0.96$, i.e. 4.5% NaCl), cold smoking (≤22 °C), drying (≤15 °C) and maturation (12-18 °C; relative air humidity 70-78%).

In home-making of cured, fermented, cold smoked and dried sausages (salamis), the production parameters also vary and are largely undocumented but, based on the epidemiological information, *Trichinella* larvae can survive in such products if they contain infected meat. For illustration purposes, typical parameters in industrial production of corresponding sausages are: rapid chilling of pork (0 °C after 24 h; pH 5.8), chopping-mixing of ingredients (55-70% lean pork, 25-40% fatty tissue, 3% curing salts (NaCl, nitrate and nitrite), 0.5% spices and flavouring), stuffing into casings, fermentation (15-40 °C; 2-5 days), cold-smoking (few hours) and drying to different extents (1-4 weeks for semidry sausages; 12-14 weeks for dry sausages).

Overall, the common scenario leading to human trichinellosis in Serbia involves consumption of home-made meat products that did not receive heat treatments (mentioned above) but which contained:

- meat from a domestic pig slaughtered under home arrangements and not subjected to *post mortem* veterinary inspection; or
- mixture of meats from multiple domestic pigs among which some were *post mortem* veterinary inspected but one was slaughtered under home arrangements and not subjected to *post mortem* veterinary inspection; or
- meat from a hunted wild boar not subjected to *post mortem* veterinary inspection; or
- mixture of meats from domestic pig and wild boar, where one (or both) of them were not subjected to *post mortem* veterinary inspection.

Obviously, the most relevant aspects of the scenario are: domestic slaughter/hunting, lack of meat examination for *Trichinella*, lack of heat treatment and, when available, use of wild boar meat.

4. Main principles of *Trichinella* controls

4.1 Global controls in wildlife

The risk caused by the wildlife reservoir is dependent on the contacts between domestic pig and wildlife. When only minimal contacts with wild omnivores/carnivores exist, as in the case of industrialised farms, the importance of the wildlife reservoir is certainly smaller than in the case of more traditional farming systems (EFSA, 2005a). With respect to surveillance of wildlife reservoirs, it is usually based on collecting and testing foxes from the area. However, there are large variations in *Trichinella* prevalence in wildlife even within a single country. Furthermore, due to mobility of wildlife and other variable factors, the geographical area to which one wishes to make inferences on the prevalence in wildlife based on wildlife surveys is difficult to define (EFSA, 2005a). Overall, monitoring of a wildlife reservoir can be helpful in assessing its potential as source for *Trichinella* exposures of domestic pigs, but there are difficulties in using wildlife monitoring to verify the absence of *Trichinella* in wildlife.

It appears, that the only measure that can be implemented to reduce the prevalence of infection among wildlife is to instruct hunters to avoid leaving animal carcasses and/or entrails in the field, a practice which increases the probability of transmission to new hosts (Gottstein *et al*., 2009). Namely, a very close relationship between the conventional hunter's practice to leave animal carcasses in the field after skinning and the prevalence of trichinellosis among wildlife has been documented in arctic, subarctic and some European regions. Furthermore, continuous rodent control in the areas/locations enabling domestic pig exposure is another very important aspect of *Trichinella* reservoir control.

4.2 Global controls in domestic pigs

Control of *Trichinella* infection in pork has traditionally been accomplished by inspection of individual carcasses at slaughter or by *post*-slaughter processing to inactivate parasites.

The changes in animal husbandry on industrial, indoor farms have virtually eradicated the domestic *Trichinella* cycle from many parts of Europe. However, in many of the new EU Member States and the EU candidate/associated countries (such as Serbia), more traditional farming practices facilitating a mixture of domestic and sylvatic cycles of *Trichinella* may be more common; the mixture represents the major infection risks to domestic pigs (EFSA, 2005a). This is indirectly confirmed by the fact that the *Trichinella*-infected pigs in the EU nowadays are primarily from backyard, free-ranging, organic or small family farm production systems (EFSA, 2005a). Therefore, the main strategies of *Trichinella* controls in domestic pigs are aimed at effective separation of the domestic and sylvatic cycles.

Declines in the prevalences of this parasite in domestic pigs in developed countries during the last 30 years, combined with improvements in pork production systems, indicated that it is possible to ensure pork safety in respect to *Trichinella* at the farm level (Gajadhar *et al.*, 2009). In practice, related measures including aspects of farm management, bio-security, feed and feed storage, rodent control programs, and general hygiene are incorporated in the good farming practices system, details of which are described in an EU scientific opinion (SCVPH, 2001) and Regulation (EC) 2075/2005 (EC, 2005). Strict application of those measures ensuring effective separation of sylvatic and domestic cycles, combined with regulatory-documented audits of the system and adequate on-farm monitoring of the parasite, makes achieving the *Trichinella*-free farm status possible. In countries effectively implementing this strategy and with an officially recognised negligible risk of *Trichinella* in domestic pigs, testing for this parasite at meat inspection is no longer mandatory for slaughter pigs reared under controlled housing conditions in integrated production. In other countries, testing for *Trichinella* of slaughtered pigs is a very important component of the control system.

4.3 Global post-harvest controls

Pork from pigs that have not been tested for *Trichinella* – for whatever reason – should be preferably processed commercially, or at least treated by the consumer, using methods proven to inactivate the parasite. As required by many regulatory authorities, ready-to-eat pork products such as dried meats and cold-smoked sausages must be processed to kill *Trichinella* larvae by heating, cooking, or curing (EFSA, 2004; Gajadhar *et al.*, 2009). This strategy is of particular interest in countries with endemic trichinellosis and higher proportion of small-scale ('backyard') pig farming. In those countries, the situation is often worsened by frequent preparation of uncooked meat products under domestic (uncontrolled) arrangements, using meat from uninspected 'backyard' pigs that can be mixed with hunted wild boar meat when available. Although all feasible efforts should be made to discourage the 'home-making of products from uninspected pork', various negative socio-economic factors can make their total elimination in the short-to-medium term unrealistic in those countries including Serbia. As an important measure in the meantime, the farmers, the hunters and the consumers should be educated and advised to freeze the pork (including from wild boars) before its further home-processing into products, or to cook the product before consumption, or both. With respect to home-made fermented, dry sausages (salamis) where cooking either before or after preparation is traditionally unacceptable for reasons of sensory quality, but where the *Trichinella*-inactivation effects of the curing are unreliable, the advice to keep the sausages in the freezer for some time before consumption would be beneficial.

5. Conclusions

What has been achieved?

Natural *Trichinella* infections in more than 100 species of mammals, seven avian species, and three reptile species have been reported, but the domestic pig is the most important source of human infection worldwide. Trichinellosis is commonly defined by two cycles, 'domestic' (in pigs on-farm) and 'sylvatic' (in wildlife). Under conditions on industrial farms (particularly indoor) with good hygienic practices and efficient management including biosecurity, and combined with effective governmental/veterinary services, *Trichinella* transmission *via* the domestic cycle is unlikely, as indicated by the lack of reports of infections on industrialised farms in developed, western countries. In countries effectively implementing these strategies and with an officially recognised negligible risk of *Trichinella* in domestic pigs, testing for this parasite at meat inspection is no longer mandatory for slaughter pigs reared under controlled housing conditions in integrated production. In other countries, testing for *Trichinella* of slaughtered pigs is a very important component of the control system.

What has been neglected?

A switch of trichinellosis from wild animals to domestic animals can occur where there is improper management in segregating husbandry and wildlife. Under conditions on small farms with pigs having access to the outdoors and where control measures are poorly implemented or are lacking, the domestic cycle can play a very important role in trichinellosis transmission. This possibility is further enhanced where socio-economic and political problems temporarily diminish the efficacy of the governmental/veterinary services. Additionally, the practice of making uncooked products from meats of uninspected domestic and/or wild pigs at home represents a major risk for human infection. These problems usually lead to increases in incidence/prevalence in susceptible food animals and humans, as illustrated by the Serbian situation in 1990s. The risk of further spreading trichinellosis is additionally exacerbated by increased globalisation including increased movements of livestock, food and people that is evident in modern time.

What needs to be done?

Traditional farming practices facilitating a mixture of domestic and sylvatic cycles of *Trichinella* need to be either modified/improved so to ensure that cycles are separated or gradually totally replaced; however, achieving the latter is not realistic in the short-to-medium term because of traditions and practical difficulties. Furthermore, hunters need to be educated to avoid leaving animal carcasses and/or entrails in the field because this increases the probability of trichinellosis spread to new hosts. Also, the farmers, the hunters and the consumers should be educated and advised to freeze the pork (including meat from wild boars) before its further home-processing into products, or to cook the product before consumption, or both, aimed at the larvae inactivation.

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Influence of climate change on diseases of wild animals

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Summary

The occurrence of transferable diseases in wild animals generally reflects the causative agent's distribution. The influence of climate change on the prevalence of pathogenic organisms is either exerted directly by causative agents with an increased survival rate as related to higher average annual temperatures, or indirectly because vectors (e.g. ticks, mosquitoes) experience better environmental conditions similarly promoting their viability and allowing them to invade new areas of circulation. The latter option explains why pathogen-carrying vectors such as ticks and mosquitoes prevail at higher sea levels than observed two decades ago. In recent years, vectors with seasonal cycles show longer periods of incidence. In addition, parasitic eggs, larvae and parasitic organisms at intermediate stages can be found at higher sea levels as they too benefit from changing climate conditions. For this reason cumulative purulent and abscess forming pneumonia is observed in chamois which originate from lungworm infections. This contribution demonstrates the relationship between climate factors and transferable diseases. The geographical dissemination and incidence of many diseases interferes with environmental changes. Additionally, risk factors increasing the diseases' prevalence and incidence are discussed. Finally, selected examples of our own findings on the impact of climate changes on diseases (tularaemia) and on habitats of alpine wild animal species (black grouse, snow grouse, chamois and ibex) are highlighted.

Keywords: climate warming, disease risks, Tularaemia, habitats

1. Introduction

Wild animals as well as infectious agents and vectors/reservoirs are greatly influenced by climate, geographical location, structure, fauna and flora and they may migrate deep into human settlement areas, depending on animal species. There are several examples for the impact of climate change on enzootic infections. Whilst this may be in accordance with ecological requirements, it also poses the danger of animal-human disease transmission. The spread of particular bacterial, viral and helminthic infections (partly with zoonotic potential) is influenced by factors of climate change, especially temperature and distribution of precipitation (Williams *et al.*, 2002; Hoberg *et al.*, 2008).

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The following variables and parameters related to climate change represent significant risk factors as regards wild animal diseases:

- influence on infectious agents (basic reproductive number, tenacity, etc.);
- increase of populations (e.g. wild boar, vectors, etc.);
- increase of temperature especially in alpine regions;
- decrease of habitat quality for alpine animal species (immunosuppression) with increased host susceptibility;
- heat waves, heat stress, water shortage;
- hygienic problems in feeding of wild animal species (red deer, row deer, wild boar), particularly at warm ambient air temperature (e.g. mild winter) and especially when feedstuff is presented on the ground;
- 'new' infectious agents (e.g. West Nile virus, hepatitis E virus, Hanta viruses, EHEC);
- changes in habitats (e.g. barrier lakes);
- global trade with animals and food of animally origin;
- increase of host range of some infectious agents?
- illegal international pick up of stray dogs and cats;
- international transport and movement of wild animals and of straying animals;
- increasing international travelling of humans.

Climate change influences the dissemination and the reproduction of both pathogenic organisms and of wild animals (Figure 1).

There is already a widespread change in natural calendars (phenology) of plants and animals, as well as change in some species distributions. Now so-called threshold changes (i.e. sudden, fundamental changes) in ecosystems are beginning to be observed in nature. This suggests a

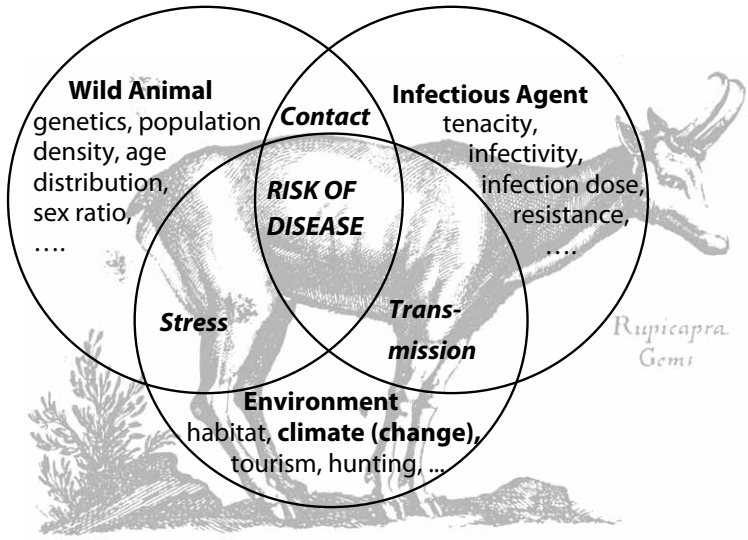

Figure 1. Interactions between wildlife, infectious agents and environmental factors (Deutz and Gressmann, 2001).

future with nature and ecosystems very much in flux, which will have major epidemiological implications (Lovejoy, 2008).

Among wild animals some species will benefit from climate change by increasing their population (e.g. wild boar) while other species may diminish or become extinct. These 'underdogs of climate change' (e.g. chamois, ibex, black grouse and snow grouse) would find themselves in suboptimal habitats whilst the infectious pressure is increasing. Additionally, it needs to be considered that the number of 'new' emerging pathogenic agents, predominantly zoonoses, occurring in Middle Europe is increasing.

Most vector-borne diseases exhibit a distinct seasonal pattern, which suggests that they are weather sensitive. Precipitation (rainfall), temperature and other weather variables in many ways affect both the vectors and the pathogens that these transmit. High temperatures can increase or reduce survival rate, depending on the vector, its behaviour, ecology and other factors. Thus, the probability of transmission may be increased by higher temperatures. Moreover, the tremendous growth in international travel increases the risk of importation of vector-borne diseases, some of which can be transmitted locally under suitable circumstances and can become autochthonous (Gubler *et al*., 2001).

The complex interaction of factors like environmental and ecological changes, social factors, decline of health care, human demographics and behaviour influences the re-emergence of such diseases. Viruses, especially RNA viruses with their ability to adapt quickly to changing environmental conditions, are among the most prominent examples of emerging pathogens (Ludwig *et al*., 2003). The effects of climate and population movements and other risk factors on emerging infectious diseases in men, domesticated animal as well as in wildlife need to be seriously reconsidered. Overall, the success of disease control measures has been disappointing. Scientists must accept the challenge of communicating effectively with the public and policy makers worldwide if success is to be achieved (Fayer, 2000).

Vector-borne diseases gained importance in men as well in animals in Middle Europe during the past years. Reports on severe diseases, formerly only known as so called 'travel sickness' from tropical countries, markedly increased in number in the recent years (Zinstag and Schelling, 2003; Ready, 2008; Schwaiger and Bauer, 2009). Climate models predict a global warming of 1.4 °C up to 5.8 °C until the year 2100 and especially arthropod-borne diseases are strongly influenced by the climate. Schwaiger and Bauer (2009) listed arthropod-borne pathogens with their reservoirs, which are relevant for Germany, and most of which have zoonotic potential (Table 1).

In a screening for West Nile virus in 2003, all samples from free-living birds and horses from Austria were negative for WNV (Weissenböck *et al*., 2003). In 2008 the first cases of West Nile virus infections in free-living birds, especially goshawks (*Accipiter gentilis*), were detected in Austria. Interventions to control West Nile virus include mosquito and bird population surveillance (Epstein, 2001).

Already in the year 1974 Thiel postulated that Q fever was found in both hemispheres within the 10 °C annual isotherms, especially where the climate was warm and dry as in the

Table 1. Arthropod-borne pathogens and their reservoir in Germany (Schwaiger and Bauer, 2009).

Viruses	Bacteria	Parasites
Tick-borne encephalitis virus	*Borrelia burgdorferi s.l.*	*Babesia* spp.
West Nile virus	*Ehrlichia chaffeensis*	*Leishmania* spp.
Bluetongue virus	*Anaplasma phagozytophilum*	*Hepatozoon canis*
Batai virus	*Ehrlichia canis*	*Dirofilaria* spp.
Tahyna virus	*Rickettsia colorii*-complex	
Uukuniemi virus	*Rickettsia slovaca*	
Eyach virus	*Rickettsia sibirica*	
Tribec virus	*Rickettsia massiliae*	
Lipovnik virus	*Rickettsia helvetica*	
Bahanja virus	*Rickettsia aeschlimanii*	
Semliki Forest complex virus	*Rickettsia akari*	
Lednice virus	*Rickettsia felis*	
Sindbis virus	*Rickettsia monacensis*	
	Rickettsia typhi	
	Coxiella burnetii	
	Francisella tularensis	
	Bartonella quintana	

Mediterranean and Black Sea areas, the steppes of central Asia, the African savannas and the grassland areas of North America and Australia (Thiel, 1974). In the last few years, Q fever cases have occurred increasingly in Middle and Northern Europe and an influence of climate change is discussed. Predisposed groups (e.g. veterinarians, farmers, slaughterhouse workers, hunters) have to recognise zoonotic risks and observe prophylactic measures (Deutz *et al.*, 2001, 2003).

A recent increase in the occurrence of *Dirofilaria repens* and *Dirofilaria immitis* has been reported from Slovakia and Hungary. In September 2007, the first autochtonous case of canine *Dirofilaria repens* in a dog was diagnosed in Austria (Duscher *et al.*, 2009; Löwenstein and Spallinger, 2009). Wild carnivores could also be a reservoir for *Dirofilaria* spp. Temperature is one of the main factors preventing the spread of leishmaniasis into Northern Europe. Evidence indicates that *Leishmania infantum* is prevalent only within the 5-10 °C January and 20-30 °C July isotherm. Human disease models predicted a dramatic increase in the incidence of visceral leishmaniasis and a slight increase in the incidence of cutaneous leishmaniasis. Climate change would most likely also effectuate parasite development and the infection rate in dogs and thus overall disease incidence (Kuhn, 1999).

Climate variables are known to affect the prevalence, intensity and regional/geographical distribution of parasites, influencing free-living larval stages or influencing invertebrate as well as vertebrate hosts. The impact of climate change appears to be more pronounced in

trematodes, and is shown by increased cercarial production (Fayer, 2000; Mas-Coma *et al*., 2008, 2009).

Parasitic eggs, larvae and parasitic organisms at intermediate stages were also found at higher sea levels as these species also benefit from changing climate conditions. As average annual temperatures in alpine space increase, the time of development and reproduction cycles of parasites is reduced and therefore more generations of parasites per year may occur. The cumulative findings of purulent and abscess forming pneumonia in chamois as a result of lungworm infections can be seen as a direct consequence of these changes (Schaumberger *et al*., 2006; Prosl, 2008). As regards trichinellosis there is a hypothesis that the distribution of T. *nativa* and *T. britovi* relates to the environmental temperature, notably the isotherm -4 °C and -5 °C isotherm in January, respectively (Pozio *et al*., 1998).

During the hot summer of 2003, an enormous heat stress in both farm animals and wild animals was triggered in Austria. Especially territorial living wild ruminants (e.g. roe deer) had severe problems to get to adequate places for their water supply. Consequently, average body weights in shot wild ruminants, were markedly reduced during this year. A higher susceptibility for diseases (e.g. paratuberculosis, endoparasites) is likely to ensue. Climate scientists predict more hot summers and higher numbers of hot days per summer (Kromp-Kolb and Formayer 2005). It is also possible, that potential vectors (e.g. specific ticks) will also suffer from hot weather conditions whilst others, particularly mosquitoes, may benefit from higher temperatures. As a result, some vectors (to date not occurring in Middle Europe) will encounter environmental conditions that are essential for their life and reproduction. As an actual example, bluetongue disease, an acute and epidemic infectious disease in sheep, goats and wild ruminants raged in many European countries (Purse *et al*., 2008).

In the following two sections selected examples of our own findings (Schaumberger *et al*., 2006; Deutz *et al*., 2009) concerning the influence of climate change on diseases of wild animals and on habitats of alpine are discussed.

2. Tularaemia in Austria

Bacterial infectious diseases, such as tularaemia, are strongly related to climate parameters as the pathogen requires specific ambient conditions. *Francisella tularensis* is a cold-resistant bacterium that is able to reproduce at moderate temperatures but will not survive at elevated temperatures. In addition, the population density of host animals and the abundance of potential disease vectors (ticks, gnats) also play a decisive role in the epidemiology of tularensis. The infection is transmitted to animals and humans by direct contact with infected animals and vectors or by inhalation of pathogens or the consumption of insufficiently cooked hare meat (Ellis *et al*., 2002). Several cases of tularaemia are recorded in Austria each year, but the number of unreported cases is likely to be markedly higher. *Francisella tularensis* is also considered as a potential biological weapon due to its low infection dose.

We aimed at investigating the impact of climate and weather on the prevalence of tularaemia in hare populations in the lowlands of eastern Austria.

3. Material and methods

A total of 271 cases of tularaemia in hares have been recorded in the area under investigation (Lower Austria, Burgenland, and Styria) in the period from 1994 to 2005, all of which were geo-referenced according to sender postal code. Temperature and precipitation data for the selected region were available from 30 meteorological stations of the Central Institute for Meteorology and Geodynamics in Vienna (Auer *et al.*, 2001). These data provided the basis for calculating an altitude dependent temperature distribution for suitable monthly mean values and period sums. The spatial distribution of precipitation was calculated using the geo-statistical universal kriging method without taking into account the influence of altitude. These data were used for a two-step analysis. The first step led to boundary values for the spatial distribution of tularaemia. These boundaries were used to estimate the distribution of tularaemia in the year 2035. The second step explained the different annual incidences using actual climate data.

4. Results

4.1 First step: finding spatial boundary values and estimation of the areal distribution of tularaemia until 2035

A high incidence probability, based on the local isoline encircling the study area, was obtained for annual precipitation totals below 720 mm, summer precipitation below 180 mm, winter temperatures above 0.5 °C and mean May temperatures above 14 °C. These limit values allowed a calculation of the diseases´ spatial distribution for current and future conditions. A warming of 2 to 4 °C having been induced by climate change was assumed for predicting the distribution area of the disease in 2035, with warming expected to be more intensive in higher altitudes than in lowlands. Figure 2 shows the possible spatial distribution of tularaemia in 2035 following a rise in mean annual temperatures. Precipitation was not taken into account

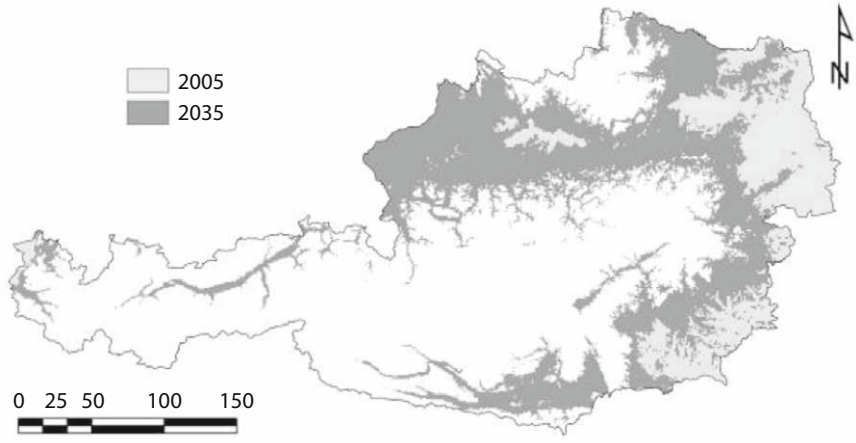

Figure 2. Possible spatial distribution of tularaemia in Austria in 2035 following a rise in mean annual temperatures.

due to the lack of a suitable scenario. Under these conditions, tularaemia will slowly spread from the eastern lowlands *via* the Danube valley to the west and southern Styria proceeding to the south. Additional incidents of the disease could also occur in inner-alpine areas providing favourable climatic conditions.

4.2 Second step: explaining coherences between climate parameters and incidence

Inside tularaemia zones a clear correlation between the two climate parameters and local disease incidence was established, which can be represented by the following linear regression model:

Number of cases per year = 52.12 + 4.08 x (average of monthly mean temperatures for December, January and February) – 3.46 x (monthly mean temperature for May) + 0.26 x (precipitation total for June and July)

This formula does not allow calculating absolute numbers of incidence in nature, because it is based on sample data of one specific region. Particularly noteworthy, however, is the highly significant ($P<0.05$) influence of the parameters selected of the incidence rate of the disease and the coefficient of determination obtained ($R^2 = 74.6\%$). It becomes clear that about ¾ of inter-year differences can be explained by temperature and precipitation conditions: warm winter temperatures result in an increase in incidence, while warm May temperatures lead to a decrease; high precipitation in summer has an increasing effect again. The ideal conditions for the spread of the disease are thus warm winters combined with low temperatures in May and high precipitation in summer (Figure 3). The result represents a feasible development of hares. Warm winter increases the population of hares. Low May temperatures and wet summers degrade the leverets. Thus, bacteria causing tularaemia find better conditions to reproduce. This correlation has been derived from observations and obviously does not apply to arbitrary temperature and precipitation values.

These findings provided the basis for specifying empirical limits for the parameters defined in the formula, which best correspond to the actual spatial distribution obtained by geographical analysis. Hence, the probability of tularaemia occurrence is high for a total annual precipitation below 720 mm, a summer precipitation rate around 180 mm, a winter temperature above 0.5 °C and a May temperature below 14 °C.

A temperature increase of 2 to 4 °C was assumed for predicting the distribution area of the disease by 2035 (Figure 4). Under these conditions, tularaemia might slowly spread from the eastern lowlands *via* the Danube valley to the west and *via* southern Styria further to the south. Additional incidents of the disease could also occur in inner Alpine areas providing favourable climatic conditions. This means that an extension of the potential tularaemia distribution area (from currently 13 to 46.5% of the Austrian territory; Figure 2) can be expected.

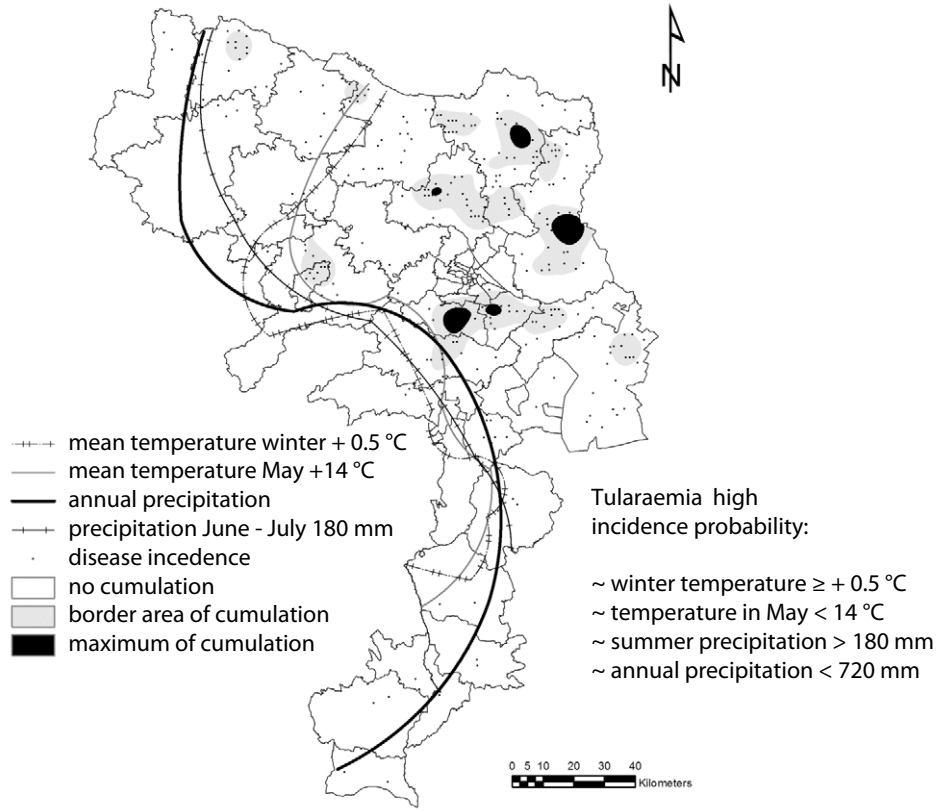

Figure 3. Isolines of analysis and high tularaemia incidence probability theory.

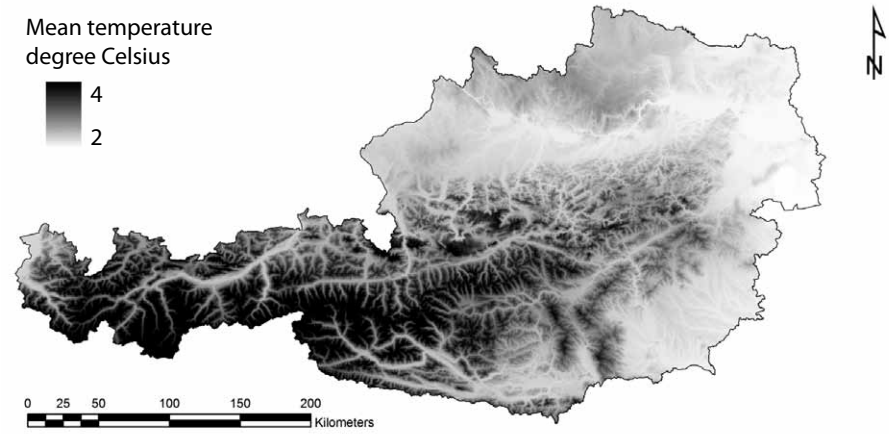

Figure 4. Estimate of future warming in Austria over the next 50 years.

5. Loss of habitats in Alpine regions

Wild animal species such as the black grouse, snow grouse, chamois and ibex have adapted to life in an Alpine environment above the tree line in the course of their evolution and thus form part of this very sensitive ecosystem. The habitat of these wild animal species might be substantially reduced by a potential upward movement of the tree line because of climate change. An attempt was made to quantify these changes with the aid of models and a geographical information system (GIS). The calculations were based on the temperature development of the past 50 years and an estimate of future warming. The scenario derived from the climate model used predicts a warming of approximately 2.2 °C for the area under investigation (Niedere Tauern, province of Styria, Austria) over the next 50 years (Figure 5).

The elevation of the tree line strongly depends on the temperature and a high correlation was found between the growth limit of trees and the 10 °C July isotherm (Daubenmire, 1954; Böhm *et al*., 2001; Grace *et al*., 2002; Holtmeier, 2003; Gobiet *et al*., 2006). The climate model shows that the relevant isotherms will rise by approx. 450 m over the next 50 years. The temperature changes, however, strongly depend on the climate model used. Without additional research work, no statement can be made on how fast the tree line is advancing towards the temperature related growth limit. Human management practices also have a substantial influence on tree line position so that future changes in the tree line cannot be derived from climatic data alone.

When accepting the theory of an elevated tree line due to climate warming, the habitat of wild animal species will be diminished massively (Figure 6 and 7). For individual species there are almost no possibilities to avoid or to cross over to other habitats. Taking into account suboptimal habitats left, disintegration of biotopes, overexploitation of remaining alpine areas due to touristic and sportive leisure time behaviour by people, endangered species will

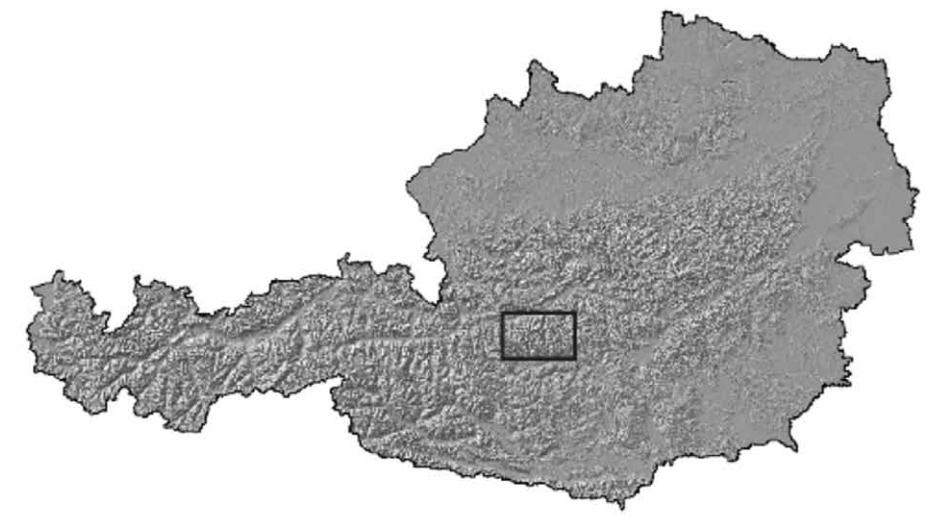

Figure 5. Area of investigation (Niedere Tauern, province of Styria, Austria).

Figure 6. Elevation of the tree line with loss of habitats for alpine wild animal species.

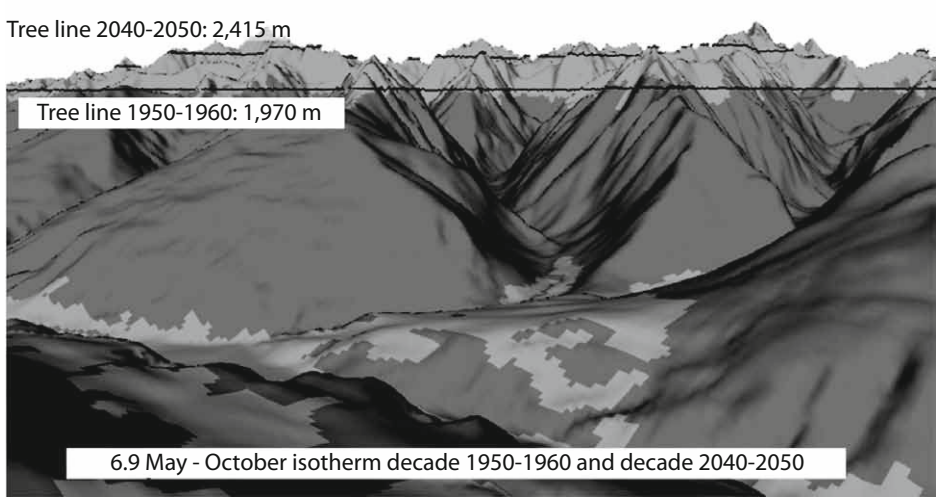

Figure 7. Tree line on base May-October isotherm decade 1950-1960 is about on 1,970 m and tree line on estimated isotherm decade 2040-2050 will be on 2,415 m.

diminish strongly. As a consequence, inbreeding will increase, genetic resources will become poor, individual immune system will weaken and parasitic and stress-related diseases but also infectious diseases will emerge.

Several populations will diminish under a critical size of their population or herds and therefore some species may become extinct. As examinations in Bighorn-Sheep (*Ovis canadensis*) showed, populations with less than 50 individuals became extinct within 50 years. Populations with more than 100 individuals were able to survive. Therefore, we can assume that at least 50 individual animals are necessary to maintain genetic variability, but for long ranging survival at least, 500 individuals should prevail. Following this approach only the effective size of the population must be considered, which means that only animals able to reproduce must be taken into account.

The current habitats of the animal species investigated were determined and mapped using knowledge based habitat model and a GIS. As an example, the present suitable habitats for snow grouse are shown in Figure 8. Assuming that the future tree line will adjust to the changed temperature of the decade 2040-2050, this shift will lead to a dramatic loss in suitable habitats, which may range from 78 to 98% depending on the season and animal species. The habitat loss to be expected for snow grouse is illustrated in Figures 8 and 9.

The present study shows, that the losses of convenient wildlife habitats is considerable for all of the four observed wildlife species. Smaller populations and flocks of these species will not survive due to loss of their living space, increasing susceptibility for diseases, rising individual losses caused by predators due to reduced clearness of habitats (higher vegetation). Most dramatic losses of habitats can be seen for the black grouse. When accepting the modelled

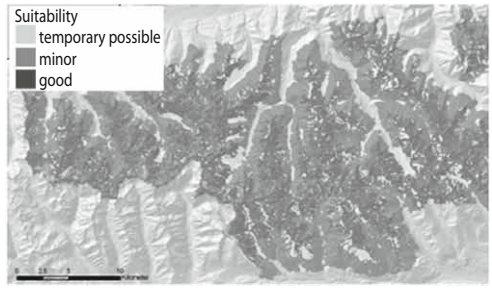

*Figure 8. Current (2010) habitat suitability for snow grouse (*Lagopus mutus helveticus*).*

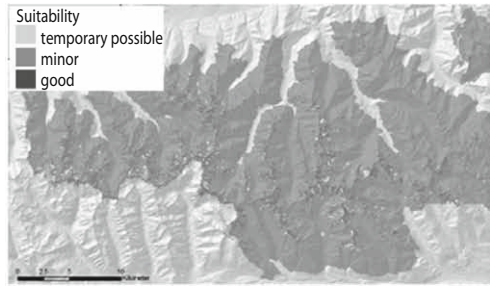

*Figure 9. Predicted habitat (decade 2040 to 2050) suitability for snow grouse (*Lagopus mutus helveticus*) assuming a temperature increase of 2.2 °C and derived shift in the tree line.*

scenario for the region 'Niedere Tauern', black grouse are in danger of extinction. Snow grouse may adapt to treeless but cragged alpine areas and therefore should be able to survive. But also populations of snow grouse would decrease. Chamois and ibex are able to migrate to woody regions and then problems with damage of trees are likely to occur.

Living in suboptimal habitats is a problem for chamois (Figure 10 and 11) and ibex as populations might diminish. Infectious diseases like scabies and ablepsia of the chamois, but also endoparasites would reduce the populations. Smaller groups of these wild animals would show extended rutting season and then bucks would be weakened. Then, some populations will be too small and genetic exhausting would be reason for inbreeding depression and for their extinction. As an example for an epidemic spread caused by changing climate conditions the outbreak of Infectious ceratoconjunctivitis in chamois (Figure 12) is presented. This outbreak occurred in 2006 and more than 80 cases of diseased animals where seen in the districts Murau, Judenburg and Liezen. In 2006, with a very mild autumn, vectors of the disease (flies) could be observed in alpine regions at the beginning of December 2006. It can be concluded that the time span, during which an infection is possible, is prolonged.

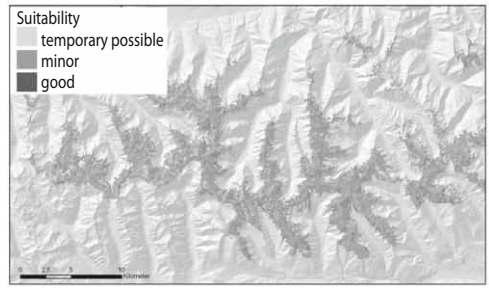

*Figure 10. Current (2010) habitat suitability for chamois (*Rupicapra rupicapra*).*

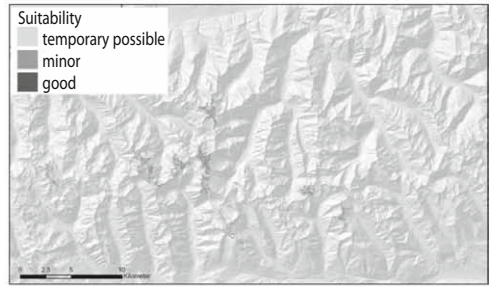

*Figure 11. Predicted habitat (decade 2040 to 2050) suitability for chamois (*Rupicapra rupicapra*) assuming a temperature increase of 2.2 °C and derived shift in the tree line.*

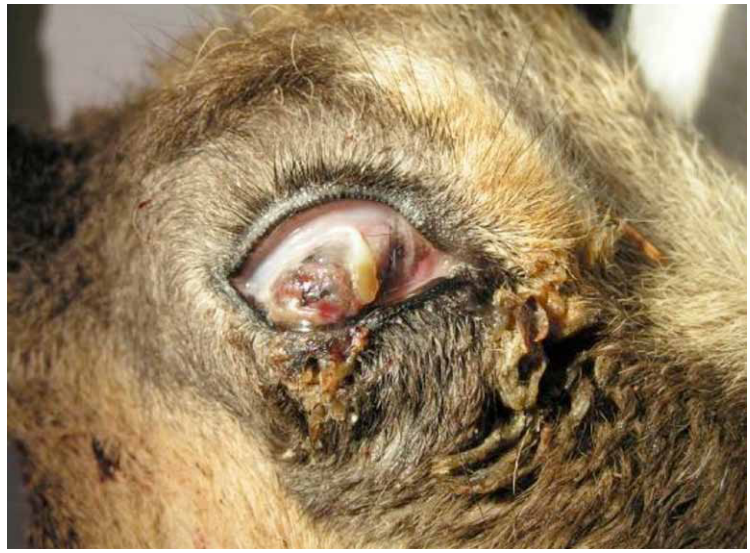

Figure 12. Infectious ceratoconjunctivitis, Chamois.

6. Conclusions

What has been achieved?

It has been established that global changes, including an increase in trade and global warming, which act on the environment, are likely to influence the evolution of pathogens and hence of diseases. To anticipate the risks created by this new situation, two methods have been developed (Dufour *et al.*, 2008): the first step is to indentify the diseases the incidence or geographical distribution of which could be affected by global warming, and the second step is to evaluate the risk of each of these diseases. Further recommendations were to develop epidemiological surveillance, to increase knowledge of epidemiological cycles, to develop research concerning these diseases and to pool cross-border efforts to control them.

What has been neglected?

In summary it can be stated that climate is the key parameter for explaining the distribution of tularaemia in the past and that limit values for the individual climate parameters can be identified. The expected warming could result in a massive expansion of the potential tularaemia distribution area. Yet, to date, little if any attention has been paid to informing risk groups (hunters, foresters, farmers, laboratory staff, taxidermists, housewives, etc.) beyond current prevalence regions and recommending them to take preventative measures of working hygiene (protective gloves, moistening the fur when skinning hares, insect protection, face masks in the lab) when handling hares and rodents, and following good kitchen hygiene practices when preparing and cooking hares.

What needs to be done?

It is a matter of urgency, that veterinarians, physicians, wild life biologists and epidemiologists consider new emerging diseases. Surveys among wild animal species should be enforced and serological databases should be developed. Thus, changes in incidences could be detected earlier and affected regions but also time spans of outbreaks could be pre-estimated. The knowledge and the vigilance concerning new diseases must be promoted by constant and up to date information including all involved persons. In addition, examination centres and laboratories have to be watchful that the spectrum of pathogenic agents will change. West Nile Virus, Usutu Virus or Louping Ill, Hepatitis E, Krim-Kongo-Fever and Ehrlichiosis display only a few examples for new diseases. In Middle Europe, there is also a massive increase in human infections caused by Hanta Virus, which is now spread also by insectivores (Soricidae) in Austria.

'A stitch in time saves nine' and so it will be necessary to reduce the risks for infection and to reduce the infection pressure by improving the habitats of wild animals. For preventing diseases it also will be essential to remove infected animals or in some cases to reduce appropriate wildlife stocks in a risk-free way. The consecutive monitoring of the wild animals' health by examination of perished animals and by taking samples from unsuspicious animals is the basis for early recognising and avoiding diseases in endangered wild animals species.

Finally, the following list of actions is proposed:

- Surveys, surveillance and monitoring of wild animal diseases.
- A stronger intersectoral collaboration between public health and veterinary institutions al local, national and international levels.
- Adaptation of population size of wild animal species at suitable habitats.
- Information and training of hunters concerning important wild animal diseases.
- Disease awareness relating to 'new infectious diseases' (75% of these diseases are zoonoses!).
- Further investigations concerning ecology and biology of infectious agents, vectors and intermediate hosts.
- Animal diseases are often media sensations. This circumstance often interferes with competence and problem solving. Hence, appropriate ways of approaching the media should be elaborated.

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Dynamics of infectious diseases according to climate change: the Usutu virus epidemics in Vienna

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Summary

As an example of the dynamics of infectious diseases in mid-latitudes, so far mainly observed in the subtropics and tropics, we discuss the Usutu virus (USUV) epidemics in Vienna, Austria. The USUV is an arbovirus, which is closely related to the West Nile virus. It caused mass mortalities mainly of blackbirds (*Turdus merula*). Infections of mammalian hosts or humans are rare. The USUV flavivirus persists in a natural transmission cycle between vectors (mosquitoes) and host reservoirs (birds) and leads – once endemic in a population – to periodic outbreaks. Following the recent work of Rubel *et al.* (2008) and Brugger and Rubel (2009), we present an epidemic model to explain the USUV dynamics in Austria. Within the model framework the USUV dynamics is mainly determined by an interaction of bird immunity and environmental temperature. We demonstrate that the USUV model is able to simulate the observations from the dead-bird surveillance 2001-2005. To investigate future scenarios, we entered temperature predictions from five global climate models into the USUV model and also considered four different climate-warming scenarios defined by the Intergovernmental Panel on Climate Change, IPCC (20 different model-scenario combinations). Long-term simulations cover the period 1901-2100. The results indicate that USUV will persist in the host population after the epidemic peak observed in 2003, but the next major outbreak is expected to occur not before 2019. USUV-specific annual blackbird-mortality time series predict that the outbreak frequency increases successively from the beginning to the end of the century. Additionally, we calculated the annually averaged basic reproduction number for the period 1901-2100. The latter depict that undetected major outbreaks before 2000 were unlikely, whereas it is likely that the USUV becomes endemic after 2040.

