The risk of STEC (Shiga toxin-producing *Escherichia coli*) contamination in wild venison

Food Standards Scotland
For safe food and healthy eating

Scottish Government
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Moredun Research Institute

ROSLIN

Scottish Quality Wild Venison

THE UNIVERSITY of EDINBURGH
Royal (Dick) School of Veterinary Studies

the british deer society

NHS Lothian

LDNS
lowland deer network scotland

SCOTTISH VENISON

Association of Deer Management Groups

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Abbreviations and Glossary

Abbreviations

ACC - Aerobic Colony Count

ADMG – Association of Deer Management Groups

AGHE - Approved Game Handling Establishment

BASC - British Association for Shooting and Conservation

BDS – British Deer Society

CFU – Colony Forming Unit

CI – Confidence Interval

DCS - Deer Commission for Scotland

DMG – Deer Management Group

EU – European Union

HACCP - Hazard Analysis and Critical Control Point

HUS – Haemolytic Uraemic Syndrome

FES – Forest Enterprise Scotland (now Forestry and Land Scotland)

FLS – Forestry and Land Scotland (formerly Forest Enterprise Scotland)

FSA – Food Standards Agency

FSS – Food Standards Scotland

LDNS - Lowland Deer Network Scotland

PCR – Polymerase Chain Reaction

PHE – Public Health England

PT – Phage Type

SERL – Scottish *E. coli* O157/STEC Reference Laboratory

SMAC – Sorbitol MacConkey Agar

Abbreviations and glossary

SNH - Scottish Natural Heritage

SGA - Scottish Gamekeepers Association

SQWV – Scottish Quality Wild Venison

STEC - Shiga toxin-producing *Escherichia coli*

SVA – Scottish Venison Association, formerly Scottish Venison Partnership

VDL - Venison Dealer License

Glossary

Animal by-products - entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption

Approved game-handling establishment (AGHE) - any establishment approved by the Food Standards Agency (in England, Wales or Northern Ireland) or Food Standards Scotland in which game and game meat obtained after hunting are prepared and health marked for placing on the market in the UK

Audit - a systematic and independent examination to determine whether activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives

Brisket - a cut of meat from the breast or lower chest deer carcass. The brisket muscles include the superficial and deep pectorals

Carcass dressing - removal of the hide (skin), the head, the legs below the elbows and hocks, as well as removing most of the viscera

Carcass - the body of an animal after killing it for human consumption and dressing it

Closed season - the time of the year during which hunting an animal of a given species is contrary to law

Contamination - the presence or introduction of a physical, chemical or biological hazard (such as microbial faecal contamination, hair, bile, soil, grass, leaves or excessive dried blood)

Deer stalking - the stealthy pursuit of deer on foot with intention of killing the deer for meat, for sport, or to control the numbers

Establishment - any unit of a food business

Final consumer - the ultimate consumer of a foodstuff who will not use the food as part of any food business operation or activity

Food business - any undertaking, whether for profit or not and whether public or private, carrying out any of the activities related to any stage of production, processing and distribution of food

Food business operator (FBO) - the natural or legal persons responsible for ensuring that the requirements of food law are met within the food business under their control

Food hygiene included in this document as "hygiene", - the measures and conditions necessary to control hazards and to ensure fitness for human consumption of a foodstuff taking into account its intended use

Fresh meat - meat that has not undergone any preserving process other than chilling

Gralloching - the process of removing abdominal and pelvic viscera of hunted wild deer

Hazard - a biological, chemical or physical agent in, or condition of, food or feed with the potential to cause an adverse health effect

Hock - the joint in a quadruped's hind leg between the knee and the fetlock (ankle), the angle of which points backwards

Inspection - the examination of establishments, of animals and food, and the processing thereof, of food businesses and their management and production systems, including documents, finished product testing and feeding practices, and of the origin and destination of production inputs and outputs, in order to verify compliance with the legal requirements in all cases

Larder - a storage facility where deer (primary products) are prepared (by cutting parts of the body such as feet, the head), kept stored and possibly processed into venison or otherwise transported for further cutting into game meat

Lowland deer – deer managed in non-upland areas of more fragmented land ownership and use

Meat preparations - fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat. Examples include meatballs, seasoned meat, raw sausages, and burgers

Meat products - processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat. Examples include: smoked, cured, dried products that may still require cooking

Minced meat - boned meat that has been minced and contains less than 1% salt ("ground meat" in the USA).

Placing on the market - the holding of food for the purpose of sale, including offering for sale or any other form of transfer, whether free of charge or not, and the sale, distribution, and other forms of transfer themselves

Primary product - refers to a deer carcass which may have had the head, feet and viscera removed but which is still in the hide

Primary production - hunting and harvesting of deer for human consumption

Red pluck or pluck - the heart, lungs, trachea and liver.

Retail - the handling and/or processing of food and its storage at the point of sale or delivery to the final consumer, and includes distribution terminals, catering operations, factory canteens, institutional catering, restaurants and other similar food service operations, shops, supermarket distribution centres and wholesale outlets.

Risk - a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard

Rodding - the procedure of sealing the oesophagus either by tying a knot or by constricting it with a plastic/metallic ring

Sticking - the operation of slitting the neck or penetrating the chest with a sharp knife with the purpose of bleeding the animals

Traceability - the ability to trace and follow a food, food-producing animal or substance intended to be or expected to be incorporated into a food through all stages of production, processing and distribution

Trained Person or **Trained Hunter** – An individual who hunts wild game with a view to placing it on the market for human consumption with 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

Upland deer – deer managed on predominantly upland areas which have large management units that are well suited to collaborative deer management, mainly of red deer

Venison - meat resulted from a deer carcass after the hide has been removed. In the UK, the deer species used for venison production are mainly red deer followed by roe, sika and fallow deer. Limited amounts of venison is also produced from muntjac deer.

Viscera - the organs of the thoracic, abdominal and pelvic cavities

Wild Deer Best Practice Guides - guides developed within Scotland's deer sector to provide information on wild deer management[1].

Wrapping - the placing of a foodstuff in a wrapper or container in direct contact with the foodstuff concerned, and the wrapper or container itself

Zoonosis - any disease or infection that is naturally transmissible between vertebrate animals and humans

[1] http://www.bestpracticeguides.org.uk/

Executive Summary

Background

Shiga toxin-producing *Escherichia coli* (STEC) bacteria are important human pathogens. They cause gastro-intestinal disease in humans through the production of Shiga toxins (Stx), which cause haemorrhagic colitis, and, in severe cases, potentially life-threatening haemolytic uremic syndrome (HUS).

Cattle are thought to be the major reservoir of STEC, although other ruminants can also act as reservoirs of infection. Transmission to humans occurs as a result of contact with ruminant faecal material containing STEC, for example by handling animals, through exposure to contaminated soil or vegetation, or from contaminated water or food. Milk and dairy products can be contaminated during milking, and meat during the slaughter process.

The STEC serogroup responsible for most human cases in the UK is O157; however other serogroups can also cause disease. Most research to date has focused on understanding the prevalence of these bacteria within cattle populations. There is limited information on the prevalence of STEC in other ruminants, and how they contribute to infections in humans.

In 2015 a severe outbreak of STEC O157 occurred in Scotland involving twelve human cases. The outbreak was linked to consumption of venison products supplied to retailers by a single approved game handling establishment (AGHE). It was concluded that the most likely source of the infection was from a heavily-contaminated deer carcass or carcasses. The investigation highlighted a number of knowledge gaps about the risk of STEC infection from venison, including a lack of information about:

(i) The prevalence of STEC serotypes in Scottish deer.

(ii) Whether deer, like cattle, can act as STEC 'super-shedders' (individuals which shed high numbers of STEC in their faeces).