Keywords: arbovirus, basic reproduction number, simulation, epidemic model, global warming

1. Introduction

The incidence and spread of previously tropical infectious diseases considerably increased under the current situation of climate change (Harvell *et al.*, 2002; Pfeffer and Dobler, 2009; Gale *et al.*, 2010) and global animal trade and migration (Pfeffer and Dobler, 2010). As an example of how climate change may affect the emergence (or re-ermergence) in wild birds of zoonoses that may have or gain public health relevance, the temperature dependent dynamics of the Usutu virus (USUV), recently investigated by Rubel *et al.* (2008) and Brugger

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and Rubel (2009), are presented here. USUV was detected the first time outside Africa in 2001 in Austria (Weissenböck *et al.*, 2002). It is a member of the flaviviridae family of the Japanese encephalitis virus complex and closely related to the more common West Nile virus (WNV), dengue virus (DENV), Japanese encephalitis-virus (JEV) and yellow-fever virus (YFV) (Weissenböck *et al.*, 2002). USUV became well-known, because it caused mass mortalities of blackbirds (*Turdus merula*) in and around the capital city of Austria, Vienna. The established monitoring programme of the University of Veterinary Medicine Vienna confirmed that USUV overwintered in Austria (Chvala *et al.*, 2007). Although the initial number of dead blackbirds was very low in 2001 and 2002, an epidemic peak was observed during the extraordinary hot summer 2003 (Schönwiese *et al.*, 2004). In the meantime, USUV was also observed in other Central European countries such as Switzerland, Hungary (Bakonyi *et al.*, 2007), and Northern Italy (Lelli *et al.*, 2008; Manarolla *et al.*, 2010). Although it was assumed that USUV does not cause severe or fatal disease in humans, in August 2009, two human cases of Usutu virus neuroinvasive infection were documented in Italy (Pecorari *et al.*, 2009; Cavrini *et al.*, 2009).

Arthropod-borne viruses (arboviruses), like USUV, persist in a natural transmission cycle between vectors (here, mosquitoes of the *Culex pipiens* complex) and avian hosts. Once endemic, it might cause periodic disease outbreaks. Rubel *et al.* (2008) developed a new epidemic model for explaining the multi-seasonal dynamics of the USUV infection in Austria. This model considers both the density-dependent seasonal dynamics of mosquitoes and birds and the USUV bird-mosquito infection cycle. The dynamics of the mosquito population and the virus reproduction rate depend strongly on the environmental temperature. Efficient USUV transmission requires warm temperatures (which increase the reproduction and biting rates of mosquitoes and decrease the extrinsic incubation period). To demonstrate the relationship between warm temperatures and USUV, Figure 1 depicts the total number of dead blackbirds associated with USUV infections (period 2001-2005) together with the

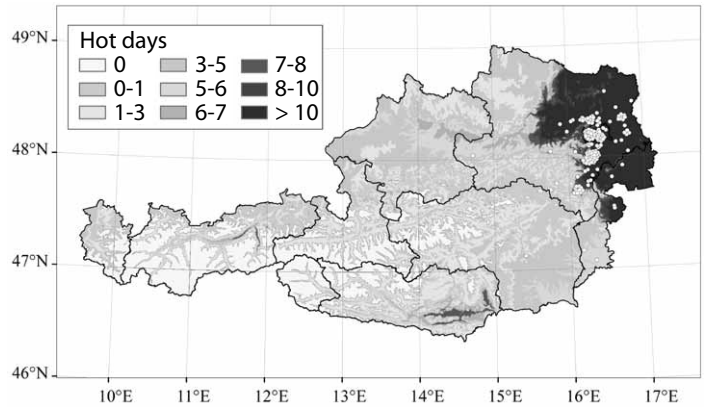

Figure 1. Spatial distribution of the average number of hot days per year in Austria for the climate reference period 1961-1990 (Auer et al.*, 2001) and blackbirds dead from USUV (white dots; Chvala* et al.*, 2007). Among some other species, about 140 blackbirds dead from USUV infections have been collected between 2001 and 2005.*

spatial distribution of the average number of hot days (days with temperature ≥30 °C) in Austria. Conspicuously, the emergence of USUV infections is mainly related to those regions around Vienna where the number of hot days exceeds 10 days/year. Most of the dead birds were found in the most populated region in the district of Lower Austria, and only few in the surrounding agricultural areas. A more detailed discussion on the dead-bird monitoring is in Weissenböck *et al.* (2003) and Chvala *et al.* (2007).

Here we will respond to the following questions: is it possible to develop an epidemic model, which can accurately simulate the observed USUV epidemics in Vienna? How might USUV around Vienna develop according to global warming? Should we expect large-scale declines in several bird populations as observed for the related WNV in North America (LaDeau *et al.*, 2007)? To answer the first question, we explain and discuss the USUV model developed by Rubel *et al.* (2008), which calculates (once initialised with some USUV-positive mosquitoes and exclusively forced by environmental temperature) time series of bird and mosquito populations of various health states. It is shown that the epidemic model is able to reproduce the observed numbers of dead birds very well. Therefore, the epidemic model will be forced with long-term climate projections to answer the second and third question.

The applied climate dataset comprises temperature predictions from five different global climate models (GCMs), to incorporate the uncertainty across climate models. However, each GCM was based on the same initial conditions. We used a combination of these five climate models with the four emission scenarios (defined by the Intergovernmental Panel on Climate Change, IPCC) resulting in a total of 20 temperature predictions for the period 2006-2100 to force our USUV model. The results of these multiple model runs are interpreted like results from a stochastic model. Finally, we used temperature observations from a weather station in Vienna to run the USUV model for the period 1901-2005. The major result from the epidemic model runs is a time series of the basic reproduction number of the Usutu virus for the period 1901-2100. It depicts the possibility for a major outbreak as a function of environmental temperature and herd immunity of birds.

2. The epidemic model

Our SEIR (susceptible, exposed, infectious, recovered) model simulates the seasonal lifecycles of blackbirds (*Turdus merula*) and mosquitoes (*Culex pipiens*) and the inter-species USUV infection cycle between birds and mosquitoes (Figure 2). The model has nine compartments (i.e. health states): susceptible birds (S_B), latent-infected or exposed birds (E_B), infectious birds (I_B), recovered or immune birds (R_B), dead birds (D_B), mosquito larvae (L_M), susceptible mosquitoes (S_M), latent-infected or exposed mosquitoes (E_M) and infectious mosquitoes (I_M). The movements between health states are defined by 12 parameters, of which seven depend exclusively on the environmental temperature. These are the birth and mortality rates of larvae and (adult) mosquitoes, the infection rates between infectious mosquitoes and susceptible birds and *vice versa* (cross-infection), and the reciprocal of the extrinsic incubation period (here defined as infectivity rate of mosquitoes). Further parameters are the birth rate of birds and the proportion of mosquitos' non-diapausing, which are functions of the Julian calendar day and the geographic latitude. Constant parameters are the mortality rate of birds,

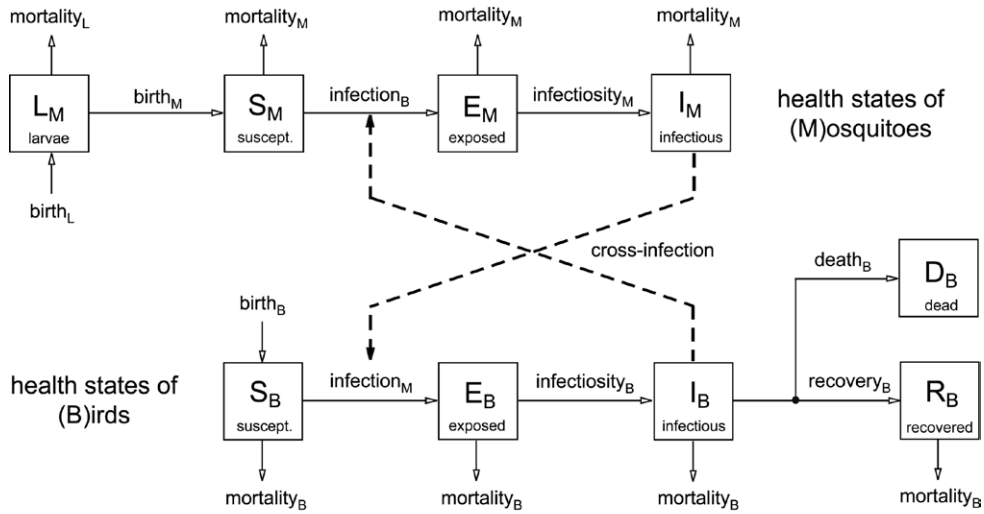

Figure 2. Block diagram of the epidemic model applied to simulate the Usutu virus dynamics in Vienna. The birth and mortality rates of mosquitoes ($birth_L$, $mortality_L$, $birth_M$, $mortality_M$), the infection rates ($infection_B$, $infection_M$) and the infectivity rate of mosquitoes ($infectivity_M$) are considered to be temperature dependent.

the reciprocal of the intrinsic incubation period, and the infectivity rate of birds (i.e. the rate of transition within birds from latently infected to infectious), the recovery rate of birds and the death rate of birds. For more details on the mathematical parameter definition see Appendix 1.

Based on the same initial conditions we used a combination of these five climate models with the four IPCC emission scenarios resulting in a total of 20 temperature predictions for the period 2006-2100 to force our USUV model. The results of these multiple model runs are interpreted like results from a stochastic model. Finally, we used temperature observations from a weather station in Vienna to run the USUV model for the period 1901-2005. The major results from the epidemic model runs are time series of the basic reproduction number of the health states of birds and mosquitoes, respectively. They depict the possibility for past and future outbreaks as a function of environmental temperature.

In doing so, the numbers of species in specific health state are calculated following the main principle of building budgets. The future number of infectious mosquitoes, for example, is calculated from actually available infectious mosquitoes minus the mosquitoes dying within some specified time step (determined by the $mortality_M$ rate) plus the newly infectious mosquitoes (determined by the $infectivity_M$ rate). Thus, the dynamics of the 9 bird and mosquito health states is mathematically formulated by 9 ordinary differential equations (not shown), which are solved numerically using daily time steps (R source code see Appendix 2).

3. Parameter estimation

The extent to which an epidemic model simulates reality depends strongly on the assumed parameters (birth, mortality and transmission rates). Note that the model parameters are generally defined per capita and per day and will be described in detail in the following sub-sections.

3.1 Bird parameters

Because more than 90% of all birds, which died from USUV infections, were blackbirds (*Turdus merula*), we focus on this species. Blackbirds are widespread in woodland, but also one of the most striking birds in urban gardens. Their average life expectancy is about 2 years, but may exceed 20 years in exceptional cases. Knowing the lifetime of blackbirds, the mortality rate can be estimated as its reciprocal value. Hatchwell *et al.* (1996) specified annual mortality rates of 0.34 per year and 0.52 per year in woodland and farmland, respectively. For the land cover of the area around Vienna we applied a mean mortality rate of 0.43 per year corresponding to $mortality_B = 0.0012$ days^{-1}.

In Central Europe blackbirds deposit eggs two to four times per year. Schnack (1991) investigated the breeding success and clutch sizes of blackbirds in 17 city parks in Vienna and in an adjacent lowland forest. On average a clutch size of 4.1 eggs was estimated for the city of Vienna and 4.6 eggs for the woodland. The breeding success (the number of fledged nestlings per eggs laid) was 22.4% for urban blackbirds and 30.7% for forest blackbirds. Similar results were documented by Tomialojc (1993 and 1994) for blackbirds in Poland (mean clutch size of 4.5 eggs, emerging nest losses of 50-92% with mean 68%). On averaged 2.5 young per pair fledged yearly, whereas the most successful pairs reared 8-9 young (Tomialojc, 1994). We assume 2.5 young per pair as bird birth rate. This yields a per capita birth rate of 1.25 per year or $birth_B = 0.00342$ days^{-1}.

Although the mortality rate is assumed to be uniformly distributed over the year, a seasonal cycle was fitted to the observed birth rate. Figure 3 (left) depicts the observed frequency distribution of the blackbird nestlings (bars), as compiled from counts of blackbird clutches in Poland (Tomialojc, 1994), and the fitted theoretical distribution (line). Originally, Tomialojc (1994) published absolute numbers of clutches for two observational periods, which were averaged and shifted by 10 days to account for breeding. As depicted in Figure 3 (left), the distribution is skewed to the right. Therefore, a gamma distribution was selected to describe the observations. By multiplying it with the average annual birth rate, we derived the birth rate as a function of the calendar day as shown in Figure 3 (right).

The breeding density in the city of Vienna varies in the range of 10-210 pairs/km^2 (Schnack, 1991), corresponding to a bird density of 20-420 birds/km^2. Two typical black bird habitats outside the city of Vienna were investigated by Wichmann and Zuna-Kratky (1997). The first habitat is an area covered with vineyards and fallow lands, and the bird density was 77-122 birds/km^2. The second habitat, a mixed forest with embedded grasslands, showed a slightly higher bird density of 104-155 birds/km^2. These densities are much higher than the large-scale blackbird density for central Europe, for example, of 25 birds/km^2 as estimated for the entire

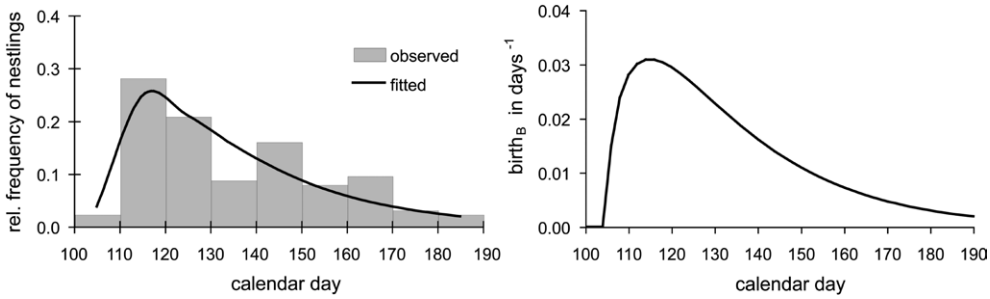

Figure 3. Observed relative frequency of blackbird nestlings (Tomialojc, 1994) with fitted gamma distribution (left) and bird birth rate as function of the Julian calendar day (right).

region of Germany (Schwarz and Flade, 1989). Considering both the large-scale density and the local densities of favoured blackbird habitats, we assumed an average blackbird density for the area of USUV emergence of about 50 birds/km^2. We used this value to define the carrying capacity of birds.

An accurate estimation of the bird mortality due to USUV infections is difficult. From the data of the dead-bird surveillance we estimated that about 30% of the infected blackbirds died (Weissenböck *et al.*, 2002).

3.2 Temperature-dependent transmission parameters

According to the cross-infection process depicted in Figure 2, two transmission parameters, the forces of infection, must be determined. Here, the forces of infection are abbreviated by the terms $infection_B$ and $infection_M$, which are functions of the mosquito biting rate k and the probabilities p_B and p_M that the USUV is transmitted by a bite. As for WNV, *Culex* mosquitoes are mainly responsible for USUV transmission. On average these mosquitoes are biting every 1-5 days, corresponding to biting rates of k = 0.2-1 days^{-1}; see for example the overview given by Cruz-Pacheco *et al.* (2005). Temporal changes in the effectiveness of transmission essentially delineate the seasonality of WNV and USUV activity and are triggered by the environmental temperature. For a description of this process, the temperature dependence of the duration of the gonotrophic cycle (i.e. the development cycle of mosquitoes comprising blood meal as well as development and deposition of eggs) as presented by Reisen *et al.* (2006) was used. The function describing the biting (i.e. contact) rate was fitted to the reciprocal of the duration of the mosquito gonotrophic cycle (Rubel *et al.*, 2008). The functional relationship is similar to Figure 4A, because the birth rate of the larvae ($birth_L$) is assumed to be a multiple of the biting rate k. The biting rate increases with increasing temperature according to a logistic function. The infection rates are proportional to the product of the biting rate and the transmission probabilities. Typical WNV probabilities as proposed by various authors vary of 0.02-0.24 (virus transmission by infectious birds to susceptible mosquitoes) and 0.8-1.0 (virus transmission by infectious mosquitoes). We determined the unknown transmission probabilities of USUV by fitting the model to observations, resulting in a transmission

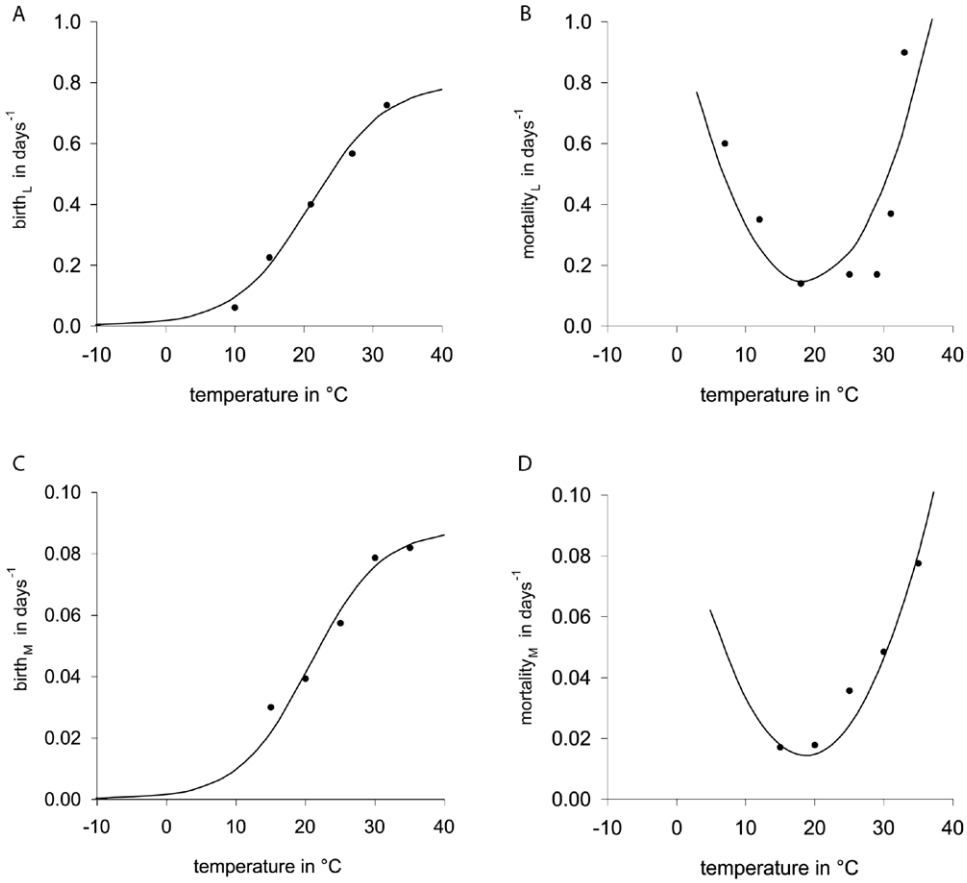

Figure 4. Observed mosquito birth and mortality rates as function of temperature. A. Function birth$_L$ fitted to data from Reisen et al. *(2006) and adjusted after Rubel* et al. *(2008). B. Function mortality$_L$ fitted to data from Bailey and Gieke (1968). C. Function birth$_M$ fitted to data from Reisen (1995). D. Function mortality$_M$ fitted to data from Reisen (1995). Note, that the parameter functions have been selected to yield parameters for larvae that are exact one order of magnitude higher than for adult mosquitoes.*

probability by infectious birds of $p_B = 0.125$ and by infectious mosquitoes of $p_M = 1.0$ (both are within the range given for WNV).

3.3 Temperature-dependent mosquito parameters

The temperature dependence of mosquito birth and mortality rates (of both larvae and adult mosquitoes) was investigated mainly in the 1960s and 1970s. Unfortunately, most of these studies do not provide functions as required for process models. Therefore, one of our goals was to find general functions describing the relationship between mosquito population

parameters and environmental temperature. A selection of functions fitted to data sets published by various authors is depicted in Figure 4.

Figure 4A shows the birth rate of larvae $birth_L$, a synonym for the egg-deposition rate, which is modelled by the scaled reciprocal of the gonotrophic cycle after Reisen *et al.* (2006). We selected the scaling factor so that the average birth rate $birth_L = 0.537$ days^{-1}, as proposed by Wonham *et al.* (2004), is reached at T = 25 °C. Typical mortality rates of larvae are shown in Figure 4B, where a function was fitted to data from Bailey and Gieke (1968). Alternative functions (not shown) were used for example by Eisenberg *et al.* (1995) or Shaman *et al.* (2006). Most studies are available for the temperature dependent birth rate of adult mosquitoes $birth_M$ (that is, the development rate of immatures). These studies comprise laboratory experiments for *Culex quinquefasciatus* and *Aedes aegypti* (Rueda *et al.*, 1990), a regression line for *Culex annulirostis* (Rae, 1990) as well as discrete values for *Culex tarsalis* (Reisen, 1995) and for *Culex pipiens molestus* (Olejnícek and Gelbic, 2000). Logistic functions $birth_M$ were fitted to all of these data sets and subsequently were evaluated in sensitivity studies (results not shown). As an example, the function fitted to the data published by Reisen (1995) is depicted in Figure 4C. Finally, Figure 4D shows the mortality rate $mortality_M$, again as fitted to observations from Reisen (1995).

From inspection of Figure 4, it became clear that that the functions for the population parameters of the mosquito larvae are of similar shape, but about one order of magnitude higher than those for the adult mosquitoes. Using this allowed us to generalise the mosquito birth rates by using the same logistic (S-shaped) function for both, larvae and adult mosquitoes. Again, only one U-shaped function was selected to describe both mortality rates (Figures 4B and 4D), which differ by a factor of ten.

3.4 Temperature-dependent extrinsic-incubation period

The extrinsic-incubation period (i.e. the time from an infectious blood meal until the mosquito can transmit an acquired arbovirus infection) is an important parameter determining the vector capacity. It is the reciprocal of the rate of virus replication within an infected mosquito vector, here called infectivity rate. The parameter $infectivity_M$ is highly temperature-dependent (Cornel *et al.*, 1993; Dohm *et al.*, 2002; Turell *et al.*, 2002; Reisen *et al.*, 2006).

Because the virus-replication rate for USUV is unknown, we relied on the results from WNV. We used data from Reisen *et al.* (2006) to fit the functions depicted in Figure 5. Thus, the extrinsic-incubation period decreases with increasing temperature. Long periods of warm temperatures amplify flavivirus transmission. Vice versa, low temperatures can reduce the flavivirus transmission or even interrupt it when the extrinsic-incubation period exceeds the mosquito lifetime.

3.5 Diapause (hibernation of mosquitoes)

Only non-diapausing mosquitoes contribute to the reproduction and the USUV transmission cycle. Therefore, it is of fundamental importance to know the fraction of non-diapausing mosquitoes. Quantitative investigations on the diapause of *Culex* mosquitoes are rare because

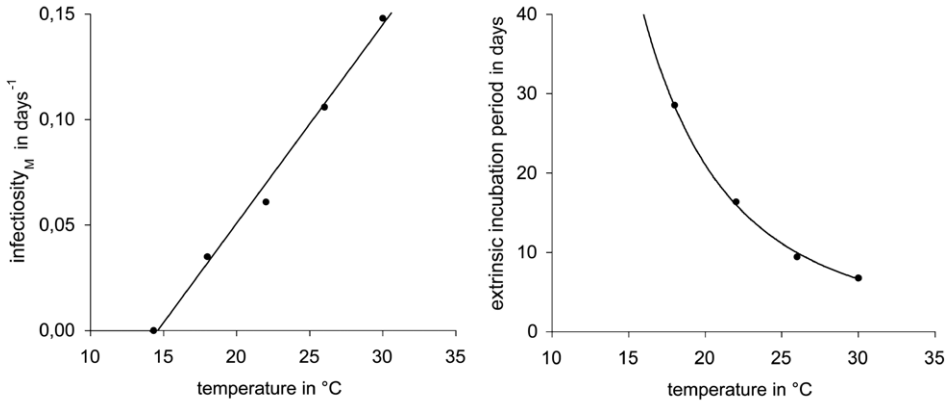

Figure 5. Observed infectivity rates (dots) and fitted function for infectivity$_M$ (left) and its reciprocal, the extrinsic-incubation period (right). Both are functions of the environmental temperature and fitted to data by Reisen et al. *(2006).*

they had not played an important role as vector until the 1999 WNV outbreak in New York (USA). One study was published by Eldridge (1968), who depicted the proportion of non-diapausing mosquitoes as a function of photoperiod (daytime length) and temperature (Vinogradova, 2000). A second paper was presented by Spielman (2001), who re-analysed 50-year-old-mosquito data from Boston (300 km north of the WNV outbreak in New York) to deduce a relationship between diapause and photoperiod (dots in Figure 6, left).

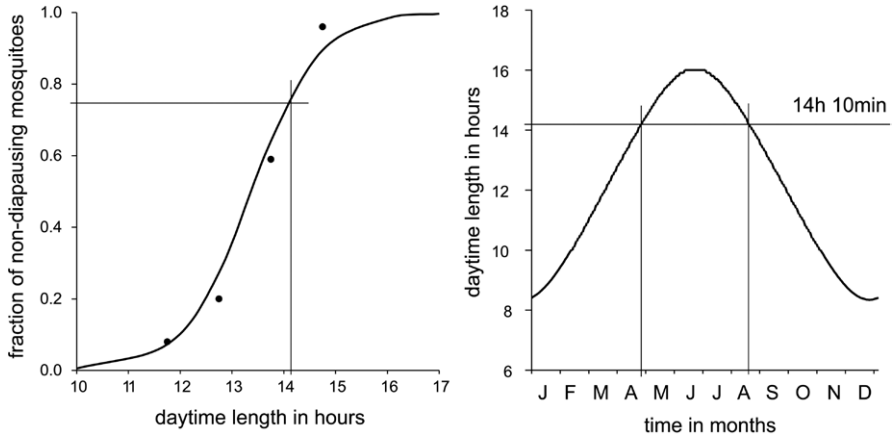

Figure 6. Observed fraction of diapausing Culex pipiens *mosquitoes as function of the daytime length after Spielman (2001) with fitted function (left) and annual cycle of the daytime length in hours for the geographical latitude of Vienna, Austria (right). At daytime lengths above 14 hours and 10 minutes (May to August) more than 75% of the mosquitoes are active.*

We fitted functions to both data sets: A 2-dimensional function (not shown) to the data of Eldridge (1968) and a 1-dimensional function to the data of Spielman (2001). Simulations demonstrated that their application yielded similar model results. This is not astonishing because of the natural correlation between the annual temperature cycle and the photoperiod. We applied the simpler relationship after Spielman (2001). The logistic function for the description of the fraction of active mosquitoes, i.e. the non-diapausing mosquitoes, is depicted in Figure 6 (left). It depends on the daytime length in hours, which is calculated from the astronomic declination (a function of the calendar day) and the geographic latitude. The annual cycle of the daytime lengths in Vienna is depicted in Figure 6 (right).

4. Climate forcing of the observational period 2001-2005

As discussed above, the epidemic model is forced by temperature data, which determine the transmission rates $infection_B$ and $infection_M$ via the mosquito biting rate k as well as the mosquito parameters $birth_L$, $mortality_L$, $birth_M$, $mortality_M$ and $infectivity_M$.

The air-temperature measurements from the automatic weather station located at the University of Veterinary Medicine, Vienna, were used. These measurements are available for the period 1997 to present and are representative for the study area around Vienna. The temporal resolution of the measurements is 10 minutes, providing the opportunity to run the epidemic model with time steps corresponding to this resolution.

On the other hand, the numbers of dead birds collected during the USUV monitoring program (Chvala *et al.*, 2007) are only representative on a weekly or (better) monthly time scale. Our goal was to reproduce and explain the observed multi-seasonal dynamics of USUV infections. Therefore, to account for the resolution of the observations, the model was forced by monthly averaged temperature data. Nevertheless, to assure numerical stability, the model must run with time steps of 1 day or less. We used spline functions to interpolate the monthly data to the model time step. In fact, smoothed data were used to force the model. Figure 7 (upper panel) depicts the time series of the temperature observations in Vienna for the study period 2001-2005, whereas the grey line represents the daily and the dark line the monthly averaged (smoothed) temperature. Figure 7 (lower panel) points out the temperature anomaly: the deviation from the 10-year mean 1997-2006. Conspicuously positive temperature deviations during the mosquito-activity period were observed for May to July 2002 and for May to September 2003, whereas no longer periods of extraordinary high temperatures were observed during the other years. Subject to the temperature dependence of mosquito parameters discussed in the previous section, high USUV activities were expected for 2002 and 2003. Most interesting is the year 2003, where the June to August temperatures exceed the long-term mean by more than 3 °C.

5. Short-term simulation results for the period 2001-2005

The first results of the model simulations were time series of health states of birds and mosquitoes. Figure 8 (upper panel) depicts the normalised numbers of mosquito larvae and

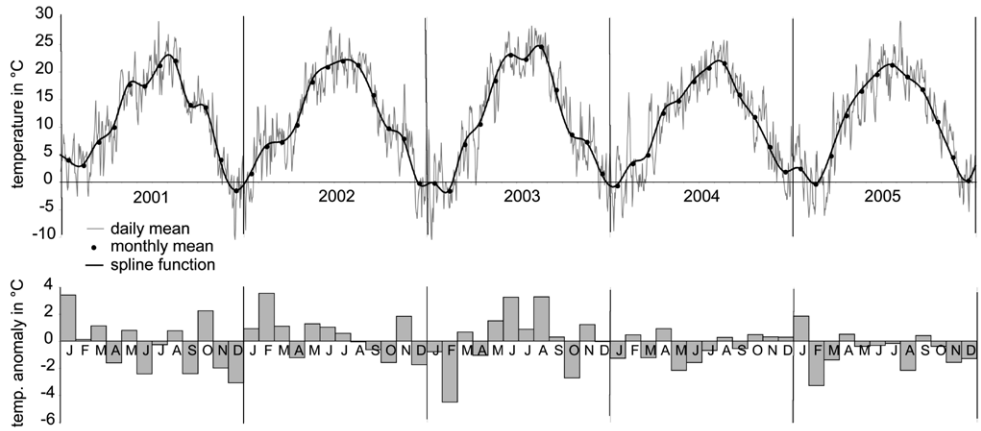

Figure 7. Observed daily and monthly averaged temperatures (upper panel) and monthly temperature anomalies (lower panel) in ° C. Location Vienna, period 2001-2005. Note the positive anomalies in spring and summer 2003.

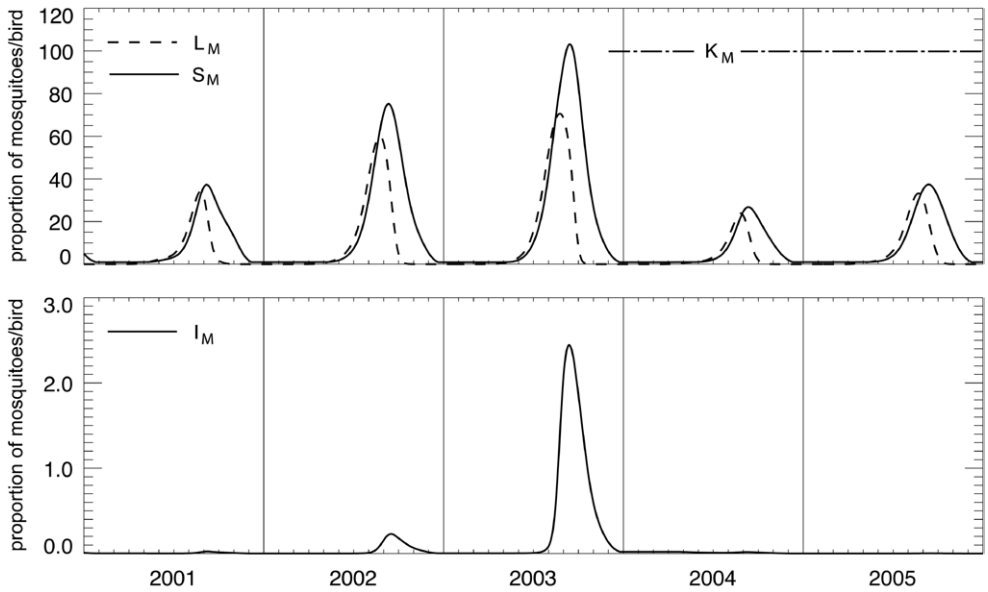

Figure 8. Simulated time series of mosquitoes for the period 2001-2005 (proportion of mosquitoes/bird). Upper panel: larvae L_M (dotted line) and susceptible mosquitoes S_M (solid line) for carrying capacity K_M = 100 mosquito larvae/bird. Lower panel: infectious mosquitoes I_M.

adult mosquitoes for the investigation period 2001-2005. While the mosquito population during the years 2001, 2004 and 2005 seemed typical, it was 2-3 times higher in 2002 and 2003. The latter were caused by the long warm periods in these years (Figure 7). The normalised

number of infectious mosquitoes, I_M (Figure 8, lower panel) was influenced by the extrinsic-incubation period, which decreases with increasing temperature (Figure 5).

Figure 9 (upper panel) depicts the proportion of birds in the health states susceptible S_B, immune R_B, as well as the total population N_B. A strong increase of the immune birds R_B = 0.7 (70%) was simulated for 2003 in correspondence to the epidemic peak in the same year. Subsequently, R_B decreases to 45% until the end of 2004 and to 30% until the end of 2005. Analogous to the illustration of the time series of mosquitoes, Figure 9 (lower panel) depicts the proportion of infectious birds with the same epidemic peak in 2003.

For a verification of simulations with observations (Figure 10), the normalised model results were scaled to fit the observations. This has been done by multiplying the model output, i.e. the proportion of birds in the various health states (Figure 9), by a factor of 385 birds. This factor is the carrying capacity of birds $K_{B,obs}$ = 385, which is called *observed*, because it follows from the number of observed dead blackbirds. After this scaling, our model described the observations quite well. Note that the dead-bird monitoring was organised only for the summer months June to August, while the model simulates continuously in time. Feasible comparisons may therefore only be done for the three summer months. Nevertheless, the model simulations indicate that blackbirds die from USUV during the whole mosquito activity period May to October. Based on the estimated blackbird density of 50 birds/km^2 (see Section 3.1) and an investigation area around Vienna of about 3,500 km^2, the true carrying capacity of blackbirds is about $K_{B,true}$ = 175,000 birds. From the fraction $K_{B,obs}/K_{B,true}$ it follows that only 0.2% of the USUV positive birds were detected by the dead-bird monitoring program.

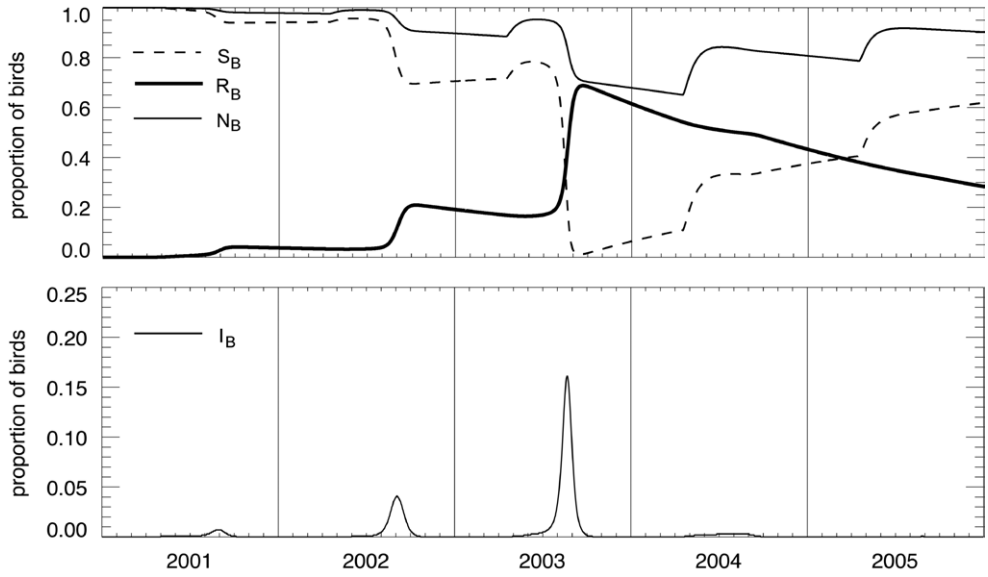

Figure 9. Simulated time series of blackbirds for the period 2001-2005 (proportion of birds related to K_B). Upper panel: susceptible birds S_B (dotted line), immune birds R_B (bold solid line) and total birds N_B (thin solid line) for K_B = 1. Lower panel: infectious birds I_B.

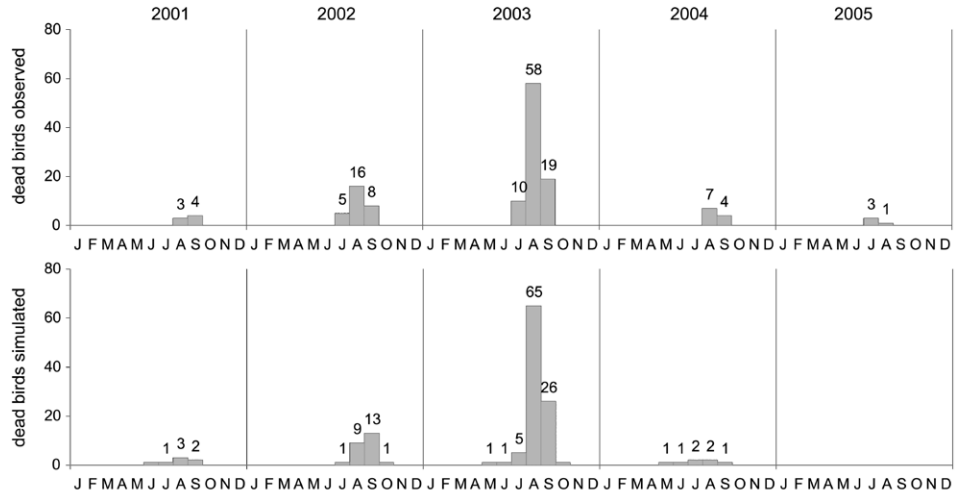

Figure 10. Time series of monthly averaged dead blackbirds for the period 2001-2005. Upper panel: observed; lower panel: simulated by the epidemic model (scaled with $K_{B,obs}$ = 385). Note that the number of dead birds modelled for 2005 is below 1, but non-zero.

6. Climate forcing for the extended period 1901-2100

To simulate future USUV outbreaks, we selected the Tyndall Centre for Climate Change Research dataset, TYN SC 2.0 (Mitchell and Jones, 2005). It is based on 4 scenarios defined by IPCC and described in the Special Report on Emission Scenarios (SRES). This dataset provides the 5 climate parameters temperature, diurnal temperature range, precipitation, water-vapour pressure, and cloud cover; but we only used the temperature data. Information is presented on a 0.5° grid, equidistant in geographical latitude and longitude that covers the world land surface and is available with a monthly time-step for the period 2001-2100. Thus, the TYN SC 2.0 dataset comprises a total of 20 climate-change model runs, combining 4 possible future worlds using SRES scenarios with 5 state-of-the-art climate models (Mitchell *et al*., 2004).

The emission scenarios (Figure 11) were developed in the mid 1990s and are based on 4 different narrative storylines to describe consistently the relationships between the forces driving emissions and their evolution and to add context for the scenario quantification (IPCC, 2000). The four SRES storylines represent different world futures in two dimensions: a focus on economic or environmental concerns, and global or regional development. The variables in each model include population growth, economic development, energy use, efficiency of energy use, and mix of energy technologies, respectively. The TYN SC 2.0 dataset considers the scenarios A1 (exactly A1FI), A2, B1 and B2. On the other hand, five general circulation models were used to simulate climatic changes: the Hadley Centre Coupled Model Version 3 (HadCM3), the National Center for Atmospheric Research-Parallel Climate Model (NCAR-PCM), the Second Generation Coupled Global Climate Model (CGCM2), the Industrial Research Organization – Climate Model Version 2 (CSIRO2) and the European Centre Model Hamburg Version 4 (ECHam4).

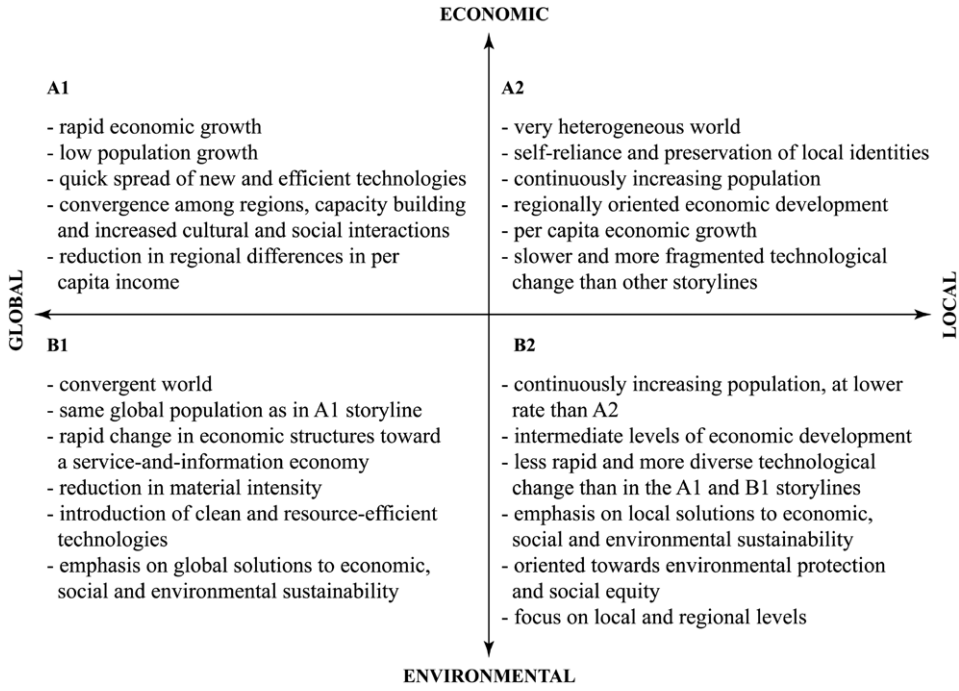

Figure 11. Brief characterisation of the main Special Report on Emission Scenarios (SRES) storylines after IPCC (2000) and Arnell et al. *(2004).*

We used only time series of monthly temperatures of the grid-point 392/277 of the TYN SC 2.0 dataset (the so-called *grid-point Vienna*), which is representative for the geographical region between 16.0-16.5° longitude and 48.0-48.5° latitude. Figure 12 depicts time series of the average annual temperature for the grid-point Vienna, grouped by scenarios (upper panel) and climate models (lower panel). All predicted temperatures show a clear trend toward warming. The average predicted increase of the temperature during the 100-year period, calculated from the linear trend, ranged from 2.6-6.1 °C (across emission scenarios) and from 2.3-6.8 °C (across climate models). For the 20 model-scenario combinations, the range in average annual predicted warming across the century was 0.8 °C (PCM model with B1 scenario) to 11.6 °C (ECHam4 model with A1 scenario). The lowest temperature increase of 2.3 °C (0.8-3.8 °C) is simulated by the B1 scenario. It represents an environmentally oriented world with emphasis on global solutions to economic, social and environmental sustainability.

Two specific features of climate model predictions have to be considered when using it to study the evolution of biological systems (e.g. epidemic outbreaks). Firstly, climate predictions for the next 100 years merely simulate a possible temperature based on a scenario which is also a prediction. Uncertainties of these climate predictions are made evident by the range of prediction, depending on the scenario and model used. Secondly, climate models are representative for a specific spatial resolution, here of 0.5° equidistant in geographical latitude and longitude. Thus, the temperature at grid point Vienna is representative for an area of about

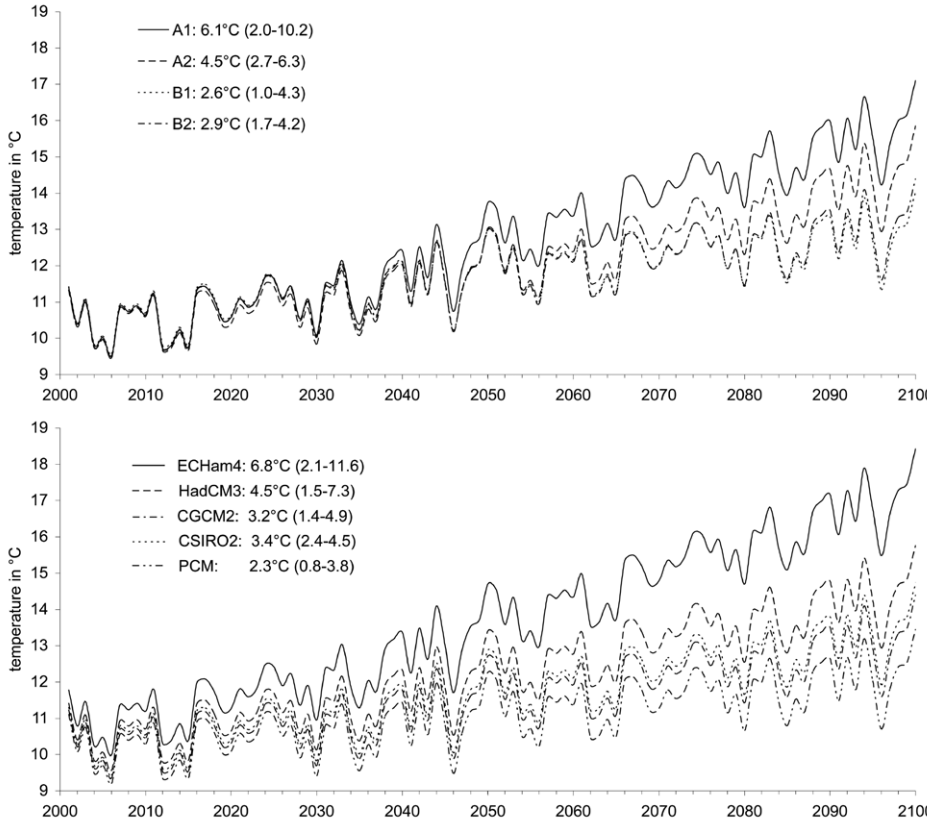

Figure 12. Time series of average annual temperature predictions for different emission scenarios (upper panel) and climate models (lower panel). Numbers in brackets denote the temperature range predicted by different climate models (upper panel) and different emission scenarios (lower panel). Grid-point Vienna, period 2001-2100.

70×50 km^2 with a mean height above sea level of 261 m. The grid-point Vienna comprises, for example, the foothills of the Alps, the Vienna Forest, and planes like the Tullner Feld (i.e. the resolution is too coarse for our study area). USUV cases have been observed only in the flat country where temperatures are high. These temperatures were measured by our weather station located at 153 m above sea level. Therefore, the temperatures simulated by climate models were adjusted to the measured temperatures (downscaled) to describe this small-scale region (Rubel *et al.*, 2008). Note that it is essential to use the (generally slightly higher) downscaled temperature predictions to force the USUV model. Otherwise the effect of the climate warming signal would be compensated by the underestimation of the temperatures predicted by the large-scale climate models.

7. Long-term simulation results for the period 1901-2100

The time series of predicted annually averaged numbers of birds dead because of USUV is depicted in Figure 13. The upper panel shows the mean proportion of dead birds together with the 95% confidence interval (grey areas) calculated from 5 model runs forced by the GCM predictions for the worst-case emission scenario A1. The lower panel shows the same for the best-case scenario B1.

The main result is that USUV was predicted to survive in almost all model simulations except those simulations where the USUV model is driven by temperatures from PCM using the A1 and A2 scenarios. This virus extinction leads to the higher variation of the proportion of dead birds in the A1 scenario. After the epidemic peak 2003, only minor outbreaks are simulated until the next major outbreak in 2019. The amplitude of the outbreaks, here the annually accumulated proportions of dead blackbirds, vary accordingly to the predicted temperatures. The higher temperature increase of the A1 scenario results in the higher predicted annual USUV specific mortality risk in the blackbirds. Moreover, forcing the USUV model with

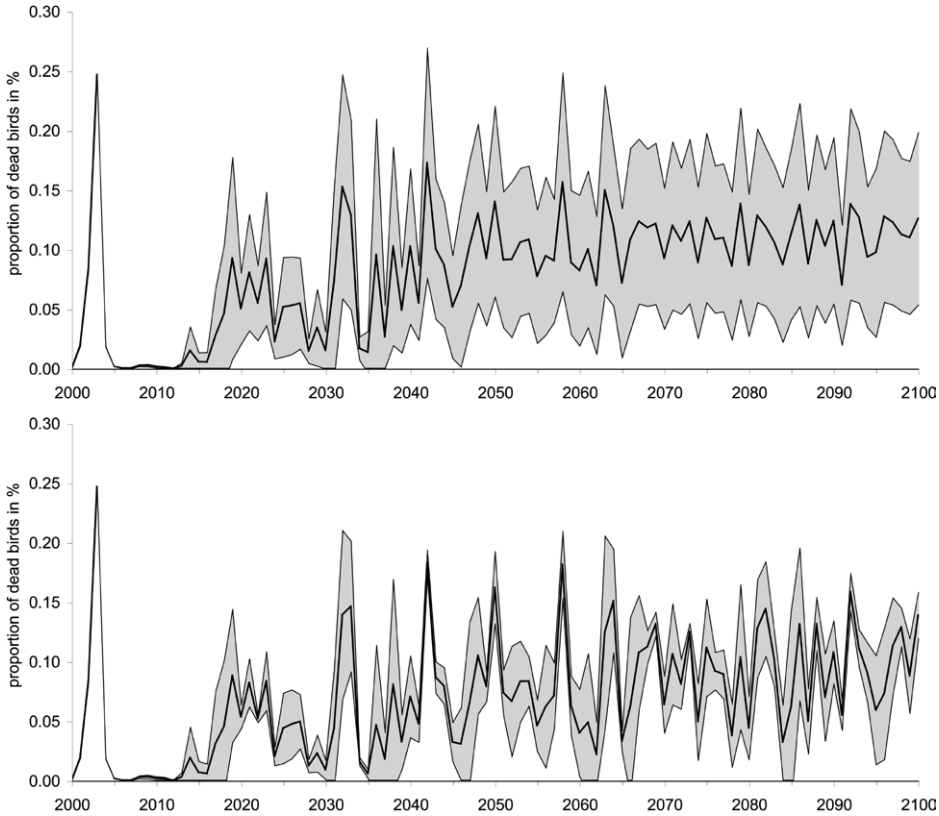

Figure 13. Average (central line) and 95% confidence interval (grey areas) of USUV-specific mortality of blackbirds around Vienna. Emission scenario A1 (upper panel) and B1 (lower panel).

temperatures from two climate models (ECHam4 and HadCM3 with A1 scenario) result in an endemic situation with a constant level of about 11% dead birds in the year 2050. Typically, a cyclic annual mortality pattern was predicted through the years – but the predicted amplitude of the cycle of annual mortality decreases later in the century especially of the A1 emission scenario. For the A1 scenario on average 60% of all birds acquire immunity until the end of the century. But also for the best-case scenario B1, a proportion of 50% of the birds acquire lifelong immunity.

Additionally, we depict the USUV dynamics by the annual basic reproduction number R_0 as derived and discussed by Rubel *et al.* (2008). Figure 14 shows the time series of R_0 for the period 1901-2100. While IPCC scenarios have been used to predict the future, observed temperature values were taken to calculate a projection to the past. Major outbreaks may only occur for R_0 >1, firstly calculated for the observed USUV epidemics 2001-2005. Thus, our backward projection using observed temperatures shows that USUV was not able to cause major outbreaks in the past. Nevertheless, minor outbreaks below some detection threshold were possible, because of the annual cycle of R_0 (not shown).

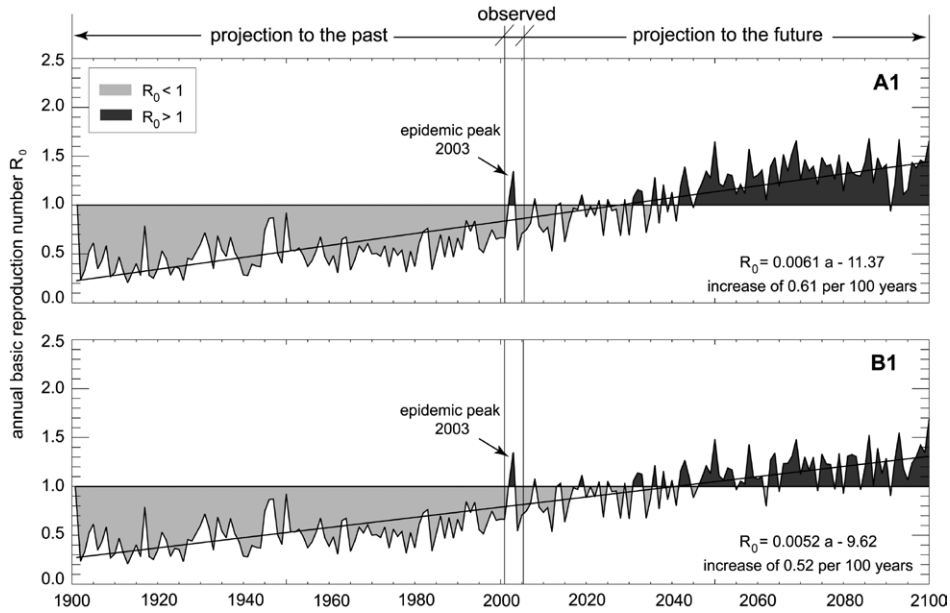

Figure 14. Mean annual basic reproduction numbers for the IPCC worst-case scenarios A1 (upper panel) and best-case scenarios B1 (lower panel). Linear trends for the period 1901-2100 indicate an increase of R_0 = 0.61 per 100 years for the A1 and R_0 = 0.52 per 100 years for the B1 scenario.

8. Conclusions

What has been achieved?

As an example for viral zoonoses affecting wildlife and domestic animals, the dynamics of the well-documented USUV epidemics in Vienna was investigated. After the epidemic peak, more than 70% of the bird population acquired immunity. However, the immunity decreases very quickly and already some years after the epidemic peak in 2003 a new major outbreak can occur, depending only on the environmental temperature. Other environmental parameters such as precipitation or flooding seem to play only a minor role (because our model fitted well without them). The 100-year flood in Vienna in August 2002, for example, had no effect on the USUV epidemics. Similar observations were made in the U.S. in connection with the hurricane Katrina and WNV (Farnon, 2006). The so-called floodwater mosquitoes are minor effective vectors for WNV and USUV.

From 20 scenario-model combinations we examined, only two were predicted to lead to extinction of USUV (PCM, A1 and A2 scenarios). The other 18 combinations lead to endemicity in Central Europe through the end of the century. It is therefore very likely, that the virus survives in Central Europe until the end of the century. We do not further investigate the possibilities for local extinction because we assume that the USUV is continuously introduced by migratory birds. Thus, the most important epidemiological quantity determining major outbreaks is the basic reproduction number R_0. It predicts optimal environmental conditions for USUV outbreaks due to global warming in about 10 years. On the other hand, undetected USUV outbreaks before the observed outbreak in 2003 are very unlikely.

Our model explains the USUV transmission by combining both the effect of the immunity status of the bird population and effects of the environmental temperature. Additionally, the parameter estimation was confirmed by the results of a Bayes analysis (Reiczigel *et al.*, 2010). Thus, we propose the application of the model presented here for studies on WNV transmission in North America.

What has been neglected?

Our estimations seem realistic when compared to the observed large-scale declines in the bird populations caused by WNV infections in North America (LaDeau *et al.*, 2007). Possible effects of global warming concerning bird and mosquito populations due to changes in land cover, availability of food or other environmental factors have not been considered; i.e., the carrying capacity of birds K_B and mosquitoes K_M were assumed to be constant during the investigation period 2001-2100. Further, we did not consider either vertical transmission of USUV in the mosquito vectors or horizontal transmission in susceptible birds. This decision was made because there exist no data at all on these particular transmission scenarios in USUV infections. In addition, these modes of transmission have been experimentally shown in infections with the related WNV, but their relevance in field infections seems negligible (Baqar *et al.*, 1993; Komar *et al.*, 2003).

What needs to be done?

A comprehensive verification of the arbovirus model might be realised in a few years when longer time series of observations are available. Especially, the population dynamics of mosquitoes of the *Culex pipiens* complex have to be verified. Unfortunately, there are no current entomological field studies in Austria. Also, the assumption that the extrinsic incubation period of USUV (which has never been investigated in laboratory experiments) is similar to that of the closely related WNV has to be verified. Other recently published verification datasets comprise the abundance of blackbirds. Steiner and Holzer (2008), for example, investigated the decline of blackbirds at 6 locations in Vienna. They found big local differences in time and extent of mass mortality in blackbirds. While the decline in bird populations was about 58% in some regions near the Danube River, in other regions more than 94% of blackbirds disappeared. This disagrees with the experience of G. Loupal (personal communications), who stated that the blackbird population was not dramatically influenced by the USUV epidemics, as it has also been confirmed by observations.

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Appendix A The USUV model

Model equations

$$\frac{dS_B}{dt} = (b_B - (b_B - m_B)\, N_B/K_B)\, N_B - \delta_M\, \beta_M(T)\, I_M\, S_B/K_B - m_B\, S_B \quad (1)$$

$$\frac{dE_B}{dt} = \delta_M\, \beta_M(T)\, I_M\, S_B/K_B - \gamma_B\, E_B - m_B\, E_B \quad (2)$$

$$\frac{dI_B}{dt} = \gamma_B\, E_B - \alpha_B\, I_B - m_B\, I_B \quad (3)$$

$$\frac{dR_B}{dt} = (1 - \nu_B)\, \alpha_B\, I_B - m_B\, R_B \quad (4)$$

$$\frac{dD_B}{dt} = \nu_B\, \alpha_B\, I_B \quad (5)$$

$$\frac{dL_M}{dt} = (b_L(T)\, \delta_M\, N_M - m_L(T) L_M)(1 - L_M/K_M) - b_M(T)\, L_M \quad (6)$$

$$\frac{dS_M}{dt} = -\delta_M\, \beta_B(T)\, S_M\, I_B/K_B + b_M(T)\, L_M - m_M(T)\, S_M \quad (7)$$

$$\frac{dE_M}{dt} = \delta_M\, \beta_B(T)\, S_M\, I_B/K_B - \gamma_M(T)\, E_M - m_M(T)\, E_M \quad (8)$$

$$\frac{dI_M}{dt} = \gamma_M(T)\, E_M - m_M(T)\, I_M \quad (9)$$

Health states

S_B	susceptible birds
E_B	latent-infected or exposed birds
I_B	infectious birds
R_B	recovered or immune birds
D_B	dead birds
L_M	larvae mosquitoes
S_M	susceptible mosquitoes
E_M	latent-infected or exposed mosquitoes
I_M	infectious mosquitoes

Main parameters

$birth_B$	birth rate birds	$b_B = 0.073\,((d-105)/19.3)^{-0.52}\,exp(-(d-105)/19.3)$
$mortality_B$	mortality rate birds	$m_B = 0.0012$
$infection_B$	force of infection	$\lambda_B = 0.04175/[1 + 1.231\,exp(-0.184\,(T-20))]$
$infectivity_B$	1/intrinsic incubation period	$\gamma_B = 0.667$
$recovery_B$	recovery rate birds	$\alpha_B\,(1-\nu_B) = 0.1274$
$death_B$	death rate due infection	$\alpha_B\,\nu_B = 0.0546$
$birth_L$	birth rate larvae	$b_L = 0.7998/[1 + 1.231\,exp(-0.184\,(T-20))]$
$mortality_L$	mortality rate larvae	$m_L = 0.0025\,T^2 - 0.094\,T + 1.0257$
$birth_M$	birth rate mosquito	$b_M = b_L/10$
$mortality_M$	mortality rate mosquito	$m_M = m_L/10$
$infection_M$	force of infection	$\lambda_M = 0.344/[1 + 1.231\,exp(-0.184\,(T-20))]$
$infectivity_M$	1/extrinsic incubation period	$\gamma_M = 0.0093\,T - 0.1352 \quad (T > 15°C)$

Additional parameters

k	biting rate of mosquitoes	$k = 0.344/[1 + 1.231\,exp(-0.184\,(T-20))]$
p_M	probability transm. M → B	$p_M = 1.0$
p_B	probability transm. B → M	$p_B = 0.125$
β_M	transmission rate M → B	$\beta_M = k\,p_M$
β_B	transmission rate B → M	$\beta_B = k\,p_B$
K_B	carrying capacity, birds	
K_M	carrying capacity, mosquitoes	
N_B	total bird population	
N_M	total mosquito population	
δ_M	non-diapausing mosquitoes	$\delta_M = 1 - 1/[1 + 1775.7\,exp(1.559(D-18.177))]$
D	daytime length	$D = 7.639\,arcsin[tan(\epsilon)\,tan(\varphi) + 0.0146/(cos(\epsilon)\,cos(\varphi))]$
ϵ	declination	$\epsilon = 0.409\,sin[2\,\pi(d-80)/365]$
d	calendar day	
φ	geographic latitude	

Appendix B R-Source-code for the USUV model

```
## R source code for USUV-model                                                    ##

quartz(height=6, width=4); par(mfrow=c(4, 1), mar=c(4, 4.5, 0.5, 0.5))

# input data: monthly temperatures for Vienna 2001-2005

T_month=c(4.0,3.0,7.2,9.9,17.7,17.4,21.1,22.0,14.0,13.6,4.0,-1.6,
    1.5,6.4,7.2,10.3,18.1,20.9,22.0,21.2,15.8,9.7,7.9,-0.2,
    -0.2,-1.6,6.8,10.5,18.4,23.1,22.3,24.5,16.7,8.6,7.3,1.5,
    -0.7,3.4,4.9,12.5,14.7,18.2,20.7,21.5,15.9,11.8,6.3,1.8,
    2.4,-0.4,4.7,12.1,16.5,19.5,21.2,19.1,16.8,10.9,4.5,0.2)
T_sp=spline(T_month,n=30*length(T_month)) # interpolation to daily values
T<-as.vector(unlist(T_sp[2]))
l=length(T)
T<-T[c((l-15):l,1:(l-14))]

n=length(T)-1
t=seq(0,n)


# parameter

k=seq(0, n); k=0.344/(1+1.231*exp(-0.184*(T-20)))
bL=seq(0, n); bL=2.325*k; bM=bL/10
mL=seq(0, n); mL=0.0025*T^2-0.094*T+1.0257; mM=mL/10
phi=48.21*pi/180
day=c(0, rep(seq(1, 365), 6))
epsilon=seq(0, n); epsilon=0.409*sin(2*pi*(day-80)/365)
D=seq(0, n); D=7.639*asin(tan(epsilon)*tan(phi)+0.0146/(cos(epsilon)*cos(phi)))+12
dM=seq(0, n); dM=1-1/(1+1775.7*exp(1.559*(D-18.177)))

KM=100
KB=1

gammaM=seq(0, 0, length=n+1)
for (i in 1:n){
  if (T[i]>15) gammaM[i]=0.0093*T[i]-0.1352
}

bB=seq(0, 0, length=n+1)
for (i in 1:n){
  x=(day[i]-105)/10
  if (x>0) bB[i]=0.125*(x/1.93)^0.52*exp(-x/1.93)/(1.93*0.887)
}

mB=0.0012
alphaB=0.182
gammaB=0.667
nuB=0.3

NMmin=1.0
pM=1.0
pB=0.125

betaM=seq(0, n); betaM=k*pM
betaB=seq(0, n); betaB=k*pB


# initialisation

LM=seq(0.001, 0.001, length=n+1)
SM=seq(5, 5, length=n+1)
EM=seq(0, 0, length=n+1)
IM=seq(0.01, 0.01, length=n+1)
NM=seq(5.01, 5.01, length=n+1)
```

```
SB=seq(1, 1, length=n+1)
EB=seq(0, 0, length=n+1)
IB=seq(0, 0, length=n+1)
RB=seq(0, 0, length=n+1)
DB=seq(0, 0, length=n+1)
NB=seq(1, 1, length=n+1)


# epidemic model

for (i in 1:n){
  lambdaB=dM[i]*betaB[i]*IB[i]/KB
  lambdaM=dM[i]*betaM[i]*IM[i]/KB

  LM[i+1]=LM[i] + (bL[i]*dM[i]*NM[i]-mL[i]*LM[i])*(1-LM[i]/KM)-bM[i]*LM[i]
  SM[i+1]=SM[i] - lambdaB*SM[i]+bM[i]*LM[i]-mM[i]*SM[i]
  EM[i+1]=EM[i] + lambdaB*SM[i]-gammaM[i]*EM[i]-mM[i]*EM[i]
  IM[i+1]=IM[i] + gammaM[i]*EM[i]-mM[i]*IM[i]
  NM[i+1]=SM[i+1]+EM[i+1]+IM[i+1]
  if (NM[i+1]<NMmin) {SM[i+1]=SM[i]; EM[i+1]=EM[i]; IM[i+1]=IM[i]}

  SB[i+1]=SB[i] + (bB[i]-(bB[i]-mB)*NB[i]/KB)*NB[i]-lambdaM*SB[i]-mB*SB[i]
  EB[i+1]=EB[i] + lambdaM*SB[i]-gammaB*EB[i]-mB*EB[i]
  IB[i+1]=IB[i] + gammaB*EB[i]-alphaB*IB[i]-mB*IB[i]
  RB[i+1]=RB[i] + (1-nuB)*alphaB*IB[i]-mB*RB[i]
  DB[i+1]=DB[i] + nuB*alphaB*IB[i]
  NB[i+1]=SB[i+1]+EB[i+1]+IB[i+1]+RB[i+1]
}


# output graphics

plot (t/365, LM, type="l", xlim=c(0,n/365), ylim=c(0, 120), xlab="time (years)",
ylab="mosquitoes", lty=2, lwd=1.5)
lines(t/365, SM, lty=1, lwd=1.5)
abline(h=100, lty=4); text(4.5, 108, "KM")
plot (t/365, IM, type="l", xlim=c(0,n/365), ylim=c(0, 3), xlab="time (years)",
ylab="mosquitoes", lty=1, lwd=1.5)

plot (t/365, SB, type="l", xlim=c(0,n/365), ylim=c(0, 1), xlab="time (years)", ylab="birds",
lty=2, lwd=1.5)
lines(t/365, RB, lty=3, lwd=1.5)
lines(t/365, NB, lty=1, lwd=1.5)

plot (t/365, IB, type="l", xlim=c(0,n/365), ylim=c(0, 0.25), xlab="time (years)",
ylab="birds", lty=1, lwd=1.5)
```

The utility of GIS in studying the distribution of Bovine Tuberculosis in wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in Central Portugal

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Summary

Tuberculosis in game animals has gained, in the past few years, an increasing significance in the central region of Portugal, mainly in wild ungulates such as red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*). Geographical Information Systems (GIS) technology is becoming an essential component of modern disease surveillance systems. In this study GIS was used to evaluate the geographical distribution of Bovine Tuberculosis (BT) in large game species in Central Portugal. Sampling plots for GIS were mapped by means of a GPS (Global Positioning System) receiver in Idanha-a-Nova county (lat 39° 55' N: long 7° 14' W) from November 2008 to February 2009. Over this period, 526 animals (337 red deer (C*ervus elaphus*), 142 wild boars (*Sus scrofa*), 29 fallow deer (*Dama dama*), and 18 mouflon (*Ovis musimon*)) were analysed for BT lesions, during meat inspection. From these harvested animals, 73 (13.88%) carried BT compatible lesions, which were later confirmed by laboratorial analysis. Data collected during fieldwork were assigned to each sampling plot location, in order to enable geostatistical analysis. The analyses of the BT intensity map, created by GIS, for wild boar and red deer hunted in Central Portugal, allow the conclusion that the main BT-afected areas were located at the south-east area of the county. These areas should be the first ones under veterinarian scrutiny with a view to control or reduce disease spread and prevalence. GIS provided an important tool to define objective strategies for preventing the spread of infectious disease.

Keywords: tuberculosis, wild ungulates, *Mycobacterium bovis*, zoonoses, GIS

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1. Introduction

Zoonoses are a matter of concern to the public health and to the economy, and the role of wildlife in the epidemiology of these diseases is an issue of increasing interest and importance and its surveillance among wildlife is a research and public health challenge (Childs *et al*., 2007). According to Kruse *et al.* (2004), zoonoses with a wildlife reservoir represent a major public health problem, affecting all continents. Also, the possibility of persistent cycling of infection in wildlife reservoirs could compromise the success of domestic animals disease control programmes, with additional concern to veterinary authorities, wildlife conservationists and managers (Bengis *et al*., 2002; Gortázar *et al*., 2007). Classical Swine Fever and Brucellosis in wild boars and Bovine Tuberculosis (BT) persistence in badgers are well-known examples (Bengis *et al*., 2002; Meldrum, 2003). With respect to BT, it is known that this disease is becoming an increasingly important throughout Europe in major game animals, like wild boars and red deer. On the Iberian Peninsula, according to Hermoso de Mendoza *et al*. (2006), wild stock (e.g. wild boar and red deer) shows evidences of sharing tuberculosis with domestic cattle, thus endangering human health and leading to economic losses. As already reported by Santos (2006) and Duarte *et al.* (2008), BT in major game (mainly in red deer and wild boar) is often found in central Portugal. This requires a rapid intervention by the veterinary competent authority in order to reduce the prevalence. Cost effective prevention and control of BT requires an interdisciplinary and holistic approach and international cooperation, where surveillance, laboratory capability, research, training and education, and communication are key elements (Kruse *et al*., 2004).

Geographical Information Systems (GIS) enable the incorporation of different spatial data like geographical, farm locations and diseases distribution due to its capability to record information from different sources and its potential for data management and manipulation (Antenucci *et al*., 1989; Goodchild *et al*., 1992). GIS also facilitates the epidemiological relationship analysis among these variables, which is of major importance to the epidemiological investigation of wildlife diseases (Clark *et al.,* 1996; Kistemann *et al*., 2002; Schröder, 2006). In addition, output data generated by GIS in map format has the particular advantage of allowing implicit representation of spatial dependence relationships (Clark *et al*., 1996; Schröder, 2006; Xavier *et al*., 2007) in an intuitive manner. Advances in technology and decreasing associated costs has lead to an increasing use of GPS (Global Positioning System) as a tool for mapping wild animals moving across natural areas or for geo-referenced data collection (Schlecht *et al*., 2004; Gorman *et al*., 2008). Thus, GPS has been used for mapping large game hunting activity and as a GIS data source.

Although some studies indicate that GIS is an important tool in wild stock management (e.g. Radeloff *et al*., 1999), no references were found about the GPS-GIS use in animal disease analysis and control. Hence, GIS and GPS technologies were used in this study so as to evaluate the geographical distribution of Bovine Tuberculosis in large game species in Central Portugal.

2. Material and methods

2.1 Sampling area

The study was performed in Idanha-a-Nova county placed in the central-east part of Portugal (lat 39° 55'N: long 7° 14'W) with 1,412.7 km^2 and 10,561 inhabitants. Located in a plateau region, it has a large border line with Spain defined by the river Erges on the east and the river Tagus in the south.

This is a typical Portuguese rural area with approximately 50% of land dedicated to agriculture (43% dry agriculture, 9% watered agriculture, and 5% graze land), 30% representing forested areas (mainly oaks) and 13% shrub land and sparse vegetation.

Idanha-a-Nova is considered to be one of the best hunting areas in Portugal with numerous game estates, many of which also breed cattle, sheep in an extensive free ranging system, grazing in pastures which are also used by wild artiodactyls.

2.2 GIS methodology

Using information produced and presented by Portuguese cartographic authorities (National Geographic Institute: www.igp.pt; Military Geographic Institute: www.igeoe.pt) such as topographic plans, contours and CORINE Land Cover, a GIS (ArcGis 9.x, Arcinfo version) was created.

Sampling plots were mapped by means of a GPS (Global Positioning System) receiver in Idanha-a-Nova county from November 2008 to February 2009, in order to geo-reference all large game events, suitable to be used for updating the GIS. Within this period, a total of 526 animals (337 red deer (C*ervus elaphus*), 142 wild boars (*Sus scrofa*), 29 fallow deer (*Dama dama*), and 18 mouflon (*Ovis musimon*)) hunted in 20 organised drive hunts, were analysed during *post mortem* meat inspection for Bovine Tuberculosis compatible lesions. Compatible BT lesions were defined as described by Martín-Hernando *et al*. (2007).

Samples with BT compatible lesions were collected and sent to the Laboratório Nacional de Investigação Veterinária (LNIV: National Veterinary Research Laboratory) for confirmation by means of histopathology and other tests like PCR-REA system (Niemann *et al*., 2000). Using GPS collected positions, a vector file (point shape file) was created with all sampling plots locations. Data collected during fieldwork (e.g. tuberculosis compatible lesions) were assigned to each sampling plot location, in order to enable descriptive statistics calculation and geostatistical analysis (Schröder, 2006). Using the percentage of tuberculosis compatible lesions calculated for each sampling plot and geostatistical analysis (performed using Geostatistical Analyst 2.0 for ArcGIS 9.x. ArcInfo version) a continuous map related to disease spread was created, enabling extending the results to the entire study area thus creating continuous disease intensity maps represented by a colour intensity gradation (Schröder, 2006).

3. Results and discussion

From the total of 526 animals hunted and examined during *post mortem* meat inspection, 73 (13.88%) presented BT compatible lesions: 45 red deer and 28 wild boar. Figure 1 and 2 represent two examples of BT compatible lesions observed during this study and located in mesenteric lymph nodes and lungs, respectively.

Figure 1. BT compatible lesions (white arrow) located in mesenteric lymph node of one red deer.

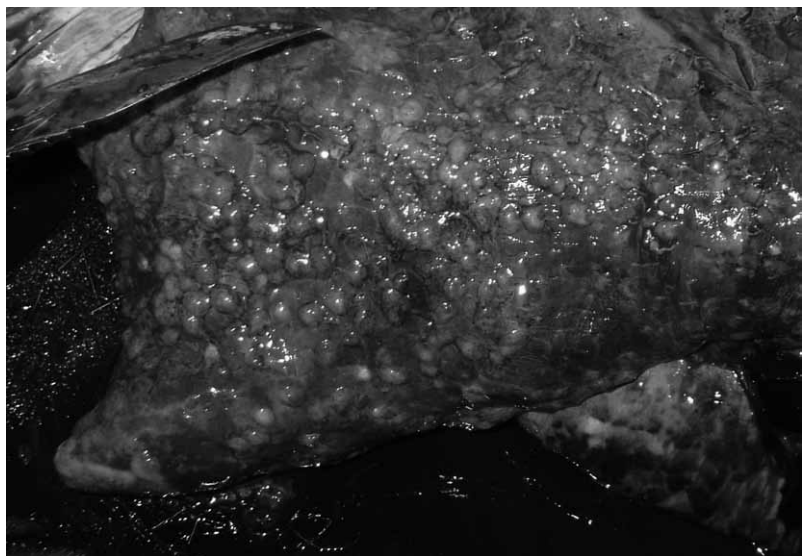

Figure 2. BT compatible lesion located in lung of one red deer.

GIS analyses of the field work allowed the creation of a continuous disease intensity maps represented by a greyscale intensity degree scale as presented in Figure 3. The percentage values represent the affected BT animals in the total of hunted animals per drive hunting day.

Figure 3 shows, that tuberculosis in wild boars (Figure 3, left side) is scattered throughout the county (circle dimension). After geostatistical calculation (grey gradient), it is possible to see a major vector from the south-south-east in a northward direction, indicating an important level of tuberculosis spread. Tuberculosis in red deer (Figure 3, right side) is confined to the south-south-east area. Both maps indicate that the main areas affected with tuberculosis in wild boar and red deer are located in the south-east area of the county. These areas should be the first to be put under veterinary surveillance and for which strategies to reduce disease spread and prevalence should be developed.

According to Curtis (1999), one of the main advantages of using GIS in a disease surveillance programme is that this technique can be used as a first step for identifying areas that need improvement in their surveillance scheme. Also, the simple use of GIS as a map of known cases allows identifying the location of those environments considered relevant to the disease or health risk. This allows mental/intellectual exploration of the whole ecological situation, but especially in space and time, which are the most important dimensions of the diseases of wildlife (Pfeiffer and Hugh-Jones, 2002).

Our study shows that the south-south-east is home to much higher densities over a larger and more continuous area than is the case for the north regions. Also, more hunted animals are processed in the same area and the offal and by-products are often thrown out to feed the vultures in places equally accessible to other species like wild boar. We believe that this irresponsible act is responsible for perpetuation of this situation.

A National Eradication Programme for bovine tuberculosis has been in operation since 1987 and is presently based on systematic tuberculin testing and slaughter with bacteriological

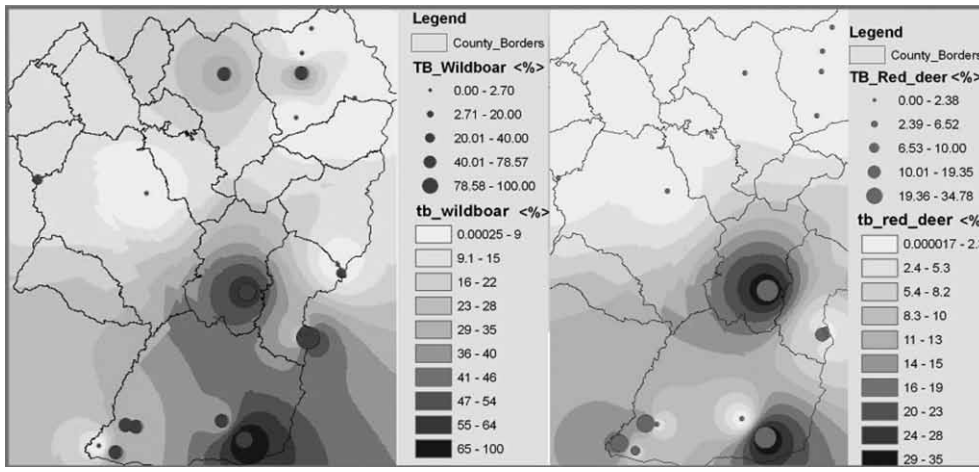

Figure 3. Bovine tuberculosis intensity map for wild boar and red deer hunted in Central Portugal.

diagnosis carried out at the National Reference Laboratory (NRL-LNIV) and permanent abattoir surveillance (Duarte *et al*., 2008). However, this programme can be undermined by game species if they act as reservoirs and spreaders of the agent. It is very important to make a GPS-GIS surveillance and control scheme and link it with the location of cattle farms so as to optimise resources in the right places and at the right time. A country scale reporting system, assessing an acceptable level of health in game species is recommended in a EU directive (92/45/CEE) (Artois, 2003).

4. Conclusions

Investigation of diseases in wild animals presents a special challenge to veterinary epidemiologists as the locations of individual animals within the populations at risk are much less predictable than are domestic animals. GIS can be considered an important tool to define objective strategies to prevent the spread of the infectious disease.

The following areas in which GIS and special GIS-functions could be incorporated include: recording and reporting information, epidemic emergency, cluster analysis, modelling disease spread, and planning control strategies.

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Section 3
Risk assessment and management

Risk Management of game: from theory to practice

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Summary

Risk Management is an integral part of Risk Analysis. It cannot be conducted in isolation from Risk Assessment and Risk Communication. Although it is generally assumed that formal Risk Management is carried out by government officials, in reality this need not be the case – risk can be managed within the industry as long as the process is functionally separated from Risk Assessment. The United Nations Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) have jointly produced the Food Safety Risk Analysis 'A guide for national authorities' (FAO/WHO, 2006), which provides a generic approach to Risk Management and is essential reading for risk managers. Responsibility for Risk Management lies with everyone in the food chain – in the case of game meat – 'from forest to fork'. This can include environmentalists, conservationists, hunters, food producers and processors, transporters, retailers, consumers and officials. Live animals and game meat may carry a great variety of biological, chemical and physical contaminants. These need to be judged in the context of their significance and the risks they pose to those handling or consuming game meat. At each stage of game meat production, every day, risks are managed by food producers and official controllers fulfilling their legal obligations. There is no prescribed rule as to when there is a need for formal Risk Management. Each case is different. Thus formal risk analysis may be required to deal with an actual or perceived risk to consumers when, for example, new emerging risks arise, or the evidence suggests that the current control measures are either ineffective or not cost-effective. Risk managers face many challenges during this process, of which perhaps the most difficult is 'to translate' and 'define' food safety issues in easily understood language and to provide evidence based answers from which effective, yet environmentally sustainable and proportionate measures can be developed.

Keywords: generic risk assessment, hazards, food handling, farmed game, wild game, United Kingdom

1. Introduction

There are many definitions applicable to Risk Management in general and to game meat production. These may be found in various dictionaries, in the Codex Alimentarius, in European Commission literature and in the laws of respective countries. For the purpose of this chapter consideration has been given to those found in the Codex Alimentarius (Box 1) and within European Union (EU) legislation. Game is defined as either 'wild' or 'farmed' in Regulation (EC) No. 853/2004 (EC, 2004):

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- *'wild game'* is defined as: 'ungulates and lagomorphs, as well as other land mammals 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.' It also includes 'wild birds that are hunted for human consumption'.
- '*farmed game*' includes 'farmed ratites and farmed land mammals'.

Box 1. Definitions of Risk Analysis terms related to food safety, according to FAO/WHO Rome 2007 'Working Principles for Risk Analysis for Food Safety for Application by Governments'.

Hazard – A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.
Risk – A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.
Risk Analysis – A process consisting of three components: Risk Assessment, Risk Management and Risk Communication.
Risk Assessment – A scientifically based process consisting of the following steps: (1) hazard identification; (2) hazard characterisation; (3) exposure assessment and, (4) risk characterisation.
Risk Management – The process, distinct from Risk Assessment, of weighing policy alternatives, in consultation with all interested parties, considering Risk Assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.
Risk Communication – The interactive exchange of information and opinions throughout the Risk Analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of Risk Assessment findings and the basis of Risk Management decisions.
Risk Assessment Policy – Documented guidelines on the choice of options and associated judgements for their application at appropriate decision points in the Risk Assessment such that the scientific integrity of the process is maintained.
Risk Profile – The description of the food safety problem and its context.
Risk Characterisation – The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment.
Risk Estimate – The quantitative estimation of risk resulting from risk characterisation.
Hazard Identification – The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.
Hazard Characterisation – The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable.
Dose-Response Assessment – The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response).

Exposure Assessment – The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.
Food Safety Objective (FSO) – The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).
Performance Criterion (PC) – The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO.
Performance Objective (PO) – The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable.

This chapter, in very broad terms, describes the main risks to the handlers and consumers which may arise from handling and consumption of game meat, the theory and practical means of managing those risks and the roles and responsibilities of all concerned. Its purpose is to provide an overview of the main hazards posed by game meat to humans as well as some practical suggestions for managing those risks. It does not intentionally go into the details of particular hazards, their prevalence in live animals and meat. The chapter also provides the non-exhaustive list of simple questions that will need answers when conducting Risk Management activities bearing in mind that each case is different.

Although not within the remit of this chapter, risk managers should be aware of their role in providing food which is not only free from contamination but also nutritionally safe. Game meats are recommended as preferable to conventional livestock by, for example, the World Cancer Research Fund and the American Institute for Cancer Research who state that 'if eaten at all red meat to provide less than 10% of total energy but consumption of meat from non-domesticated animals is preferable.'

2. Hazards in game meat (wild and farmed)

2.1 Which hazards are relevant in game meat?

Hazards here are divided into biological, chemical and physical hazards. Theoretically, there may be many hazards in or on live animals or game meat and this needs to be assessed as to the risk they pose to the public either through handling or eating game meat. Some examples of hazards are:

- Biological: *Salmonella* spp., *Campylobacter* spp., *Clostridia* spp., *Listeria,* entero-haemorrhagic *Escherichia coli* O157*, Staphylococcus aureus, Yersinia enterocolitica, Chlamydia* spp., *Francisella tularensis, Brucella* spp., *Mycobacterium bovis/avium,* aflatoxin, some viruses (e.g. avian influenza), yeasts, fungi, parasites (e.g. *Trichinella, Toxoplasma gondii*), etc.
- Chemical: residues of antimicrobials, antiparasitics, pesticides, heavy metals (e.g. lead, cadmium, mercury, etc.), other environmental chemicals (e.g. dioxins), hormones or hormone like substances, mycotoxins, etc. and radioactive isotopes.
- Physical such as foreign bodies (bone, glass, metal, plastic, etc.).

2.2 How do we know about hazards?

The majority of hazards are invisible and may cause no damage to the animal but sometimes they will cause observable symptoms and/or lesions. This is particularly true for the major biological contaminants causing food poisoning, e.g. *Salmonella, Campylobacter,* or chemical contaminants such as lead, mercury, etc. In many cases the hazards may not be obvious and we need to rely on reports, surveillance data, animal and human data, and other sources of data, e.g. from the environment.

2.3 How do hazards enter the live animal?

This depends on the type of hazard, whether it is biological, chemical or physical. Animals are exposed to risk through direct and indirect contact with the environment which may include equipment, other animals (including vectors) and interaction with humans or human activities, e.g. feeding. Whilst this may be accidental, in some cases, as for example in the irresponsible disposal of chemical waste to the environment, it may approach the deliberate introduction of hazards to live animals.

There are many factors which may influence whether or not contaminants may harm animals. This, for example in the case of microbial hazards, will depend on many interrelated factors such as virulence, pathogenicity, pathway and susceptibility of animals. Exposure to chemical hazards usually but not invariably involves ingestion, as for example in the case of game being shot when physical hazards (pellets, dirt, etc.) are introduced to live animals.

Because wild animals graze and browse freely and may not be killed until many years old they are especially susceptible to contamination from heavy metals such as cadmium, and from radioactive fallout such as that which was released following the accident at Chernobyl. These may accumulate during the lifetime of the animal to reach levels deemed toxic.

2.4 How do hazards enter meat?

Chemical hazards such as lead and mercury may be accumulated in offal, e.g. liver, and kidneys, and to a lesser extent in meat, while animals are alive. Physical hazards are usually introduced when animals are shot.

Biological hazards may be present in the meat before shooting as in parasites such as *Trichinella.* Bacteria are normally present in the intestines or on the skin of animals and may be introduced onto or into the meat during the shooting, or at the first stage of dressing and evisceration in the field. In small wild game it is also possible that hazards may be introduced from the mouth of dogs retrieving the game.

In all subsequent stages of the production cycle – transport, storage, dressing, cutting and processing – bacteria or fungi may contaminate the surface of the meat. The survival and further multiplication of the microbes will depend on the availability of food, temperature and water activity. Bacteria and fungi may also contaminate meat during food preparation in the kitchen.

3. Risk Management: from theory to practice

3.1 Risk Management principles

Risk and Risk Management terms are defined by WHO/FAO as follows:

- '*Risk*: a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.'
- *'Risk Management:* the process, distinct from Risk Assessment, of weighing policy alternatives, in consultation with all interested parties, considering Risk Assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control.'

The Codex Alimentarius Commission paper (CAC, 2007) provides 'Working Principles for Risk Analysis for Food Safety for Application by Governments'. They advocate applying the following principles when managing risk:

1. National government decisions on Risk Management, including sanitary measures taken, should have as their primary objective the protection of the health of consumers. Unjustified differences in the measures selected to address similar risks in different situations should be avoided.
2. Risk Management should follow a structured approach including preliminary Risk Management activities, evaluation of Risk Management options, implementation, monitoring and review of the decision taken.
3. The decisions should be based on Risk Assessment, and should be proportionate to the assessed risk, taking into account, where appropriate, other legitimate factors relevant for the health protection of consumers and for the promotion of fair practices in food trade, in accordance with the Criteria for the Consideration of the Other Factors Referred to in the Second Statement of Principles as they relate to decisions at the national level. National Governments should base their sanitary measures on Codex Standards and related texts, where available.
4. In achieving agreed outcomes, Risk Management should take into account relevant production, storage and handling practices used throughout the food chain including traditional practices, methods of analysis, sampling and inspection, feasibility of enforcement and compliance, and the prevalence of specific adverse health effects.
5. Risk Management should take into account the economic consequences and the feasibility of Risk Management options.
6. The Risk Management process should be transparent, consistent and fully documented. Decisions on Risk Management should be documented so as to facilitate a wider understanding of the Risk Management process by all interested parties.
7. The outcome of the preliminary Risk Management activities and the Risk Assessment should be combined with the evaluation of available Risk Management options in order to reach a decision on management of the risk.
8. Risk Management options should be assessed in terms of the scope and purpose of Risk Analysis and the level of consumer health protection they achieve. The option of not taking any action should also be considered.
9. Risk Management should ensure transparency and consistency in the decision-making process in all cases. Examination of the full range of Risk Management options should,

as far as possible, take into account an assessment of their potential advantages and disadvantages. When making a choice among different Risk Management options, which are equally effective in protecting the health of the consumer, national governments should seek and take into consideration the potential impact of such measures on trade and select measures that are no more restrictive than necessary.

10. Risk Management should be a continuing process that takes into account all newly generated data in the evaluation and review of Risk Management decisions. The relevance, effectiveness, and impacts of Risk Management decisions and their implementation should be regularly monitored and the decisions and/or their implementation reviewed as necessary.

3.2 Generic Risk Management framework

Risk Management, in general terms, is described in the Food Safety Risk Analysis 'A Guide for national authorities' (FAO/WHO, 2006). The generic Risk Management Framework (RMF) is a structured process for food safety regulators consisting of four major phases and numerous specific activities (Figure 1). The four phases are:

1. Preliminary Risk Management activities.
2. Identification and selection of Risk Management options.
3. Implementation of Risk Management decision.
4. Monitoring and review.

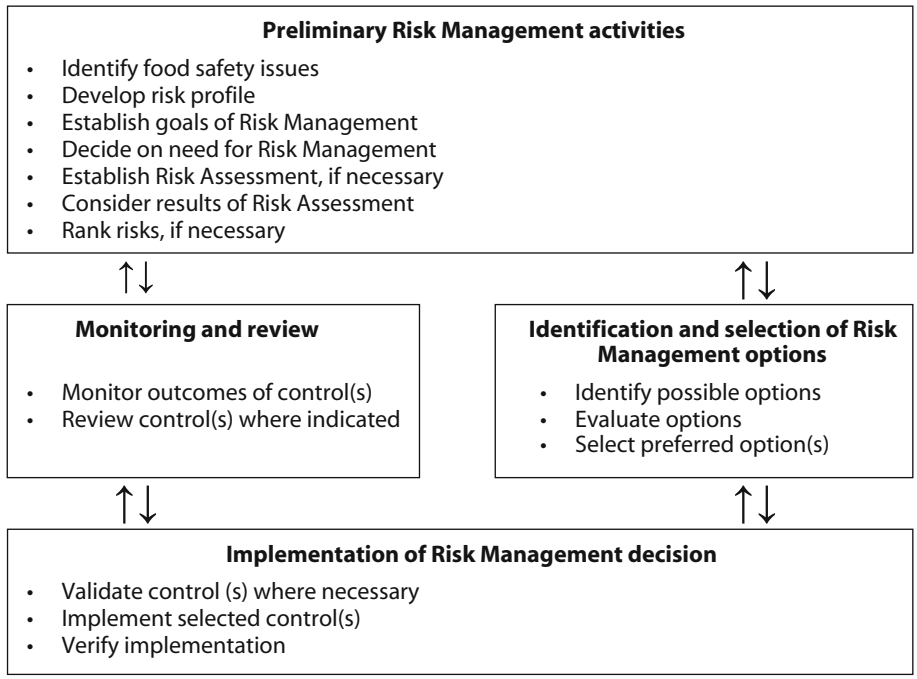

Figure 1. Generic framework for Risk Management (FAO/WHO, 2006).