(iii) Which stages of processing present the greatest risk of cross-contamination.

To address these knowledge gaps, this project was commissioned by the Scottish Government and Food Standards Scotland to better understand the risk of STEC contamination of wild venison. The work involved three objectives:

Objective 1: To map the venison industry in Scotland: A detailed report on the structure of the deer industry was generated, and the steps involved in venison production from the hill to the end product described.

Objective 2: A field survey to assess STEC prevalence in wild deer in Scotland: A study was performed on deer entering the human food chain to estimate the prevalence of STEC O157, and levels present in positive samples. A preliminary survey of non-O157 STEC was also conducted.

Objective 3: A review of cross-contamination risks in the slaughter and processing stages of wild deer from the field to the larder: A systematic review of existing literature relating to cross-contamination of venison was conducted and a field study of contamination during production undertaken.

Results

Mapping of the venison industry in Scotland

Scotland produces an estimated 3,000 – 3,500 tonnes of venison per annum, of which around 90 tonnes (~2.5%) comes from farmed deer and ~98% from the cull of wild deer (all species). A proportion of the cull is not reported, so in fact actual output may be closer to ~3,800 tonnes. For comparison; beef and lamb production in Scotland is around 170,000 and 60,000 tonnes, respectively.

Approximately one third of UK-produced venison is exported to Europe. Approximately 1,000 tonnes of venison consumed in the UK is imported, mainly farmed deer from New Zealand, which accounts for around one quarter of the UK's venison consumption. However, this figure has declined as New Zealand has prioritised other global markets for venison.

UK venison consumption has increased over the last 10 years but is now steady and restricted by available product, wild and farmed, UK produced and imported. To meet market demand, venison imports remain important in the medium term. Deer farming is being actively promoted by the Scottish Government's *Beyond the Glen strategy*[2] which aims to increase farmed venison production 8-fold by 2030 to 850 tonnes per annum.

The impact of changes in venison imports on zoonotic disease risk is unclear as there is a lack of information on the relative prevalence of zoonotic diseases in Scottish *vs.* imported venison. The exception is *Mycobacterium bovis* which is present in New Zealand (the main source of imported venison) but not Scotland.

Wild deer in the uplands are managed by voluntary Deer Management Groups (DMGs), which exist across most of Scotland's upland deer ranges, and by Forestry & Land Scotland. A less structured approach to deer management exists in the low ground.

Wild venison production involves killing in the field by a free bullet. Once shot, deer are bled out on the hill, stomach and intestines removed (gralloching), and the carcass transported to a larder. Here the sternum is split to enable the removal of the 'red pluck' (lungs, heart and liver) and the head and feet. The carcass is then refrigerated at the larder until it is transported to the AGHE, where final processing and packaging is performed.

A voluntary Quality Assurance (QA) scheme exists for wild venison production which is underpinned by Best Practice Guidance. The scheme is delivered by Scottish Quality Wild Venison (SQWV). It is estimated that around 75% of wild venison from Scotland is produced under the SQWV QA scheme, and this is rising.

While legislation is in place for larger scale wild venison production and farmed venison production, exemptions from the regulations exist for small scale operators or those supplying venison for private consumption. There is a lack of understanding of what proportion of wild venison consumed originates from small scale operators, particularly as returns from venison dealers are not actively requested.

[2] https://scotlandfoodanddrink.blob.core.windows.net//media/1555/venison-strategy-brochure.pdf

Traceability of wild venison could be improved through increased uptake of quality assurance schemes, and a better understanding of the volume of wild venison produced under exemptions to the legislation. However, the lack of official traceability of deer carcasses from small scale operators is mitigated by the short supply chains for these carcasses.

Prevalence and characterisation of STEC in wild Scottish deer

A prevalence study was conducted on wild Scottish deer between July 2017 and June 2018. Samples represented all four wild deer species in Scotland (Red, Roe, Sika and Fallow deer) from all regions of Scotland. Out of a total of 1087 faecal samples analysed, three were positive for STEC O157. The mean estimated prevalence of STEC O157 in wild Scottish deer was 0.34% (95% Confidence Intervals = 0.02 – 6.30). Faecal samples from the three positive deer contained high levels of STEC O157 (>10^4 CFU/g faeces).

STEC O157 strains isolated from the deer faecal samples were sequenced to allow comparisons with STEC O157 strains isolated from UK human STEC cases, in order to estimate their human pathogenic potential. Deer STEC O157 strains were also compared with strains isolated from UK cattle populations to determine if they are likely to circulate between cattle and deer populations.

All three deer STEC O157 strains contained the gene encoding Stx subtype 2a (*stx2a*) and were positive for the virulence factor *eae*. Two of the deer STEC O157 strains, both isolated from Red deer, were phage-type (PT) 21/28. The third isolate was a PT8 strain from a Sika deer. While both phage types have been associated with human disease, PT21/28 is associated with more severe disease, as is the presence of both *stx2a* and *eae* in the same strain. This suggests the three deer strains identified have human pathogenic potential.

Whole genome sequence comparisons of the strains identified allowed comparisons with cattle strains and other human outbreak strains, including the strain involved in the 2015 venison outbreak. This demonstrated a close relationship between the deer strains, and human and cattle STEC O157 isolates, with one of the PT21/28 STEC O157 deer strains being closely related to the 2015 venison outbreak strain. This provides further evidence that the deer strains identified during this study are pathogenic to humans, and that STEC O157 in deer may be circulating between deer and cattle populations.

To investigate the prevalence of non-O157 STEC, faecal samples were screened for the presence of genes encoding Stx type 1 (*stx1*) or Stx type 2 (*stx2).* A high proportion of faecal samples (~70%) had detectable *stx*1 and/or *stx*2 genes, of which ~25% were also positive for *eae*. There was no obvious association between positive samples and deer species or sampling location. Isolation of STEC strains from *stx2*+/*eae*+ samples was performed on a subset of these samples. From a total of 74 samples tested, STEC strains were isolated from a total of 60 faecal samples, with two STEC strains isolated from 8 samples. These strains were all *stx2* positive but *eae* negative, suggesting they are less likely to cause more severe human disease.

Overall, the results from the field sampling indicate that prevalence of STEC O157 in wild Scottish deer is low. However, when found, the levels of bacteria in positive faecal samples were high and of strain types associated with more severe human disease. Furthermore, *stx* genes were found in a high proportion of deer faeces tested, with 68 non-O157 STEC strains isolated from a subset of the faecal samples representing a diverse deer species and geographical range.

Risk factors associated with *E. coli* contamination of wild deer carcasses

This work involved both a systematic review of the existing literature and a prospective study to determine risk factors associated with *E. coli* contamination at different stages of wild venison production from cull to the final venison product.

(i) Systematic literature review

From the literature review the following risk factors were identified:

• The health status of the animal, with unhealthy animals potentially posing greater risk of STEC O157 contamination (although unhealthy animals should not enter the food chain).

• Hygiene practices in the field from the time of killing to gralloching to transportation to the larder or AGHE, as poor hygiene practices will allow bacteria such as *E. coli* to transfer onto the carcass from faecal or environmental contamination.

• Maintenance of the cold chain from larder to final product. Critically, maintaining temperatures below 7°C would help to limit growth of bacteria on the carcass.

• Handling and hygiene procedures involved in further skinning, cutting and processing of the venison.