Most stages of Risk Management cannot be seen in isolation and will require extensive communication, coordination and collaboration between risk managers, risk assessors and stakeholders. Whilst the responsibility for formal Risk Management usually lies with the managers of the national food safety authority, in practice managers from industry may serve as risk managers.

Each formal Risk Management case is different. An approach to each case will depend on many factors such as the nature of the hazard, the actual and/or perceived risks to consumers, the economic situation, etc. Throughout the Risk Analysis process the risk managers need to make many subjective judgements and find answers to a number of specific questions. Under each heading below there are examples of some simple questions that will need answers. However, on a case-by-case basis there may be many more questions. Transparent Risk Communication with all stakeholders starts at the beginning of the process and lasts till the end of Risk Analysis. With increased awareness of the importance of minimising 'carbon dioxide footprint', Risk Management must always assess environmental sustainability in evaluating what is proportionate.

3.2.1 Preliminary Risk Management tasks

Identify and describe the food safety issue – What is the problem? What is an issue? Why is it necessary to manage the risk? Develop the risk profile – What do we know about the problem so far? What evidence is available? What is the situation? How are handlers and consumers exposed to the hazards? What are the possible risks? What are the actual and the perceived risks? What are consumers' perceptions? What are current control measures? How effective are current control measures? Establish the goals of Risk Management – What needs to be achieved in broad terms? Decide on the need for Risk Assessment – Is formal Risk Assessment needed? Why is formal Risk Assessment needed? How much will it cost? Establish the Risk Assessment policy – What needs to be assessed? Will it be quantitative or qualitative Risk Assessment? Commission a Risk Assessment, if necessary – Who will make the Risk Assessment? How will the independence of the risk assessor be judged? Will risk assessors be functionally independent? Consider the results of Risk Assessment – What are the strengths/weaknesses of Risk Assessment? What are the uncertainties raised in Risk Assessment? Is there any (immediate/short/long term) action required? Rank the risks, if necessary – What is the relative importance of a risk?

3.2.2 Identification and selection of Risk Management options

Identify the possible options – What needs to be done to control risks? Is immediate/short/long term action required? Evaluate the options – What is the effectiveness, including cost effectiveness, of the different options in achieving objectives? Who can conduct the Risk Management options? Is any action justified? Select the preferred option(s) – Which options are effective and cost effective?

3.2.3 Implementation of Risk Management decision

Validate the control(s) where necessary – Will controls achieve the objectives that are set up? Who will do the validation? Implement the selected control(s) – Who will implement the chosen options? Verify implementation – How is it implemented?

3.2.4 Monitoring and review

Monitor outcomes of control(s) – What evidence exists to support the effectiveness of chosen options? Review control(s) where indicated – Are the controls effective? Would new Risk Assessment be useful?

4. Wild and farmed game meat production

4.1 Wild game meat production

Wild game meat may be sold directly to the final consumer by the hunter or through the shops and supermarkets. It is estimated that in the EU over 50% of wild game meat goes through an unapproved food chain with no official veterinary meat inspection being carried out. Initial *post mortem* examination is performed in the field by a trained hunter who must sign a declaration about any abnormal behaviour that may have been observed before killing and relevant *post mortem* findings. It is an EU legal requirement that hunters must be trained in health and hygiene, so they must have sufficient knowledge of the pathology of wild game to undertake initial examination of wild game on the spot.

Each wild game species has 'seasons' within which they can legally be hunted. The British Association for Shooting & Conservation (BASC) lists the hunting seasons for the species found in the UK (see http://www.basc.org.uk//en/departments/game-and-gamekeeping/game-shooting/shooting-seasons.cfm). The vast majority of wild game animals are killed by shooting, with the exception of some small wild game (rabbits and hares) which can be trapped before being dispatched by a blow to the back of the head. Game birds, wild waterfowl and small wild game are shot using cartridges that contain a number of small pellets (some may be lead pellets although substitutes are being introduced, especially for waterfowl), whereas large wild game, e.g. wild deer and wild boar, are shot with a bullet. Deer are generally gralloched (eviscerated) on the hill by trained hunters and an initial *post mortem* inspection carried out by trained hunters who will sign a declaration that will accompany the carcase to the processing plant where final veterinary *post mortem* inspection will take place.

There is variation in the time between animals being killed and their arrival at the plant for processing. Animals are generally shot 24 hours before being transported for processing, but on occasions the carcases may be held for several days in refrigerated facilities at the hunting ground before transportation to the processing plants. This period will depend on the remoteness of the hunting ground and the proximity of game handling establishments. It would be environmentally unsustainable and uneconomic to send a refrigerated vehicle many miles for small numbers of game carcasses. Carcasses should be suspended during

transport, however game bird carcasses and waterfowl carcasses may be laid down on vehicle floors provided they are not warm. They should not be heaped up to a great depth. After killing and arrival at the processing plant the carcases are chilled within a reasonable period of time: large wild game to less than 7 °C and small wild game to less than 4 °C.

Traditionally in Britain wild game birds, as well as hares and deer, may be 'hung' in well ventilated refrigerated spaces for a period of several days to tenderise the meat and enhance the flavour. Game birds are then usually de-feathered 'dry' using plucking machines, although some processing plants may immerse duck or geese carcases in hot wax to aid feather removal. Carcases may be eviscerated manually, or by machines which act by sucking out intestines. If breast meat only is required, as for example is commonly the case with woodpigeons, birds are not de-feathered prior to removal of the meat.

Large wild game (deer and boar) are usually skinned at the plant and most of them require trimming due to contamination during shooting. When animals have been poorly shot in the abdominal area this can result in excessive contamination and total condemnation of the carcase. In approved processing plants *post mortem* inspection of wild game carcases and available offal are carried out as per regulation.

4.2 Farmed deer meat production

Farmed deer are usually killed at the place of rearing. Carcases are processed and meat sold to the consumer through an established route similar to that of conventional domesticated livestock. There is no hunting season; farmed deer can be killed throughout the year. *Ante mortem* inspection is carried out by a veterinarian within 72 hours of death and where the deer are to be killed in a field by free bullet this is best conducted by observing the entire herd at rest. Farmed deer may be killed in the field by a free bullet to the head or upper neck. They may also be brought into handling facilities or a conventional abattoir for stunning with a captive bolt to the frontal region and bleeding. Meat inspection is carried out by qualified meat inspectors and the premises are audited by qualified veterinarians. Farmed deer meat must be cut and processed in approved cutting plants in the same way as the meat of conventional livestock.

5. Practical Risk Management in game meat

5.1 An assessment of hazards and risks from game meat

Farmed game meat production in the UK has been, for many decades, subject to veterinary official controls. However, prior to 2006, the majority of wild game meat produced for the UK domestic market was not subject to veterinary inspection, with the exception of some sold by larger retailers. As a part of the hygiene package the EU was, at that time, proposing three legal measures (later adopted) relevant to wild game meat production.

1. The training of hunters.
2. Audited Hazard Analysis and Critical Control Points (HACCP), and
3. Official *post mortem* inspection of all.

The UK Food Standards Agency (FSA) wished to assess, in particular, the necessity for veterinary presence and *post mortem* inspection, over and above the fully implemented controls on general hygiene (Good Hygiene Practices (GHP) and HACCP) and the removal of obviously unfit carcases by non-veterinary staff. To enable such an assessment, the FSA requested a qualitative Risk Assessment to address the following risk question:

> Under current UK law, what is the risk to human health (particularly of human infection with a foodborne pathogen) from the handling/consumption of wild game meat and how would the currently proposed EU hygiene proposal affect the risk ?

A qualitative Risk Assessment of hazards in wild game was made in 2003 (Coburn *et al*., 2005). It summarises the risk from the identified hazards and concludes that veterinary *post mortem* inspection alone will not significantly decrease the risk in the majority of cases as long as an effective application of procedures based on HACCP principles and removal of obviously unfit carcases takes place.

Different factors affect the risk from each hazard and these have been discussed within the Risk Assessment. Hazards that have been identified as potentially harmful for human health were those commonly found in different species: *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157, *Clostridium botulinum*, *Yersinia pseudotuberculosis*, *Mycobacterium avium/bovis* and lead shot. Where data could not be identified, expert opinion has been elicited and relevant data from other countries or species has been used.

Four categories of wild game were considered with potential risk pathways: (1) wild game birds; (2) waterfowl; (3) large wild game; and (4) small wild game. Regardless of different species each pathway has the same processing structure and similar risks (Figure 2). An assessment of consumers' kitchen hygiene and malpractices has also been made (Table 1).

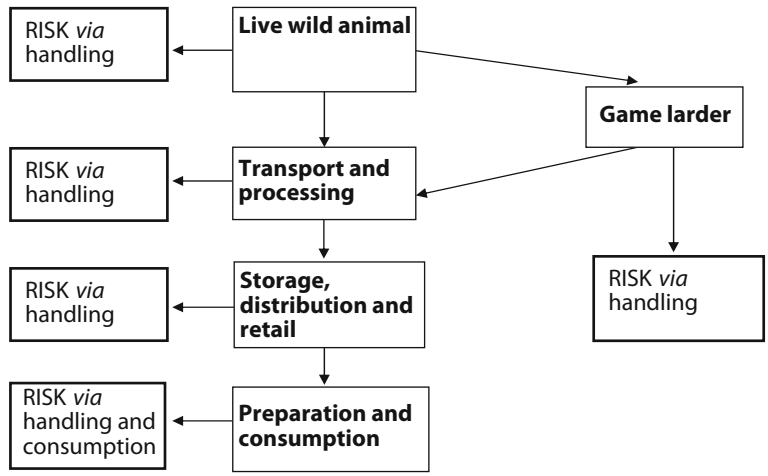

Figure 2. Live wild animal-to-fork pathway for wild game animals.

Table 1. Incidence of food handling malpractices (adapted from Worsfold and Griffith, 1997)

Preparation stage	Behaviour	% Occurrences	Result
Transport	Transported food subject to temperature abuse	45	Possible growth of organism
Refrigerated storage	Chilled ingredients stored above 5 °C	58	
Handling and preparing raw foods	Hands not washed prior to preparation	66	Possible cross-contamination and contamination of hands
	Hands not washed after handling raw animal ingredients	58	
	Meat/poultry packaging not removed from preparation area	18	
	Raw poultry washed in sink	33	
	All ingredients cut on a single board	60	
	Failure to use recommended method of cleaning cutting board	25	
Cooking	Product not cooked to internal temperature of at least 74 °C	15	Possible survival of organism
Post cooking handling	Cooked product held at ambient temperature for more than 90 minutes	35	Possible growth of organism
	Cooked food cut on board not cleaned according to recommended method	8	Possible cross-contamination and contamination of hands
	Cooked food handled directly	9	
	Cooked product stored in refrigerator at a temperature above 5 °C	17	Possible survival of organism
	Product not reheated to an internal temperature of at least 74 °C	11	Possible survival and growth of organism
	Product reheated more than once, with intervening periods at room temperature	6	

In general, if the organism is present there is plenty of opportunity for growth and cross-contamination, and particularly contamination of hands. The percentage of cooking steps that fail to heat food adequately is also not negligible; therefore if the organism is present there is a low, but not negligible probability that it remains viable. This coupled with the probable growth and cross-contamination of organism if present, means that it is quite likely to grow and spread.

6. Conclusions

What has been achieved?

There are potentially a number of risks associated with handling and eating game. An assessment of the most obvious bacterial species and lead found that there was a low risk to human health from the handling and consumption of game species in the UK. Proper handling and cooking of game meat will destroy most microbial organisms; however, this is not always the case. Every day Risk Management in game meat production is achieved by all involved 'from forest to fork'. Food producers are legally obliged to apply Good Hygiene Practices (GHP) and procedures based on Hazard Analysis and Critical Control Points (HACCP) principles continuously, properly and proportionately. Official controllers, on the other hand, are required to perform auditing and inspection tasks (*ante* and *post mortem* inspection, when appropriate) in order to verify food producers' legal obligations. There is also guidance available to help everyone in the food chain to manage risks appropriately on a daily basis. More comprehensive general guidance, from WHO/FAO, is available for managers when conducting formal Risk Management activities.

What has been neglected?

It is important to acknowledge that handling and/or consumption of game meat may also result in human exposure to novel diseases. The knowledge about these agents or diseases is sparse in wild game meat species. It is also true that more research and data is needed to assess the risk from handling and consuming game meat outside approved food chain and from different species. Risk Management poses many challenges for risk managers, risk assessors and risk communicators, of which perhaps the most difficult is 'to translate' and 'define' food safety issues in easily understood language and to provide evidence based answers from which effective and proportionate measures can be developed. This can only be done by people who are properly trained.

What needs to be done?

Each Risk Management case is different and must be done in a systematic well documented and transparent way. It cannot be done in isolation from Risk Assessment and Risk Communication. An approach to each Risk Management case will primarily depend on whether it is necessary to deal with: (1) an actual and/or perceived risk to handlers or consumers; (2) new unknown emerging risks; and (3) known risks where the evidence suggests that the current measures are not effective in controlling the risks or are unnecessary. More training, better collaboration and exchange of information is required from all involved handling and consuming game 'from forest to fork'.

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The monitoring of selected zoonotic diseases of wildlife in Lombardy and Emilia-Romagna, northern Italy

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Summary

Several zoonotic agents that infect wildlife may be transmitted to humans through contaminated game meat. *Toxoplasma gondii* and *Trichinella* spp. infect the muscular tissue at some stage of their biological cycle and consequently contaminate meat, while enteric bacteria (e.g. *Salmonella* spp., verocytotoxic *Escherichia coli* (VTEC), *Yersinia enterocolitica*) may contaminate the carcase after the animal's death when the intestine is ruptured by shot pellets or during evisceration. Gutting and skinning of carcases may lead to contamination of humans with *Mycobacterium bovis*, *Brucella* spp., and *Francisella tularensis*, the etiological agents of bovine tuberculosis, brucellosis and tularaemia, respectively. We report here the occurrence of the above mentioned organisms over a 5-year period in some species of wild mammals of Lombardy and Emilia-Romagna, northern Italy. Trichinellae were recovered in 0.15% and 0.13% of samples from red foxes and wild boars, respectively. Antibodies to *T. gondii* were detected in a percentage ranging from 23.3% to 33.3% of wild ruminants and wild boars, and the parasite was also directly detected in European hares. Several serotypes of *Salmonella* spp. were recovered from cervids, red foxes and wild boars with prevalences from 1.46% to 18.7%. Faecal samples from roe deer tested negative for VTEC, while *Y. enterocolitica* was detected in about 8% samples from roe deer and from a few wild boars. *M. bovis* was rarely recovered from red deer and red foxes, and occasionally from wild boar, where *M. microti,* another species belonging to the *Mycobacterium tuberculosis* complex, occurred much more frequently. Antibodies to *Brucella* spp. were very rarely detected only in European hares, while *F. tularensis* was detected by PCR in about 7% European hares. This monitoring

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programme was conceived and implemented by the regional and local administrations in close collaboration with the official veterinary services and the hunters' associations.

Keywords: wild mammals, infectious disease, parasitic disease, zoonosis

1. Introduction

In the last few years, wildlife health has progressively become a cause for common concern for different stakeholders, including veterinary services, public administrations, wildlife conservationists, hunters, gamekeepers, farmers and for the whole community as well. In Europe, changes in agricultural land use and in wildlife management practices have recently and dramatically influenced the population dynamics of wildlife, often leading to an overabundance of species (e.g. wild boars and roe deer) in several areas, which may in turn impact on the health status of the populations (Gortázar *et al*., 2006).

Wild animals naturally harbour a number of agents of infectious and parasitic diseases which may be transmitted to livestock and humans (Simpson, 2002; Bengis *et al*., 2004; Kruse *et al*., 2004; Böhm *et al*., 2007). Transmission of diseases from wildlife to livestock and *vice versa* is related to several variables, such as animal movements, restocking and environmental changes, that lead to increased contact opportunities between animal populations, and allow pathogenic organisms to cross species barriers. The risk of exchange of pathogens and transmission of diseases between domestic and wild animals increases significantly when contacts occur in common grazing areas, in open-air livestock rearing conditions and in organic livestock production systems, and can be also mediated by vectors (Lanfranchi *et al.,* 2003; Gortázar *et al.,* 2007; Kijlstra *et al*., 2009). Climate change also influences the development of infectious and parasitic diseases in free-ranging animals by affecting the distribution of vectors and the migratory routes of wildlife (Gilbert *et al*., 2008). As to the relevance of wildlife as a source of disease for humans, it should be noted that about 60% of emerging infectious diseases globally detected in human populations between 1940 and 2004 were zoonotic, i.e. acquired from animals and about 70% of them originated from a wildlife reservoir (Jones *et al*., 2008). As a consequence, the interest for the detection of pathogens in wildlife has considerably grown in the last few years, leading to the development of disease monitoring programmes in several European countries (Artois *et al.,* 2001; Mörner *et al*., 2002).

In Italy, infectious and parasitic diseases of wildlife are monitored through laboratory testing carried out by different institutions. Among them, departments from several universities and a network of region-based governmental laboratories (the Istituti Zooprofilattici Sperimentali) are prominently involved. The National Reference Centre for Wildlife Diseases (Ce.R.M.A.S.), based in Aosta, northwestern Italy, provides the coordination of the activities of the regional laboratories and the transmission of data of the wildlife health monitoring programmes to the World Organisation for Animal Health (OIE). In Lombardy and Emilia-Romagna, two regions in northern Italy, the monitoring of diseases of wildlife has been systematically carried out for more than 10 years, and is based on the collection of data on the occurrence of infectious and parasitic diseases which directly impact the health status of the animal populations, or are shared between livestock and wildlife, or are zoonotic.

In this contribution, we present data on selected zoonotic diseases that can be transmitted from wildlife to humans through contact with contaminated game meat either by ingestion or handling during gutting and skinning procedures preliminary to consumption. Data refer to the results of laboratory tests carried out through a five-year (2005-2009) monitoring programme, and refer both to the direct detection of selected pathogenic organisms, as well as to indirect tests for the detection of specific antibodies.

2. Study area

The data reported in this communication refer to wildlife of the territories of Lombardy and Emilia-Romagna, two regions in northern Italy. Lombardy covers an area of 23,861 km^2 and is bordered by Switzerland to the north and by other Italian regions to the west, east and south. Its northern part is a mountainous area limited by the Central Alps chain whose altitude ranges from 1,000 to 4,020 m. Moving south, the mountains (40.6% of the territory) slope into the hills (12.4%) and then to the plains of the Po Valley (47%). Emilia-Romagna is a region located south of Lombardy and bordered on all sides by other Italian regions and by the Adriatic Sea. The region covers an area of 22,124 km^2 and approximately half of it, in its northern part (48% of the territory), consists of the plains of the Po Valley, while the remaining part of the region is covered by hills (27%) and mountains (25%), up to an elevation of 2,121 m.

The territories of both Lombardy and Emilia-Romagna are characterised by natural and semi-natural habitats with different levels of human activities. Both regions are highly industrialised, yet intensive farming is well developed. In addition, limited traditional farming activities are still maintained in some marginal hilly and mountainous areas where, in summertime, large flocks of sheep and goats are taken up to the alpine pastures.

Wildlife in the two regions include several species of wild ruminants, some of which – the Alpine chamois and the Alpine ibex – are only found in the alpine area of Lombardy. Roe deer and red deer are found in both regions, with a larger population of the former species in Emilia-Romagna. Wild boar, red fox and several species of mustelids, lagomorphs, and rodents are also widespread. In addition, the wolf (*Canis lupus*) and the bear (*Ursus arctos*) have sporadically reappeared following reintroduction projects or for natural radiation. Due to a combination of changes in agricultural land use, and in forest and wildlife management practices, all populations of traditionally hunted wild mammals, particularly wild ungulates, have significantly increased over the last twenty years to the sizes presented in Table 1. This has been favoured by the reduction of traditional farming practices, which has made available to wildlife vast areas formerly exploited by humans.

Wildlife disease monitoring programmes in the two regions were initially – around 1998 – implemented by the official veterinary services at the request of the hunters' association. Since then, at the beginning of each year, the veterinary services in collaboration with the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER), the regional governmental laboratory, have been planning the activities. Several zoonotic diseases are included in the monitoring programmes, along with some livestock diseases for which wild animals may act as a reservoir (e.g. classical swine fever and pseudorabies).

Table 1. Estimated population sizes (as to 2008) for some wild mammals in Lombardy and Emilia-Romagna.

Species	Region	
	Lombardy	Emilia-Romagna
Roe deer (*Capreolus capreolus*)	26,000	90,000
Red deer (*Cervus elaphus*)	7,300	4,000
Alpine chamois (*Rupicapra rupicapra rupicapra*)	19,500	-
Alpine ibex (*Capra ibex ibex*)	2,600	-
Mouflon (*Ovis musimon*)	800	250
Wild boar (*Sus scrofa*)	3,300 (hunting bag/year)	17,000 (hunting bag/year)

Sources: Regione Lombardia, Direzione Generale Agricoltura [http://62.101.84.225/agrinet/fauna/rapportofauna2008.htm]; Regione Emilia Romagna, Servizio Territorio Rurale [http://www.ermesagricoltura.it/Box-Informazioni/Politiche-Faunistiche-e-Venatorie/Osservatorio-Faunistico-Venatorio/Dati/Ungulati].

Hunters are asked to collect samples (blood, faeces, swabs, and/or viscera) from animals harvested during the regular hunting seasons, and both hunters and gamekeepers are instructed to deliver the carcases of dead animals to the laboratory for collection of samples at necropsy. In few cases, samples are also collected from injured animals temporarily hosted in rehabilitation centres. Investigations are carried out in several departments of IZSLER for the detection of organisms that infect the muscle tissue (*Trichinella* spp., *Toxoplasma gondii*), or that may contaminate the carcases due to intestinal rupture (enteric bacteria: *Salmonella* spp., verocytotoxic *Escherichia coli* (VTEC), *Yersinia enterocolitica*) or due to contact with other infected tissues or organs during skinning and gutting (other bacteria: *Francisella tularensis*, *Brucella* spp., *Mycobacterium bovis*). Bacteriological, parasitological and serological examinations are carried out following the procedures recommended in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2009a) as summarised in Table 2.

All results are presented in Table 3.

3. Diseases

3.1 Trichinellosis

Trichinellosis is a zoonotic parasitic disease caused by nematodes of the genus *Trichinella* that mainly infect carnivorous and omnivorus mammals with cannibalistic and scavenging behaviour. Some trichinellae also infect birds and reptiles (Pozio, 2005). All *Trichinella* species are transmitted directly by ingestion of parasitised muscle tissue and are maintained in nature in sylvatic or domestic cycles, the latter mainly involving domestic pigs. Humans are accidental hosts of trichinellae and are infected following consumption of contaminated raw or undercooked meat (Pozio, 2000).

Table 2. Organisms considered and detection methods employed in the monitoring programme.

Organism	Detection methods	
	Direct method	Serological test
Trichinella spp.	artificial digestion	nd
Toxoplasma gondii	PCR	ELISA
Salmonella spp.	culture, serotyping	nd
Verocytotoxic *Escherichia coli*	culture, PCR	nd
Yersinia enterocolitica	culture	nd
Mycobacterium bovis	PCR, culture	nd
Brucella spp.	PCR	CFT, RBT
Francisella tularensis	PCR, culture	AT

Legenda: PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; AT = agglutination test; CFT = complement fixation test; RBT = Rose Bengal test; nd = not done

In Europe, four species of trichinellae have been detected in wild and domestic animals: *T. spiralis*, *T. britovi*, *T. nativa* and *T. pseudospiralis*. The first two species are the most widespread and occur up to 60-61° latitude north, while *T. nativa* is only found in arctic and subarctic regions, and limited data are available as to the overall distribution of *T. pseudospiralis* (Pozio *et al.,* 2009). *T. britovi* is known to occur in Italy in several wild species, including red foxes and wild boars, and *T. pseudospiralis* has been detected in nocturnal birds of prey (Pozio *et al*., 1999; Pozio, 2007).

According to Regulation (EC) No. 2075/2005 of the European Union legislation (EC, 2005), carcases of farmed and wild animal species susceptible to *Trichinella* infestation shall be systematically examined for trichinellae. Data from the monitoring programme in wildlife is required when applying for *Trichinella*-free status for a pig herd (EC, 2005).

Our data refer to the testing of samples of muscular tissue collected from the diaphragm and the forearm muscles, which are the predilection sites for the localisation of trichinellae in the wild boar and red fox, respectively, and processed by artificial digestion according to the EU regulation (EC, 2005).

Trichinellae were detected in the muscular tissue of several wild boars (n=36) sampled in Lombardy in 2007 and 2008, and in a red fox and a wild boar sampled in Emilia-Romagna in 2008 and 2009, respectively (Table 3). Typing was carried out by multiplex PCR (Pozio and La Rosa, 2003) only for the latter two trichinellae, and confirmed the occurrence of *T. britovi* in the fox, while the isolate from the wild boar was identified as *T. pseudospiralis*, a species previously identified in wild boars of France, Finland, Sweden and the Netherlands, as well as in other mammals and birds (Pozio and Zarlenga, 2005). The detection of *T. britovi* in the red fox confirms the circulation of this *Trichinella* species in the study area (Pozio, 2007), while

Table 3. Test results of a 5-year wildlife disease monitoring programme for selected diseases in Lombardy and Emilia-Romagna.

Disease or infection	Animal species	Detection method[1]	Year					Total Prevalence % (95% CI)
			2005 positive/tested	2006 positive/tested	2007 positive/tested	2008 positive/tested	2009 positive/tested	
Trichinellosis	wild boar	AD	0/191	0/1,196	11/6,111	26/11,106	1/10,468	38/29,072 0.13 (0.09-0.18)
	red fox	AD	-	0/21	0/87	1/204	0/336	1/648 0.15 (0.01-1.00)
Toxoplasmosis	roe deer	ELISA	-	-	-	-	122/460	122/460 26.52 (22.59-30.85)
	wild boar	ELISA	4/23	-	63/281	7/13	-	74/317 23.34 (18.88-28.47)
		PCR	-	-	0/53	-	-	0/53 0 (0.00-5.49)
	European hare	PCR	1/1	1/10	1/5	3/71	3/120	9/207 4.35 (2.14-8.36)
	mouflon	ELISA	-	-	-	-	5/19	5/19 26.31 (10.12-51.42)
	red deer	ELISA	-	-	-	-	5/15	5/15 33.33 (12.99-61.31)
Brucellosis	wild boar	CFT	0/152	0/162	0/433	0/307	0/192	0/1,246 0 (0.00-0.24)
	roe deer	RBT, CFT	0/224	0/24	0/136	0/13	0/142	0/539 0 (0.00-0.55)
		PCR	-	-	0/19	-	0/58	0/77 0 (0.00-3.8)
	European hare	RBT, CFT	0/14	0/109	0/93	2/195	0/99	2/510 0.39 (0.06-1.29)
	red deer	CFT	-	0/52	0/9	-	0/164	0/225 0 (0.00-1.32)
	chamois	CFT	-	0/191	-	-	-	0/191 0 (0.00-1.55)

Salmonellosis	wild boar	C	-	92/311	123/666	79/643	147/736	441/2,356 18.7 (17.41-20.10)
	roe deer	C	-	-	2/110	2/104	1/16	5/230 2.17 (0.8-5.2)
	red deer	C	-	-	1/67	0/36	2/102	3/205 1.46 (0.37-3.93)
	red fox	C	-	0/17	2/17	-	0/3	2/37 5.13 (0.86-15.92)
	chamois	C	-	-	-	-	0/10	0/10 0 (0.00-25.88)
Infection by VTEC	roe deer	C, PCR	-	-	0/43	0/81	-	0/124 0 (0.00-2.38)
Yersiniosis by *Y. enterocolitica*	roe deer	C	0/10	0/6	10/114	9/107	-	19/237 8.02 (5.02-12.43)
	red deer	C	-	0/11	-	-	-	0/11 0 (0.00-23.84)
	chamois	C	-	0/10	-	-	-	0/10 0 (0.00-25.88)
	wild boar	C	-	4/5	-	-	-	4/5 80 (29.88-98.95)
Tularaemia	European hare	AT	8/2,394	12/2,658	15/1,693	2/1,980	20/2,376	57/11,101 0.51 (0.39-0.67)
		PCR	8/134	0/91	0/43	3/34	8/92	19/260 7.31 (4.58-11.36)
	wild boar	AT	0/231	-	-	0/14	7/372	7/617 1.13 (0.50-2.43)
		PCR	-	-	-	-	0/35	0/35 0 (0.00-8.20)
	roe deer	AT	0/248	-	-	0/22	0/312	0/582 0 (0.00-0.51
	chamois	AT	-	0/85	-	-	-	0/85 0 (0.00-3.46)

[1] Detection methods used: AD = artificial digestion; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; CFT = complement fixation test; RBT = Rose Bengal test; C = culture; AT = agglutination test.

the occurrence of *T. pseudospiralis* in the wild boar adds new information, as this species had never been reported before in Italy in a mammalian host.

3.2 Toxoplasmosis

The protozoan parasite *Toxoplasma gondii* infects all warm-blooded animals (mammals and birds). It multiplies asexually in several mammal and bird species, which are intermediate hosts, and reproduces sexually only in domestic and wild felids, which act as definitive hosts. In intermediate hosts, *T. gondii* forms tissue cysts that can be mainly found in neural and muscular tissues, and are occasionally detected in visceral organs such as lung, liver, kidneys, while definitive hosts shed the oocysts of the parasite in their faeces. Transplacental infections may also occur in several animal species (Dubey and Beattie, 1988). Wild ruminants are infected through ingestion of *T. gondii* oocysts shed by felids, which is the case also for the wild boar that can be infected also through consumption of parasitised muscle tissue (Gauss *et al.*, 2005, 2006).

Antibodies to *T. gondii* in wildlife have been found in several surveys carried out in European wildlife, with the highest levels of seropositivity and antibody titers in animals living in mountainous areas with shade and high humidity, which are environmental conditions that favour the survival of *T. gondii* oocysts (Gauss *et al.*, 2006). In Europe, seropositivity to *T. gondii* has been detected in all tested wild ruminant species, i.e. moose, reindeer, red deer, roe deer, fallow deer, mouflon, ibex (Kapperud, 1978; Oksanen *et al.*, 1997; Vikøren *et al.*, 2004; Aubert *et al.*, 2006; Gaffuri *et al.*, 2006; Gamarra *et al.*, 2008). Antibodies have been detected more often in roe deer compared to other cervids, with percentages of seropositivity that tend to increase with the age of the sampled animals (Gauss *et al.*, 2006). In addition, antibodies have been often found in wild boars (Edelhofer *et al.*, 1996; Hejlícek *et al.*, 1997; Lutz, 1997; Gauss *et al.*, 2005; Bártová *et al.*, 2006; Ruiz-Fons *et al.*, 2006; Antolová *et al.*, 2007; Richomme *et al.*, 2009).

Several sera collected in our study area from roe deer, wild boar, red deer and mouflon were tested for specific antibodies to *T. gondii* with a commercial ELISA (ID Screen® Toxoplasmosis Indirect ELISA, IDVET, Montpellier, France). An overall seropositivity ranging from 23.3% to 33.3% was detected (Table 3). In order to further investigate the seropositivity of wild boars and directly detect the parasite, a PCR-RFLP assay targeting the 18S small-subunit ribosomal gene of *T. gondii* (Magnino *et al.*, 1998) was subsequently carried out on samples of heart tissue of a subset (n=53) of seropositive animals, but all samples tested negative. *T. gondii* was also detected by PCR-RFLP in visceral samples of European hares, which are highly susceptible to the infection and often die of acute fatal toxoplasmosis (Gustafsson *et al.*, 1988; Luppi *et al.*, 2009).

The finding of antibodies to *T. gondii* in a significant proportion of samples collected from several wild species suggests that the parasite is widespread in the study area. The risk of infection through consumption of undercooked meat of *T. gondii*-infected game from the area should be taken into account, as reports of human infection linked to consumption of game meat have been published (Sacks *et al.*, 1983; Ross *et al.*, 2001). As to measures for reducing the risk, thorough cooking (to 67 °C) as well as freezing (to -13 °C) of meat are

suitable treatments for ensuring the inactivation of *T. gondii* tissue cysts. In addition, since evisceration and handling of *T. gondii*-infected game may also represent a risk of infection to humans, hands and all materials coming in contact with uncooked meat should be washed with soap and water for inactivating the parasite (Dubey and Beattie, 1988; Dubey, 1994).

3.3 Salmonellosis

Salmonellae are enteric bacteria which are able to infect a large number of animal hosts, including all wild animals (Millán *et al*., 2004). Wild mammals can act as reservoirs or carriers of different serotypes of *Salmonella*, including the ones that infect birds and cold-blooded animals. Although animals are the major reservoir of Salmonellae, environmental contamination from human activities, including livestock farming and waste disposal, is likely to be an additional source of infection also for wildlife (Murray, 2000). Moreover, the probability of being infected by *Salmonella* is also related to the feeding habits of each species, which makes omnivores and carnivores more susceptible to the infection.

In our study area, most samples were collected from wild boars and to a lesser extent from roe deer, red deer and red foxes. Caecal or rectal contents were either directly sampled by the hunters or collected during necropsy and cultured according to standardised procedures for the detection of Salmonellae (ISO 6579:2002 Annex D).

Salmonellae were often isolated from wild boars (Table 3) in a percentage of samples ranging from 3.9% (Confidence Interval (CI), 2.76-5.67) to 26% (CI, 23.86-28.25), in relation to the geographical area of sampling. Serotyping was performed according to the Kauffman-White typing scheme. The most prevalent serotypes were *S.* Coeln (81 isolates), *S.* Typhimurium (74), *S.* Ball (43), *S.* Thompson (37), *S.* Veneziana (37), *S.* Enteritidis (18), *S.* Infantis (5). In addition, cultures yielded several (40) isolates of *S. enterica* subsp. *diarizonae*, a subspecies of *Salmonella* that is typically found in reptiles and other cold-blooded animals.

Salmonellae were also occasionally detected in the intestinal content or faeces of roe deer, red deer and red foxes (Table 3). As to the serotypes, *S.* Typhimurium, *S.* Napoli, *S. enterica* subsp. *enterica*, *S.* Veneziana and *S.* Mishmarhaemek were detected in red deer and roe deer, and *S.* Virchow in foxes.

The recovery of a large number of *Salmonella* isolates from wildlife, mainly from wild boars, is related to the wide occurrence of these bacteria in the environment and in a number of animal hosts. The high variability in the prevalence and serotypes detected in different geographical areas of sampling could not be clearly referred to differences in contamination sources from human activities, including livestock farming and waste disposal, and should be further investigated.

3.4 Infection by verocytotoxic *E. coli* (VTEC)

Several serotypes of VTEC, including *E. coli* O157:H7, are associated with disease in humans, with clinical presentations ranging from diarrhea to hemorrhagic colitis and hemolytic uremic syndrome, a severe condition which may be fatal (Karmali *et al*., 2010).

Healthy ruminants, mainly cattle, harbour more than 400 different serotypes of VTEC in their intestine, as part of their gut flora (Blanco *et al.*, 2004) and shed the organism in their faeces. VTEC have been occasionally detected also in wildlife, e.g. in faecal samples from white-tailed deer (*Odocoileus virginianus*) in the United States (Rice *et al.*, 1995; Sargeant *et al.*, 1999; Fischer *et al.*, 2001; Renter *et al.*, 2001; Dunn *et al.*, 2004), moose (*Alces alces*) in Canada (Todd *et al.*, 1999), reindeer (*Rangifer tarandus*) in Norway and Finland (Lahti *et al.*, 2001; Aschfalk *et al.*, 2003), sika deer (*Cervus nippon*) in Japan (Fukuyama *et al.*, 1999), as well as in the intestinal content of red deer and roe deer in Italy (Conedera *et al.*, 2004) and in rectal swabs from red deer in Spain (García-Sánchez *et al.*, 2007). The occurrence of VTEC in wildlife has been linked to transmission from farm animals through faecal contamination of shared pastures (Keene *et al.*, 1997; Sargeant *et al.*, 1999). On the other hand, the importance of wild animals for the transmission and/or persistence of VTEC within farms or between farms is unknown (Nielsen *et al.*, 2004).

Faecally-contaminated bovine foods and dairy products have been identified as important vehicles of VTEC to humans in a number of outbreaks and sporadic cases of disease (Rangel *et al.*, 2005; Yoon and Hovde, 2008), and contaminated game meat as well has been linked to human disease (Keene *et al.*, 1997; Rabatsky-Ehr *et al.*, 2002; Ahn *et al.*, 2009). Game meat from caribou (*Rangifer tarandus*), deer and roe deer has been occasionally found contaminated with VTEC (Milley and Sekla, 1993; Thoms, 1999; Nagano *et al.*, 2004; Lehmann *et al.*, 2006), and a recent study further indicates that game animals are an additional reservoir for human pathogenic VTEC (Miko *et al.*, 2009).

Our data refer to the testing of a population of roe deer in the province of Reggio Emilia, Emilia-Romagna (Spaggiari *et al.*, 2009). Faecal samples were examined for VTEC by selective culture and PCR assays targeting intimin (*eae*) and verotoxin (VT) genes (García-Sánchez *et al.*, 2007). Fifteen out of 124 (12.1%) *E. coli* isolates tested positive for the *eae* gene, but none of them carried VT genes.

Failure to detect VTEC in the study area might have been related to the small number of tested samples, as well as to the restriction of testing to one species only (roe deer). Previous surveys in other wildlife populations either failed to detect VTEC as well (Wahlström *et al.*, 2003; Lillehaug *et al.*, 2005) or detected low prevalences, ranging from 0.3% in roe deer in northeastern Italy (Conedera *et al.*, 2004) to 1.5% in red deer in Spain (García-Sánchez *et al.*, 2007) to 0.25%, 0.6% and 2.4% in different surveys in white-tailed deer populations in the United States (Sargeant *et al.*, 1999; Fischer *et al.*, 2001; Renter *et al.*, 2001).

3.5 Yersiniosis by *Y. enterocolitica*

Y. enterocolitica is an enteric bacterium associated with foodborne disease in humans (Bottone, 1999). The organism has been detected in wildlife, e.g. in Norway in 29/2,243 (1.3%) faecal samples from reindeer (Kemper *et al.*, 2006) and in 8/170 (4.7%) faecal samples of red deer (Aschfalk *et al.*, 2008), in the rectal content of 41/131 (31%) wild boars from Japan (Hayashidani *et al.*, 2002) and from the Canton of Geneva in Switzerland, with a significantly higher positivity detected in tonsil samples than faecal samples (Fredriksson-Ahomaa *et al.*,

2009). Recent surveys also detected *Y. enterocolitica* on the surface of game meat in Bavaria, Germany (Bucher *et al.*, 2008).

In our survey, caecal content samples from wild ruminants and wild boars were tested for *Yersinia* spp. by the cultural method, employing selective media and special procedures, including cold enrichment. Typing was carried out according to the procedure described by Bottone (1999). Overall, *Y. enterocolitica* was detected in a limited percentage (7%) of samples, while other species (*Y. bercovieri*, *Y. kristensenii*, *Y. frederiksenii*, *Y. intermedia*) were detected more frequently (13% of samples). All isolates were recovered from wild boars and were typed as biotype 1A, which includes *Y. enterocolitica* strains that are considered non-pathogenic, although recent evidence suggests that some of them can actually be virulent (Tennant *et al.*, 2003).

3.6 Tuberculosis

Tuberculosis (TB) by *M. bovis* is a disease that occurs worldwide in a wide range of domestic and wild animals (Michel *et al.*, 2010). Affected wildlife may show no clinical signs of disease, or just exhibit a loss of weight. The occurrence of TB in wildlife may interfere with eradication programmes of the disease in livestock, and may also pose a direct risk to human health (De Lisle *et al.*, 2002).

M. bovis belongs to the *Mycobacterium tuberculosis* complex (MtbC), which includes *M. tuberculosis*, *M. caprae*, *M. microti* and other species. Humans may get infected with *M. bovis* and the other species belonging to MtbC following direct contact with infected animals, mainly through inhalation of contaminated aerosols, and consumption of milk. Meat consumption has not been documented as a way of transmission of MtbC to humans, but direct contact with organs and tissues of infected animals during skinning and evisceration procedures might lead to infection by hand-to-mouth contact (De la Rua-Domenech, 2006).

Samples collected in Lombardy from wildlife were submitted for isolation of mycobacteria and for detection and differentiation of MtbC. Retropharyngeal and submandibular lymph nodes were collected from carcases of wild boar (n=4,200), red deer (n=208), roe deer (n=152) and red foxes (n=53) and examined by macroscopical inspection. Upon detection of TB-like lesions consisting in small rounded, necrotic and often calcified foci, samples of tissue were decontaminated and cultured for mycobacteria in a solid medium (Lowenstein-Jensen-ST) and a liquid culture system (BACTEC™ MGIT™ 960; Becton, Dickinson and Company). Samples were concurrently processed for the detection of MtbC by DNA extraction and PCR assay targeting the IS6110, an insertion element (IS) present in the genome of the MtbC group (Haddad *et al.*, 2004). In case of growth, cultures were identified by molecular and biochemical tests (Boniotti *et al.*, 2009). Raw samples that tested positive with the IS6110 PCR but negative by culture were retrieved and processed for the direct identification of MtbC by a PCR-restriction fragment length polymorphism assay targeting the *gyrB* gene (*gyrB* PCR-RFLP), which allows the differentiation of MtbC species (Niemann *et al.*, 2000). Typing of mycobacteria strains was carried out by spoligotyping, a method based on PCR amplification of a highly polymorphic direct repeat locus (DR) (Kamerbeek *et al.*, 1997) and by analysis of the VNTR loci as described by Boniotti *et al.* (2009).

A limited percentage (333/4,200; 7.9%) of wild boar lymph nodes showed macroscopic lesions consistent with TB infection; out of these samples, 228 were positive to IS6110 PCR but only 180 were confirmed by *gyrB* PCR-RFLP. Surprisingly, in most samples (n=170) the main etiological agent was identified as *M. microti*, a mycobacterial species that is commonly isolated from rodents (mice and voles) and has been also detected in larger mammals such as cats, pigs and llamas as well as in humans (Van Soolingen *et al*., 1998; Xavier Emmanuel *et al*., 2007), while *M. bovis* was only detected in 10 samples. Cultures yielded 20 isolates of *M. microti* and 4 isolates of *M. bovis*. Genetic profiles of all *M. bovis* isolates were found to differ from those of isolates recovered in recent outbreaks of TB in cattle from the same areas (Pacciarini *et al*., 2006).

Although no macroscopic lesions were found in the lymph nodes of red deer, roe deer and red foxes, it was decided to analyse all samples by both IS6110 PCR and culture. Few samples tested positive at PCR, namely 7/152 from roe deer, 5/208 from red deer, and 3/53 from red foxes. Subsequent analysis by *gyrB* PCR-RFLP confirmed the detection of *M. microti* in the red deer (n=2/208) and roe deer (n=1/152), and of *M. bovis* in the red deer (n=3/208) and red foxes (n=2/53), but no isolate could be recovered in culture.

Overall, our data do not suggest a significant role for wild mammals as a reservoir for bovine tuberculosis in the study area.

3.7 Brucellosis

Brucellosis is a disease caused by bacteria of the genus *Brucella*, that currently includes nine species, namely *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. pinnipedialis*, *B. ceti* and *B. microti* (Whatmore, 2010). The first three mentioned species are the agents of bovine brucellosis, small ruminant brucellosis and swine brucellosis, respectively, which are diseases that cause huge economic losses and important morbidity in domestic and wild animals. Brucellae are transmitted to humans either by direct contact with infected animals, or by ingestion of contaminated animal products, usually milk and dairy products, while inhalation of contaminated aerosols is an important route of infection for occupationally exposed people, such as workers in laboratories and slaughterhouses (Seleem *et al*., 2010).

In wildlife, *B. abortus* and *B. suis* have been isolated in a number of species, such as bison (*Bison bison),* deer, wild boar, African buffalo (*Syncerus caffer*), European brown hare, with the infection being endemic in some of these species worldwide (Godfroid, 2002). As to European wildlife, *B. melitensis* has been recovered sporadically from chamois and ibex in the Italian and French Alps and its occurrence has been interpreted as a spill-over from infected small ruminants (Garin-Bastuji *et al*., 1990; Ferroglio *et al*., 1998). In addition, *B. suis* is known to occur in Central Europe in the wild boar and in the European hare, which are both considered to be reservoir of *B. suis* biovar 2 (Garin-Bastuji *et al*., 2000; Godfroid, 2002; Al Dahouk *et al*., 2005). High values of seropositivity to brucellae and the isolation of *B. suis* biovar 2 have been also recently reported in wild boars in Piedmont, a region west of Lombardy (Bergagna *et al*., 2009).

In our study area, antibodies to brucellae were not detected in wild boars nor in wild ruminants. To this regard, it should be noted that brucellosis was eradicated from livestock of the study area in 1996. In addition, in a serological survey carried out between 1995 and 2002 in roe deer and chamois in the central Italian Alps in Lombardy, all samples tested negative for antibodies to brucellae (Gaffuri *et al*., 2006). Antibodies to brucellae were only detected in the European hare, where two samples tested positive (Table 3) by the Rose Bengal test (RBT) and complement fixation test (CFT).

As to direct testing by real-time PCR (Bogdanovich *et al*., 2004), no sample from any species was positive for brucellae. The low (0.39%) seropositivity detected in hares suggests a very limited circulation of brucellae in this species. Nonetheless, since there is a risk of introduction of *B. suis* with imported hares from Eastern Europe, monitoring for brucellosis in the two regions is set to continue both in the European hare and the wild boar, which are the main reservoir hosts of *B. suis*.

3.8 Tularaemia

Francisella tularensis is the etiological agent of tularaemia, a zoonotic bacterial disease that mainly affects rodents (mice, voles) and lagomorphs (hare, rabbit), and occasionally other mammals and birds (Foley and Nieto, 2010; Padeshki *et al*., 2010). Currently, four subspecies of *F. tularensis* that differ in virulence and geographical distribution are recognised, namely: *tularensis, holarctica*, *mediaasiatica* and *novicida*. *F. tularensis* subsp. *tularensis* (type A), which is more virulent, is found in North America, while *F. tularensis* subsp. *holarctica* (type B) is less virulent and occurs in the Old World and occasionally in North America and *F. tularensis* subsp. *mediaasiatica* is moderately virulent and has been only isolated in Central Asia. *F. tularensis* subsp. *novicida* has low virulence in humans, and has been isolated from clinical cases only in a few occasions (Johansson *et al*., 2000). *F. tularensis* is listed among the most dangerous organisms (also known as 'Category A agents') that can be used for bioterrorism purposes, due its low infectious dose, ease of dissemination and high case-fatality rate (Davis, 2004). The organism can be transmitted to humans by direct contact with infected animals (mostly hares), by tick or insect bite, and by exposure to contaminated aerosols, e.g. to aerosols generated when grass is cut in areas contaminated with carcases of infected animals (Feldman *et al*., 2001; Foley and Nieto, 2010). Outbreaks of human disease may also follow the ingestion of contaminated water as occurred in the past in several countries, including Bulgaria (Christova *et al*., 2004), Norway (Brantsaeter *et al*., 2007), Turkey (Willke *et al*., 2009), and also recently in Tuscany, Italy (Fabbi *et al*., 2009).

Although the transmission of *F. tularensis* following ingestion of meat is considered as exceptional, humans may get infected preliminary to the preparation and consumption of meat, i.e. when skinning and gutting contaminated carcases, as also recently reported in outbreaks of disease occurred in Germany, Spain and France (Hofstetter *et al*., 2006; Schätzle and Schwenk, 2008; Mailles *et al*., 2009; Aldea-Mansilla *et al*., 2010). The Oltrepo' Pavese, an hilly area in the province of Pavia, in the southern part of Lombardy, has been an endemic area for tularaemia since the 1960s, when the first documented cases of disease in Italy were reported both in hares (Rinaldi *et al*., 1964) and humans (Bianchi, 1966).

During the monitoring activity, *F. tularensis* was occasionally detected in European hares by two PCR assays, one that allows the identification of the genus *Francisella* and the other specific for *F. tularensis* subsp. *holarctica*, and by culture on special media enriched with cysteine (Forsman *et al.*, 1994; Johansson *et al.*, 2000; Foley and Nieto, 2010). The infection was demonstrated both in indigenous hares found dead in Lombardy in the province of Pavia and in Emilia-Romagna in the provinces of Piacenza, Parma and Bologna, and in hares imported from Hungary, Slovakia and Romania for restocking. All isolates were typed as *F. tularensis* subsp. *holarctica*. The organism was detected by PCR in Emilia-Romagna also in a few coypus (*Myocastor coypus*), which might be frequently exposed to water-borne infections due to their habit of living in burrows along river banks. Overall, antibodies to *F. tularensis* were very rarely detected in indigenous hares, which is probably due to the short course and high fatality of the disease. On the other hand, high antibody titers (1:640 and higher) were occasionally found in a few imported hares from Eastern Europe, where the disease is endemic. In addition, antibodies to *F. tularensis* were detected in a few sera collected from wild boars.

It should be noted that the handling and especially gutting and skinning of hare carcases may be associated to a risk of infection by *F. tularensis*, due to the occurrence of the organism in hares in the two regions. The presence of *F. tularensis* in the environment, as well as in vectors and wildlife, is often revealed when cases of disease are diagnosed in humans, as recently reported in Italy (Castro *et al.*, 1999), Czech Republic (Cerný, 2001), Spain (Martín *et al.*, 2007; Allue *et al.*, 2008), Germany (Kaysser *et al.*, 2008) and France (Mailles *et al.*, 2009). Tularaemia has been diagnosed also recently in hares in several European countries, including Austria (Hofer, 2002), Germany (Müller *et al.*, 2007), France (Mailles *et al.*, 2009) and Spain (Aldea-Mansilla *et al.*, 2010). Direct methods for detecting and monitoring tularaemia in the European hare should be preferred to serological methods as mortality rate in this species is often high and death occurs before the development of a detectable antibody response.

4. Conclusions

What has been achieved?

The encroachment of humans and domestic animals into wildlife habitats and the growing interest in and popularity of wildlife has globally led to enhanced monitoring and increased detection of diseases in wild animals (Rhyan and Spraker, 2010). In this regard, the monitoring and surveillance of wildlife for the prevention and control of diseases is considered by OIE 'a crucial component of the safeguarding of global animal and public health and related agriculture and trade issues' (OIE, 2009b).

The wildlife disease monitoring programme that has been established in Lombardy and Emilia-Romagna has been conceived and implemented by the regional and provincial administrations and the official veterinary services in close collaboration with major partners in the field, i.e. hunters and gamekeepers. Over the years, the programme has grown to become an ongoing system for assessing trends of infectious and parasitic diseases of wildife and has allowed documenting the occurrence of most zoonotic pathogens in several wild species of Lombardy and Emilia-Romagna.

What has been neglected?

In spite of the commitment of hunters and gamekeepers, the quality of a few samples was affected due to a failure to comply with the given instructions regarding the choice and range of tissues and organs to be submitted for analysis. In addition, inconsistent handling and poor storage and transport conditions of the samples occasionally led to their extensive contamination or to inactivation/loss of viability of the associated infectious organisms. As a consequence, a few samples were either deemed unfit for the laboratory tests or yielded questionable results, which precluded their inclusion in the analysis.

What needs to be done?

The focus of the monitoring programme needs to be expanded in the future in order to tackle emerging zoonotic infections. For this purpose, specific instructions in the collection of samples and building of technical capacity at the laboratory level will have to be provided. In addition, training courses for hunters and gamekeepers will need to reflect any relevant update in the epidemiology of zoonotic diseases that may be acquired from wildlife.

Special attention will also need to be given to the harmonisation of the sampling schemes and laboratory methods with other monitoring programmes implemented at regional and national levels, as that will allow the direct comparison of data referred to different wildlife populations.

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Assurance of food safety along the game meat production chain: inspection of meat from wild game and education of official veterinarians and 'trained persons' in Austria

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Summary

Wild game harvested by common hunting methods represents a sustainable source of meat. Most hunting practices fulfil animal welfare requirements and the meat has usually a high nutritive value and favourable sensory characteristics. The mode of production, however, differs from that of farmed animals, and prompts taking specific measures along the food chain. Among these measures, meat inspection and hygiene control are necessary to assure that meat from wild game is 'safe' for the consumer. In 1994, Austria implemented an inspection system in compliance with EU directive 92/45/EEC (EC, 1992), which involves three categories (hunter, trained person and official veterinarians), and provides continuous training and evaluation of these people in a consistent and logical way. With the 'new' food hygiene legislation entered into force in 2006, only minor adaptations had to be made. In compliance with EU recommendations, 'Guides to Good Practice' form an important element not only in training of inspection personnel, but they also serve as references when game carcasses or food businesses trading meat from wild game are inspected.

Keywords: official veterinarian, trained person, wild game, *Trichinella*

1. Introduction

Meat from wild game is a highly valued product with favourable composition and sensory characteristics (Hoffman and Wiklund, 2006). It is also attractive for consumers who are concerned about ethical and sustainable aspects of food production, similar to products from organic farming (Winkelmayer and Paulsen, 2008a). Careful inspection of game before killing and *post mortem* examination of carcass and organs, as well as the strict adherence to certain rules of good hygiene practice along the food chain ('from forest/field to fork') are necessary to assure that all quality traits are preserved and that game meat is not a source of hazards to the consumer. Although the quantity of meat produced by hunting is small compared to

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pork or beef, it is estimated that in Austria the total carcass weight (eviscerated, skin-on) of wild game is ca. 9 million kg/year, see Table 1.

In this field of meat production, self-checks form an essential part of food safety. In Austria, considerable effort is spent on training and motivation of hunters and trained persons, also by providing continuous education and voluntary evaluation schemes. This system allows the risk-based use of (limited) inspection capacities of the competent authority.

In comparison with farm animals, meat from wild game constitutes a minor fraction of the average meat supply. However, this does not mean that the inspection of wild game should be less strict than that of farm animals. Based on a calculation scheme given by Fehlhaber (2007), the number of consumers possibly exposed when a hazardous game carcass is not examined at all or passes all examinations and is processed into portions can be estimated. Calculations were done based on 2007 data from a major Lower Austrian game-handling establishment. Thus, roe deer (15.3 kg, uneviscerated, skin-on; average of 18,000 carcasses), red deer (87.8 kg, average of 2,900 carcasses) and wild boar (44 kg, average of 3,600 carcasses) would yield 37, 265 and 147 portions, respectively (Winkelmayer and Paulsen, 2008b). In the most extreme case the number of affected consumers could be as high as the number of portions. This is a clear indication that each single game carcass has to undergo thorough inspection in order to minimise health hazards to the consumer (Table 2).

2. Inspection system for wild game in Austria (excl. *Trichinella*)

2.1 Pre-1994 situation

Before 1994, no specific regulations existed in Austria with respect to the inspection of wild game. The Food Act of 1975 stated that hazardous or spoiled food must not be placed on the market. The Austrian Food Codex, Chapter B14 (Anonymous, 2010) required that game meat must undergo a veterinary meat inspection before being processed to meat products, which was practically only possible for meat from farmed game.

Table 1. Quantity of wild game[1] harvested in Austria in 2007 (Pontasch, 2008).

Species	Animals (ca.)
Roe deer	260,000
Red deer	48,000
Chamois	22,000
Wild boar	25,000
Brown hare	180,000
Pheasant	160,000
Wild duck	70,000

[1] Represents a total carcass weight of ca. 9,000,000 kg; the average game meat consumption is ca. 0.5 kg/person/year.

Table 2. Average number portions/servings of meat and meat products per carcass (serving sizes according to Fehlhaber, 2007).

Species	Average carcass weight (skin-on)[1] in kg	Meat cuts in kg	Meat for processing in kg	Number of portions			Max. number of consumers
				Meat cuts	Meat products	Total	
Red deer	88	40	3	235	30	265	265
Wild boar	44	20	3	117	30	147	147
Roe deer	15.5	5.5	0.5	32	5	37	37

[1] Rounded to 0.5 kg.

At EU level, a directive of 1992 (EC, 1992) laid down the principles for the inspection and hygiene of meat from wild game, with the exception of marketing of carcasses or meat cuts from the hunter to the final consumer or to local food businesses which supply directly to the consumer.

2.2 Development from 1994 to 2004/2006

Austria became member of the European Ecomonic Area in 1994 (member of the European Union in 1995), and hence, had to implement Directive 92/45/EC (EC, 1992). While this caused no substantial problems with respect to hygiene requirements for game larders and game-handling establishments, mandatory game meat inspection presented a challenge. The inspection scheme had not only to meet EU directive requirements for placing safe game meat on the market, but there were numbers of additional requirements and practical considerations:

1. Common hunting strategies in Austria: the majority of large game is harvested by still hunting, outside typical working hours or at weekends. Hunting grounds can be remote from settlements and also quite distant from (the relatively few) game-handling establishments. Carcasses are delivered to game larders (i.e. cooling facilities shared by a number of hunting grounds), from where they are picked up at regular intervals by wholesalers.
2. Hunting traditions: offals, e.g. liver are traditionally consumed after the hunt and would thus not be available for a veterinary inspection at game larders or at game-handling establishments.
3. Availability and costs of inspection personnel: inspection of wild game immediately after the hunt or at game larders exclusively by meat-inspecting veterinarians was not a realistic option with respect to both costs and limited capacities.
4. Applicability for all branches of marketing: *ante* and basics steps of *post mortem* inspection should be identical for all branches of marketing, from local 'direct' marketing, trading to approved game-handling establishments, intra-community-trade and export to third countries. A modular scheme (see below) was found most suitable for that purpose.
5. Compliance of hunters and game handling establishments.

As a consequence, a three-step inspection scheme was designed (Anonymous, 2004), where hunters have to provide information on the *ante mortem* condition of hunted game and on carcass and stomach and intestines upon evisceration. 'Auxiliaries for inspection of game meat', i.e. registered hunters with special training – now termed 'trained persons' – were responsible for inspection of the carcass and edible inner organs at game larders. The examination by hunters as well as trained persons was based on a checklist (Table 3). When carcasses were delivered to game-handling establishments or serious abnormalities detected by hunters or trained persons, carcass and organs had to be presented to the official veterinarian for inspection (Figure 1 and 2).

Table 3. Inspection criteria for wild game in Austria, 1994-2004 (after Winkelmayer et al.*, 1994, based on Austrian Game Meat Regulation 1994).*

To check *ante mortem*	Examples of abnormalities
Nutritional condition	• emaciation
Behaviour, movement	• dazed state, somnolence
	• lameness
	• complicated fractures
Skin, hair	• hair loss
	• hair discolouration
Orifices	• exudates (blood, foam)
	• diarrhoea
Atypical sounds	• cough
	• groan/rattle
To check *post mortem*	**Examples of serious abnormalities**
Outer surface, orifices, nutritional condition	• emaciation
	• wounds, when numerous or inflamed
	• tumours and abscesses, when numerous
	• anal region soiled by massive diarrhoea
	• compound fractures, when not caused by shooting
	• omphalitis
	• orchitis
	• swollen joint(s) (arthritis)
Thoracal and abdominal cavities	• foreign matter, esp. gut contents or urine (not when caused by shooting), when pleura or peritoneum are discoloured
	• pus and other septic masses
	• signs of acute pleuritis or peritonitis
Organs and pleura/ peritoneum	• considerable changes in: colour, consistency, odour
	• tumours and abscesses, when numerous or disseminated
	• abnormities of liver (with exception of parasites) and spleen
	• considerable gas formation in guts esp. when organs are discoloured (greening)
	• enteritis (serositis, peritonitis)
Visible muscle surfaces	• considerable changes in: colour, consistency, odour
	• tumours and abscesses, when numerous or disseminated

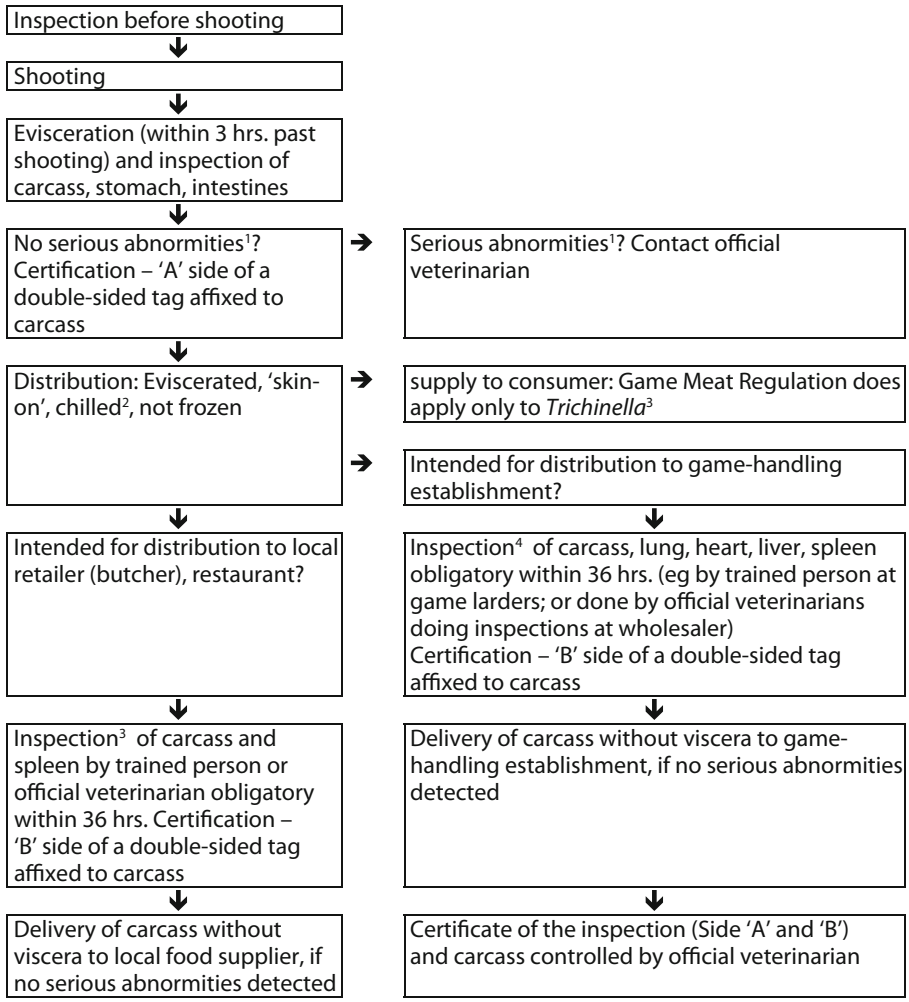

Figure 1. Meat inspection of wild ungulates (adopted from Paulsen and Winkelmayer, 2000) in Austria 1994-2004/2006.

[1] see Table 3.

[2] Storage of eviscerated, hide-on ungulates at -1 to +1 °C max. 15 days, +1 to +7 °C max. 7 days.

[3] Microscopical examination of wild boar meat for *Trichinella* sp. obligatory. Can be performed by trained person with additional education. For this type of *Trichinella* examination, 5 specified muscles are sampled (diaphragm, intercostal muscle, leg muscle, tongue, *M. masseter*) and a total of 14 subpieces is inspected via trichinoscopic method.

[4] Examination of wild boar meat for *Trichinella* sp. by digestion method obligatory

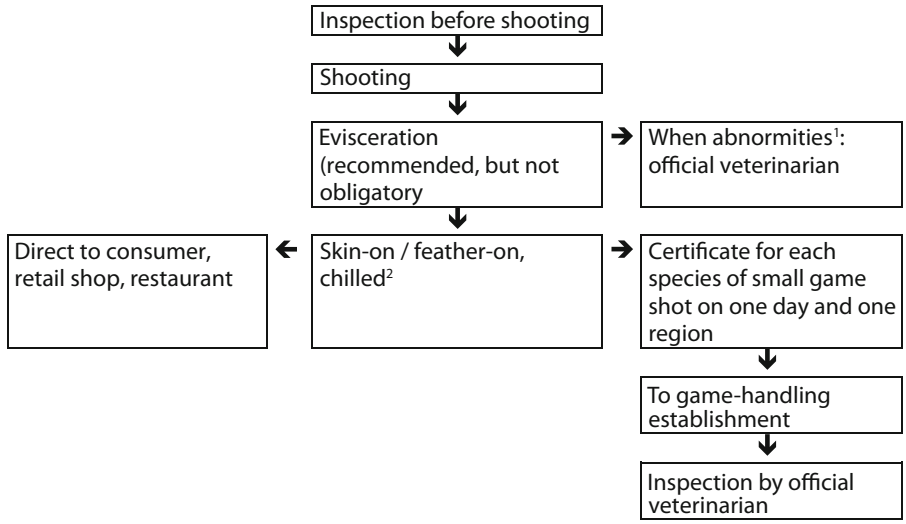

Figure 2. Meat inspection of small game (i.e. birds, rabbit, hare) in Austria, 1994-2004/2006 (adopted from Paulsen and Winkelmayer, 2000).

[1] see Table 3.

[2] storage at -1 to +4 °C max. 15 days.

2.3 Current system

2.3.1 Role of trained persons

Only minor modifications were necessary to ensure compliance with the 'new' EU 'hygiene package'. The inspection system involves three categories of personnel (Table 4), and their involvement depends on:

- the way of marketing; and
- the presence of certain abnormalities detected during *ante* and *post mortem* inspection; or
- the suspicion of environmental contamination.

In 2008, there were ca. 110,000 licensed hunters in Austria, ca. 25,000 of which were registered as trained persons.

For education and training, a series of textbooks exist and they provide the basis for uniform and consistent training courses in Austria. The basic training (mandatory for all hunters to pass the exam for the first hunting license) includes diseases of wild game and good hygiene practice during primary production (evisceration, cooling, and storage of the carcass) and is addressed in the 'Österreichischer Jagdprüfungsbehelf' (a guide to preparation for hunter´s examination in Austria; Sternath, 2006).

For trained persons, a textbook on game meat inspection ('Wildbrethygiene'; Winkelmayer *et al.*, 2008) and one on hygiene during primary processing and fresh meat production

Table 4. Ways of marketing of meat from wild game and involved inspection personnel[1].

	hunter (Step 1)	trained person (Step 2)	official veterinarian (Step 3)
Self-supply	-	-	**
Local trade[2]	+	+	**
Intra-community trade	+	+	+
Export	+	+	+

[1] Inspection: - = none; + = mandatory; ** = only in case of serious abnormities, as defined in Regulation (EC) No. 854/2004, Annex 1, Sect. IV, A, Par. 3., lett. (a), (d), (e).

[2] i.e. direct supply to final consumers or to food businesses supplying directly to the final consumer (local, small quantities)

('Wildbretdirektvermarktung'; Winkelmayer *et al*., 2007) are provided. The latter textbook also serves as a 'Guide to Good Practice' for processing of game meat.

Training of official veterinarians in hygiene of meat from wild game is done in the course of the periodical 'evaluation', with training material provided by the Federal Ministry of Health ('Food Safety Module D').

Notably, all abovementioned training materials originate from the same team of authors, which contributes to consistency in contents as well as presentation. This consistency forms an important part of the 'forest/field to fork' principle.

2.3.2 Examination performed by the official veterinarian

A simple sensory examination will give a first indication if spoilage has begun, and more specific techniques as known from meat inspection of slaughter animals can be applied, if deemed necessary. Details for the examination are defined in Regulation (EC) No. 854/2004, Annex 1, Sect. IV, A (EC, 2004a) and additional national legal texts ensure uniformity in inspection procedures.

The official veterinarian also considers the information on the *ante mortem* condition of the game and on abnormalities detected during evisceration. This information is provided on a double sided tag ('Wildfleischanhänger') affixed to large game carcasses or on an accompanying document ('Begleitschein') for small game. It is the responsibility of the hunter to document the *ante mortem* inspection results and abnormalities detected during evisceration (i.e. on the carcass and the intestines), whereas the examination by the trained person focuses on the carcass and the (edible) inner organs. This document also assures traceability in the food chain. When game carcasses are delivered to game handling establishments with missing or incomplete certificates, these carcasses will not pass the examination of the trained veterinarian.

2.3.3 Legal framework according to marketing branch

In principle, Regulations (EC) No. 178/2002 (EC, 2002), No. 852/2004 (EC, 2004b), No. 853/2004 (EC, 2004c) and No. 854/2004 (EC, 2004a) apply to the production of meat from wild game. Additional Austrian legislation comprises a food safety act (Anonymous, 2006a), acts on meat inspection, an act on direct marketing of certain foods (Anonymous, 2006b) and on the local trade of certain meat products (Anonymous, 2006c) and a number of either binding texts (e.g. on labelling of food, personal hygiene) or recommendations.

Regulation (EC) No. 853/2004 (EC, 2004b) does neither apply to domestic use of game meat by the hunter nor to small quantities that are traded locally either directly to the consumer or local food businesses (butchers, retailers, restaurants, canteen, etc.) supplying directly to the consumer. This sector of 'direct marketing' is governed by national legislation (e.g. Anonymous, 2006b). Table 5 gives an overview on relevant legal texts.

3. *Trichinella* inspection

3.1 Epidemiological situation

The epidemiological situation in Austria has been reviewed by Duscher *et al.* (2005). In brief, the red fox is a wildlife reservoir for *Trichinella britovi*, and reservoirs are mainly located in Western provinces. All reported cases of human trichinellosis (incidence <0.1/100,000 inhabitants/year) originate from foods consumed outside Austria or self-imported food.

Table 5. Legal texts relevant for production and processing meat from wild game[1].

	Marketing	Reg. (EC) No. 178/2002 (EC, 2002)	Reg. (EC) No. 852-854/2004 (EC, 2004a,b,c)	Lebensmittel-Direkt-vermarktungsVO (Anonymous, 2006a)	Lebensmittel-EinzelhandelsVO (Anonymous, 2006b)
Meat from large and small game	Own private use	-	-	-	-
Carcasses, eviscerated, skin-on; not deep-frozen	Local, direct, small quantities	+	-	+	-
Carcasses, skinned, meat cuts	Local, direct, small quantities	+	852/2004	+	-
Meat products	Local, direct, small quantities	+	852/2004 853/2004	+	+

[1] - = not applicable; + = applies.

The last autochtonous case of trichinellosis in a domestic pig dates from before 1970. In wild boar, there are only sporadic cases, mostly with not well-documented history, at a rate of ca. 1 case/10 years. Ca. 50% of hunted wild boars are marketed *via* game-handling establishments and tested by the digestion method. To date, *Trichinella pseudospiralis* has not been detected in wild boars in Austria.

3.2 Inspection by official veterinarians

Trichinella testing of wild boars in game-handling establishments is done by official veterinarians by digestion method according to Regulation (EC) No. 2075/2005 (EC, 2005).

3.3 Inspection by trained persons – Lower Austrian model

Regulation (EC) No. 2075/2005 (EC, 2005) does not apply to meat from wild boars supplied from the hunter directly to the final consumer or to food businesses supplying directly to the final consumer ('direct marketing'). Federal legislation allows *Trichinella* testing of wild boars destined for local trade by trichinoscopy done by specially educated trained persons. The decision to allow this option was motivated by (a) the relatively stable epidemiological situation and (b) the absence of *Trichinella pseudospiralis*.

This option has been, however, not implemented by all provinces. In addition to a primary training course, voluntary evaluation schemes/proficiency tests are offered. If these possibilities are not used, the competent authority will evaluate the trained person.

3.3.1 EU and national legislation

Lower Austria contributes ca. 2/3 to the hunting bag of wild boars in Austria. Not all carcasses are collected and processed by game handling establishments, but a substantial fraction is marketed by the hunters directly to the consumer or to local food retailers supplying directly to the consumer ('local trade'; Winkelmayer and Paulsen, 2008a).

With the exception of animals hunted for private use, wild boars undergo an *ante* and *post mortem* inspection and an examination for *Trichinella* sp. For carcasses collected by game handling establishments, EU law applies and these carcasses are tested by the digestion method according to Regulation (EC) No. 2075/2005 (Stangl and Paulsen, 2005).

Inspection of carcasses for 'local' trade is regulated by national legislation. While the methods in *ante* and *post mortem* inspection are identical to those applied for carcasses under EU law, it is usually sufficient that the hunter and a trained person perform this examination. With respect to *Trichinella* examination, wild boar carcasses entering a game handling establishment must be examined by the digestion method.

As 'direct marketing' of wild boars, and thus *Trichinella* inspection for such carcasses, is not covered by EU legislation, the Austrian provinces have been given the option to implement *Trichinella* examination by trained persons using the trichinoscopic method. This option exists since 1994. Minor adaptations were necessary to ensure compliance with Regulation

(EC) No. 2075/2005 (EC, 2005) (number of samples taken from the carcass and number of examined subsamples). The restrictions laid down in Article 16 of this regulation apply (i.e. max. 10 carcasses/day; digestion method not available; meat is marked with a health mark that is clearly different from the common 'oval' health mark provided for in Article 5(1)(a) of Regulation (EC) No. 853/2004 (EC, 2004b), and that the meat is not used for the production of products where the production process does not kill *Trichinella*).

Lower Austria has taken this opportunity, and established a basic training course, and several options for periodical (voluntary or mandatory) evaluation. If more than 10 carcasses are to be examined by one trained person, or if the meat is to be used for products where the production process does not kill *Trichinella*, then trained persons have to take samples and send them to an accredited laboratory where *Trichinella* testing is done by the digestion method. Also, if *Trichinella* is detected (irrespective of the method used), an alert procedure comes into force which makes digestion method mandatory also for carcasses intended for 'direct marketing'.

3.3.2 Requirements for trained persons performing Trichinella *inspection*

Trained persons already active in inspection of game can apply for additional training in *Trichinella* inspection. After passing a basic training course, they have to register at the veterinary district administration. Trained persons have to be evaluated by an official veterinarian on a regular basis. In Lower Austria, the frequency and extent of such controls will be based on evidence of successful attendance of advanced courses and proficiency tests (Kahrer, 2009).

3.3.3 Basic training course

The basic training course is an obligatory one-day course and consists of theoretical parts (4 hours) and 2 practicals (4 hours).

In the first practical, the participants practice
- Working with the microscope.
- Trichinoscopic examination with meat containing inactivated larvae.
- Sampling of wild boar carcasses.

The second practical is a sort of proficiency test. Each participant has to examine 7 numbered fields in a previoulsy prepared compression glass. The only information given to the participant is that at least one field will contain trichinellae. The results are reported and in case of discrepancies, the examination is, at the end of the training session, repeated together with an experienced veterinarian. This step has been found to be very effective in improving the relative sensitivity, and subsequently, relative accuracy of the trichinoscopic examination (Table 6).

Based on the results, the competent authorities receive an annual summary report. Individual result forms of the trichinoscopic examination are collected and can be presented to the competent authority upon request.

Table 6. Efficacy of Trichinella examination by freshly trained ('basic') and experienced participants.

	'Basic' (497 samples tested by 71 participants)	'Experienced' (695 samples tested by 174 participants)
Relative sensitivity, %	83.1	98.4
Relative specifity, %	93.1	94.4
Relative accuracy, % ('agreement')	88.1	96.5

3.3.4 Advanced courses

Advanced courses are voluntary half-day events and can be attended only by trained persons already registered for *Trichinella* examination. Participants are requested to bring their microscope and all necessary tools with them.

The theoretical part consists of 1.5 hours (update on legislation and possibility for discussion on practical and administrative issues); 2.5 hours are provided for practicals.

The first practical is the self-evaluation of the microscope according to a checklist addressing completeness and functionality of equipment and the major mechanical or optical problems related to microscopes used in a rugged environment. In case of unsatisfactory results, small repairs or cleaning jobs are done directly in the course.

For the second practical, each participant receives four coded meat samples (1-4 of the samples may contain inactivated *Trichinella* larvae) and has to examine the samples microscopically. The results are reported and discrepancies are discussed.

Advanced courses started in 2008, with a total of 174 participants, and are repeated on an annual basis, with a total capacity of 40-60 participants/year (i.e. 2 courses). Based on the results, the competent authorities receive an annual summary report. The overall performance is demonstrated in Table 6. Completed self-evaluation forms and results of the trichinoscopic examination are collected and can be presented to the competent authority upon request.

3.3.5 Proficiency tests

The first proficiency test has been completed in 2001. Basically, each participant received two coded samples and had to examine them and report the results (Paulsen *et al.*, 2003). As only two meat samples were distributed per participant, the power of this test was low (i.e. the chance to report two correct results by simply guessing was 25%). On the other hand, participants had not been informed about the test in advance, and 91% responded, which indicated a very high compliance for a voluntary proficiency test.

Similar to the experiences in the training courses, the relative accuracy was 86.3% for the first set of samples. When participants with discrepant results received a second sample set, relative accuracy improved to 97.1%.

4. Conclusions

What has been achieved?

In 1994, Austria implemented an inspection system in compliance with EU directive 92/45/EEC, which involves three categories (hunter, trained person (former: auxiliaries) and official veterinarians), and provides continuous training and evaluation of these people in a consistent and logical way. The Austrian game meat inspection system relies on motivated trained persons and recognises the self-responsibility of the primary producers (i.e. the hunters).

With the 'new' food hygiene legislation of 2006, only minor adaptations had to be made. This system is augmented by 'Guides to Good Practice'. Grants are available from hunting associations for research targeted to practical game meat hygiene issues.

What has been neglected?

The current game meat inspection scheme includes periodical re-training, but there is only limited possibility to check if the routine activity of trained persons and hunters is always in full compliance with legislation. Practically, routine control of the health certificates affixed to large game carcasses and comparison to the findings on the carcass should give an indication if trained persons perform correctly. This is done on a routine basis in game-handling establishments and on a risk-based plan in game larders. However, a real re-evaluation scheme under field conditions should be developed.

What needs to be done?

In the game food chain, the use of lures, baits, winter feeding, etc. has received too few attention and the primary producers must be made more aware that they are responsible for the safety and traceability of feedstuff used for wild game (Deutz, 2010).

Between EU member states, there are also some unresolved items:

- Currently, it is unclear under which provisions wild game game carcasses (skin-on) can be transported directly from hunting grounds or from game larders to game-handling establishments in other EU countries.
- In Austria, it is principally possible that trained persons, who have received their training and examination in other EU countries, register in Austria. A harmonisation of education schemes for trained persons in all EU countries is necessary.

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Structure and legal framework for the direct local marketing of meat and meat products from wild game in Austria: the Lower Austrian model

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Summary

The 'new' EU food hygiene legislation prompted for an update of the Austrian legislation on the marketing of meat or meat products from wild game from the hunter directly to the consumer or local food suppliers. The Lower Austrian implementation is an integrated model ('forest-to-fork', Winkelmayer, 2006) which recognises the self-responsibility of the hunters supplying meat from wild game and which encourages this food sector to establish an own codex of good practice (in the sense of a 'guide to good practice'). Core elements are a well-established educational and training concept, templates for documentation of good manufacturing practice and an evaluation system including microbiological examination of the products.

Keywords: wild game, meat, own-checks, direct local marketing, Good Hygiene Practice, microbiology, forest-to-fork

1. Introduction

From official statistics, it can be estimated that in Austria 1/3 to 2/3 of the yearly hunting bags are – either as fresh meat or as meat products – marketed from the hunter directly to the consumer or to local food retail establishments (Kainz and Paulsen, 2005). It is conceivable that the fraction of game meat traded *via* this 'local marketing' had been in the same order of magnitude before a legal framework was established. This indicates that there must already be considerable experience in handling of meat from wild game. As there are no records that such meat or meat products had been involved in foodborne disease incidents, these empirical practices seem to have provided a certain level of safety. The establishment of a legislation specifically addressing this food sector started in 1994, and underwent a revision in 2006, to ensure that direct marketing was regulated in compliance with the EU 'hygiene package'.

Concurrently with this specific legislation, empirical meat handling practices have been evaluated along the production chain 'from forest-to-fork' and adjusted to comply with science- and risk-based food safety. This included, for example: location of the shot wound (Winkelmayer *et al.*, 2005); time to onset of cooling of eviscerated large game carcasses (Paulsen and Winkelmayer, 2004), relation of visible contamination to the microbial

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contamination of wild game carcasses (Paulsen *et al*., 2003), storage of uneviscerated pheasant (Paulsen *et al*., 2008), application of Good Hygiene Practice (GHP) in cutting and deboning of pheasants (El-Ghareeb *et al*., 2009), and optimising safety and quality traits in fermented sausage from wild game (Bauer *et al*., 2007).

It is not surprising that most of the research work is funded by hunters associations, as these associations have a vital interest that game meat is 'safe' food.

2. Current legal framework in Austria

Only parts of EU legislation apply to local marketing of fresh meat from wild game, most relevant being Regulation (EC) No. 178/2002 (EC, 2002) and, when carcasses are broken down into meat cuts, Regulation (EC) No. 852/2004 (EC, 2004a). Austrian legislation provides an act on direct marketing. This act governs the hygienic conditions for direct and local marketing of small quantities of certain primary products of vegetable (berries, mushrooms) or animal origin, such as milk, eggs, carcasses of rabbit, poultry, wild game; in addition, this legislation covers also meat cuts from wild game.

Said legislation is very 'lean', in the sense that only few binding limits are specified and it is obvious that it appeals to the self-responsibility of the food business operators. For example, the term 'local' is not explicitly defined, which means that 'local market' could be the entire area of Austria; and small quantities are explicitly defined only for rabbits and poultry, which are slaughtered at and sold directly from the farm (5,000 and 10,000 per year, respectively).

For meat products, another act sets parts of Regulation (EC) No. 853/2004 (EC, 2004b) into force. The main items addressing wild game are given in Table 1.

3. Achievements to date

Lower Austria is the largest province in Austria, also in terms of contribution to the annual hunting bag. The hunting association of Lower Austria, in cooperation with external expert and the veterinary authority has developed a training and evaluation concept which is based on four pillars:

1. Already existing infrastructure in terms of trained persons since 1994.
2. Training courses on meat processing with practical in approved cutting rooms under veterinary supervision.
3. Checklists for self-checks and GHP compliance.
4. Microbiological own-checks.

3.1 Good Hygiene Practice (GHP) and documentation

Sector-specific 'Guides to Good Practice' are suggested in EU food hygiene legislation for the different branches of food industry. These guides can be designed by food industry and are then subject to approval of competent authorities. They are a recognised tool to

Table 1. Legal framework applying to the local, direct trade of meat and meat products from wild game.

Product	Subtypes	Requirements as in the act on direct marketing[1]	Other requirements[2]
Fresh meat from wild game	carcasses, skin-on, cooled	• marketed by the hunter • has undergone *ante* and *post mortem* inspection[3] • evisceration and cooling to max. 7 °C or 4 °C for large and small game, respectively, without undue delay • transportation and storage must not cause contamination of meat and temperatures must be maintained • must be marketed with 7 days *post mortem*	• Regulation (EC) No.178/2002 • national guide to personal hygiene (based on Regulation (EC) No. 852/2004, Annex II, Chapter VIII)[4] • national Act on animal-by-products[5]
	meat cuts, cooled or deep-frozen; also packaged; (frozen carcasses)	as above, and in addition: • meat must be labelled ('Wildfleisch aus Direktvermarktung' plus indication of region of origin)	as above, and • Regulation (EC) No. 852/2004 (Annex II) • national legislation on labelling of foodstuffs[6] • Austrian Food Codex[7]
Meat products		Act on direct marketing does apply only implicitly, but an act on local trade of foods[8] sets into force parts of Regulation (EC) No. 853/2004.	as above, and • muscle tissue must comply with requirements as above • all other ingredients must be food grade

[1] 108. Verordnung der Bundesministerin für Gesundheit und Frauen über die Direktvermarktung von Lebensmitteln (Lebensmittel-Direktvermarktungsverordnung), BGBl. II, Nr. 108/2006, as amended; the underlying Austrian Food Safety Act applies also.

[2] Not exhaustive.

[3] Also *Trichinella*-inspection, as applicable.

[4] 'Leitlinie zur Sicherung der gesundheitlichen Anforderungen an Personen beim Umgang mit Lebensmitteln'. Available at: http://www.bmgfj.gv.at/cms/site/attachments/5/9/5/CH0819/CMS1073644716719/gesundheits-anforderungen1.pdf.

[5] Verordnung der Bundesministerin für Gesundheit, Familie und Jugend über nähere Bestimmungen zum Umgang mit tierischen Nebenprodukten (Tiermaterialien-Verordnung), BGBl. II Nr. 484/2008.

[6] Verordnung über die Kennzeichnung von verpackten Lebensmitteln und Verzehrprodukten, BGBl.Nr. 72/1993, as amended.

[7] Codex Alimentarius Austriacus, 4th Ed., chapter B14. Available at: http://www.bmg.gv.at/cms/site/attachments/4/9/6/CH0832/CMS1167207128242/b_14_fleisch_und_fleischerzeugnisse.pdf.

[8] 92. Verordnung der Bundesministerin für Gesundheit und Frauen über Lebensmittelhygieneanforderungen an Einzelhandelsunternehmen (Lebensmittel-Einzelhandelsverordnung), BGBl. II, Nr. 92/2006, as amended.

establish GHP, and also for HACCP (Hazard Analysis and Critical Control Points) based food safety assurance systems. GHP and HACCP implementation guides have been issued in several EU countries. Recently released textbooks in Austria on inspection and on processing of wild game were designed to address all relevant issues laid down in EU food hygiene legislation (Winkelmayer *et al*., 2007, 2008). Particular emphasis is put on the requirements for food premises which are temporary or used primarily as a private dwelling house (in the sense of Regulation (EC) No. 852/2004, Annex II, Chapter III (EC, 2004a)). The minimum documentation recommended for hunters operating a temporary food premise is: (1) a signed copy of the guide for personal hygiene; (2) a master data sheet; and (3) a two-pages checklist for each workday which includes personal hygiene, condition and cleanliness of the premise and food-contact surfaces, condition of cooling facilities and maintainance of the required temperatures, results of the inspection of the game carcasses, water quality and disposal of waste/by-products (Winkelmayer *et al*., 2007). Hunters operating permanent food premises are offered separate checklists addressing individually the chapters in Annex II of Regulation (EC) No. 852/2004 (EC, 2004a). These checklists are based a system already operational for meat industry (slaughterhouses, butchers, etc.) in Austria.

3.2 Education and training

Food safety is the primary responsibility of the producer. In order to enable the producer to fulfill this obligation, training courses for direct marketing of fresh meat and meat products from wild game are offered in Lower Austria (Table 2). These courses have a modular structure and rely on a basic training in game inspection and hygiene (Winkelmayer *et al.,* 2011). For the practicals, a network of agricultural schools (equipped with approved cutting rooms, cooling rooms) provides a reliable infrastructure.

3.3 Survey on structure and product range

From December 2008 – April 2009, a survey was conducted on the direct markting of meat and meat products from game in Lower Austria (Fettinger and Paulsen, 2009). All hunters which had undergone training courses on direct marketing, or which had registered as direct suppliers of game meat, received a questionnaire, to collect basic information of infrastructure, hygiene level and products (fresh meat or meat products), see Table 3. These ca. 420 persons represent ca. 1.4% of licensed hunters in Lower Austria (Pontasch, 2008). All participants submitting a completed questionnaire were entitled to send up to 15 samples (fresh meat or products from wild game) for microbiological examination in the year 2009 (Fettinger *et al*., 2010).

A total of 109 completed questionnaires (ca. 26%) have been returned. 77% of the hunters operated temporary game processing premises, and 23% had permanent facilities. Selected findings are presented in Table 3. Such data can be particularly useful when the Competent Authority has to establish risk-based inspection schemes.

Table 2. Training courses for local, direct trade of meat and meat products from wild game.

	Theoretical lessons lectured by official veterinarians	Practicals under supervision of a specialised butcher and an official veterinarian
Fresh meat (ungulates)	• legislation • required infrastructure • hygiene during meat processing • humans and animals as source of foodborne disease • factors affecting food safety and shelf life, • documentation	• skinning of carcasses • preparation of boneless cuts • vacuum-packaging • declaration • assortment of meat cuts
Meat products	as above, plus • Austrian food codex • food declaration incl. QUID • good manufacturing practice in meat products • meat technology (ingredients, specific machinery for meat products; mincing, curing, smoking and heating) • temperature control as a part of HACCP based food safety • documentation	as above, plus • production of 2-3 different pasteurised cured meat products (e.g. frankfurter-type sausage and paté) • food labelling
Small game	see 'fresh meat'	

3.4 Microbiological own-checks

Microbiological self-checks can be a useful tool to document compliance with GHP and to assess product quality and safety. The prerequisite is, of course, that there are standardised procedures for evisceration, cooling, storage, cutting, etc. (Fettinger *et al*., 2010).

Based on the fact that meat from game can be marketed in the same way as that from farm animals, and that both types of meat can be displayed in the same shelf in butchers shops, it is reasonable to suggest the same microbiological limits for game meat as for meat from farm animals, provided that GHP procedures are strictly adhered to. This assumption has been substantiated in various studies (e.g. Paulsen *et al*., 2003; Paulsen and Winkelmayer, 2004; El-Ghareeb *et al*., 2009).

To check this hypothesis, submitted game meat and meat product samples were categorised (Fettinger *et al*., 2010) and then examined according to a product-specific catalogue consisting of food safety criteria as defined in Regulation (EC) No. 2073/2005 (EC, 2005) and other criteria laid down in specifications of fresh meat from slaughter animals (AMA, 2004) and a collection of microbiological limits for meat products (Eisgruber and Bülte, 2006).

Table 3. Results of a survey on the structure of local marketing of meat from wild game in Lower Austria (data in %), 2008-2009 (after Fettinger et al.*, 2010).*

Specific education of hunters*	specific training courses	83
	professional education (butcher, etc.)	17
	self-study of textbooks, etc.	36
Assistance of	none	42
	butchers or other professionals	38
	non-professionals	20
Premises	permanent	23
	temporary	77
Cooling facilities*	own cool room for game carcasses	59
	shared cool rooms	41
	additional refrigerator for meat cuts and meat products	47
Production of*	fresh meat	100
	meat products	43
Game species*	roe deer	97
	wild boar	70
	red deer	41
	brown hare	43
	pheasant	35
Meat traded*	refrigerated	93
	deep frozen	54
	vacuum-packed	71
Meat supplied to*	self-supply	86
	final consumer	79
	local butcher	8
	restaurants	32

* multiple answers possible

To date, 13 of 109 interested hunters have submitted a total of 55 samples, 22 of which were fresh meat. The pathogens *Salmonella* spp. and *Listeria monocytogenes* were not detected in any sample. From interviews conducted with a number of participants, this overall moderate acceptance was most probably caused by the discontinuous (seasonal) and low-scale mode of production.