This literature review identified a number of limitations including the lack of studies on both O157 and non-O157 STEC in deer, including the lack of prevalence data in deer faeces, hides and carcass and a lack of studies identifying risk factors associated with STEC contamination at various stages of wild game meat production along the food chain. Information on the survivability characteristics of STEC serotypes of deer origin on surfaces, hands and equipment was also lacking.

ii) Field study on *E. coli* contamination from different stages of wild venison production

The field study involved sampling of wild deer carcasses at all stages of production, from hill to end-product, to identify the risk factors associated with microbiological condition of wild deer carcasses. Both numbers of coliforms (bacteria present at high levels in the intestinal tracts of mammals and used as an indicator of faecal contamination) and *E. coli*, (a specific coliform species) were measured on the hide and internal cavity prior to skinning, and on the carcass surface after skinning.

Phase I of this study involved collection of samples from 14 carcases at the point of cull and subsequently at the larder. Both coliform and *E. coli* counts were much lower compared to those found previously at AGHEs. Counts collected from the carcass at the point of cull were slightly lower than those collected at the larder two to four hours later but were all significantly lower than the acceptable limits for similar groups of bacteria described for domestic ruminants. These results demonstrate that levels of bacterial

contamination of carcasses of culled wild deer can be limited to a standard suitable for human consumption if carcasses are processed appropriately.

In phase II of this study, deer carcasses were sampled from six AGHEs across Scotland including Red, Roe and Sika deer. Seven meat samples were also collected from four of the AGHEs. Regardless of deer species, levels of both coliforms and *E. coli* were highest in the internal cavities of carcasses and lowest on hides. Levels of bacteria were lower in Roe deer compared to Red and Sika deer. Average levels of coliforms on carcases were between 3.9-4.3 log higher than the acceptable limits for similar groups of bacteria described for ruminant livestock (as defined in Regulation (EC) No. 2073/2005); however, *E. coli* levels on carcasses were within acceptable limits for all deer species tested, although two of the seven meat samples tested had levels of *E. coli* higher than limits set for domestic ruminant products.

A risk factor analysis was performed using the field study data from Red and Roe deer, (the number of Sika deer tested not being adequate for robust analysis). This identified the following risk factors associated with increased contamination of carcasses:

Risk factors associated with coliform contamination (Roe deer only)
- Time in storage ≥6 days
- Longer distance between cull location and AGHE

Risk factors associated with *E. coli* contamination (Red and Roe deer)
- Warmer environmental temperatures, above 7ºC (Roe only)
- High levels of visible faecal contamination (Roe and Red) or dirty skin (Red)
- Wet and slimy carcasses (Roe only)
- Males (Roe only)

Conclusions

This report represents a comprehensive evaluation of the wild venison industry in Scotland and provided an evidence base to assess the risk of human STEC infections through consumption of wild venison.

It is concluded that the venison industry has the potential to grow with consumer demand expected to increase year-on-year. However, it is restricted by available product. Despite initiatives to increase deer farming, venison production from the wild cull is likely to remain the important source of venison in the medium term. The industry is well organised into Deer Management Groups and has produced Best Practice Guides for wild venison production, which are followed by at least ~75% of wild venison producers. There is a lack of traceability of deer carcasses from small scale operators which are exempt from regulations, however in terms of investigating disease outbreaks the lack of official traceability is mitigated by the short supply chains for these carcasses.

STEC O157 prevalence was found to be low in wild Scottish deer, with only 3 out if 1087 samples being positive for the bacteria. However, as the STEC O157 strains were of a type associated with severe disease in humans, and were present in high levels in deer faeces, it concluded that there is a real risk of future human STEC O157 infections arising from consumption of venison contaminated with deer faeces. Furthermore, a

number of non-O157 STEC strains were isolated from deer faeces, although the risk they pose to humans is currently unclear.

Risk factors for contamination of venison carcasses with *E. coli* and coliforms included visual contamination of the skin with faeces or dirt, wet and slimy carcasses, environmental temperatures >7˚C and increased distance between cull location and AGHE. Minimal *E. coli* contamination of carcasses was demonstrated when good hygiene practices on venison processing were observed. This information will be useful for those involved in wild venison production and should be integrated into current Best Practice guides. Strict hygiene precautions aimed at avoiding faecal contamination of the carcass during processing are expected to minimise the risk of human STEC infections from wild venison.

Lay Summary

E. coli bacteria are very common in the environment, and many types of *E. coli* live in the guts of mammals. Some types of *E. coli* cause disease, some are harmless and some can even be beneficial. Shiga toxin-producing *E. coli* (STEC) are a particular type that can cause human disease as a result of the Shiga toxins they produce during infection. The most important type of STEC in the UK is O157, although a number of other types of STEC can also cause disease in humans.

Cattle are thought to be the main source of STEC infection for humans, although other animals including deer and sheep can also shed the bacteria in their faeces. The bacteria do not cause disease within these species themselves.

In 2015 an outbreak of human STEC infections occurred which was linked to consumption of venison, most likely from a wild deer. This prompted the Scottish Government and Food Standards Scotland to commission a study to provide information on the Scottish venison industry and the risk of further STEC infections arising from consumption of Scottish wild venison.

The work in this project had three main objectives:

(i) To review the Scottish venison industry and venison consumption in Scotland.

(i) To estimate how common STEC O157 and other STEC infections are in wild Scottish deer.

(iii) To identify risk factors associated with contamination of deer carcasses with *E. coli* and other gut bacteria.

The project found the following:

• Venison production is relatively small compared to beef and lamb production, but consumption is increasing. Most deer in Scotland is produced from wild deer which are culled in the field and processed in approved game handling establishments. Some are processed locally by retailers (hotels, butchers) or eaten at home.

• The wild venison industry is organised into deer management groups across Scotland, and the industry has detailed Best Practice guides to ensure that wild venison is produced in a hygienic manner and is safe to eat.

• STEC O157 is present in very few wild deer with only 3 out of 1087 samples tested being positive for it. However the types of STEC O157 found were closely related to those which cause severe human disease. A number of non-O157 STEC were also present in deer faeces. This shows that the faeces of deer can contain bacteria which are potentially dangerous to human health.

• The risk of deer carcasses becoming contaminated with *E. coli* was increased if: the carcasses were stored for longer before being processed to venison, the distance from the site where the deer was killed to the place it was being processed increased, the carcasses were wet and slimy or obviously dirty or contaminated with faeces, or if the temperature was higher.

Lay summary

• The conclusions of the study are that, while STEC O157 is present at very low levels in wild deer, contamination of venison carcasses with potentially dangerous STEC O157 is possible. The risk factors for carcass contamination identified in this study should be taken into account when producing venison for human consumption.

Recommendations and future work

From the results of this project a number of recommendations can be made:

(i) There is a lack of understanding of what proportion of wild venison consumed in the UK originates from small scale operators exempt from current regulations. Proactive requests for venison returns from venison dealers would improve knowledge of the volume of wild venison being sold and consumed through small scale operators, which would improve traceability of venison products. Better uptake of the voluntary Quality Assurance scheme for wild venison production would also improve carcass traceability.

(ii) Due to increasing demand for venison, it is likely that imports of venison into the UK will increase. Better knowledge of zoonotic pathogens carried in imported venison, together with knowledge of existing zoonotic pathogens other than STEC in UK venison, is required to understand the food safety implications of increasing venison importation.

(iii) Although the prevalence of STEC O157 was low in wild Scottish deer, the bacteria were present at high levels and were of strain types associated with more severe forms of human disease. Therefore, adherence to strict hygiene practices from cull to final product is strongly recommended.

(iv) Risk factors for *E. coli* contamination of wild deer carcasses (e.g. dirty hides, faecally contaminated or wet and slimy carcasses, higher environmental temperatures, and longer distances from cull sites to AGHE) should be relayed to those involved in wild venison production and integrated and emphasised in Best Practice Guides. This would allow those involved in venison production to consider how best to minimise the risks of *E. coli*-contaminated venison entering the human food chain.

The following future research is also recommended to better understand the risk of zoonotic disease posed by venison:

(i) Characterisation of non-O157 STEC strains isolated from deer in this study to determine their human pathogenic potential.

(ii) A risk factor analysis to determine what factors are associated with the presence of non-O157 STEC in deer. For example, is contact with other ruminants an important factor in non-O157 STEC presence in deer?

(iii) An investigation of STEC prevalence in farmed deer. The current project focussed on wild deer; however, it is possible that STEC are better able to persist in deer populations kept in more intensively farmed systems. This is particularly important given the drive to increase the number of farmed deer in Scotland.

(iv) Studies to estimate the prevalence of zoonotic pathogens, including STEC, in imported venison should be performed to better determine the food safety implications of increased importation of venison.

1. General Introduction

Shiga toxin producing *Escherichia coli* (STEC) are important human pathogens of global importance which cause gastro-intestinal disease in humans with potentially life-threatening consequences. These bacteria cause human disease due to the production of Shiga toxins (Stx) which cause haemorrhagic colitis and in severe cases potentially life-threatening haemolytic uremic syndrome (HUS) (Melton-Celsa *et al*., 2012). It is considered to have a low infectious dose in humans, with fewer than 10 to 100 colony forming units (CFU) of STEC thought sufficient to cause infection (Hara-Kudo *et al.* 2011). Almost 50% of cases in Scotland are in children under 16 years old, and 85% of HUS cases occur in this age-group (VTEC/*E. coli* O157 Action Plan for Scotland 2013-2017[3]).

Stx toxins have been classified into two major groups Stx1 and Stx2, with Stx2 toxins further divided into subtypes Stx2a – g (Melton-Celsa *et al.*, 2012; Scheutz *et al*., 2012). Strains encoding subtype Stx2a were more likely to be associated with HUS, including in clinical cases in the UK (Brandal *et al*., 2015; Buvens *et al*., 2012; Dallman *et al*., 2015). Cattle are considered the major reservoir of STEC; however other ruminants such as sheep, goats and deer can also act as reservoirs of infection (Beutin *et al.*, 1993; Caprioli *et al*., 2005; Rounds *et al.,* 2012). Transmission to humans can occur as a result of direct or indirect contact with STEC containing ruminant faecal material, for example by handling animals, through exposure to soil or vegetation contaminated by faeces, or from contaminated water or food. In the case of food of animal origin, contamination can occur during milking (milk and dairy products) or during the slaughter process (for meat and meat products).

The STEC serogroup responsible for most human cases in the UK and North America is O157 (Majowicz *et al*., 2014); however other serogroups are a threat to human health and there has been an increasing incidence of outbreaks associated with non-O157 STEC in recent years (Gould *et al.,* 2013). In recognition of the growing importance of non-O157 STEC serotypes, six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) were classified as food adulterants in the USA as these serogroups were historically the most important in terms of human infections (FSIS (2014) FSIS Notice 40-12[4]). However, given the diversity of non-O157 STEC serogroups which can cause human disease, a scheme has now been developed to assess the risk non-O157 STEC isolates according to their virulence gene profile, rather than their serogroup[5]).

As cattle are thought to be the major reservoir of STEC, most research has focused on understanding the prevalence of these bacteria within cattle populations, and how STEC levels in cattle can be controlled by pre- and post-harvest interventions. In Scotland, three large scale STEC O157 prevalence studies in cattle have been performed (Pearce *et al*., 2009; Henry *et al.,* 2017). From these studies, it is evident that

[3] http://www.gov.scot/Publications/2013/11/8897
[4] http://www.usda.gov/wps/portal/usda/usdahome
[5] http://www.fao.org/3/ca0032en/CA0032EN.pdf

approximately 20% of Scottish herds are positive for the organism, individual pat prevalence in positive farms is between 5-10%, and that the majority of STEC O157 shedding occurs in a relatively small number of individuals (<20%), so called 'super-shedders', that shed high levels (>10^4 colony forming units per gram of faeces (CFU/g)) of the organism and are responsible for around 80% of all animal-to-animal transmissions (Chase-Topping *et al*., 2007; Matthews *et al*., 2006). **However, we have limited information on the prevalence of STEC in other ruminant species, and what risk they pose to human health.**

In autumn 2015 a severe outbreak of STEC O157 occurred in Scotland involving a total of twelve human cases (Health Protection Scotland: National Outbreak of *Escherichia coli* O157 Phage Type 32 in Scotland[6]; Smith-Palmer *et al.*, 2018). Five of the twelve cases required hospitalisation, but went on to completely recover. The outbreak was linked to consumption of venison products (steaks, grillsteaks, sausages and meatballs) supplied to a number of retailers by a single approved game handling establishment (AGHE). While the clinical outbreak strain, a Phage Type 32 (PT32), was not isolated from these products, strong epidemiological evidence linked the clinical cases to consumption of these venison products. Following extensive investigations, it was concluded by the authorities and the company that the most likely source of the contamination was from a wild deer carcass or carcasses heavily contaminated with STEC O157. Wild deer are shot and dressed (or gralloched) on the hill and there is theoretically a greater risk of faecal contamination of wild deer carcasses compared to farmed deer carcasses which are usually processed at abattoirs. Indeed, a previous Food Standards Agency (FSA) project demonstrated that retail packs of wild venison were more likely to be contaminated with *E. coli* than venison from farmed animals (Richards *et al.*, 2011).

As a result of this outbreak, a number of actions were undertaken by the AGHE to reduce the risk of STEC contamination of venison products, including deep cleaning of the premises, reviewing Hazard Analysis and Critical Control Points (HACCP) plan and procedures for processing large wild game, and increasing the frequency of microbiological testing. However, investigation of this outbreak also highlighted a number of knowledge gaps relating to the risk of STEC infection associated with venison consumption outlined below:

(i) There is a lack of information relating to the prevalence of STEC O157 and other STEC serotypes in Scottish deer populations. In an unpublished study conducted in 2003 (Singh BK, University of Edinburgh), no STEC O157 was detected in a total of 784 faecal samples collected from mainly wild Scottish deer of a range of species including Roe, Red and Sika deer. This low prevalence level is in line with similar studies conducted in North America and Europe which estimated the prevalence of STEC O157 in deer between 0 and 2.4% (Ferens *et al*., 2011). Non-O157 STEC appear to be more prevalent in deer populations, although often lack key virulence genes associated with human disease (Miko *et al*., 2009). Information on STEC prevalence in Scottish deer, and whether these strains present a risk to human health, is urgently required to

[6] http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=2987

quantify the overall potential risk of STEC contamination within the Scottish venison production system.

(ii) It is unknown whether deer, like cattle, can act as STEC super-shedders. Super-shedding deer would theoretically have an increased risk of contaminating the carcases of both the super-shedder itself and other carcases through cross-contamination during carcass processing.

(iii) There is a lack of information on what stages of the venison processing chain (from gralloching to processing) represent the greatest risk of cross-contamination. Such knowledge would allow targeted interventions and training to reduce the risk of carcass contamination with STEC.

Study Objectives

To address these knowledge gaps, work was commissioned by the Scottish Government and Food Standards Scotland (CR/2016/26) to better understand the risk of STEC contamination of wild Scottish venison. The work involved three inter-related objectives to (i) map the venison industry in Scotland; (ii) determine the prevalence of STEC in Scottish wild deer and (iii) review cross-contamination risks in the slaughter and processing stages of wild deer from the field to the larder.

2. Objective 1: Mapping the venison industry in Scotland

Summary

The aim of this objective was to provide a detailed overview of the structure of the Scottish deer industry between 2017 and 2019, including the role of imports and exports, the main legislation relating to venison production, and the steps involved in venison production from the hill to the end product. The main findings are summarised below:

• Scotland produces an estimated 3000 - 3500 tonnes of venison for consumption per annum, of which around 90 tonnes (~2.5%) comes from farmed deer and ~98% from the cull of wild deer (all species). A proportion of the cull is not reported so the output may be closer to ~3800 tonnes. For comparison, beef and lamb production in Scotland is around 170,000 and 60,000 tonnes, respectively.