4. Conclusions

Although a training and product evaluation system for hunters supplying game meat and meat products directly to the final consumer or to local food retail establishments has been established, it has become clear that traditional training must be augmented by new ways, e.g. by contests for game meat (products) in the framework of local conferences. The submission of products for such contests would require that certain chemical and microbiological

specifications are met and that evidence is presented that GHP is adhered to. In turn, such product contests would communicate to the public that the hunters are able to supply game meat and meat products of consistent quality under observation of hygiene rules. Such a pilot conference has already been held in November 2009.

Also, the possibility to rent the infrastructure of the network of agricultural schools spread over entire Lower Austria should be improved. This would allow hunters which are not willing or able to do investments in own infrastructure to work and process meat under professional conditions.

Acknowledgements

This contribution is based on presentations given at the conference 'Hygiene of wild game meat and its safety within the food chain', Prague, 22-24 April 2009, and on the 50. Arbeitstagung des Arbeitsgebietes 'Lebensmittelhygiene' der DVG, Garmisch-Partenkirchen, 29 September - 2 October 2009.

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Approaches to game hygiene in the province Belluno (Italy): from training to meat microbiology

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Summary

In the last decades, the number of animals shot in the Italian Alps during the regular hunting seasons has dramatically increased, which is particularly the case for wild ungulates. In these areas game is considered an important source of meat, and an increasing interest of consumers for game meat with high added value and favourable nutritional properties is noticeable (Hoffman and Wiklund, 2006). Nevertheless, targeted measures to assure game meat hygiene are still lacking in Italy, and much work is needed to convince hunters and consumers in general of the importance of this topic. This contribution summarises various complementary approaches to game meat hygiene, as initiated in the territory of the province Belluno, in the Italian Eastern Alps, i.e. the centre of the Dolomites, where red deer, roe deer, chamois, mouflon and wild boar are hunted.

Keywords: hygiene training, meat microbiology

1. Passive sanitary surveillance

Sanitary surveillance, together with a better management of wildlife and livestock, is one of the first actions to ensure quality and safety of game meat.

To be effective, sanitary surveillance should involve different stakeholders in the territory – including hunting associations, authorities for animal and human health and for wildlife management, laboratories and research institutes – with the aim to collectively elaborate a surveillance system. In the province Belluno, a 'passive surveillance programme' has been carried out in 2009, by developing a specific *vademecum* and cards for data collection, as shown in Figure 1 and 2.

This activity also included a 2-3 days training for hunters and gamekeepers, consisting of basic instructions on:
1. game anatomy and physiology;
2. game pathology;
3. good practices in game meat handling and conservation;
4. main alterations of game meat;

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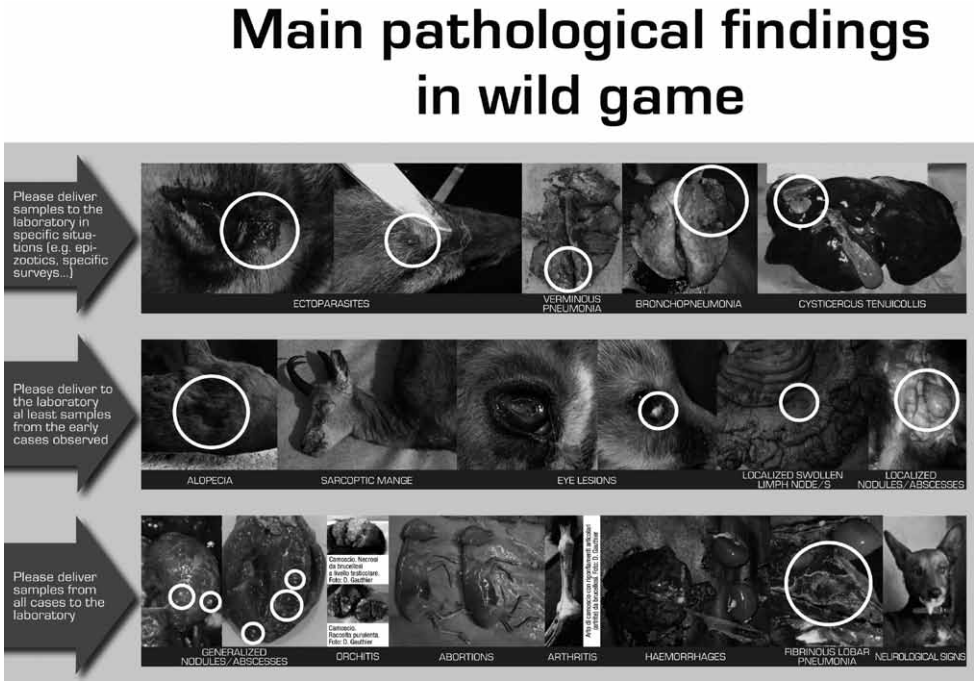

Figure 1. Vademecum *for passive sanitary surveillance in hunted game.*

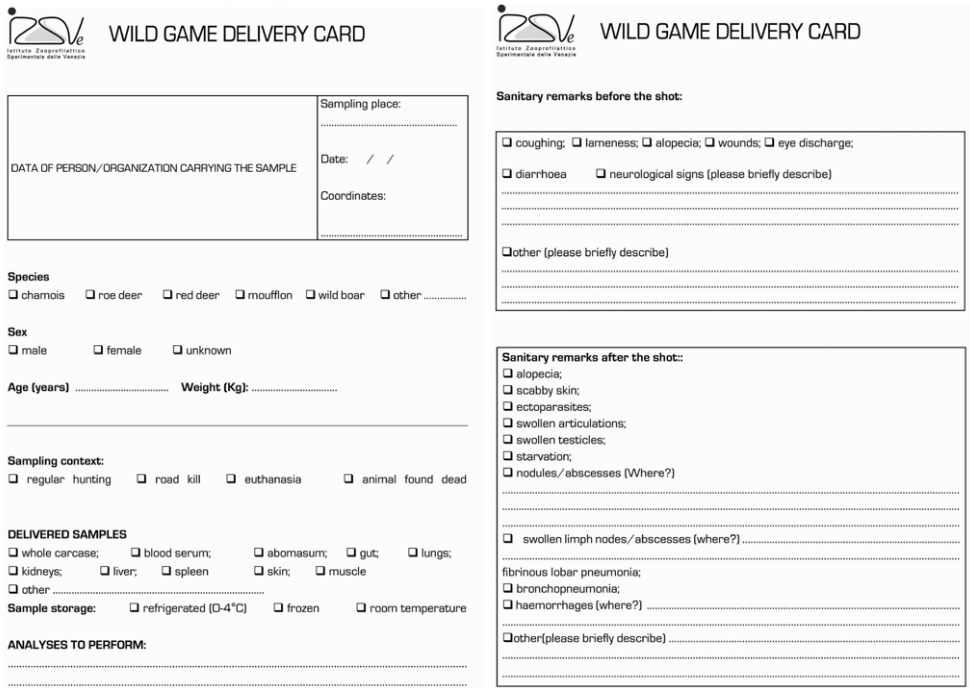

Figure 2. Cards for data collection by passive sanitary surveillance.

5. eco-epidemiology of disease in game;
6. regulations about game and game meat;
7. terminal ballistics (e.g. Figure 3).

For topics 1-3, a practical session was also held.

2. Game meat microbiology

From Belluno, data about game meat microbiology were also available (Bragagna *et al.,* 2004). Microbiological analyses were performed on 90 muscle samples from various wild ungulate species (39 roe deer, 27 red deer, 11 chamois, 8 mouflon and 5 fallow deer). The mean values (log_{10} cfu/g) of microbiological counts of core tissue samples are summarised in Table 1.

Pathogens such as *Salmonella* spp. or *Listeria monocytogenes* were not isolated. The Total Viable Count mainly consisted of Gram negative bacteria, such as faecal coliforms. A substantial difference in microbial carcass contamination was found between carcases correctly stored (i.e. in cold rooms at 0-4 °C) and carcases stored in less refrigerated places, such as cellars, where generally temperatures of 7-14 °C prevail depending on the season. In the latter case, levels of spoilage bacteria were definitely higher. Similar observations have been reported by Paulsen and Winkelmayer (2004).

Figure 3. Example of a practical session in terminal ballistics.

Table 1. Microbiological profile (mean values) of muscle core samples taken from 90 specimens of wild ruminants in Belluno province, Italy.

	$\log_{10}$ cfu/g
Total viable count	4.52
Total coliforms	4.32
Faecal coliforms	3.46
Sulphite reducing clostridia	2.11
Micrococcus spp.	3.16
Staphylococcus spp.	2.15
Lactobacillus spp.	4.55
Pseudomonas spp.	3.59
Aeromonas spp.	1.90

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Section 4
Muscle biology and meat quality

The muscle biological background of meat quality including that of game species

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Summary

A review of the major biological mechanisms determining the physical-chemical and sensory quality traits of whole tissue meat (colour and water-holding of fresh- and tenderness and flavour of cooked meat) is presented. The effect of various *ante mortem* and processing factors, affecting sensory quality traits of the various major animal meat species, are summarised and reference is made to effects reported for game meat species.

Keywords: muscle biology, meat quality, colour, water-holding, tenderness, flavour

1. Introduction

The concept of 'meat quality' includes many aspects such as hygiene and food physiological, technological and sensory properties (Hofmann, 1990). Whilst consumers can assume that the meat industry generally adheres to Good Hygiene and Manufacturing Practices which guarantee the meat safety- and allow storage of meat during a certain period, they usually are less aware of the many technologies that are primarily directed at improving sensory traits of meat.

The quality of fresh meat, as perceived when buying, preparing and consuming whole tissue cuts, is determined by *ante* and *post mortem* factors having interfered with muscle biological events in skeletal muscle. Many of these are common to all animal species and the effects of various processing technologies affecting meat quality (for instance, that of beef, pork, and poultry) have over the past decades been studied extensively. With few exceptions, game meat species have received less attention and the available information is still scarce and fragmented.

The purpose of the present contribution is first and foremost to review the major muscle biological mechanisms and processing effects underlying the sensory quality of meat in general. In addition, some data on game meat quality recently generated in our laboratory have been included.

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2. Muscle structure, composition and function

Skeletal muscle (expressed as percentage wet weight) is composed of 75% water, 19% protein, soluble organic compounds (3.5%), variable amounts of lipids (0.5-3%), carbohydrates (1-2%) and small amounts of minerals and vitamins (Lawrie and Ledward, 2006).

However, the visual appearance and the physiological and biochemical characteristics of skeletal muscles vary considerably, reflecting the proportion of fibre types present in the muscle cells. The commonly used fibre type classification is based on the contraction speed (fast or slow) and the energy metabolism (oxidative or glycolytic) of the fibres. The biochemical traits associated with different fibre types (e.g. ATP concentration, calcium-, myoglobin-, glycogen-, lipid-, proteinase and their inhibitors content, and enzyme activities) reflect the diversity of muscle fibres and are related to their physiological function exerted in the living animal (Pearson and Young, 1989). In this respect, meat quality parameters such as colour, flavour, juiciness and tenderness are fibre type dependent (for a review: see Monin and Ouali, 1991).

The coherent structure of muscle fibre bundles is maintained by the intramuscular connective tissue. Three connective tissue structures are distinguished in muscle, i.e. the epimysium (a fibrous sheath of connective tissue which surrounds the entire muscle), the perimysium (a three dimensional collagen network surrounding the muscle fibre bundles) and the endomysium (a layer of fine connective tissue fibres encircling individual muscle fibres).

Not more than 5% of the total water of muscle is directly bound to hydrophilic groups of the proteins, the rest is divided between the so-called 'free water' (i.e. immobilised by the physical configuration of the proteins but not bound to them) and the so-called 'loose water', which is expressed when the water-holding capacity drops (Hamm, 1972).

Fat content varies in quantity and composition between muscles and species, males generally having less than females. Many intracellular lipids are associated with membrane structures. In addition, considerable amounts of lipids are present in the perimysium surrounding myofibre bundles. Macroscopically this is perceived as 'marbling'.

Muscle proteins may be classified as sarcoplasmic, myofibrillar and stroma proteins. Sarcoplasmic proteins are soluble in water or salts of low ionic strength. Many of them are enzymes involved in the breakdown of glycogen. Myofibrillar proteins, which constitute the major part of the muscle's contractile mechanism, are only soluble in solvents with higher ionic strength than required for the extraction of sarcoplasmic proteins. The main myofibrillar proteins are myosin, actin, and the troponin-tropomyosin complex. Stroma proteins include collagen, elastin and reticulin, all present in connective tissue, and the proteins which are found in the membrane systems of the muscle cell organelles such as the mitochondria and sarcoplasmic reticulum.

The myofibrillar proteins are arranged in repeating units of actin and myosin. The thin myofilament is composed of two strands of ('filamentous') F-actin, which consist of polymerised ('globular') G-actin. Two strands of F-actin are coiled around each other and have a very precise length. The actin helix is associated with tropomyosin, a double helix of

two unidentical peptide chains, and the troponin complex (i.e. consisting of three polypeptide subunits C (binding calcium ions and thus effecting a change of conformation), I (inhibiting the interaction of actin to myosin) and T (the tropomyosin-binding subunit), which cements the tropomyosin to the actin helix). Figure 1A schematically presents this arrangement.

The troponin complex is thought to turn on the contraction process by binding Ca^{2+} ions that are released from the sarcoplasmic reticulum after a nervous stimulus. The thick myofilament consists of packed myosin molecules (Figure 1B) each of which has a helical tail and a head consisting of two globular units. The myosin heads contain a site with ATPase activity and a site which forms cross bridges that interact with actin to form actomyosin during muscle contraction.

Figure 2 shows a diagrammatic representation of the main (ultra)structural features of muscle. The I-band mainly consists of actin filaments that are attached to a transverse structure called Z-lines. The spacing between Z-lines is referred to as sarcomere length. Myosin fills the entire length of the A-band. A clear central H-zone contains the M-line representing the cross bridges between separate myosin filaments.

In developed striated muscle various 'cytoskeletal' proteins serve the role of maintaining cell shape and integrity and they are important for the function of movement. An example is titin, which is referred to as the 'gap-filament', anchored inside the myosin as a core protein and, running through the Z-lines, interconnects myosin filaments of adjacent sarcomeres.

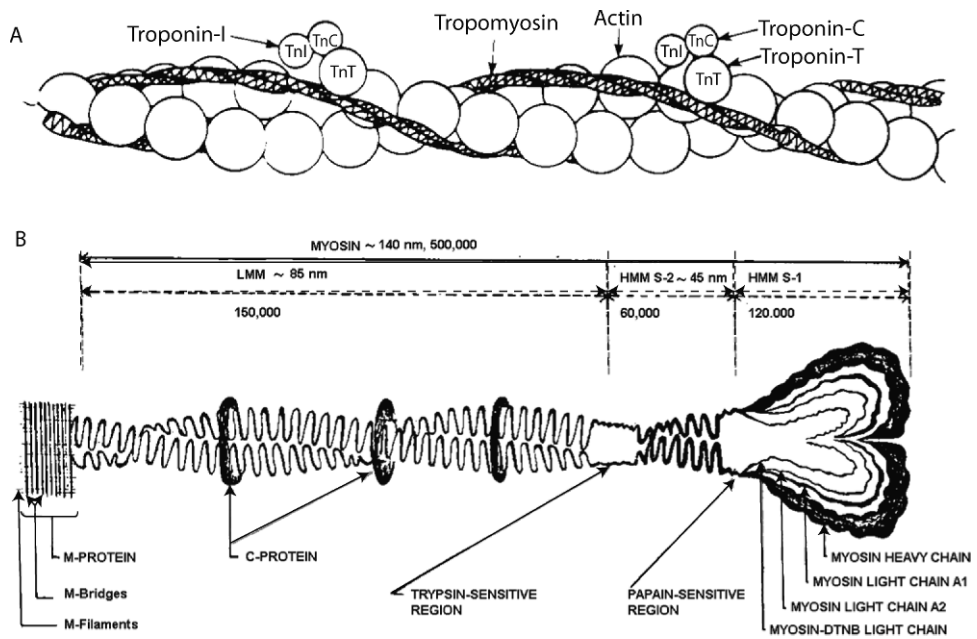

Figure 1. (A) The association of actin with the troponin-tropomyosin complex. (B) The structure of the myosin molecule (Cohen, 1975).

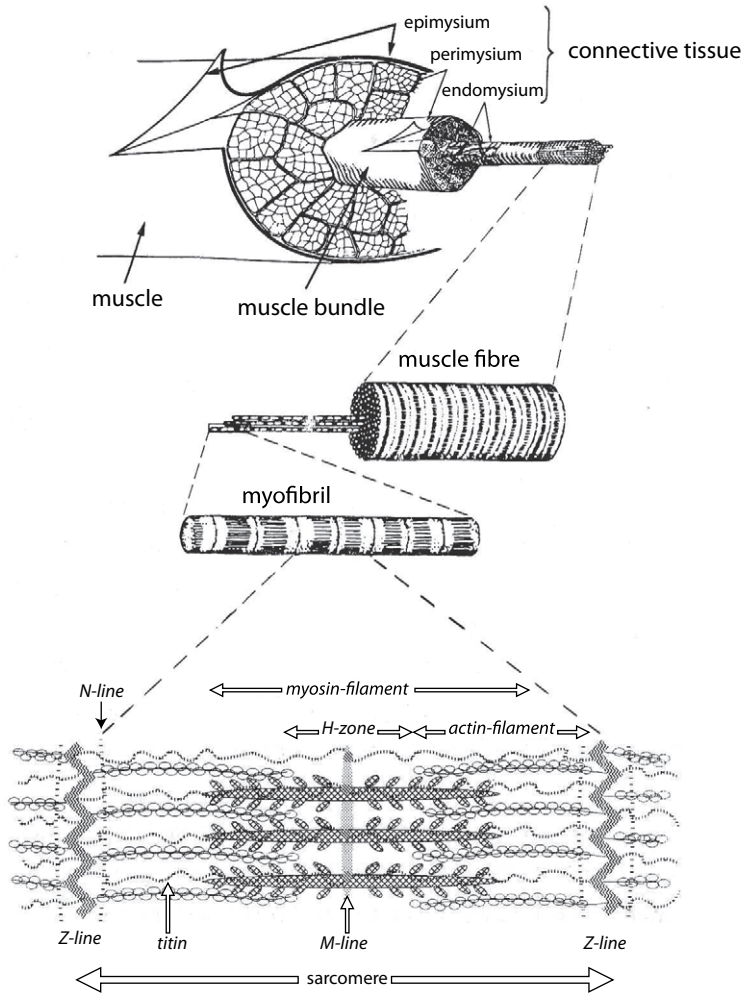

Figure 2. A schematic overview of the structure of skeletal muscle (Geesink, 1993).

3. *Post mortem* muscle physiology; the conversion of muscle to meat

Figure 3 is a diagrammatic representation of the main muscle physiological events, which are related to sensory meat quality traits.

In the living animal the blood circulation provides the muscle fibres with oxygen and glucose. Whenever muscle action is required, nervous stimuli effectuate a depolarisation of the sarcoplasmic reticulum membrane. This results in a release of Ca^{2+} ions which activate the enzymes that convert glycogen into pyruvate and eventually into carbon dioxide and water in the mitochondria (Krebs cycle). In the course of this process adenosine diphosphate (ADP) is phoshorylated to adenosine triphosphate (ATP). Initially the muscle relies on the

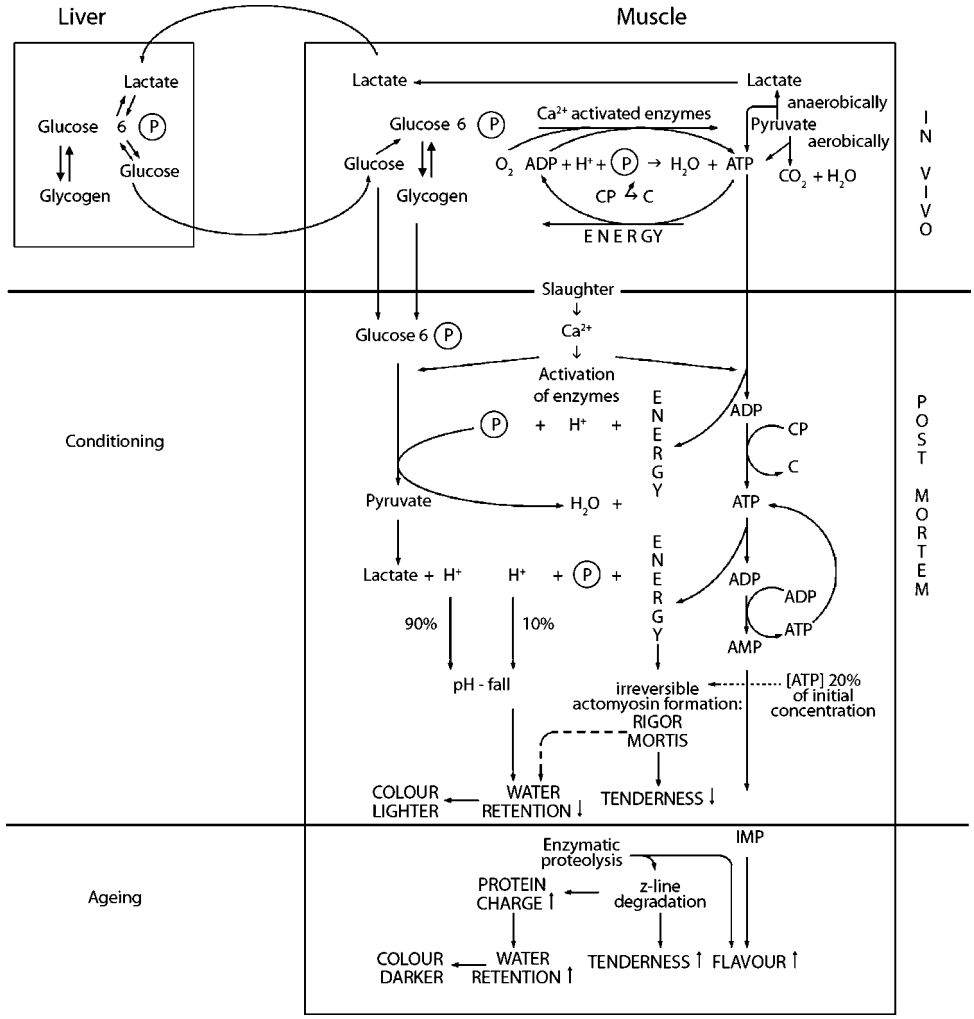

Figure 3. Diagrammatic presentation of the physiological events effecting the conversion of muscle to meat (After Smulders, 2007).

muscle specific creatin phosphate (CP) to supply the high energy phosphate. The conversion of ATP to ADP directly supplies the energy needed for muscle contraction and metabolic activities. This is affected by Ca^{2+} ions released by the sarcoplasmic reticulum, which stimulate myosin ATPase. Muscle relaxation also requires ATP, not only because the calcium pump transporting Ca^{2+} back into the sarcoplasmic reticulum is energy requiring, but also because myosin rods will only be allowed to shift out of the sheaths formed by the actin filaments provided ATP is present in its function as 'plasticizer'. Under anaerobic conditions ATP is resynthesized via the comparatively inefficient glycolysis leading to the formation of lactate, which may be resynthesised to glycogen in the liver *via* the Cori cycle (gluconeogenesis).

When an animal is slaughtered it is subjected to a state of shock which acts upon the muscle as a complex of nervous stimuli. Since carbohydrate and oxygen supply has ceased, the muscles are dependent on glycolysis for their energy synthesis from the moment the muscle specific creatin phosphate (CP) reserves have been depleted. Consequently comparatively little ATP is resynthesised. Moreover, the enzymes catalysing glycolysis and ATP breakdown are activated and lactate and metabolites such as adenosine monophosphate (AMP) are formed. As a result the muscle pH gradually falls. *Rigor mortis* sets in when too little ATP is available to keep the actin and myosin filaments apart in a relaxed state. This occurs when the ATP residue is approximately 20% of its initial concentration (Hamm, 1977).

The biochemical reactions in the period before onset of *rigor mortis* – generally referred to as the period of 'conditioning' – as well as those resulting from the subsequently occurring *post mortem* muscle proteolysis – also known as 'aging' – have a great impact on sensory meat quality characteristics.

4. Major sensory characteristics of meat

Whilst major interrelated phenomena occurring *post mortem* and valid across the various meat species have been included in Figure 3, the following section discusses in more detail the relevance of the various factors that determine the major quality traits of meat. Wherever meaningful, particular reference will be made to those factors that might have relevance to the quality of game meat. The reader is referred to another contribution to this Volume for an overview of methods to quantitatively assess these various meat quality characteristics (Hofbauer and Smulders, 2011).

4.1 Colour of fresh meat

The colour of fresh meat is determined both by light absorption (dependent on the contents of pigments (i.e. apart from the cell pigment cytochrome, particularly hemoglobin and myoglobin)) and light being reflected from water present on the meat surface (largely related to the water-holding properties of muscle proteins and the density of the myofibrillar matrix; see below). Provided an animal is properly exsanguinated, the role of hemoglobin is relatively minor: fresh meat contains no more than 0.3% residual blood.

4.1.1 Myoglobin – related factors

The pigment primarily responsible for colour is myoglobin (Mb). Like hemoglobin in blood, its physiological function is to store oxygen and deliver it to the muscle whenever necessary. Upon binding of oxygen, its colour changes from purple to an attractive cherry-red which reflects the presence of oxymyoglobin (MbO). When the heme-iron oxidises (ferrous iron, Fe^{2+}, turns into ferric iron Fe^{3+}), metmyoglobin (MMb) is formed which has lost the ability to bind oxygen. Concurrently colour turns into an unattractive greyish-brown. Only through reduction with the aid of reducing enzymes can MMb be converted back into physiologically active (oxygen-binding) Mb.

The relationship between partial pressure of oxygen (pO_2) and the chemical form of Mb (Mb, MbO or MMb) is presented in Figure 4. Depending on the presence of oxygen, be it at atmospheric- or at higher pressures as for instance prevailing in modified atmosphere packaging, the surface of any piece of meat will have a more or less thick superficial layer of MbO, which is being replaced by MMb at depths where oxygen penetration is insufficient, and finally by Mb in the core of the muscle where pO_2 is zero.

When interacting with hydrogen peroxide (H_2O_2) or hydrogen suphide (H_2S), Mb transforms into the green pigment cholemyoglobin (ChMb) or sulphmyoglobin (SMb). The formation of one or both of these pigments is responsible for the green discoloration of meats with severe bacterial contamination.

4.1.2 Fresh meat colour as affected by its water-holding properties

The ability of a muscle matrix to hold on to water has a large impact on meat colour. The lower the water-holding capacity of meat (see below) the more water molecules will be released onto- and the more light will be reflected from its surface.

The well-known Pale-Soft-Exudative (PSE) condition predominantly found in pork is largely the result of sarcoplasmic protein and myosin denaturation, caused by a combination of high carcass temperatures associated with stress (malignant hyperthermia- or porcine stress syndrome) and the resulting extremely fast pH decline. As a consequence, PSE meat binds water very poorly and the meat surface reflects light causing its appearance to be pale.

Certain stressful *ante mortem* conditions, e.g. those associated with physical exhaustion as seen after intensive hunting, can cause healthy animals to develop so-called Dark-Firm-Dry (DFD) meat, which is characterised by a high ultimate pH, a purplish-black colour, a firm

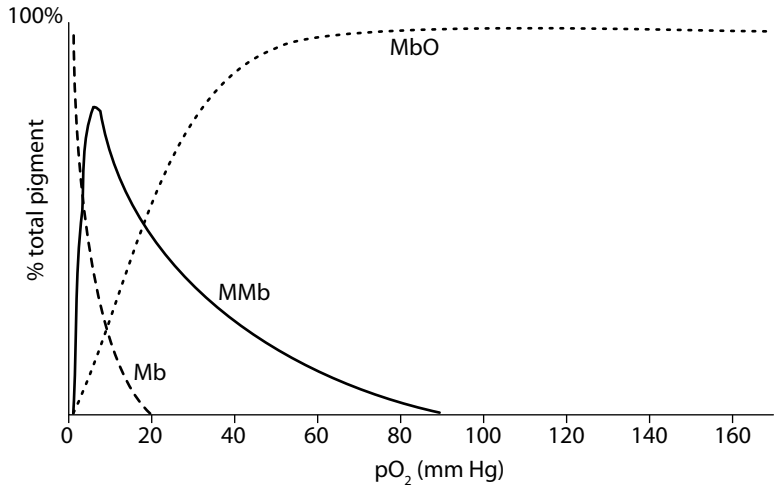

Figure 4. The effect of partial pressure of oxygen on the prevalence of various myoglobin forms (Smulders, 2007).

texture and a dry sticky surface. The condition is predominantly reported to occur in beef and pork, but is similarly relevant for game species such as deer (Wiklund and Smulders, 2011) subjected to stressful conditions, e.g. regrouping, being chased or being subjected to inappropriate transport conditions. The depletion of muscle glycogen stores caused by these activities results in a lower than normal muscle acidification (higher ultimate pH values), and consequently in unusually high electrostatic binding of water by the muscle proteins (i.e. an extremely low release of water to its surface (see Section 4.3.1)) and hence less light reflectance (explaining its darker appearance).

It is questionable if the PSE or DFD condition occurs in other species than those mentioned, although there are reports suggesting their prevalence for instance in poultry (e.g. Van Hoof and DeZeure-Wallays, 1980).

4.1.3 Colour stability of meat

In essence, the stability of the colour of fresh meat is characterised by the muscle's ability to keep Mb in the oxygenated form and to prevent the formation of MMb. Although none of them react independently of the other, several biological factors impact on meat colour stability. Major ones recognised by Faustman and Cassens (1991) are: (1) muscle pH, low values promoting the formation of MMb, (2) temperature, higher muscle temperatures favouring increased formation of MMb and dissociation of oxygen from MbO, (3) relative humidity (rh), lower rh's leading to more desiccation and a darker surface colour, (4) exposure to light (particularly the ultraviolet part) resulting in more MMb formation, (5) bacterial contamination, the exponential growth phase of the spoilage flora coinciding with the highest oxidation, (6) lipid oxidation, (7) partial pressure of oxygen (pO_2), where zero (not low) pO_2 prevents MMb formation and only pO_2's around or above 80 mm Hg promote the desirable MbO formation (see Figure 4), (8) the presence of MMb reducing enzyme systems, prolonged refrigerated storage leading to a considerable loss of reducing activity ('fading' of meat), (9) pre-slaughter stress, leading to an aberrantly fast pH decline or high ultimate pH values (see above), and finally (10) muscle dependent sensitivity to discoloration, probably related to their oxidative capacity (fibre type).

4.1.4 Ante mortem *and processing effects on meat colour – summarised*

Factors affecting the ultimate appearance (colour) of fresh meat (Smulders *et al.*, 1991) include: (1) species effects or genetic variation within species (responsible for differences in oxygen consumption rate of mitochondria which counteract MbO formation, or determining the vulnerabilty to stress and the associated PSE condition); (2) sex and age (e.g. as reflected in the darker colour of bull vs. cow meat); (3) plane and quality of nutrition (e.g. determining the degree of marbling and iron content); (4) inappropriate *ante mortem* handling of animals leading to PSE or DFD (see above); (5) accelerated carcass processing methods such as electrical stimulation (which, when not applied correctly, can lead to increased protein denaturation (e.g. Eikelenboom and Smulders, 1986)) or hot boning allowing faster cooling of primals and reduced protein denaturation (Van Laack, 1989); and, finally (6) modified atmosphere packaging (allowing an increased formation of MbO, provided high oxygen concentrations are present in the gas mixture).

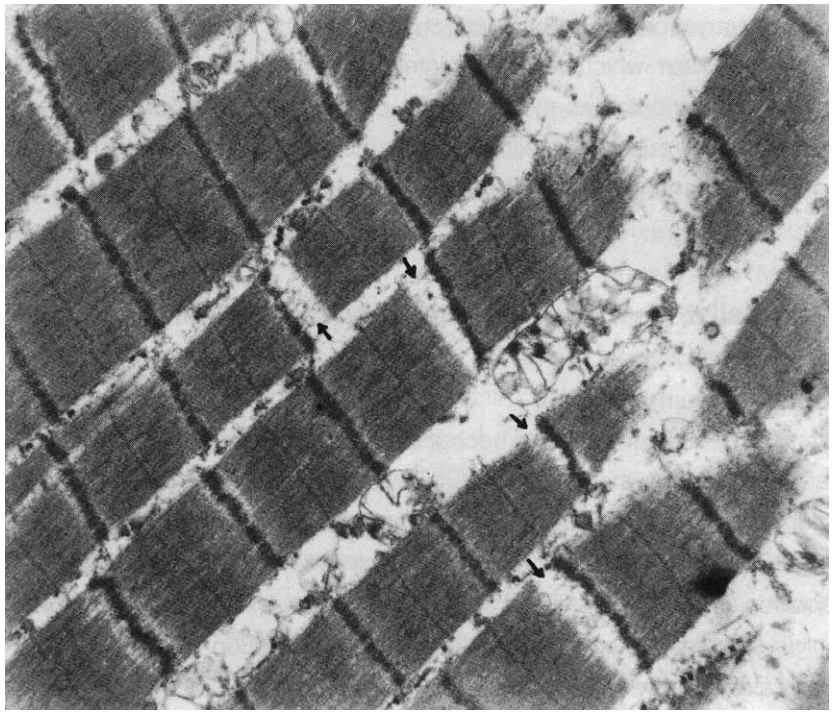

Figure 5. Fragmentation of myofibrils adjacent to Z-lines (see arrows) in bovine longissimus *muscle after 14 days of storage at 2-4 °C (by courtesy of P. Koolmees).*

4.2 The tenderness of meat

The main components thought to determine the tenderness of meat are connective tissue, myofibrillar proteins matrix and fat. As the role of fat is primarily that of a 'dilutant' of the muscle matrix (a high degree of marbling generally being associated with better tenderness and only extremely low contents <1% being reported to promote toughness) the following sections concentrate on the contribution to tenderness of the former two components.

4.2.1 The connective tissue component of tenderness

Connective tissue serves an important physiological function in muscle. It supports muscle fibres and carries over the forces generated by myofibrils to the skeleton. Connective tissue predominantly consists of collagen (65-95%). In addition, the less rigid elastin prevails in lower amounts (5-35% of the total connective tissue, dependent on the muscle), particularly in the epimysium.

Not only the collagen content, but also its type and the degree of cross-linking between the constituting tropocollagen molecules affect the tenderness of meat. Various isoforms of collagen exist. In muscles, types 1-5 are important, each type having distinct properties, For instance, type 3 is markedly more heat stable than is type 1 (McCormick, 1994). Consequently,

the distribution of collagen isoforms largely determines the solubility of connective tissue (Stanton and Light, 1987). Contrary to the myofibrillar component of tenderness, which is significantly affected during *post mortem* storage of raw meat, changes in connective tissue are only observed in the course of heating.

Although the ratio connective tissue: muscle substance decreases during growth, the degree of cross linking increases, which explains the relative toughness of meat from older *vs.* younger animals. It was once thought that connective tissue was the overriding factor explaining tenderness differences between meats. However, research in the past decades has clearly shown that its role is restricted to its determining the so-called 'background toughness', which obviously is muscle- but also species dependent and is particularly related to the content of collagen type 3 (Dransfield, 1977, Light *et al.*, 1985). Whereas in the course of storage the properties of collagen barely change, heating during meat preparation will affect its solubility markedly, provided temperatures >80 °C are reached at which collagen gelatinises and becomes soft.

4.2.2 The myofibrillar component of tenderness

Density of the myofibrillar matrix

Although a clear relationship between sarcomere length and tenderness score of meat does not always exist, there appears to be general consensus that long sarcomeres are associated with tenderer-, short sarcomeres with tougher meat (unless 'super-contraction' has occurred beyond 40% of the muscle's rest length of about 2 µm (Marsh and Carse, 1977)). However, said relationship is not linear and appears to be only valid for muscles with slow glycolysis (Smulders *et al.*, 1990). Preventing muscles to contract during the onset of *rigor mortis* (as achieved through the 'tenderstretch' process, e.g. by pelvic suspension), also leads to a tenderer end-product (Smulders *et al.*, 1992).

The degree of muscle contraction during rigor onset is markely affected by the muscle's temperature decline. Although at low temperatures ATP breakdown is slower (as the glycolytic enzymes' activity is lower during refrigeration), at critically low temperatures <6 °C mitochondrial release of Ca^{2+} increases while at the same time the calcium pump responsible for pumping Ca^{2+} ions back into the sarcoplasmic reticulum fails to function properly. The resulting increased concentration of sarcoplasmic Ca^{2+} leads to an intensive contraction known as cold shortening (Conforth *et al.*, 1980). Cold shortening occurs primarily in meat from animal species exhibiting slow muscle glycolysis (e.g. ruminants). Beef processors using fast chilling regimes therefore generally rely on electrical stimulation which accelerates glycolysis and thus prevents low temperatures being reached when muscle pH is still high (Smulders and Van Laack, 1995). In this context, Bendall (1973) reported that combinations of muscle pH >6.2 while temperatures are already below 12 °C should be considered particularly hazardous.

A phenomenon known as 'heat-shortening' or '*rigor* contracture' may become relevant when *rigor* sets in at temperatures >25 °C, e.g. when muscle is not chilled immediately after the animal's death (as is often the case in game animals left unrefrigerated for too long). This situation also explains the sometimes extremely tough meat found in PSE pork.

Myofibrillar proteolysis

In *post mortem* tenderisation, which results from a loss of the muscle's structural integrity (see Figure 5), proteolytic enzymes play a dominant role. Research has concentrated on two enzyme systems, viz. the calpains (i.e. calcium activated sarcoplasmic enzymes active at high muscle pH) and the cathepsins (lysosomal enzymes active at low pH); (for more extended reviews: see Roncalés *et al.* (1995), Smulders *et al.* (1999), Ouali (2007)).

Calpains belong to the cystein group of proteases, which have an optimum pH between 7 and 7.5 and need calcium to become active. The efficacy of the calpains' proteolytic action is based both on their breaking down of structural myofibrillar elements, and on their own autolysis. The proteolytic rate of both these processes depends heavily on pH and temperature (Dransfield, 1994). At least 3 components of the calpain system have been distinguished, i.e. the proteinases μ-calpain (active at micromolar concentrations of Ca^{2+}) and m-calpain (active at millimolar concentrations of Ca^{2+}) and the specific inhibitor calpastatin. Binding of calcium induces autolysis, by which the enzyme is both activated and ultimately broken down (Figure 6). The m-calpains concentration remains fairly constant throughout ageing, indicating its contribution to meat tenderisation is minimal (Koohmaraie *et al.*, 1987).

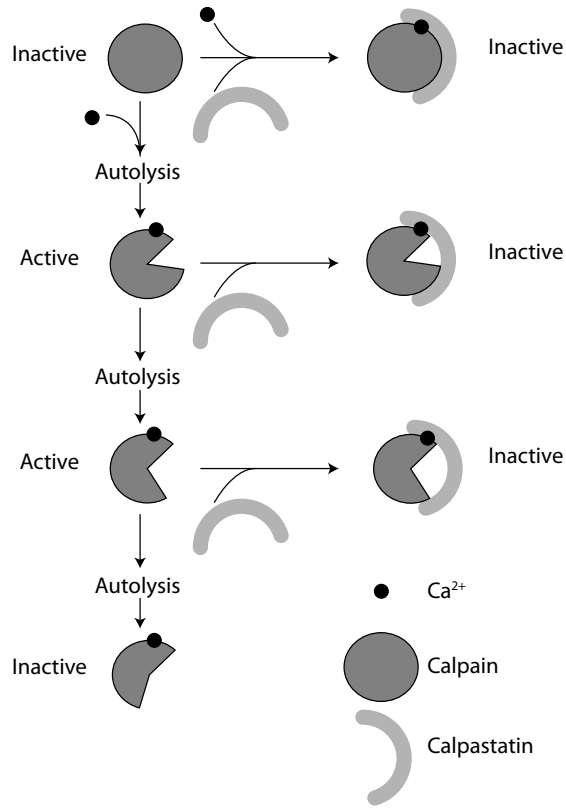

Figure 6. Schematic diagram showing the interaction of calpain with Ca^{2+} and calpastatin, and autolysis of calpain (Geesink, 1993).

Cathepsins are lysosomal endopeptidases, 8 of which have been found in skeletal muscle (cathepsin A, B, C, D, H, L, J and carboxypeptidase B). Cathepsin B, D, H and L appear to have the highest activity in skeletal muscle and are active at pH values ranging from 2.5-6.0. Cathepsins activity is regulated by a series of inhibitors, the cystatins, prevalent in the sarcoplasm. It has been reported that at least cathepsins D, H and L may break down actin and myosin, albeit only provided temperatures are higher than 2-4 °C. Hence, it is assumed proteolysis by cathepsins is probably only relevant when meat is held at elevated temperatures (Goll *et al.*, 1989).

4.2.3 Ante mortem *and processing effects on meat tenderness – summarised*

Factors affecting the ultimate tenderness of whole tissue meat as assessed after heating were listed by Smulders *et al.* (1991) and include: (1) species effects, related both to the vulnerability of muscles to phenomena like cold shortening, but also to ageing rates (these decrease in the order poultry > pork > lamb > beef); (2) sex, e.g. bulls generally rendering tougher meat than cows (a muscle dependent phenomenon also partially explained by differences in fat deposition and the age at which animals are slaughtered; see the following entry); (3) age, largely based on a decrease in collagen solubility; (4) nutrition, energy-rich rations promoting a higher degree of marbling; (5) preslaughter handling, possibly resulting in PSE or DFD meat; the latter condition yielding meat with high tenderness scores, probably because calpain activity is prolonged at high ultimate pH values; (6) rate of carcass refrigeration, possibly leading to cold (or heat) shortening; (7) accelerated processing, e.g. by relying on electrical stimulation possibly combined with hot boning, (8) tenderness promoting additional processing, such as pelvic suspension or pressure/heat treatment; and finally, (9) adhering to optimal ageing times; i.e. 5 days for pork and around 2 weeks for beef, veal, lamb and rabbit.

Not related to processing per se, but rather to domestic preparation of meat, are various methods for cooking meat, which have a marked influence on tenderness as perceived by the consumer. The reader is referred to Smulders *et al.* (1991) for further information on this important tenderness determinant.

4.3 The water holding of meat

After the carcass and muscles are cut, a red proteinaceous fluid, called drip, oozes from the cut surfaces. The mechanism of its formation is not completely revealed yet. Fluid loss can be partly explained by the decreased water-binding by proteins resulting from the *post mortem* pH fall. However, the amount of water chemically bound to proteins is too small to account for the total fluid loss. The following major mechanisms of water-holding of fresh meats have been reported and extensively discussed by Den Hertog-Meischke *et al.* (1997).

4.3.1 Shrinkage of myofibrils

Since most of the muscle water is present within the myofibrils, a general hypothesis for explaining drip loss is that it originates from the 'lateral shrinkage' of the myofibrils *post mortem* (Offer and Trinick, 1983). Lateral shrinkage is partly due to *post mortem* pH fall, as with decreasing pH the charges of the filaments and thus the negative electrostatic repulsion

between the filaments are reduced, causing the space between the filaments to diminish (Hamm, 1986).

The second factor causing ('longitudinal') shrinkage is the actomyosin formation during *rigor* onset. The shorter the sarcomere length, the smaller the myofilament lattice spacing, and a close and linear relationship between drip loss after one week of storage and sarcomere shortening has been observed (Smulders *et al.*, 1984, Honikel *et al.*, 1986).

A third factor promoting shrinkage is the denaturation of myosin, which reduces the charge of – and hence diminishes the electrostatic repulsion between the thick filaments as well as reduces the myosin head length. All these events draw the thick and thin filaments close together and thus result in higher drip loss (Offer *et al.*, 1989).

4.3.2 Denaturation of sarcoplasmic proteins

Although it has been well-established that in meat with a low water-holding capacity sarcoplasmic proteins are denatured, one must confront the fact that these proteins only hold about 3% of the water and that upon denaturation these globular proteins lose their compact folded structure and may thus entrap more rather than less water. Monin and Laborde (1985) suggest that precipitation of (denatured) sarcoplasmic proteins may contribute to the loss of electrostatic repulsion between filaments and thus to drip loss.

4.3.3 Changes in osmotic pressure

Maximal osmotic pressure reached in *post rigor* meat is highly muscle dependent and may partly account for the variability in water-holding capacity between muscles within a carcass (Monin and Ouali, 1991). As muscle pH drops, extracellular spaces become hyperosmotic, causing migration from water out of the muscle cells until the sarcolemma becomes permeable for proteins and ions.