• Approximately one third of UK produced venison (mainly from the cull) is exported to Europe.

• In 2017 approximately 800 tonnes of venison consumed in the UK was imported, mainly from farmed deer from New Zealand, which accounts for around one quarter of the UK's venison consumption. However, this figure has declined in 2018 and 2019.

• UK venison consumption has increased over the last 10 years but is now steady and determined by available product.

• To meet this market demand, venison imports remain important in the medium term. Deer farming is being actively promoted through the Scottish Government's *Beyond the Glen* strategy which aims to increase farmed venison production ~9-fold by 2030 to 850 tonnes per annum.

• The impact of changes in venison imports on zoonotic disease risk is unclear as there is a lack of information on the relative prevalence of zoonotic diseases in Scottish vs. imported venison. The exception is *Mycobacterium bovis* which is present in New Zealand (the main source of imported venison) but not Scotland.

• Wild deer in the uplands are managed by voluntary Deer Management Groups (DMGs), which exist across most of Scotland's upland deer ranges, and by Forest Enterprise (now Forestry and Land Scotland). A less structured approach to deer management exists in low ground areas.

• Detailed requirements are set out in law for larger scale wild venison production and farmed venison production. However, certain exemptions from these requirements exist for operators involved in, for example, the direct supply of small quantities of game to either the local market or direct to final consumers. In addition, food law excludes from its scope in full those hunters preparing game for private domestic use.

• There is a lack of understanding of what proportion of wild venison consumed in the UK originates from those operating under specific exemptions in law, particularly as

returns from venison dealers are not actively requested by Scottish Natural Heritage (SNH).

• A voluntary Quality Assurance (QA) scheme exists for wild venison production which is underpinned by Best Practice Guidance. The scheme is delivered by Scottish Quality Wild Venison (SQWV). It is estimated that around 75% of wild venison from Scotland is produced under the SQWV QA scheme, and this is rising.

• Traceability of wild venison could be improved through improved uptake of quality assurance schemes, and a better understanding of the volume of wild venison produced under exemptions to the legislation.

2.1. Structure of the venison industry in Scotland

This section was compiled from published reports and cull data provided by SNH.

2.1.1. Origin of venison consumed in Scotland

UK production

Scotland produces an estimated 3000-3500 tonnes of venison per annum, of which around 90 tonnes (~2.5%) currently comes from farmed deer. These estimates are based on cull returns (annual number of deer culled) collated by Scottish Natural Heritage (SNH, Figure 2.1) multiplied by the average carcass weights of each species (Appendix 1). Of the deer culled in Scotland, just under a third are culled by Forestry and Land Scotland, who cull ~20%, 40%, 50% and 20% of the total Red, Roe, Sika and Fallow deer culled each year, respectively (Appendix 2). Most (~90%) of deer are culled in season (Appendix 3). The majority (~80%) of out of season culling is by Forestry and Land Scotland (FLS, formerly Forest Enterprise) for deer management purposes, with carcasses processed for human consumption. Hunting seasons for each deer species are shown in Appendix 4.

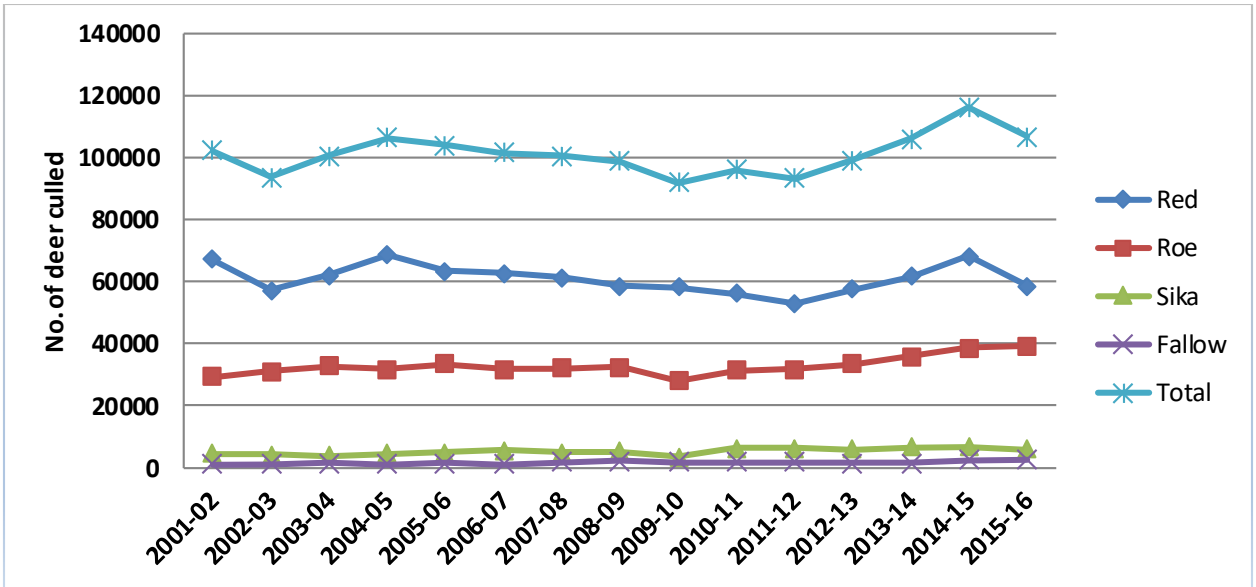


***Figure 2.1** Scotland's wild deer cull 2001 – 2016 - Source: Scottish Natural Heritage (SNH)*

The amount of venison production from the cull has remained fairly consistent over the last 15 years with most volume of production (~80%) originating from Red deer. There has been a slight upward trend in production over the last five years (Figure 2.2). Despite this, volume of UK venison production remains insufficient to meet market demand due to seasonal variation in venison production. Consequently, venison imports are sustaining a year-round UK market.

Deer farming (the practice of rearing deer in fenced areas for the purpose of venison production), while still contributing only a small amount of the total venison consumed, is increasing in the UK. Scottish deer farmers are now eligible for the Basic Payment Scheme[7], the largest of the European Union's rural grants and payments to help the farming industry. There has been a drive to encourage increased production of Scottish farmed venison and reduce the UK's reliance on imports, through schemes such as the Scottish Government funded *Deer Farm and Park Demonstration Project* (2014-2015), *Beyond the Glen strategy* for wild and farmed venison[8] and the Venison Advisory Service Ltd[9], a consultancy established specifically to provide support and advice to those considering setting up commercial deer farms. The *Beyond the Glen* strategy for venison aims to increase production of farmed venison from 100 tonnes to 850 tonnes per annum by 2030, by growing the annual number of deer slaughtered from 1,700 to 15,000 animals.

[7] https://www.gov.uk/government/collections/basic-payment-scheme
[8] https://scotlandfoodanddrink.blob.core.windows.net//media/1555/venison-strategy-brochure.pdf
[9] http://venisonadvisory.co.uk/

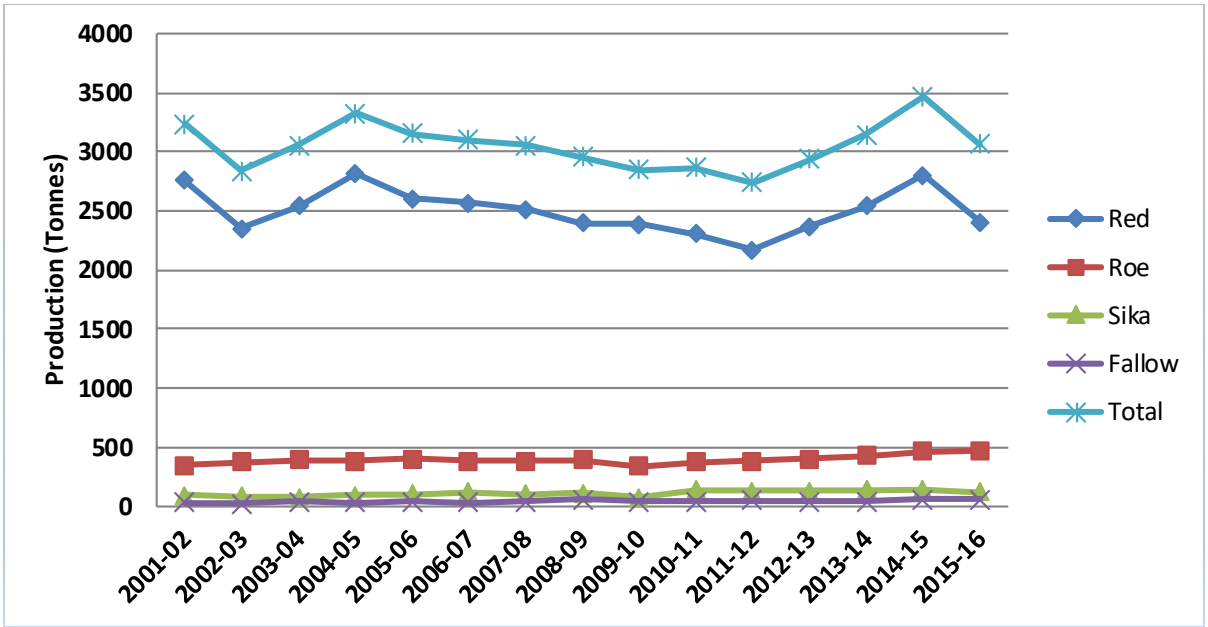


Figure 2.2 *Estimated venison production from the culls 2001-2016*

Scottish venison production is significantly lower than that for beef and lamb: In 2016 Scotland produced around 170,000 and 60,000 tonnes per annum of beef and lamb, respectively[10].

Venison imports/exports

Around one third of the venison (800 -1000 tonnes) consumed in the UK is imported in order to maintain a year-round supply not possible through the seasonal cull in Scotland. The majority of imported venison comes from farmed deer in New Zealand, with other imports from Poland, Ireland, Spain, Benelux and other European countries. The Deer Industry New Zealand figures (to year-end September 2017) show the UK as New Zealand's fifth largest venison export market accounting for around 6% (770 tonnes) of its total venison exports. A breakdown of recent venison exports from New Zealand is shown in Table 2.1 and shows a slight decline in total venison exports from 2015 to 2017. Approximately one third of venison produced in the UK is exported to Europe, which consists of mainly Roe deer venison and late season Red deer from the cull. Scottish wild venison is sold predominantly in the UK[11].

Table 2.1 *Venison exports from New Zealand – source Deer Industry New Zealand (deernz.org)*

New Zealand Venison exports	2015	2016	2017
Total venison exports (tonnes)	14,869	12,911	11,939
Total exported to UK	n/a	n/a	770
% exported to UK	n/a	n/a	6.4%

n/a = data not available.

[10] https://www2.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/agritopics/alllivestock
[11] https://scotlandfoodanddrink.blob.core.windows.net//media/1555/venison-strategy-brochure.pdf

2.1.2 Organisation of the venison industry in Scotland

Wild venison industry

Deer Management Groups
Wild deer are managed by Deer Management Groups (DMGs), which are voluntary and exist across most of Scotland's upland deer range, and by Forestry and Land Scotland, across the National Forest Estate. Organised deer management occurs in around 70% of Scotland's land area (Deer Management in Scotland: Report to the Scottish Government 2016) despite deer being present throughout Scotland. The areas covered by DMGs is shown in Figure 2.3.

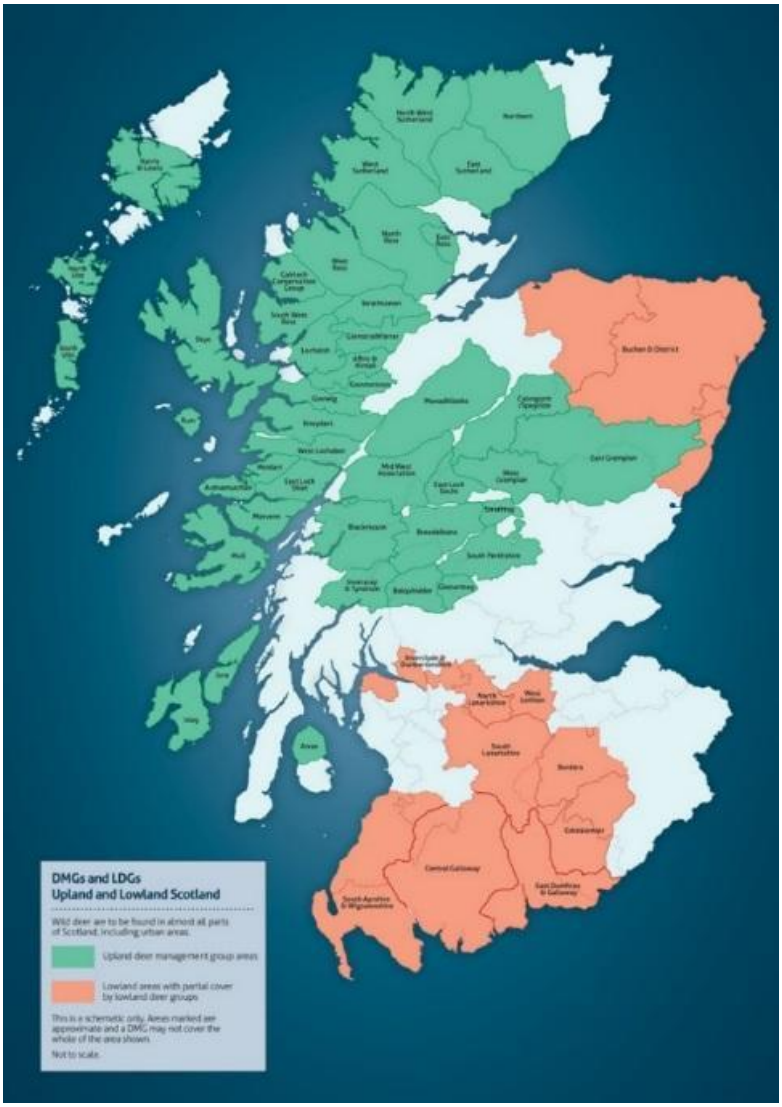


Figure 2.3. Map of Deer management groups in Scotland.
Green = upland deer management groups; Orange = lowland areas with partial cover by lowland deer groups.
Source: ADMG website http://www.deer-management.co.uk/

Deer Management Groups usually meet at least twice per year. Membership comprises land managers, including public sector land managers, farmers, private estates, non-Government organisations, Forestry and Land Scotland, and private forestry/land

management companies which own or manage land in the Group area. Representatives from Scottish Natural Heritage (SNH) also attend these meetings. Almost all upland DMGs have published their Deer Management Plans which are available via the ADMG website[12].

Wild venison quality assurance schemes
The quality assurance schemes for wild venison production and for processors are delivered by Scottish Quality Wild Venison Ltd (SQWV)[13]. SQWV is an independent company established to maintain, develop and promote quality assurance standards throughout the wild venison industry (see section 2.3.4 for more details).