4.3.4 Fluid accumulation within the muscle

Offer and Cousins (1992) studied the time course of structural changes in *post mortem* beef sternomandibularis muscle. Immediately after the animal's death, the extracellular space of a muscle primarily exists of the vascular system and connective tissue (perimysium and endomysium). After 4-6 hours *post mortem*, large gaps (presumably filled with fluid) appear between the fibre bundles. After *rigor* onset, these gaps also arise between the fibres and the endomysial network. The separation between fibres is only partial and a fibre may remain in contact with one of its neighbours (see Figure 7). At this time the sarcolemma loses its integrity and sarcoplasmic proteins can diffuse freely into the extracellular space. The fluid in the extracellular space may be the source of drip. Both perimysial and endomysial gaps form longitudinal channels, which are in connection with the cut surface of the meat. Drip probably arises predominantly by the action of gravity draining the fluid in these channels to the cut surfaces (Offer *et al.*, 1989).

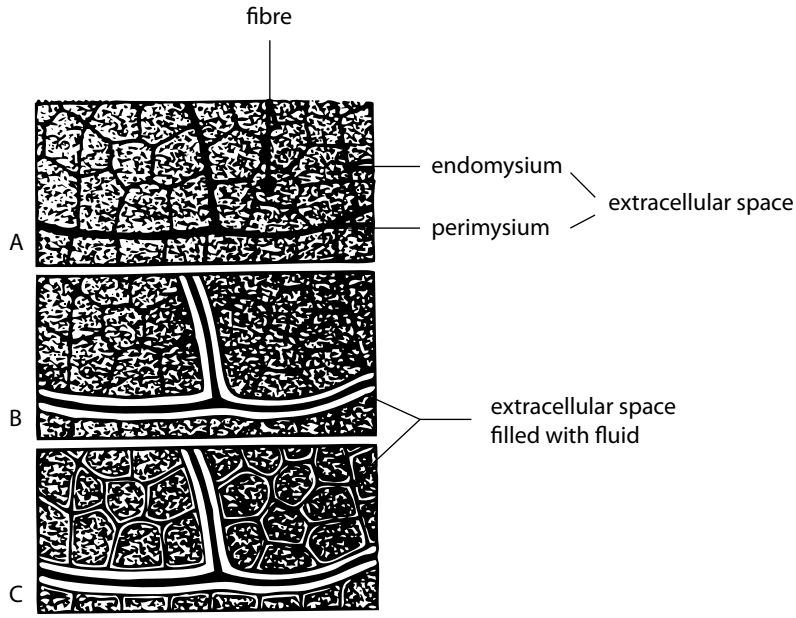

Figure 7. Schematic overview of structural changes in beef post mortem. *(A) Living muscle and immediately after slaughter; (B) 4-6 hrs* post mortem*; (C) rigor (Offer and Cousins, 1992).*

4.3.5 Ante mortem *and processing effects on the water-holding of fresh meat – summarised*

The following factors were identified by Smulders *et al.* (1991) and Den Hertog-Meischke *et al.* (1997) as affecting the water-holding properties of fresh meat: (1) animal species and breed differences, partly associated with their vulnerability to cold shortening and/or their (largely genetically determined) susceptibility to stress; (2) pre-slaughter handling, possibly leading to the PSE or DFD condition (see above); (3) stunning method (e.g. electrical stunning may cause a somewhat accelerated glycolysis); (4) electrical stimulation, leading to faster *post mortem* pH decline causing increased protein denaturation; (5) chilling rate, high chilling rates slowing down protein denaturation but – unless counteracted by appropriate measures such as electrical stimulation – also bearing the risk of inducing the cold shortening phenomenon; (6) hot boning, allowing a faster and more uniform drop in temperature and thus counteracting protein denaturation, but at the same time increasing the risk of cold shortening; (7) sample characteristics, i.e. the degree to which muscles are cut and whether the cut is longitudinal (less drip) or transversal (more drip); (8) method and material of support, suspended muscles losing less than those supported from below, and muscle portions laid on a tray losing less than those placed on absorbent paper; (9) packaging methods, the effects being largely dependent on the forces applied on the meat and the degree to which cut ends are sealed off; (10) storage temperature, probably because at higher storage temperatures fluids have a lower viscosity and will migrate more easily; (11) freezing and thawing; fast freezing resulting in the formation of many small ice crystals which cause less cell damage, and slow thawing allowing remigration of water into the intracellular space; (12) transport conditions, transport vibrations possibly

enhancing drip loss from meats with a low intrinsic water holding capacity; and finally (13) ageing time, extended storage periods possibly allowing re-uptake of drip as a result of pH increase and proteolytic changes.

4.4 The flavour of meat

Flavour is determined both by taste and olfactory sensations, although astringency, mouth-feel and juiciness also play a role. Whereas mouth receptors can assess the 4 taste sensations (sweet, salt, sour, bitter), hundreds to thousands odour compounds can be distinguished by epithelial receptors which are reached either by smelling (*via* the nose) or *via posterior* nares at the back of the nose and throat while food is being chewed in the mouth (Farmer, 1994).

Moody (1983) lists the non-volatile or water-soluble compounds with taste or tactile properties as: inorganic and sodium salts of certain acids (salty), hypoxanthine (an ATP metabolite), peptides and some amino acids (bitter), sugars and some amino acids (sweet) and acids (sour). In addition, there are thousands of low-molecular weight compounds that give rise to odour sensations. Although more than 800 volatile compounds have been identified in cooked meat aroma (Maarse, 1989), it is believed that only a relatively small number of these compounds actually play a role in the overall aroma of cooked meat and whether one of these represents a key odour impact compound depends on both its concentration and its odour threshold, i.e. how sensitive the human nose is to that particular compound. Farmer (1994) lists the major compounds and their routes of formation and she indicates two reactions that are of particular importance in meat aroma formation, i.e. the Maillard reaction (a complex network of reactions which yields both high-molecular-weight brown coloured products and volatile aroma compounds) and lipid oxidation during heating which contributes to the formation of desirable flavours. The latter author concludes that free amino acids, peptides, sugar and also phospholipids and their fatty acids are particularly significant for flavour forming reactions, that various vitamins and minerals can alter the rate and extent of these reactions and consequently that changes in composition of nutrients in meat could lead to a change in the balance of flavour forming reactions and therefore to a change in the overall aroma and flavour.

4.4.1 Ante mortem *and processing effects on the flavour of cooked fresh meat – summarised*

The following factors have been identified by Smulders *et al.* (1991) as affecting meat flavour: (1) animal species and breed, probably through the genetic control of lipid composition and metabolism; (2) sex (e.g. 'boar taint' in pork, for which compounds such as androstenon and skatole are thought to be responsible); (3) nutrition (e.g. 'boiled cabbage' off-flavours of mutton fed with rapeseed, or 'fishy' off-flavours of pork fed with polyunsaturated fatty acids which also makes this pork more prone to oxidation and hence rancidity; (4) *ante mortem* stress, for instance, the DFD condition's inherent low acidity and hence a flavour which is less 'accentuated' making DFD taste rather bland; (5) bacterial contamination, as a result of which, dependent of the bacterial ecology, 'sulphurous' off-flavours (*Pseudomonadaceae, Enterobacteriaceae*) or 'dairy/cheesy' off-flavours (*Lactobacillaceae, Brochothrix thermospacta*) may occur; (6) irradiation at high dosages (causing increased carbonyls or hydrocarbon formation); (7) lipid (per)oxidation particularly catalysed by heme iron and reported in meat

that has been size-reduced (e.g. deboned chicken and turkey); (8) phospolipid and fatty acid oxidation ('warmed-over flavour') in cooked meats, probably catalysed by non-heme iron.

5. Some data on game meat species

5.1 Introductory remarks

Whereas worldwide a vast number of wild animals are hunted – in situations where this is essential for the provision of nourishment but also to satisfy man's intrinsic desire to hunt (Winkelmayer, 2009) – the type and range of animals hunted for food vary in different parts of the world, depending on climate, animal diversity, local taste and locally accepted views about what can or cannot be legitimately hunted.

Contrary to the situation for farm animal species, on which a vast body of information is available from the literature, muscle physiological specifics of wild animals and scientific data on their meat quality are scarce, amongst other reasons because only in rare cases (e.g. Hoffman and Wiklund, 2006) there is an economic incentive to support domestic or export game meat marketing by solid research. Not surprisingly, much of the information on the sensory quality of various game species appears to be rather based on culinary tradition than on scientific investigation.

The experimental design (particularly as regards random sampling of experimental and control animals from a uniform population for which ample physiological and other background data are available) is relatively easily achieved in studies on farm animal species. However, this represents the major hurdle in studies on game species, unless these are (semi-) domesticated. The latter is for instance the case for deer and reindeer as outlined by Wiklund and Smulders (2011). In this context, one must also realise that once animals are domesticated, breeding efforts may affect the animal's physiology as evidenced, for instance, by the altered muscle fibre structure and biochemistry of the breast muscle of domesticated turkey in comparison with the wild counterpart animal from which it was bred (Sosnicki *et al*., 1991) or by the in-bred genetic stress susceptibility prevalent in Landrace pigs (Eikelenboom and Minkema, 1974).

In the framework of this contribution it would be impossible to provide a comprehensive overview of all the data available on game meat properties. Also, the methodologies used by the various scientists often differ markedly, making comparison of published results difficult. Therefore, the purpose of the following section is to provide some results of studies on meat quality characteristics of a restricted number of game meat species as conducted in our laboratory with the standardised methods described by Hofbauer and Smulders (2011).

Although this authorship has also been involved in studies of more exotic game animals (e.g. on properties of guanaco- (Gonzalez *et al*., 2004) or beaver meat (Hofbauer *et al.,* 2005)) we will in the following focus on animal species with relevance to hunting activities in Central Europe. In the absence of sufficient muscle biological background information, some of these data are inevitably of a more descriptive nature.

5.2 Ruminant species

5.2.1 Feral deer

Table 1 and 2 include results of a study of the seasonal variation in meat quality in one year old roe deer (*Capreolus capreolus*) shot (and subsequently eviscerated) in Austria's subalpine regions either in Spring or Autumn (Winkelmayer *et al.*, 2004). Whereas carcass weights differed – obviously related to the plane of nutrition available in the animals' habitat – none of the variables measured indicate a difference relevant for sensory quality as perceived by the consumer.

Table 1. Carcass weight, pH and water holding capacity (WHC, method of Grau and Hamm, 1953, procedures according to Hofmann et al.*, 1982) as assessed at 45-60 min ('1') and at 24 hrs. post mortem in semimembranosus muscle of one year old roe deer carcasses (means ± standard deviation) (after Winkelmayer* et al.*, 2004)*[1].

Quality trait	Spring (n=15)	Autumn (n=12)
Carcass weight (kg)	11.07^{b}±1.72	13.18^{a}±1.72
pH_1	6.54±0.3	6.41±0.45
WHC_1	0.69±0.17	0.79±0.14
pH_{24}	5.66±0.28	5.58±0.15
WHC_{24}	0.72^{a}±0.16	0.84^{b}±0.08

[1] Figures with different superscripts differ significantly ($P<0.05$, F- Test).

Table 2. Sensory quality traits of longissimus muscle of one-year old roe deer as assessed after 14 days of vacuum storage at 2 °C (means ± standard deviation); none of the differences were significant (P>0.10; F-test) (after Winkelmayer et al.*, 2004).*

Quality trait	Spring (n=15)	Autumn (n=12)
Drip loss (g/100 g)	3.11±1.13	2.35±1.05
Cooking loss (g/100 g)	18.70±3.1	19.67±2.95
Shear force (N/cm^2)	23.45±4.61	22.08±4.30
Sarcomere length (µm)	1.78±0.08	1.89±0.09
L* value	37.06±3.06	36.94±2.73
a* value	14.91±1.25	15.96±2.23
b* value	10.36±1.7	11.37±1.59

5.2.2 Chamois

In Table 3 and 4 the results are presented of a similar study (Hofbauer *et al.*, 2006) on seasonal meat quality variation in Chamois (*Rupicapra rupicapra*) essentially following the same procedures as those for roe deer.

Again, carcass weights reflected the expected effects of differences in nutritional input over the seasons. The contrasts in colour (L*-values) are likely to have resulted from differences in the availability of nutrients (e.g. iron), the more so because the variables related to water-holding (drip loss) indicate that light reflection in Autumn samples is superior to that in Spring samples. For the latter observation there is no adequate physiological explanation as the pH/temperature profile of Autumn and Spring samples (indicative for possible differences

Table 3. Carcass weight and pH, water holding capacity (WHC; method of Grau and Hamm, 1953, procedures according to Hofmann et al.*, 1982) and temperature as assessed in semimembranosus muscle of carcasses of one-year old chamois at 45-60 min ('1' hr) and at 24 hrs post mortem (means ± standard deviation) (after Hofbauer* et al.*, 2006)*[1].

Quality trait	Spring (n=10)	Autumn (n=8)
Carcass weight (kg)	8.39^{b}±1.36	13.94^{a}±2.18
pH_1	6.49±0.31	6.63±0.42
WHC_1	0.75±0.15	0.61±0.21
$Temp_1$	33.70±4.44	34.15±3.09
pH_{24}	5.61±0.23	5.84±0.54
WHC_{24}	0.72±0.16	0.77±0.13
$Temp_{24}$	5.12±2.88	7.08±3.41

[1] Figures with different superscripts differ significantly (P<0.05, T- test).

Table 4. Sensory quality traits of longissimus muscle of one year old chamois, as assessed after 14 days of refrigerated storage (mean value ± standard deviation) (after Hofbauer et al.*, 2006).*[1]

Quality trait	Spring (n=10)	Autumn (n=8)
Drip loss (g/100 g)	1.37^{a}±0.72	2.29^{b}±0.66
Cooking loss (g/100 g)	16.36±1.94	17.38±2.21
Shear force (N/cm^2)	41.48±23.33	33.70±11.12
Sarcomere length (µm)	1.79±0.16	1.74±0.09
L* value	39.30^{a}±4.43	31.00^{b}±2.25
a* value	15.50±1.78	16.83±2.01
b* value	11.51±1.28	10.83±1.70

[1] Figures with different superscripts differ significantly (P<0.05, T- test).

in the degree of myofibrillar lattice spacing or those in protein denaturation) and data on myofibrillar density (sarcomere lengths) are similar.

5.3 Wild boar

Hofbauer and Smulders (unpublished results) investigated wild boar (*Sus scrofa*) meat to determine whether its ageing rate is similar to that observed in muscles of domesticated pigs. To this end 10 animals were killed by hunting and transported to a chill room at 0 °C. At 3 days *post mortem longissimus* samples were excised from the carcasses and stored overnight at 0-2 °C, after which (at 4 days *post mortem*) these samples were either subjected to immediate analysis or vacuum packed and stored at 0-2 °C for a further week (i.e. 11 days *post mortem*). Table 5 includes the results.

No differences of practical relevance were observed between the two storage periods. As shear force between day 4 and 11 days *post mortem* was similar, it appears that – as is the case for domesticated pigs (for which optimum ageing times between 5-7 days are reported (Dransfield *et al.*, 1980-1981)) – maximum tenderness is already achieved by the earliest time wild boar meat is marketed in commercial practice. In essence, these results concur with those reported by Zmijewski and Korzeniowski (2001). Marchiori and DeFelicio (2003) report that, compared to commercial pork, wild boar meat has a more gradual glycolysis, lower L* and a* colour values and (at least in female animals) lower exudative (drip) losses.

5.4 Feral pheasants

Hofbauer *et al.* (2010) investigated meat quality of hunted feral pheasants (*Phasianus colchicus* L.). The righthandside superficial breast muscles of 14 birds were removed after 24 hrs of chilling for immediate analysis, the lefthandside counterpart muscles were left on the refrigerated carcass until 96 hrs *post mortem* and then analysed. Data are presented in Table 6.

Table 5. Sensory quality traits of wild boar longissimus muscle as assessed at 4 days post mortem and at 11 days post mortem *(i.e. after one week of vacuum refrigerated storage) (Hofbauer and Smulders, unpublished)*[1].

Quality trait	Day 4 *post mortem*	Day 11 *post mortem*
pH	5.47^{a}±0.04	5.51^{b}±0.04
Drip loss (g/100 g)	-	6.24±3.37
Cooking loss (g/100 g)	20.90^{a}±2.90	22.54^{b}±3.11
L* value	54.52 ±4.12	53.17±5.32
a* value	18.17±2.22	18.85±2.07
b* value	17.69±1.97	17.98 ±1.00
Shear force (N/cm^2)	40.34±9.22	43.81±10.68

[1] Figures with different superscripts differ significantly (P<0.05; T-test).

Table 6. Sensory quality traits of pheasant pectoralis superficialis *muscle as assessed at 24 and 96 hrs* post mortem[1] *(after Hofbauer* et al.*, 2010).*

Quality trait	Time *post mortem*	
	24 h p.m. (n=14)	96 h p.m. (n=14)
pH	5.62±0.05	5.64±0.08
Cooking loss (g/100 g)	11.51±4.90	12.60±1.77
Shear force (N/cm^2)	28.9±13.02	31.8±11.23
L* value	54.24^a±4.49	56.61^b±3.45
a* value	3.81±1.86	4.04±1.43
b* value	8.02^a±1.24	9.18^b±1.24

[1] Figures with different superscripts differ significantly (P<0.05; T-test).

L* values are in the same range, a* and b* values higher than- and shear forces and ageing rates similar to those recorded for other poultry species (e.g. turkey, e.g. Hillebrand *et al.*, 1991, Smulders and Hofbauer, 2003)

6. Conclusions

What has been achieved?

Targeted research conducted over the past decades has elucidated many of the muscle biological mechanisms which explain the variation in meat quality, albeit primarily that of the major farm animal species. In addition, many processing technologies allowing an improvement of sensory quality traits have been developed and recommended standardised laboratory methods to assess these have been made available.

What has been neglected?

Notwithstanding these achievements, one must confront the fact that publications on the quality assessment of feral game species are scarce and the generated information is mostly fragmented. Inevitably, available data are generally descriptive rather than interpretative.

What needs to be done?

Those factions with a vested interest in increasing consumer acceptability of game meat items and striving to extend what is currently still a 'niche market' are well-advised to base their strategies on scientific investigations, to make more research funds available allowing the generation of relevant data and, finally, to more effectively communicate to hunters and meat processors what needs to be done to assure the best possible sensory quality of game meat.

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Muscle biological and biochemical ramifications of farmed game husbandry with focus on deer and reindeer

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Summary

The deer farming industry in New Zealand and the traditional Sami reindeer husbandry culture in Fennoscandia (Sweden, Norway and Finland) lead the world in commercial venison (deer meat) production from semi-domestic or farmed deer species, i.e. red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus tarandus*), respectively. Production systems are based on natural pastures but include pre-slaughter handling routines and a slaughter process similar to that of domestic species like beef and lamb. Venison has several attributes attractive to consumers – it is tender, has low fat content, a favourable fat composition and high levels of minerals. All these attributes of venison are criteria demanded by today's discerning meat consumer. The introduction of new venison production routines such as intensive farm-based management, industrialised slaughter and meat processing, use of commercial feed mixtures and possibly new ingredients used to supplement or replace pasture can alter venison quality. One topic of central importance for venison is the image as a natural, free-range origin, clean and healthy product. Care should be taken to ensure the positive image of venison as a 'natural' and healthy product is not lost when production systems are intensified to meet the market demand.

Keywords: meat quality, venison, pH, shelf life, tenderness, colour, flavour

1. Introduction

To illustrate the worlds' most important sources of venison (deer meat), the examples in this chapter are taken from the young deer farming industry in New Zealand and the traditional Sami reindeer husbandry culture in Fennoscandia (Sweden, Norway and Finland). These two deer industries are mainly focused around venison production based on systems dependent on pastures. The red deer (*Cervus elaphus*) and the reindeer (*Rangifer tarandus tarandus*) are the most common deer species for venison production, in New Zealand and Fennoscandia, respectively.

Reindeer husbandry is performed in a less intensive way than red deer farming, with the reindeer free ranging (i.e. not enclosed in fenced areas) in forests and on the mountain tundra.

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Nevertheless, at times reindeer are fed during winter to prevent starvation and to improve body weight and condition (Staaland and Sletten, 1991). In 1986, parts of the reindeer pasture areas in Sweden were affected by radioactive fallout from the accident in the Chernobyl nuclear power plant, and therefore feeding is still used in these areas as a measure to reduce radioactive caesium in the reindeer and thereby in the meat (Åhman, 1999). Also in New Zealand, many deer farmers feed their animals a variety of supplements during winter to provide extra nutrition when pasture is inadequate.

Consumer opinion is increasingly important to meat industries worldwide and consumers value attributes such as flavour, tenderness and nutrient content when evaluating the quality of meat (Smulders *et al*., 1991; Dransfield, 2003). Although the quality preferences of different consumer groups may vary, producing consistent quality is critical: the quality of every purchase should be the same. Venison is also attractive to the health-conscious consumer for its low fat content, favourable fat composition and high mineral content (Drew and Seman, 1987).

2. Production systems for venison

2.1 Red deer

New Zealand has pioneered the development of farm-based production systems for venison. However, deer are not native to New Zealand. The first deer were brought there from England and Scotland for sport (hunting) in the mid-late 19th century. The environment proved ideal and feral deer populations grew uncontrolled. By the middle of the 20th century feral deer were regarded as a pest because of their impact on the environment and native forests. The export of venison from feral deer started in the 1960s, turning a pest into an export earner. Industry pioneers saw an opportunity to build on this base and in the early 1970s started capturing live deer from the wild and farming them. A new industry was born and rapidly spread throughout New Zealand (Deer Industry New Zealand, 2010). There are currently about 3,800 deer farms in the country, ranging in size from smaller lifestyle properties to farms carrying many thousands of deer. On these farms there are approximately 1.7 million deer (estimated in June 2006), or half the world's farmed deer population. Reflecting the original imported wild population, the majority of New Zealand's deer herd is red deer (*Cervus elaphus*) (Figure 1), the balance is predominantly elk (also called 'wapiti') (*Cervus elaphus nelson*) descending from animals imported from the USA and Canada or red/elk hybrids. There are also small numbers of fallow deer (*Dama dama*).

An important component of the research and development for the New Zealand deer industry has been to define production systems that render distinctive and high-value attributes to venison, together with *post mortem* processing systems to complement these goals. The deer industry aims to meet market demand for venison by slaughtering deer in early spring, when animals are 9-11 months old. A major strategy is to calve as early as possible and to maximise growth rates in the first 7 months of life before first winter. Currently, a small proportion of animals achieve target slaughter weight pre-winter. This provides the choice to either maintain the animals through winter (when feed is expensive) then slaughter in spring when venison

Figure 1. New Zealand has pioneered the development of farm-based production systems for venison (Photo: Eva Wiklund).

prices are highest, or the novel opportunity to slaughter prior to winter, before the animals' metabolism undergoes major photoperiod-induced changes (Wiklund *et al*., 2008).

As a specialist culinary product, venison is processed in specialised facilities licensed by the New Zealand Ministry of Agriculture and Forestry. The amount of venison produced in New Zealand in 2008/2009 was estimated at 21,000 tonnes (Deer Industry New Zealand, 2010). An animal welfare Code of Practice governs the humane treatment of deer prior to processing to ensure minimum stress and to enhance product quality. Venison is processed according to customer requirements. The trend is towards added-value (or further processed) cuts, where the bones and 'silverskin' (i.e. the connective tissue surrounding the muscles) are removed.

2.2 Reindeer

The Sami people can be regarded as the indigenous population of the Arctic region of the Nordic countries and the Kola Peninsula. Already in 1539 the well known map of Olaus Magnus, the *Carta Marina*, showed reindeer and people inhabiting this area. The oldest Sami relics derive from the Komsa culture – a people inhabiting the region between Tromsø in northern Norway and the Kola Peninsula in northwest Russia – as far back as 8,000 BC. The nomadic lifestyle of the Sami people did not evolve until the late 16th century and until the 19th

century they roamed over vast expanses with their reindeer. From this perspective, reindeer herding was very intensive and Sami families tended their reindeer herds all year round (Bäck, 1993). Scefferus (1673) reported seeing large numbers of wild reindeer in northern Sweden, and also tame reindeer herds tended by the Sami. Tame reindeer were very useful as beasts of burden and for milk and meat production (Figure 2).

The herders guarded their animals constantly. Winter time was the main slaughter period, when the castrated males were in prime condition and the cold weather made it possible to store meat over long periods. The reindeer carcass and viscera were utilised with the utmost thoroughness, supplying the herding family with far more necessities than food alone (Beach, 1981). During the 20th century, Swedish reindeer herding has gradually become more and more extensive. Sami families now live in Swedish communities and use all kind of modern technology for reindeer herding, such as helicopters, airplanes, motorbikes and snow-machines (Bäck, 1993). However, the traditional knowledge around reindeer is still very much in focus in the Sami reindeer herders' everyday life (Figure 3).

Traditionally, reindeer were slaughtered at the selection site, i.e. at various locations surrounding the reindeer herding districts of the Sami people. In Sweden, the new directives regarding reindeer meat inspection were instituted in 1993 (National Food Administration, 1993) and consequently many of the former outdoor slaughter sites were closed, the numbers of reindeer transported to slaughter increased and new mobile slaughter facilities were developed. The rules applied for animal transport, veterinary inspection of living animals and carcasses, stunning methods, slaughter hygiene, carcass grading and chilling conditions for reindeer are similar to those applied for other domestic species.

Figure 2. Tame reindeer were very useful as beasts of burden and for milk and meat production for the Sami people of northern Fennoscandia and Russia (from Scefferus, 1673).

Figure 3. The traditional knowledge around reindeer is very much in focus in the Sami reindeer herders' everyday life (Photo: Anders Wiklund).

The Swedish reindeer population has during the last 10 years varied between 200,000-300,000 head (winter stock) and on average over this time period an annual 23% of the population has been slaughtered. During the same period the proportion of slaughtered calves per total number of slaughtered reindeer has increased from 43 to 67%. Due to the small production (1,500 tonnes in 2008/2009; Sami Parliament, 2009) reindeer meat is a very exclusive gourmet product, which is in high demand and always on the menu in the more luxurious restaurants. Almost no reindeer meat is exported. The meat is consumed fresh but is also marketed as cold- or hot-smoked and dried meat products.

3. Impact of production systems on venison quality

3.1 Muscle glycogen content and meat pH and their microbiological consequences

Meat pH is related to shelf life, tenderness, colour and water-holding properties, and is therefore a good indicator of meat quality. A pH value of 5.5-5.7 is within the normal range, while values over 5.8 result in reduced shelf life, especially for vacuum-packaged meat. Meat with high pH, so called DFD (Dark, Firm, Dry) meat, is a persistent quality defect found in all meat species. Meat pH values are directly correlated to the levels of muscle energy (glycogen) at the time of slaughter (Gill and Newton, 1981; Hood and Tarrant, 1981; Tarrant, 1989). If the glycogen stores in the muscles are low, meat pH will be elevated. Low muscle glycogen stores might result from poor physical condition, intense physical activity or stress during pre-slaughter handling. It has been demonstrated that deer and reindeer in good physical

condition produce meat with optimal pH values, whether they were fed a commercial feed mixture or grazed (Wiklund *et al*., 1996a; Wiklund *et al*., 2000).

Two comprehensive surveys of meat pH for deer (n=3,600; New Zealand) and reindeer (n=3,400; Sweden) demonstrated DFD frequencies (i.e. meat pH >6.2 measured in the *M. longissimus* at the last rib) of 1.5% for red deer and 6% for reindeer (Table 1). In the New Zealand study (Pollard *et al*., 1999) there was no indication of a relationship between pH and the stress parameters studied (fighting or agitation in lairage or unsettled behaviour in the lead-in race to stunning). It was suggested that other factors besides physical exertion at the slaughter plant affected meat pH; for example effects of transport, yarding and handling at the farm were not studied. On the contrary, the Swedish results (Wiklund *et al*., 1995; Wiklund *et al*., 1996b) clearly showed that selecting reindeer for slaughter using a lasso had the most negative effect on meat pH. In both surveys it was highlighted that there are possibilities to improve pre-slaughter handling routines for reindeer and red deer to further reduce the frequency of DFD carcasses.

Figure 4 and 5 (Wiklund and Smulders, 1997) illustrate the microbiological effects of distinct ultimate pH values on shelf life and safety of reindeer meat. Whilst at 3 days *post mortem* Aerobic Colony Counts (ACC) tended to be higher in high pH meat ($P<0.10$), all samples reached microbial numbers around 6 log units after 2 weeks of vacuum storage. However, at that time Enterobacteriaceae Colony Counts (ECC) of samples with high pH were approximately one log unit higher than those with intermediate and low pH. Such observations have been explained by Gill and Newton (1981) to be the result of low glycogen stores favouring the predominance of proteolytic microbial flora rather than the general positive effect high pH values have on microbial growth.

3.2 Tenderness, proteolytic enzymes and muscle fibre composition

Variation in meat pH and glycogen content can give rise to considerable variation in meat tenderness in species such as beef and lamb (Watanabe *et al*., 1996; Purchas *et al*., 1999), and similar results have been found for red deer venison. Within the normal pH range, values around 5.5 have been reported to yield more tender venison than those in the 5.8-6.0 range (Stevenson-Barry *et al*., 1999). This intermediate pH venison was tougher than normal pH even after ageing and also more variable in tenderness (i.e. of less consistent quality) than normal pH (deer) venison.

*Table 1. Average ultimate pH values (pH_u) and frequencies of intermediate DFD ($5.8 \leq pH_u \leq 6.2$) and DFD (pH_u >6.2) in red deer, fallow deer and reindeer venison (*M. longissimus*).*

	Ultimate pH (overall means)	Intermediate DFD (%)	DFD (%)	Reference
Red deer (n=3,500)	5.64	9.1	1.5	Pollard *et al*., 1999
Fallow deer (n=100)	5.93	68.3	1.0	Pollard *et al*., 1999
Reindeer (n=3,400)	5.67	23.1	6.0	Wiklund *et al*., 1995

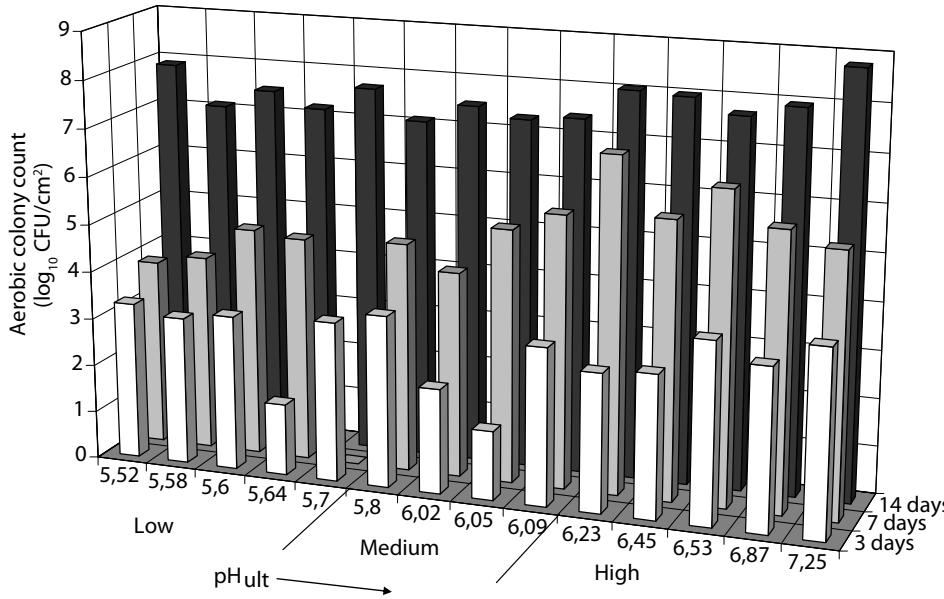


Figure 4. Aerobic Colony Count of individual reindeer longissimus *samples of various ultimate pH, as assessed after 3,7 and 14 days of refrigerated vacuum storage (Wiklund and Smulders, 1997).*

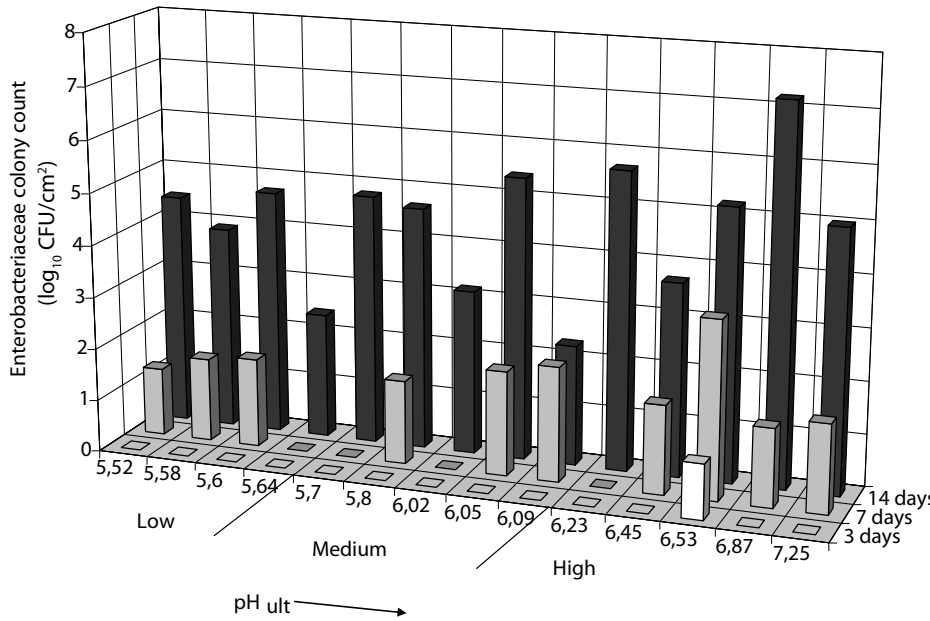
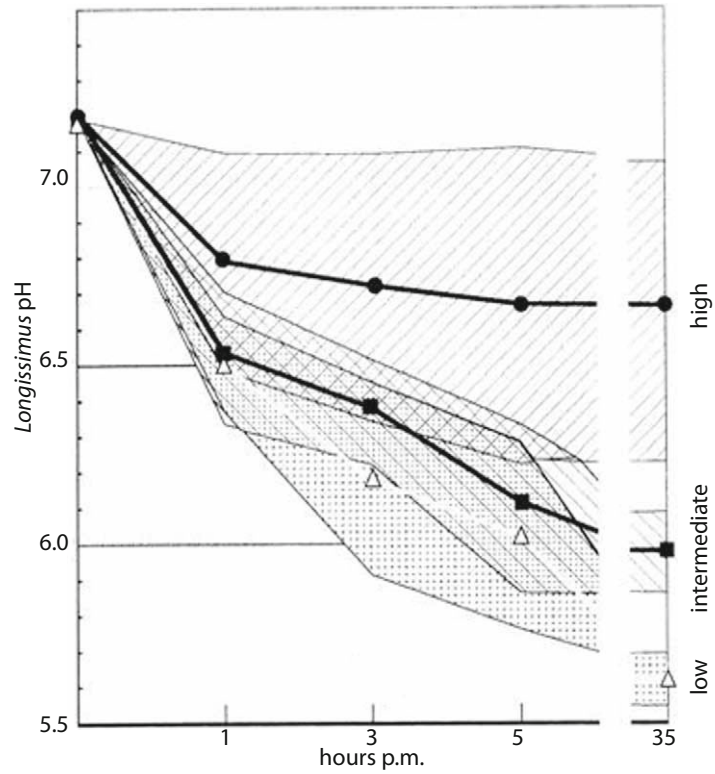


Figure 5. Enterobacteriaceae Colony Count of individual reindeer longissimus *samples of various ultimate pH, assessed after 3, 7 and 14 days of refrigerated vacuum storage (Wiklund and Smulders, 1997).*



	high	35.4-39.0	10.3-16.2	5.9-9.6	4.9-6.7
Range in core temp.	interm.	36.3-37.9	11.8-19.0	6.7-9.8	5.6-6.1
	low	38.1-39.2	12.9-17.9	7.1-11.8	5.2-5.9

Figure 6. Early post mortem *pH and temperature decline in lumbar* longissimus *muscle of 15 refrigerated reindeer carcasses with various ultimate pH values (Smulders and Wiklund, 1998; also see Table 2).*

In contrast, reindeer venison has been found to be extremely tender regardless of meat pH (Wiklund *et al.*, 1997). Figure 6 and Table 2 (Smulders and Wiklund, 1998) illustrate the rate of pH and temperature decline in reindeer lumbar *longissimus* muscle and the effect of ultimate pH on major physical/chemical traits related to the sensory quality of reindeer meat.

The darker appearance of DFD meat is reflected in the higher L values observed in samples with pH >6.1. Although water-holding capacity was found to be decidedly higher than that usually observed for ruminant meat species (i.e. drip loss values in the range of 5 to 7% as reported by Den Hertog-Meischke *et al.*, 1997a,b), the values for the various pH groups were remarkably similar. However, cooking losses (indicating both the water loss from intra- and extracellular matrix and heat-induced changes in protein structure) were significantly lower in high pH reindeer meat as is generally the case for all meat species (Honikel, 1992). Shear force values – although rather low in all samples regardless of pH – decreased with higher ultimate pH and extended storage caused further tenderisation and a gradual shift in contrasts between the various pH groups.

Table 2. The effects of ultimate pH of reindeer longissimus muscle on physical-chemical variables related to eating quality (n=5 in each subgroup; least square means and standard error); (After Smulders and Wiklund, 1998).

Trait	Low pH	Medium pH	High pH
Minolta L*			
3 days	30.1^{a}±0.9	26.0^{b1}±1.2	26.5^{b}±0.9
7 days	30.4^{a}±0.9	5.7^{b12}±1.2	26.8^{b}±0.9
14 days	30.3^{a}±0.9	25.1^{b2}±1.2	26.7^{b}±0.9
Minolta a*			
3 days	11.4±1.0	13.0±1.3	11.2±1.0
7 days	11.5±1.0	13.1±1.3	10.8±1.0
14 days	11.4±1.0	13.1±1.3	11.3±1.0
Minolta b*			
3 days	5.8±0.8	5.1±1.0	3.8±0.8
7 days	6.2^{a}±0.8	5.3^{ab}±1.0	3.7^{b}±0.8
14 days	1.0±0.2	5.2^{ab}±1.0	3.9^{b}±0.8
Drip loss (%)			
3 days	1.1±0.1	1.1±0.2	1.1±0.1
7 days	1.2±0.1	0.9±0.2	1.1±0.1
14 days	1.1±0.2	0.9±0.2	1.1±0.2
Cooking loss (%)			
3 days	18.8^{a}±2.3	14.0^{ab}±3.0	10.4^{b}±2.3
7 days	17.8^{a}±2.4	14.6^{ab}±3.0	10.6^{b}±2.3
14 days	18.6^{a}±2.4	14.1^{ab}±3.2	11.8^{b}±2.4
Shear force (kg/cm^2)			
3 days	5.3^{a1}±0.6	4.8^{a1}±0.6	2.4^{b}±0.6
7 days	4.0^{a2}±0.7	3.8^{ab12}±0.6	2.1^{b}±0.6
14 days	4.0^{a2}±0.6	3.1^{ab2}±0.6	2.0^{b}±0.6

Within rows, means with superscripts not containing a common letter differ significantly ($P<0.05$); Within columns and traits, means with superscripts not containing a common number differ significantly.

The observation that shear force values of venison are relatively low (indicating high tenderness) has been confirmed in more recent studies on both red deer by Farouk *et al.* (2009) and on reindeer by Rincker *et al.* (2006), who report that venison is generally much more tender than beef, which is explained both by its small muscle fibre size (Taylor *et al.*, 2002) and its higher activity of proteolytic enzymes as compared with beef (Barnier *et al.*, 1999; Farouk *et al.*, 2007a) (Table 3).

In all species raised for meat production, genetic selection and improvement in nutrition and breeding conditions has led to a tremendous increase in the efficiency of animal production and improvement in carcass composition by decreasing fatness and increasing muscle yield (Lefaucheur, 2010). Comparisons between wild and domesticated animals within different

Table 3. Tenderness (shear force) measured in beef, red deer, fallow deer and reindeer M. longissimus *in non-electrically stimulated carcasses from animals aged 1.5-2 years.*

	Shear force (kgF) 1-3 days *post mortem*	Shear force (kgF) 1 week *post mortem*	Reference
Beef (n=8)	11.7	9.8	Barnier *et al*., 1999
Red deer (n=7)	11.4	8.2	Wiklund *et al*., 2001a
Fallow deer (n=8)	5.4		Sims *et al*., 2004
		4.5	Volpelli *et al*., 2003
Reindeer (n=8)	2.9	2.6	Wiklund *et al*., 1997

species suggest that selection for increased growth rate and lean meat content has shifted muscle metabolism/fibre type composition towards a more white/glycolytic and less red/oxidative type (Ashmore, 1974; Ruusunen and Puolanne, 2004). White/glycolytic muscle fibres are bigger than red/oxidative fibres, and several studies have found a negative relationship between increased muscle fibre size and meat tenderness for a number of animal species (Lefaucheur, 2010).

A pilot study was carried out to compare quality attributes in meat from fast growing young red deer stags slaughtered prior to winter (which had reached their slaughter weight at 7 months of age) with that of slower growing young red deer stags slaughtered more traditionally, at 12 months of age (Wiklund *et al*., 2008). Overall, this pilot study highlighted relatively minor differences in quality attributes of venison from fast and slow growing red deer stags, indicating that as the deer farming industry heads towards more efficient venison production systems there are unlikely to be any major negative impacts on product quality (Wiklund *et al*., 2008). However, further studies of the impact of growth rate on venison processing, packaging and storage techniques should be conducted before making recommendations to the venison industry. We are not aware of any further studies on how the selection for increased growth rate and lean meat content in deer would affect deer muscle fibre composition – and possibly venison tenderness – but it is reasonable to believe that intensified genetic selection for meat production would eventually affect deer in a similar way to the other animal species studied.

3.3 Meat colour and colour display life

Colour is an important attribute affecting the decision by the consumer at the point of purchase whether to buy meat on retail display or not (Bekhit and Faustman, 2005). Consumers judge the acceptability of meat colour by how bright red the meat looks on display, and a strong correlation exists between redness (a*), hue angle and consumer colour acceptability (Farouk *et al*., 2007b). Hue angle is also a good indicator of colour stability of meat on display. The browning of meat, which determines the colour display life, is due to the reaction where red oxymyoglobin is oxidised to brownish metmyoglobin. The speed of this oxidation process

is dependent on several factors like antioxidant content, oxygen consumption and reducing enzyme activity in the meat (Faustman and Cassens, 1990).

Venison contains a higher concentration of myoglobin (Young and West, 2001) and pro-oxidants such as iron and copper (Drew and Seman, 1987; Stevenson-Barry *et al*., 1999) than beef. This fact probably explains the overall poorer colour of venison relative to beef. In addition, the higher enzymatic activities of venison compared to beef may have resulted in venison losing its metmyoglobin reducing activity, resulting in faster and irreversible oxidation than usually observed in beef and consequently leading to a decreased colour display life (Farouk *et al*., 2007a). Wiklund *et al*. (2006) demonstrated a significantly better colour display life of red deer and fallow deer (Wiklund *et al*., 2005) venison from grazing animals compared to deer fed a pelleted feed or whole barley, and suggested that this difference was related to the higher content of natural antioxidants (vitamin E) in the pasture compared to the grain-based feed (Figure 7 and 8). In a recent study it was suggested that the colour display life of red deer venison was related to a seasonal variation in pasture quality (Wiklund *et al*., 2010). Meat from animals slaughtered in the spring had a significantly better colour stability compared with meat from animals slaughtered in the summer, autumn and winter

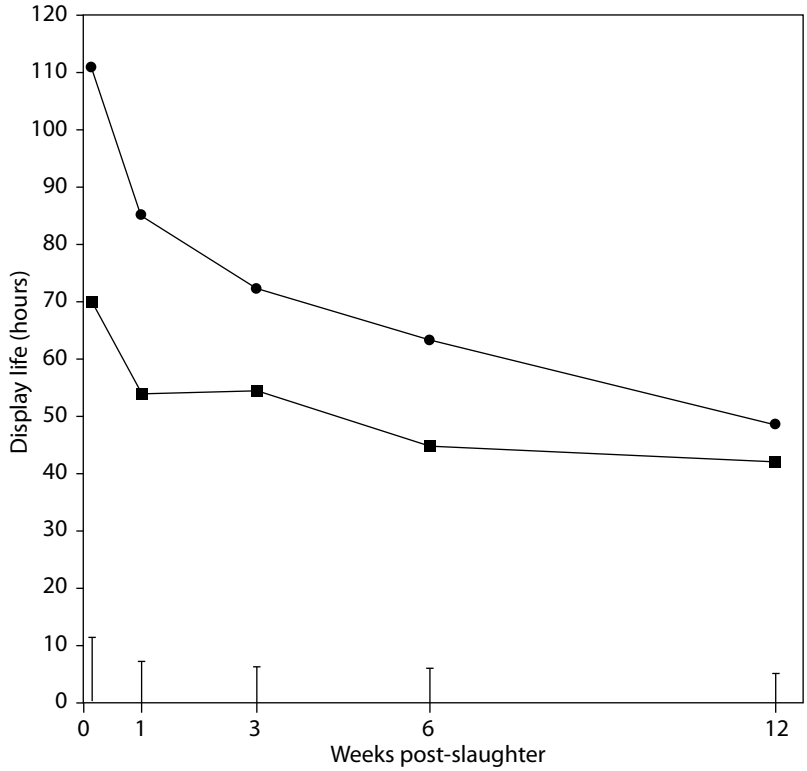


Figure 7. Mean display life (hours of Minolta a value ≥12) in* M. longissimus *from the red deer (*Cervus elaphus*) from two treatments (• pasture grazing and ▪ pellet fed, n=8 in each group) included in the study, measured at 1 day, 1, 3, 6 and 12 weeks of refrigerated storage (-1.5 °C), with error bars indicating standard error of difference (S.E.D) (from Wiklund* et al.*, 2006).*

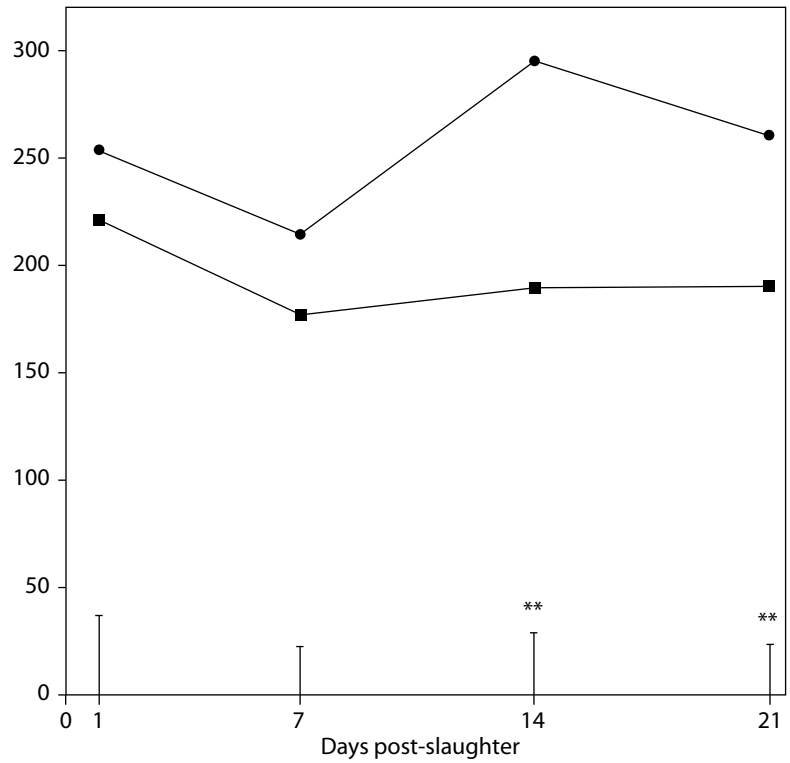


Figure 8. Mean display life (hours of Minolta a value ≥12) in* M. longissimus *from the fallow deer (*Dama dama*) from two treatments (• pasture grazing and ▪ barley fed, n=12 in each group) included in the study, measured at 1 day, 1, 2, and 3 weeks of refrigerated storage (+2 °C), with error bars indicating standard error of difference (S.E.D) (from Wiklund* et al.*, 2005).*

which was explained by a higher antioxidant content of the superior quality pasture growing in the spring.