Best Practice Guides
Best Practice Guides underpin many aspects of the SQWV quality assurance schemes. Best Practice Guides are produced in partnership with a steering group comprising of SNH, ADMG, British Association for Shooting and Conservation (BASC), British Deer Society (BDS), Scottish Forestry, LANTRA (the National Training Organisation for the Land Based Industries), and the Scottish Gamekeepers Association (SGA)[14]. These guides cover all stages of wild venison production from cull to processing within larders (see section 2.3.4 for more details).

Deer farming industry

As at 2018 there were 97 registered deer farms in Scotland farming only red deer. Farmed deer are usually killed at less than 27 months of age (Scottish Venison[15]). This is in contrast to wild deer which are culled at different ages from <1 year old through to adult (≥ 2 years old) (based on the mean weights supplied by Forestry and Land Scotland for each season from 2007-08 to 2015-16).

Approved Game Handling Establishments (AGHEs)

AGHEs process carcasses from both wild and farmed deer, with the exception of wild venison produced by small scale wild game processors which are exempt from the specific requirements relating to wild venison set out in food hygiene regulations (see section 2.3.2). As of November 2019, there are 11 AGHEs in Scotland which can process deer carcasses[16].

Scottish Venison Association

The Scottish Venison Association (SVA)[17], formerly the Scottish Venison Partnership, is a pan-sector group that promotes wild and farmed Scottish venison and represents its producers. SVA is a not-for-profit organisation currently funded by a levy paid by producers of wild venison from all deer species processed through two SQWV assured

[12] http://www.deer-management.co.uk/dmgs
[13] http://www.sqwv.co.uk/
[14] http://www.bestpracticeguides.org.uk/
[15] http://www.scottish-venison.info/index.php?page=Deer-farming-in-Scotland
[16] http://www.foodstandards.gov.scot/ publications-and-research/approved-premises-register, Appendix 5
[17] http://scottish-venison.info/

game dealers/processors. SVA also receives an annual grant from Forestry and Land Scotland.

In addition to promoting Scottish venison as a product, the SVA is involved in promoting Best Practice for wild venison production, through training events and educational videos and other projects such as the application for Protected Geographical Indication (PGI) status for Scottish Wild Venison[18].

2.2. Recent trends in UK venison consumption

UK consumption of venison could rise from ~3800 to more than 5000 tonnes by 2021, based on SVA estimates of around a 10% increase year on year from 2017-2019. However, the UK market is reliant on imports, especially from New Zealand, and volumes of imports have been contracting over the past two years. Market analyst Mintel reported an increase in UK game meat sales from £98 to £106 million from 2015 to 2016, with game meat sales forecast to hit £143 million by 2020. Sainsbury's has reported an increase of 115% in venison sales from 2014 to 2016[19]. While there is a lack of primary data, it appears that venison consumption would increase in the short to medium term subject to available product, albeit this may stall if there is a reduction in imported volume. The effect of Exit from the EU on the UK venison export market may also be a factor with regard to future growth.

2.3. Wild venison production in Scotland from cull to end product

2.3.1 Legislation relating to the wild venison industry in Scotland

Food safety legislation relating to the production of wild venison in Scotland is summarised below.

- Regulation (EC) 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, including establishing traceability of food producing animals.
- Regulation (EC) No. 852/2004 – the hygiene of foodstuffs.
- Regulation (EC) No. 853/2004 – specific hygiene rules for food of animal origin.
- Regulation (EC) No. 2017/625 – specific rules for the organisation of official controls on products of animal origin intended for human consumption.

The hygiene regulations are implemented in Scotland by the Food Hygiene (Scotland) Regulation 2006 (SSI 2006/3) (as amended).

[18] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/775607/pfn-scottish-wild-venison-spec.pdf
[19] https://meatmanagement.com/meat-industry-enjoys-a-successful-festive-season/

These regulations apply to any hunter, estate, middleman, or transporters supplying venison to an AGHE. These operators must do the following:

- Register the business with the Environmental Health department of their Local Authority.
- Comply with traceability requirements of Regulation (EC) No. 178/2002.
- Exercise appropriate temperature control, hygienic transport and preparation, in line with Regulation (EC) No. 852/2004.
- Ensure the game is examined by a trained person who completes a hunter declaration form (Appendix 6). This declaration provides details of the type of deer culled, the cull location, and a declaration in accordance with Regulation (EC) No. 853/2004 that no abnormal behaviour was observed before killing, and that the carcass exhibited no gross abnormalities or evidence of environmental contamination.

2.3.2 Exemptions in hygiene regulations

Importantly, a number of exemptions to certain elements of the regulations exist for small scale wild game operators, such that game carcasses or venison produced from the cull **may not pass through AGHEs**. These exempt categories of operations are required in EU regulation instead to be covered by national law to ensure that public health is protected.

There are a number of exemptions from the more detailed requirements set out in regulation 853/2004 and these include exemptions for:

(a) Primary production for private domestic use (and domestic preparation for private consumption)

(b) The direct supply by hunters who supply small quantities of b(i) wild game, or b(ii) wild game meat directly to the final consumer or to local retail establishments directly supplying the final consumer. Definitions of 'local' and 'small quantities' can be found in the FSS/FSA Wild Game guide (revision 2018)[20].

For exemption (a) the food hygiene regulations do not apply. For exemption b(i), producers are not subject to the requirements of the EU Food Hygiene Regulations and are therefore not required to follow a HACCP plan. For exemption b(ii) (hunters supplying wild game meat), whilst exempt from regulation 853/2004 the full requirements of regulation 852/2004 apply, including HACCP requirement. All retailers are required to comply with traceability requirements of Regulation (EC) No. 178/2002. Only retailers in exemption b(ii) are required to register with the Environmental Health department of their Local Authority and have a HACCP plan in place, exercise temperature control, hygienic transport and preparation, in line with Regulation (EC) No. 852/2004.

[20] https://www.foodstandards.gov.scot/downloads/The_Wild_Game_Guide_1.pdf

In order for retailers to be eligible for exemptions, venison sold has to be local, the quantities of venison need to be 'small'. The definitions of 'small quantities' and 'local' are set out in FSS guidance[21]. However it should be noted that anyone placing any food on the market must ensure that it is safe in accordance with the requirements set out in the Food Safety Act 1990[22].

As a result of these exemptions, knowledge of wild venison production and traceability of venison carcasses will be incomplete.

2.3.3 Current recording of the wild venison cull and sales

Statutory annual cull returns

All landowners and occupiers asked to do so must provide information about all of the deer they have shot that year to Scottish Natural Heritage (SNH) under the Deer (Scotland) Act 1996[23]. The annual cull return should record for each individual landholding the number and sex of each species of deer culled from 1 April to 31 March.

Venison dealer returns

To sell venison, you must be registered as a venison dealer and hold a venison dealer licence (VDL) from your local authority[24]. Only wholesalers are required to be licensed; there is no need for every butcher and retailer selling venison in Scotland to be licensed so long as the venison on sale has been purchased from a licensed dealer so that at least one component of the food chain holds a VDL.

Registered venison dealers should submit venison dealer returns to SNH for all purchases and sales of venison made during the year from 1 April to 31 March, although currently there is no statutory requirement to send in these records.

Records of transactions involving venison must contain:

- when the venison was bought or received
- name and address of the person who supplied the venison
- number of carcases, their sex and if possible deer species

Dealers are required to:

- Keep records of each transaction and any relevant documents (e.g. receipts, invoices) for 3 years
- present records for inspection by SNH, a police officer or anyone authorised in writing by the council on request
- allow SNH or a police officer to make copies of your records

[21] https://www.foodstandards.gov.scot/downloads/The_Wild_Game_Guide_1.pdf
[22] http://www.legislation.gov.uk/ukpga/1990/16/contents?view=plain
[23] http://www.legislation.gov.uk/ukpga/1996/58/section/15
[24] https://www.gov.uk/venison-dealer-licence-scotland

Prior to 2010, the Deer Commission for Scotland (DCS) actively requested annual returns from venison dealers. However, in August 2010 the functions of the DCS were transferred to SNH under section 1 of the Public Services (Reform) (Scotland) Act 2010 and the Commission was dissolved. SNH does not actively request annual returns from dealers, and therefore data on venison dealer returns from 2010 onwards is incomplete.