3.4 Chemical composition and flavour

Natural or managed pastures (grasses, herbs and bushes) contain high levels of poly-unsaturated fatty acids (PUFA) and are also rich in different antioxidants. Grain-based feeds are higher in saturated fatty acids (SFA), and antioxidants like vitamin E are often added to commercial feed mixtures. When animals are grazing pasture or if they are fed grains, the fatty acid composition in their muscles/meat will change towards the composition of their feed (Wood and Smulders, 1999; Wood *et al.*, 2008). Fatty acid profiles in red deer and reindeer venison related to feed-type have been thoroughly investigated and linked to venison flavour. Similar results have been found for both species; deer/reindeer grazing pasture produced PUFA-rich venison with more 'grassy', 'gamey' and 'wild' flavours, while the grain fed animals gave meat with significantly less PUFA and a 'mild' and 'beef-like' flavour (Wiklund *et al.*, 2003a,b) (Table 4). These flavour differences were demonstrated using both trained sensory

Table 4. Mean values for fatty acid composition (% of total fatty acids) in M. longissimus *from pasture and pellet-fed reindeer (*Rangifer tarandus tarandus*) and red deer (*Cervus elaphus*).*

Fatty acid	Reindeer[1]			Red deer[2]		
	Pasture (n=6)	Pellets (n=9)	Degree of sign.[1]	Pasture (n=7)	Pellets (n=7)	Degree of sign.[3]
Polar lipids						
SFA[4]	25.4	26.3	n.s.	25.9	24.4	n.s.
MUFA	17.3	16.0	*	13.8	12.4	n.s.
PUFA (n-6)	31.9	39.4	***	29.3	41.9	***
PUFA (n-3)	14.2	7.5	***	14.2	4.5	***
(n-6)/(n-3)	2.2	5.3	***	2.1	9.6	***
Neutral lipids						
SFA	53.0	54.6	n.s.	54.7	50.6	**
MUFA	37.6	39.2	*	36.4	39.8	*
PUFA (n-6)	2.6	2.3	n.s.	4.3	6.6	**
PUFA (n-3)	1.4	0.3	***	2.5	0.6	***
(n-6)/(n-3)	1.9	8.1	***	1.7	12.3	***

[1] Wiklund *et al*., 2001b.

[2] Wiklund *et al*., 2003b.

[3] n.s. = $P>0.05$; * = $P\leq0.05$; ** = $P\leq0.01$; *** = $P\leq0.001$.

[4] Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

panels and consumer tests. New formulations for grain-based reindeer feed mixtures, where ingredients like linseed cake (Sampels *et al*., 2006) and fish meal (Finstad *et al*., 2007) were included, verified that the higher PUFA content in the feed was reflected in the meat, so that the fat composition of reindeer venison from animals fed the new formulations was very similar to that of reindeer venison from grazing animals.

From a human health perspective, a reduction in SFA and an increase in PUFA intake have been recommended to combat cardiovascular disease and cancers. Within PUFA, the omega-3 fatty acids are preferred over the omega-6 because they confer positive nutritional and physiological advantages (Williams, 2000). Today's Western diets have an omega-3/omega-6 ratio between 15 and 20, whereas it is assumed that it was close to 1 during human evolution (Simopoulus, 2002). From a nutritional point of view it is suggested that the omega-3/omega-6 ratio should be below 4, and it is therefore important to include sources rich in omega-3 PUFA in the daily diet. The venison industries are in a unique position to take advantage of the fact that grass-fed venison has naturally high levels of omega-3 PUFA and antioxidants (e.g. vitamins C and E, carotenoids and flavonoids).

4. Ethics and image of venison

Production systems like reindeer husbandry and deer farming, where the animals graze during most of the year, are usually considered more animal-friendly and ethical compared with the standard commercial production of beef, pork or poultry and venison is a product that meets most of the criteria demanded by today's discerning meat consumer (Hoffman and Wiklund, 2006). Most of the basic principles (effects of gender, age, region, etc.) and practices (nutrition, pre-slaughter handling, transport, lairage, stunning, electrical stimulation, etc.) that influence meat quality and composition that are applicable to more traditional red meat species are also applicable to deer (Hoffman and Wiklund, 2006).

In more intensive production systems the use of strategic feeding and new feed ingredients, herd health vaccination programmes, artificial insemination and even embryo transfer can be a part of normal practise. Some studies have reported on the use of growth hormones to improve deer productivity (Mulley *et al*., 1996), although hormones to increase meat production have been totally rejected by the deer industries world-wide. It is logical to wonder whether including too many of these 'standard commercial production practises' in the deer farming systems will in the long run damage the consumers' perception of venison being different from other types of meat. Care should be taken to ensure the positive image of venison as a 'natural' and healthy product is not lost when production systems are intensified to meet the market demand. In addition, the deer industries use the natural, healthy and ethical image as their central message in any promotion and marketing of venison.

5. Conclusions

What has been achieved?

The introduction of new venison production systems such as intensive farm-based management, industrialised slaughter and meat processing, use of commercial feed mixtures and possibly new ingredients used to supplement or replace pasture can alter venison quality.

What has been neglected?

One topic of central importance for venison is the image as a natural, free-range origin, clean and healthy product. Concerns about how this image could change when new feeding regimes are introduced have to be balanced against the need of using these feeds as supplements or replacements when pastures cannot provide enough nutrition for maintenance and growth.

What needs to be done?

The deer studies from New Zealand and the reindeer studies from Sweden suggest that there may be several pre-slaughter conditions that could be improved for deer and reindeer, leading to a more consistent venison quality. It is also of interest for the venison industries world-wide to recognise the quality differences related to different feeding regimes. For example, venison

with more or less 'wild' flavour could be directed to specific markets based on information about production system/feeding regime and consumer preference.

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A summary of methods to assess major physical-chemical and sensory quality traits of fresh (whole tissue) meat

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Summary

This contribution summarises the standard methodologies used to assess meat quality characteristics as applied at the University of Veterinary Medicine Vienna, Austria. It also compares the utility and validity of the various approaches to measure sensory quality of fresh meat in scientific experiments and indicates where major references for further consideration can be found.

Keywords: meat quality, fresh meat, sensory traits, methodologies

1. Introduction

Product quality as defined by ISO is 'the totality of features and characteristics of a product that bear on its ability to satisfy stated or implied needs' (ISO, 1986). Relevant to the sensory quality of meat are only those features and characteristics that determine the acceptability by the consumer (Smulders *et al*., 1991). By using physical or chemical methods or relying on the senses of analytical ('expert') panels, the major sensory quality traits can be measured in numerical terms. Apart from its relevance for predicting how successful (acceptable) a meat item may potentially be on the consumer market, this serves the important purpose of identifying the biological mechanisms that underlie the variability in quality characteristics of fresh meat of various animal species as influenced by fresh meat processing (see Hofbauer and Smulders, 2011). In the following, the major methods commonly used in meat quality research laboratories to assess the sensory quality of meat are summarised.

2. pH and temperature decline

The pH of *post mortem* muscle is a central characteristic explaining a number of muscle biological events. Both the speed of early *post mortem* pH decline (in concert with the temperature at which this takes place as this, for instance, determines the muscle's vulnerability to protein denaturation) and the ultimate pH value (dependent on residual *post mortem* glycogen stores) are important indicators explaining possible effects on water-holding capacity, colour and tenderness. Hence these variables are recorded in meat research, as well

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as for purposes of (on line) industrial quality management. Generally, either the traditional glass- or more robust steel-covered pH probes (e.g. the K21 electrodes allowing up to 600 measurements per hour at temperatures ranging from 0-80 °C and hence more suitable for on-line use) and digital steel thermometers are applied for this purpose.

3. Waterholding capacity (drip loss; cooking loss)

It is crucial to distinguish the concepts of 'water-holding' and 'water-binding' capacity. Whereas the former is relevant to assess the muscle's intrinsic ability to hold on to its own water, the water bound in the course of meat products manufacture (i.e. generally implying the addition of salts in products to be cooked) reflect the potential of the muscle matrix to bind added water (brine).

Various methods exist to quantify water-holding such as the 'press-method' (i.e. pressing a standard meat sample between filter paper, leaving a ring of water the diameter of which is related to water-holding as described by Grau and Hamm (1953); see Figure 1), centrifugal methods (at 5.000 to 40.000 g), capillary suction by gypsum discs as in Hofmann's capillary volumeter (Hofmann, 1975, particularly suitable to distinguish PSE from normal meat), measuring weight increases of analytical filter paper placed on the meat's surface (Kauffman *et al.*, 1986) or even through costly NMR analysis (Den Hertog-Meischke, 1997).

The utility of the various methods has been extensively discussed by Trout (1988). For instance, whereas the press method appears to be very practicable, its accuracy is not, as

Figure 1. Measurement of the water holding capacity of fresh meat by the press-method (Grau and Hamm, 1953).

such is heavily dependent on the homogeneity of the rather small size muscle sample and related to difficulties in ensuring standardisation of the pressure applied. Consequently, recent years have seen most researchers refraining from this simple piece of equipment and rather relying on procedures recommended by an OECD working group (Barton-Gade *et al*., 1993). These are based on weight loss measurements over a standard time (usually 24 hrs) with muscle samples being suspended in an inflated polyvinyl bag at 5 °C, carefully avoiding the sample making contact with the bag (Figure 2). The same sample can be used for further drip loss measurements (e.g. after 2, 7 days, etc.), but in every case the initial sample weight is used as reference point.

Heating of fresh meat results in water ('cooking') loss from the intra- and extracellular muscle matrix (i.e. through cell membrane damage, shrinkage of muscle fibres, aggregation of sarcoplasmic proteins and particularly by shrinking of connective tissue; Offer, 1984). Honikel (1998) describes the standard procedure for measuring cooking loss in whole tissue meat. It relies on placing a weighed and standardised meat slice of max. 50 mm thickness in thin-walled plastic bags in boiling water until the sample's internal temperature has risen to 75 °C while making sure the bag opening extends above water level (Figure 3) and subsequently cooling down the sample in an ice slurry and holding it in chill conditions (1 to 5 °C) until equilibrated, whereupon the sample is blotted dry and reweighed. Occasionally water bath temperature is held at maximum of 80 °C (in such cases sometimes the term 'heating loss' is used) to facilitate keeping sample core temperatures at maximum 75 °C (e.g. Eikelenboom and Smulders, 1986).

Figure 2. Assessment of water holding capacity by measuring weight loss over time in suspended fresh meat samples.

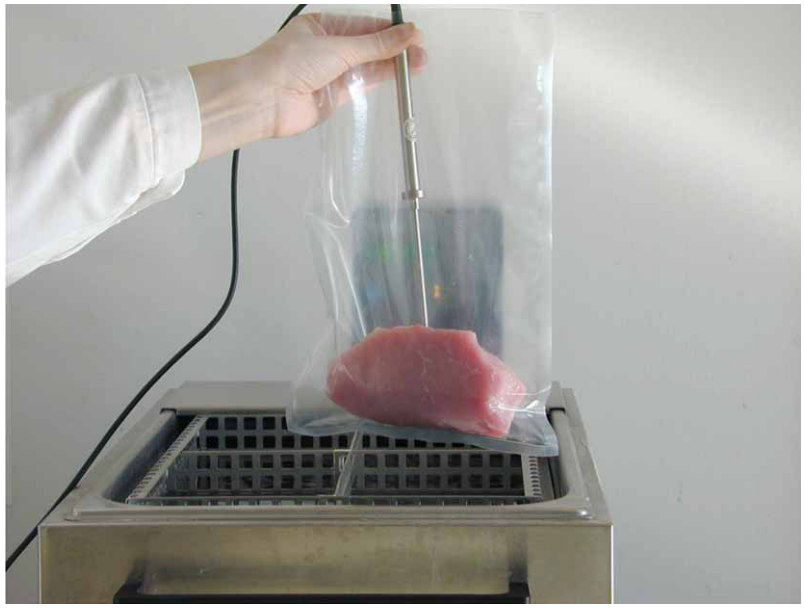

Figure 3. Assessment of cooking loss by measuring weight differences before and after heating in a water bath.

Water-holding capacity being heavily dependent on the integrity of muscle proteins (as for instance markedly influenced by the PSE condition in meat), sometimes additional chemical tests are performed. These include determining the degree of protein solubility with classical or more modern methods or by measuring bound phosphorylase (Hart, 1962; Den Hertog-Meischke *et al.*, 1997). These additional tests allow for distinguishing protein denaturation effects from those caused by other chemical or physical events and thus enable a more mechanistic interpretation of the results. In this context it should also be noted that information on the density of the muscle matrix (measurable by assessing sarcomere length; see below) is important to determine the physical potential of meat to contain ('structurally bound') water.

4. Fresh meat colour

Colour as perceived by the consumer is the physical modification of light by colorants as observed by the human eye and interpreted by the brain. For a person to see the colour of an object there needs to be light illuminating, and being reflected from, that object. Absolute colour scores cannot be provided by visual assessment since humans have a poor colour 'memory'. Guidelines for human evaluation of meat colour have been published by AMSA (1991).

To monitor changes in meat colour over time, instruments are commonly used to measure reflection and absorption. The principles of these 'objective' colour measurements have been

described by Klettner and Stiebing (1980). The frequently used, so-called CIELAB system is based on the L*, a*, b* colour space suggested by the Commission Internationale de l'Eclairage (CIE, 1976). In this system L* denotes the lightness (black/white) coordinate, a* the red/green coordinate and b* the blue/yellow coordinate. Mathematical transformations of these coordinates, notably Chroma (or saturation, i.e. the square root of $(a^*)^2+(b^*)^2$, indicating the degree to which colour differs from grey) and Hue (or colour tint, i.e. the tangent of the ratio b*:a*, describing the names of the colours red, via yellow and green, to purple) describe colour changes in more imaginable terms (Figure 4).

The following factors determine muscle colour variation by affecting light absorption, i.e. (1) pigment content (notably Myoglobin); (2) oxygenation and oxidation of Mb (resulting in formation of Oxymyoglobin and Metmyoglobin, respectively) and by affecting light reflection i.e. (3) water on the meat's surface, which is dependent on its water-holding properties (see Hofbauer and Smulders, 2011)

The recommended procedure for the instrumental measurement of fresh meat colour in numerical terms has been described by Honikel (1998). It includes casting light from a defined source (D65 with illumination/viewing system as 45/0 or 0/45 or diffuse /8 (d/8)) on the meat surface with a preferred observer angle of 10° and relying on the L*a*b* colour scale. Apart from ensuring that the measuring instrument (e.g. Hunter or Minolta equipment) is adequately calibrated using black (L* = 0) and white (L* = 100) colour standards, the meat must be allowed to 'bloom' for 1-2 hrs prior to measurement so as to ensure its surface is fully oxygenated (Figure 5).

Standardised colour scales with an appearance mimicking a meat surface and with a 1-6 scale have also been made available for qualitative visual colour classification to be applied on-line, such as the Japanese pork colour scale developed by Nakai *et al.* (1975), allowing for a rapid distinction between PSE, normal and DFD pork (Figure 6).

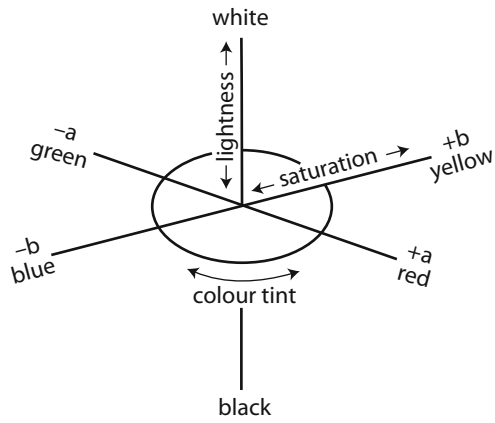

Figure 4. The colour space: L, a* and b* values (Smulders, 1986).*

Figure 5. Colorimetry of fresh meat cut surface after 1-2 hours of exposure to air.

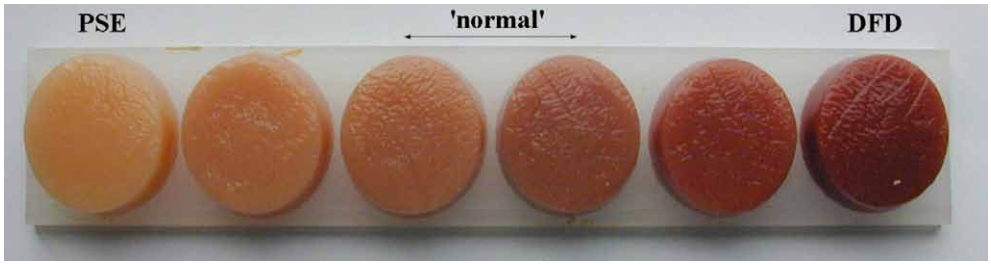

Figure 6. Visual scale for measuring fresh pork colour (Nakai et al.*, 1975).*
PSE: Pale-Soft-Exudative; DFD: Dark-Firm-Dry.

5. Tenderness traits

To study the significance of *ante* and *post mortem* factors affecting tenderness methods are required that allow some sort of quantification of the effects. In marketing and trading of carcasses and fresh meat determining the 'coarseness of grain' (i.e. the fibre size as assessed by running the ball of the thumb over a transverse muscle section and feeling its smoothness) is still often used by 'experts' as a tenderness indicator. Although such a procedure has been suggested to be related with tenderness (Hammond, 1952) it is extremely subject to personal interpretation. Moreover, to distinguish the distinct roles of the various tenderness determinants fine-tuned methodologies are required.

Many mechanical devices have been developed to simulate the shearing, penetrating, biting mincing and compression actions of the human teeth. Pearson (1963) lists their inherent

errors and limitations and suggests that the correlation coefficient between shear values and sensory tenderness of the widely used Warner-Bratzler shearing device (found in various studies to vary between 0.60 and 0.85 (average 0.75)) is quite satisfactory, considering the variability within sensory panels alone.

The Warner-Bratzler device consists of a 1.2 mm thick stainless steel blade with a hole in which the sample is placed. The blade is led through a slid between two shear bars and the amount of shearing force is recorded with the aid of a draw bench (e.g. the Instron apparatus; see Figure 7). More closely related to the biting action of the teeth is the Volodkevich device. That consists of rounded wedges or bars between which the meat sample is squeezed and sheared until it finally breaks. The so-called 'initial yield' (the first inflexion in the force-deformation curve, which, however, is not always clearly observable) is generally associated with the myofibrillar contribution to tenderness, whereas the 'peak force' reflects the combination of myofibrillar and connective tissue strength (Figure 8). The MIRINZ tenderometer used in most studies from New Zealand appears to primarily measure the former.

Honikel (1998) discusses the recommended procedures in using both these pieces of equipment. They include detailed descriptions of muscle selection and preparation (e.g. preferably lumbar longissimus muscle), muscle excision (perpendicular to the muscle's longitudinal axis), muscle size (at least a 50 mm slice), heating procedure (in essence as described under waterholding; see above), number of core samples to be tested (at least 10), fibre orientation and size of core samples (10×10 mm cross section strips of at least 30 mm length with fibre direction parallel to longitudinal axis) and the draw bench's shearing speed and direction (at 50-100 mm/min at right angles to the fibre axis).

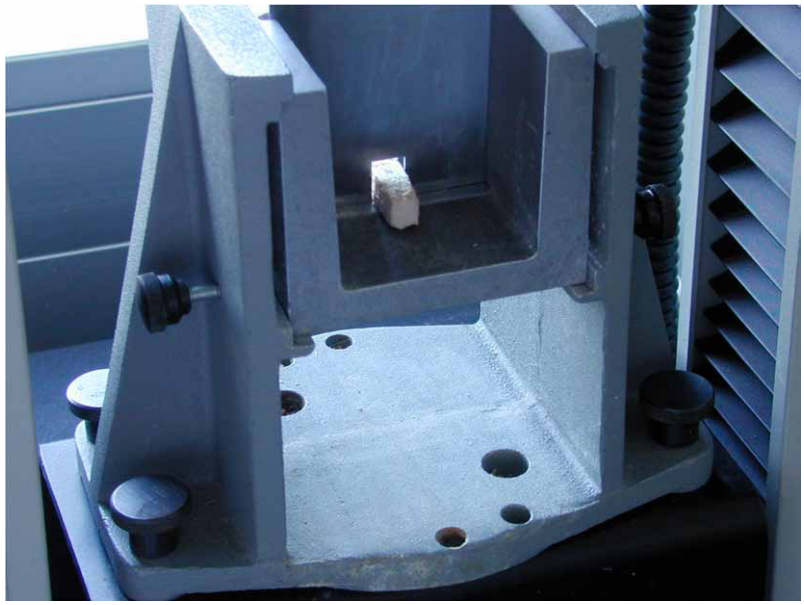

Figure 7. Draw bench fitted with a Warner-Bratzler shear device.

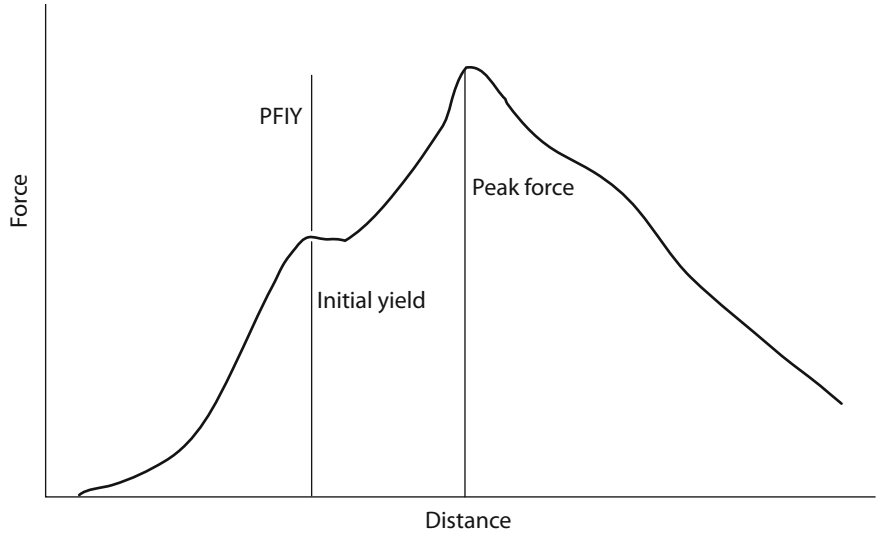

Figure 8. Force deformation curve of the Warner-Bratzler shear force measurement (Honikel, 1998).

Usually, concurrent with mechanical shearing, additional tests are conducted which assess the influence on tenderness of muscle contraction. As muscle contraction may considerably increase myofibrillar density (and consequently the substance to be sheared in a core sample), sarcomere length is measured, either microscopically (a rather time-consuming procedure) or *via* laser diffraction (Figure 9). The recommended procedures for the latter method have been described by Koolmees *et al.* (1986).

Such and further tests (for instance those indicative for the degree of meat ageing, or even more specifically for the activity of the enzymes and their inhibitors responsible for proteolysis) are helpful when one is particularly interested in why meat is tender or tough rather than how tender or tough it is.

'Subjective' measurements of tenderness, i.e. by a panel of individuals relying on their physical senses, are often renounced because the training and testing of the panellists is time-consuming and costly. Yet, instrumental analysis of tenderness cannot be considered a complete substitution for subjective evaluation. Should one consider that taste panel studies are necessary, it is essential to realise that for its reliable quantification by panellists only trained experts should be relied on, Provided these are available their number can be limited. In 'consumer acceptance studies' (i.e. assessing the marketing possibilities of a certain product) trained panellists are useless as the purpose of the exercise is to invite the more qualitative opinions of inexperienced consumers representing a true cross section of the target group. For consumer acceptance studies a panel size of at least 50 individuals has been suggested. A detailed description of recommended procedures for sensory panel studies on meat and meat products has been made available by Cross *et al.* (1978).

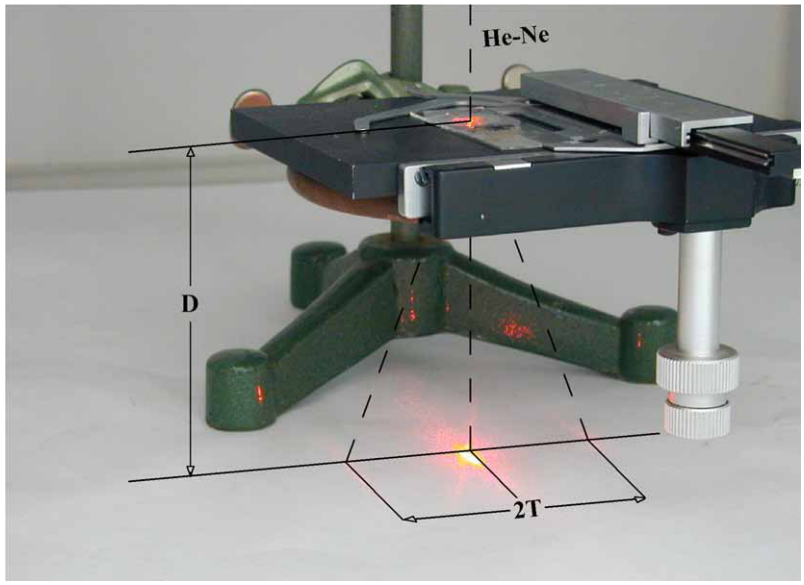

Figure 9. Measuring sarcomere length (SL) by laser diffraction. A Helium-Neon laser beam (wave length 632.8 nm) is sent through a fibre specimen (fixed by glutaraldehyde);

$$SL\ (in\ \mu m) = \frac{632.8 \times 10^{-3} \times \sqrt{(D^2+T^2)}}{T}$$

6. A remark on problems associated with sample size: animal species differences

Whereas most of the procedures described above are indeed suitable for use in major production animal species such as beef, lamb and pork, some animal species simply do not yield enough muscle substance to perform all the tests according to the above recommendations so choices on the variables to be tested and/or numbers of animals necessary for a justified experimental design must be made. This is particularly relevant in the sensory analysis of small (including game) birds. Also, since – in contrast to the economically important muscles from mammalian species – avian musculature has a very distinct distribution of fibre types (compare the almost entirely white breast muscle with the largely red leg musculature) results should be interpreted with care.

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Evaluation of some parameters of *post mortem* changes of pheasant (*Phasianus colchicus*)

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Summary

Post mortem changes were evaluated in muscle tissues of 99 male, uneviscerated pheasants (*Phasianus colchicus*) stored for up to 14 days at 0 and 4 °C. The control group (Group I) included regularly slaughtered and bled pheasants (n=33). From approximately 350 pheasants hunted on a single occasion, specimens were taken and, on the basis of X-ray examination performed from *latero-lateral* and *dorso-ventral* body projection of hunted pheasants, two groups were constituted: Group II included pheasants (n=33) with one or multiple shots in the muscle tissues but not in the body cavity, whereas Group III included pheasants (n=33) with shots in both muscle tissues and the body cavity. Each group was divided into two subgroups, in which uneviscerated pheasants were stored at 0 °C and 4 °C, respectively, for a maximum of 14 days. Randomly selected pheasants from each subgroup (n=4), were examined on days 0, 3, 7, and 14. The following *post mortem* changes in breast and thigh muscles were evaluated: pH value (in watery extract), lactic acid and ammonia concentration. Generally, low pH values were associated with higher concentrations of lactic acid, most pronounced in breast muscle. Significant differences ($P<0.05$) in pH – both at 0 and 4 °C storage temperature – were found for breast muscles between Groups I and II, albeit average differences were merely in the range of merely 0.1 to 0.2 units. Thigh muscles of Group I had significantly lower pH (0.2 to 0.4 units) than Groups II and III, for all study days. A statistically significant difference ($P<0.05$) was found between the lactic acid concentrations in breast muscles (4 °C) between groups II and III. The highest average concentrations of ammonia in the breast muscle (day 14) were determined in the Group III (4 °C), and the lowest in the group of slaughtered pheasants (4 °C). The highest average concentrations of ammonia in the thigh muscles were in Group III (0 °C), and the lowest in the group of slaughtered pheasants (0 °C). This difference was statistically significant ($P<0.05$). In summary, muscles from pheasants being slaughtered instead of hunted were characterised by lower pH, higher lactic acid and lower ammonia concentration at the end of the storage period.

Keywords: pheasant, *post mortem* changes, lactic acid, pH, ammonia

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1. Introduction

The conversion of muscle to meat has been extensively described by Hofbauer and Smulders (2011). It starts by the conditioning period, i.e. the time period between slaughtering of the animal and the onset of *rigor mortis*, during which muscle glycogen reserves are broken down anaerobically, resulting in the intramuscular accumulation of lactic acid and the concurrent decline in pH values. During the subsequent ageing period muscle proteolysis occurs, which may after extended ageing involve the formation of alkalic substances such as ammonia with concurrent elevation of pH values (Lawrie and Ledward, 2009; Hofbauer and Smulders, 2011). In this context, these events to some extent reflect the progress of proteolytic changes.

By measuring intramuscular pH changes and simultaneously assessing the lactic acid and ammonia generation one may monitor glycolytic and proteolytic changes. Measuring intramuscular pH values by probes is not uncomplicated, as the interface of the probe's acid-sensitive membrane and the muscle surface liquids may vary, dependent for instance on the location of measurement. For example, subsequent pH measurements on the same site where the initial incision was made occasionally generate slightly different pH values than those conducted at fresh incision sites. To limit such effects, intramuscular pH is alternatively measured in watery suspensions of muscle samples taken from the carcasses under scrutiny (e.g. Smulders *et al.*, 1990).

The objective of this study was to compare *post mortem* changes (pH, lactic acid, ammonia) of exsanguinated or hunted pheasants over a 14 day storage period at two different temperatures, i.e. 0 and 4 °C.

2. Material and methods

2.1 Origin of the pheasants

The *post mortem* changes were evaluated in 99 pheasants (*Phasianus colchicus*) from the hunting grounds of the University of Veterinary Medicine and Pharmacy in Košice, 66 of which were randomly collected from approximately 350 pheasants hunted on a single occasion. Animals were hunted or slaughtered according to local animal welfare regulations.

2.2 Constitution of the experimental groups

The study design has been described earlier (Paulsen *et al.*, 2008). In brief, male pheasants were divided into three basic groups. The control group (Group I) included regularly slaughtered and exsanguinated pheasants (n=33) (Figure 1). Hunted pheasants were subjected to X-ray examination in *latero-lateral* and *dorso-ventral* body projection and assigned to one of the following groups. Group II included pheasants (n=33) with one or multiple shots in the muscle tissues, but none in the body cavity (Figure 2), group III included pheasants (n=33) with shots in both muscle tissues and the body cavity (Figure 3). Each group was divided into two subgroups, i.e. pheasants stored uneviscerated at 0 or 4 °C, for a maximum of 14 days.

Figure 1. Pheasant after evisceration – Group I (slaughtered pheasants).

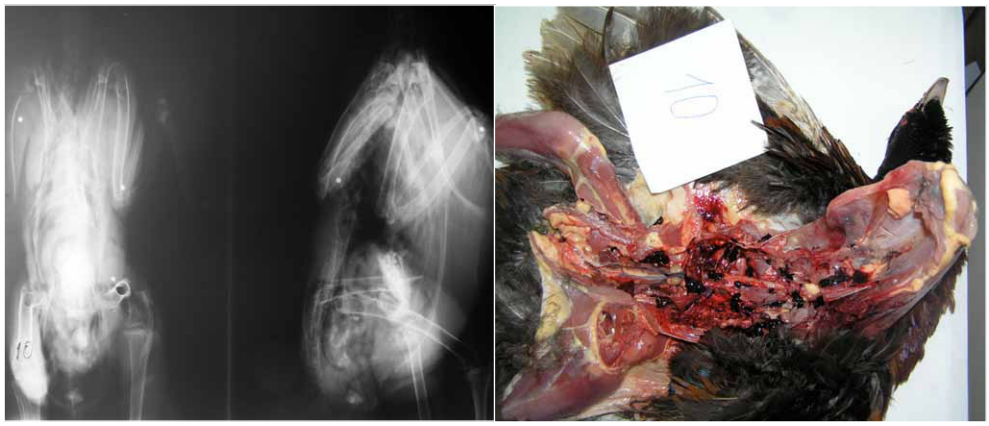

Figure 2. X-ray and pheasant after evisceration – Group II (shots only in muscle tissue).

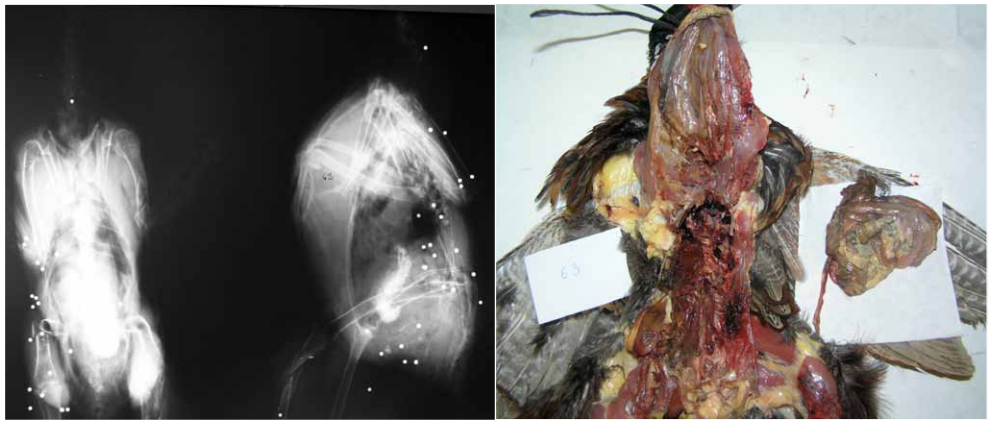

Figure 3. X-ray and pheasant after evisceration – Group III (shots in both muscle tissue and body cavity).

On days 0, 3, 7, and 14, pheasants from each subgroup (4 animals) were chosen randomly, and breast and thigh muscles were removed for laboratory examinations described below.

2.3 Evaluated parameters

Post mortem changes in the muscles of pheasants were evaluated with respect to pH value (in watery extract), amount of lactic acid, and ammonia content. The pH values of breast and thigh muscles (in watery extract) were measured by digital pH meter and electrode (both WTW, Germany). The amount of lactic acid was determined by methods of analytical capillary isotachophoresis (Isotachophoretic analyser ZKI – 001, Labeco Spišská Nová Ves, The Slovak Republic). Ammonia content was determined by Conway's method (Conway, 1947). The obtained results were tested by one way ANOVA. Comparison of slaughtered and hunted groups was performed using t-test.

3. Results and discussion

3.1 pH

The average pH values measured in the breast muscles were lower than the values in the thigh muscles. This confirms previous reports from Richter *et al.* (1992), Paulsen *et al.* (2008) and Hofbauer *et al.* (2010). The latter authors hypothesised that this might be due to thigh muscles of flightless birds being more active than wing muscles, and consequently that *ante mortem* activity will leave less carbohydrate for *post mortem* lactic acid formation.

At day 0, pH values were similar in all three groups. During storage, the highest average pH values (breast muscle) were found in the Group III (4 °C). Marked differences in ultimate pH values at day 14 were not observed. The highest average pH values (day 14) determined in the thigh muscles were found in Group II (4 °C), the lowest in Group I (0 °C).

Significant differences ($P<0.05$) between pH values in breasts muscles of Group I animals (slaughtered pheasants) and those of Group II (without shots in body cavity) were found. Also, pH values of the thigh muscles stored at both 0 and 4 °C differed significantly for Group I as compared to Group II, and Group I compared to Group III (Table 1).

3.2 Lactic acid

In breast muscles lactic acid concentrations and pH values were highly correlated at both storage temperatures: at day 3 pH values decreased as lactic acid concentration increased, at day 7, pH values increased and lactic acid concentration decreased, at day 14, both values changed negligibly. Average concentrations of lactic acid were higher in breast than in thigh muscles. At day 14, the highest average concentration in the breast muscles was measured in Group I (4 °C), the lowest levels in Group III (0 °C).

Table 1. Comparison of average pH values in breast and thigh muscles of pheasants stored uneviscerated at 0 °C and 4 °C, respectively.

	Group I		Group II		Group III	
	0 °C	4 °C	0 °C	4 °C	0 °C	4 °C
Breast muscle						
Day 0	5.79	5.79	5.76	5.76	5.84	5.84
Day 3	5.71	5.61	5.68	5.55	5.62	5.82
Day 7	5.98	5.72	5.90	5.65	6.00	5.71
Day 14	5.82	5.84	5.67	5.72	5.66	5.83
Thigh						
Day 0	6.12	6.12	6.61	6.61	6.68	6.68
Day 3	6.15	6.15	6.61	6.57	6.57	6.49
Day 7	6.31	6.13	6.58	6.50	6.59	6.37
Day 14	6.04	6.21	6.64	6.45	6.36	6.53

In thigh muscles, the highest average lactic acid concentration was determined in Group I (4 °C), the lowest one in Group II (0 °C). At day 14, lactic acid concentrations were statistically different in the breast muscles (4 °C) of Group II (without shots in the body cavity) *vs*. Group III (shots in the body cavity) (Table 2).

Table 2. Comparison of average lactic acid values (g/kg) in breast and thigh muscles of pheasants stored uneviscerated at 0 °C and 4 °C.

	Group I		Group II		Group III	
	0 °C	4 °C	0 °C	4 °C	0 °C	4 °C
Breast muscle						
Day 0	2.13	2.13	2.13	2.13	2.47	2.47
Day 3	5.20	2.60	5.15	2.93	4.60	3.60
Day 7	3.00	3.48	3.16	3.12	4.10	3.35
Day 14	2.45	3.65	2.02	2.93	1.70	3.27
Thigh						
Day 0	1.57	1.57	1.60	1.60	1.57	1.57
Day 3	4.20	2.50	3.00	2.27	2.95	2.60
Day 7	1.98	2.12	2.13	1.86	3.10	2.45
Day 14	1.70	1.80	1.20	1.77	1.10	1.33

3.3 Ammonia

The highest average concentrations of ammonia in the breast muscle (day 14) were determined in Group III (4 °C), and the lowest in Group I (4 °C). At day 14, the highest average concentrations of ammonia in the thigh muscles were in Group III (0 °C), and the lowest in Group I (0 °C). Statistically significant differences were observed between Groups I and III (thigh muscles) stored at 0 °C (Table 3).

Table 3. Comparison of ammonia content (mg/kg) in breast muscles of pheasants stored uneviscerated at 0 °C and 4 °C.

	Group I		Group II		Group III	
	0 °C	4 °C	0 °C	4 °C	0 °C	4 °C
Breast muscle						
Day 0	3.00	3.00	3.13	3.13	3.05	3.05
Day 3	2.30	2.48	2.53	2.69	2.83	2.83
Day 7	3.78	3.99	3.88	4.11	5.14	4.03
Day 14	3.58	3.31	3.78	4.13	3.52	4.15
Thigh						
Day 0	2.58	2.58	3.28	3.28	3.02	3.02
Day 3	2.09	2.62	2.55	2.54	2.79	2.47
Day 7	4.37	4.67	4.49	3.9	5.32	4.21
Day 14	3.57	3.64	4.64	4.21	6.88	4.28

4. Conclusions

Our results indicate that *post mortem* changes in pheasant meat are influenced by method of killing and storage conditions. In summary, muscles from pheasants being slaughtered instead of hunted were characterised by lower pH, higher lactic and lower ammonia concentration at the end of the storage period.

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John Fletcher graduated BVMS from Glasgow University in 1970. Subsequently, he studied the reproductive physiology and behaviour of red deer on the the Isle of Rum, Scotland receiving a PhD from Cambridge University in 1974. He established Europe's first commercial venison unit in Auchtermuchty, Scotland in 1973. John Fletcher was the first vice chairman of the British Deer Farmers' Association and was subsequently chairman. He was instrumental in founding and is past president of the Federation of European Deer Farmers' Associations and the British Veterinary Deer Society. He has contributed to many scientific journals including Nature and chapters to several academic books. He has written widely for the general public in journals such as the New Scientist, Country Life, The Times, and published a full length book, Fletcher's Game in 2001. A further book on the history of British deer parks is in press.

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implementation of EU directive 92/45/EEC in Austria. He designed a three-step inspection system which was later adopted in EU food hygiene legislation. Other duties include: chairman of the Austrian standing committee of hygiene and member of the chapter 'meat and meat products' of the Austrian Food Codex.

Eva Wiklund graduated from the Swedish University of Agricultural Sciences in 1990 and then obtained a PhD in Food Science/Meat Science from the same university in 1997. She has specialised in venison (deer meat) research for her entire research carreer and her work has been very much focused around contacts with the industry, meaning the meat industry in general and reindeer/deer industry in particular. This research profile has provided the opportunity to build contact net-works with the world's most important venison industries and deer researchers in Fennoscandia, New Zealand, Australia and Alaska. Over the last 10 years she has worked for extended periods in all these countries. In 2009 she moved from her position as a Senior Meat Scientist at AgResearch Limited, New Zealand to the Swedish Reindeer Association (Svenska Samernas Riksförbund, SSR). She also holds a position as an Affiliate Associate Professor at University of Alaska Fairbanks, USA.

Rudolf Winkelmayer is a veterinarian who graduated from the University of Veterinary Medicine from which he received the Doctorate degree in 1981. From 1981 to date he combines a private small animal practice (recognised as Specialist since 1988) with a function as Official Veterinarian in Lower Austria. He is a licensed hunter and has initiated and continues to coordinate transnational scientific projects on wildlife and game (e.g. since 2000 the 'INTERREG' project on the prevalence of *Fascioloides magna* in Austria, Hungary, the Czech Republic and Slowakia), to which end he cooperates with various universities in these countries in the fields of game meat hygiene, game diseases and animal welfare. In 2000, he received the Josef Bayer-award from the University of Veterinary Medicine at Vienna for his contributions to the promotion of game meat hygiene. In 2005, he was certified as Diplomate of the ECVPH. In 2006, Dr. Winkelmayer was given the title 'Professor' by the Federal Austrian Government, and in 2009 he became Chief Veterinary Counseler ('wirklicher Hofrat'). Over the past decades Rudolf Winkelmayer has been active in the training of (thousands of) hunters, for which purpose he has authored or co-authored a large number of books, some of which have been translated in various languages. For their book Wildbret-Hygiene (Winkelmayer, Paulsen, Lebersorger, Zedka) Winkelmayer et al. received, in 2009, the Literary Prize of the 'International Council for Game and Wildlife Conservation' (CIC).

Maria Grazia Zanoni graduated in 1994 in Veterinary Medicine from the University of Milan, Italy, and holds a specialist degree in Hygiene and Technology of milk and dairy products from the University of Milan, Italy. She has been working since 1999 as Head Veterinarian in the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), in the central diagnostic laboratory of Brescia. Her professional activity focuses on the diagnosis of animal bacterial and parasitic diseases, with special reference to the diagnosis of infections by *Mycobacterium bovis*.

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