Estimated shortfall in venison dealer returns

The difference between the annual cull returns and venison dealer returns, if accurately recorded, should provide an estimate of the number of carcasses which are exempt from the regulations set out in section 2.3.2, i.e. carcasses which are either privately consumed or supplied to local retailers in small quantities.

From DCS data from 1999 to 2009 shown in Table 2.2, there was a shortfall in cull vs. venison returns of up to 37.8% and 30.0% for Red and Roe deer, respectively. During this same time-period, an average short fall in cull vs. venison returns of 38% for Sika and Fallow deer (Scottish Venison, an industry review 2010).

As SNH, which took over responsibility for collating deer returns in 2010, do not actively pursue venison dealer returns, there is currently limited information on the shortfall in cull vs. venison returns from 2010 onwards. **Consequently, we have little current understanding of how much wild venison consumed in Scotland originates from small scale wild game operators who are exempt from venison specific requirements in food hygiene legislation**.

Table 2.2 *Shortfall in cull vs. venison returns for Red and Roe deer from 1999-2009 – adapted from Scottish Venison, an industry review 2010*

Year	% shortfall in cull vs. venison returns for Red deer	% shortfall in cull vs. venison returns for Roe deer
1999-2000	12.5	4.0
2000-2001	18.6	11.0
2001-2002	13.0	16.0
2002-2003	19.0	7.8
2003-2004	30.0	21.0
2004-2005	21.7	20.0
2005-2006	37.8	32.0
2006-2007	25.9	27.0
2007-2008	36.2	30.0
2008-2009	31.7	15.0

2.3.4 Overview of venison production in Scotland

Wild venison production

Wild deer lawfully entering the human food chain are killed by a free bullet. Hunters (individuals who shoot wild game) supplying wild venison to AGHEs (i.e. those not operating under exemptions outlined in section 2.3.2) need to be qualified to shoot deer (i.e. trained hunters) or be supervised by a qualified person (see section 4.2.1 for more details. Qualification requires knowledge in the practice of stalking and deer ecology. Competence can be demonstrated by the attainment of Deer Stalking Certificates 1 and 2 from DMQ Ltd, the British Association for Shooting and Conservation (BASC), the British Deer Society (BDS). Further qualification in Game Meat Hygiene is provided by Scottish Qualifications Authority (SQA).

Once shot, the deer is exsanguinated (bled) and the abdominal cavity opened and abdominal viscera (stomach and intestines) removed (gralloching) (more details are provided in section 4.3.2.) At this point, an initial inspection of the carcass is made and a trained hunter's declaration is completed (Appendix 6). The carcass is then transported to a larder, either by pony, an all-terrain vehicle (ATV), or carried in the case of smaller roe deer. If the deer is shot it may need to be dragged (ideally in a 'drag-bag') to a more accessible point before loading onto an ATV or a pony. Larders can be either co-located at AGHEs or separate to AGHEs, in which case the larder has to be registered with the local authority where hygiene controls are enforced by Environmental Health Officers. Larders should allow carcass temperature to be brought down to and maintained at below 7ºC throughout the meat as quickly as possible, with a suggested temperature range of between 1-3ºC.

At the larder, the sternum is split to enable the removal of the 'red pluck' (lungs, heart and liver), and the head and feet removed. The carcass is then refrigerated until it is transported to the AGHE. Deer culled by Forestry & Land Scotland are currently all processed at one AGHE (Highland Game Ltd).

At AGHEs, trained hunter declarations are checked and stamped with a health mark. The carcass is then skinned, dressed (by trimming dry or visibly contaminated areas) and transferred to a chiller where only skinned carcasses are held. Final processing and packaging of the carcass is then performed. Further information on carcass processing at AGHEs is provided in section 4.6 of this report.

Quality assurance scheme for wild venison

Voluntary quality assurance schemes are owned and run by SQWV Ltd[25] and cover aspects of wild venison production from the point of cull to final product[26]. Two schemes exist:

[25] http://www.sqwv.co.uk/
[26] https://services.acoura.com/media/doc_65738073/SQWV%20Producer%20Standards%20-%20Issue%2010%20%2005%20May%202017%20V5.pdf?pbc=1010947

- *The SQWV Stalking and Carcass Handling Assurance Scheme.* This covers all the stages between wild deer being shot through to the storage of skin on carcases that have been gralloched and are awaiting collection by the game dealer or processor.
- *SQWV Primary Processor scheme*. This is for AGHEs being used for the skinning of game and cutting of carcases into meat. Game Dealers and Game Processing Plants are also assured under the *Primary Processor scheme*.

Both schemes require regular inspections by qualified independent inspectors (currently Acoura[27]). Members of the *Stalking and Carcass Handling Scheme* are currently inspected at intervals between 12 and 18 months. Members of the *Primary Processing Scheme* are inspected between 6 and 18 months.

SQWV schemes cover the following specific areas of wild venison production:

- Deer management and control
- Stalking proficiency
- Larder management
- Carcass inspection
- Processing (transport, dressing, cutting, packaging and labelling) of wild venison
- Product specification
- Hygiene standards
- Traceability

Importantly, as part of the *SQWV Stalking and Carcass Handling Assurance Scheme* carcasses are labelled at the larder with SQVW labels. These labels include information on the hunter, the cull site, the larder address and the date and time of the cull, thus allowing accurate traceability of the carcasses from point of cull to end product.

All carcasses from FLS shot deer, which culls approximately one third of the total number of wild deer culled in Scotland, are covered by these quality assurance schemes. It is not known what proportion of wild deer not culled by FLS adheres to SQWV quality assurance schemes.

Best Practice Guides for wild venison production

Best practice guides[28] underpin many aspects of the SQWV Quality Assurance schemes. In addition to providing clarity on the law relating to wild venison production, the guides have three central aims: safeguarding public safety; ensuring food safety; and taking full account of deer welfare. In relation to venison production, the guides cover processes from the point of cull to venison supply, including culling, gralloching,

[27] https://www.acoura.com/
[28] https://www.bestpracticeguides.org.uk/guides/

extraction to larders, carcass inspection, hygiene principles, processing with larders, and supply of venison to consumers, retailers or venison dealers.

2.4. Farmed venison production

Unlike other farmed livestock it is permissible to shoot farmed deer as they graze in the field rather than transport them by road to an abattoir for slaughter, although supermarkets generally purchase farmed venison from abattoir-killed carcasses. There is no legal requirement to identify farmed deer until they are transported, at which point they must be ear tagged. Deer movements in Scotland are recorded on Animal Transport Certificates. Deer hauliers are controlled in that journeys over 65 km require authorisation of the transporter under Council Regulation (EC) No. 1/2005.

Farmed deer can only be killed by a person in possession of a Certificate of Competence under the Welfare of Animals at the Time of Killing (Scotland) Regulations 2012. Unlike wild deer, all farmed deer must be examined *ante-mortem* by a veterinary surgeon within the 72 hours preceding slaughter in accordance with Regulation (EC) 853/2004. This is a clinical inspection in which the veterinary surgeon certifies that in their opinion the animal was not affected with any disease or condition liable to render the whole carcass unfit for human consumption or that could be transmitted through the meat to humans. All farmed deer carcases must also be processed through AGHEs. The carcases must also be inspected post-mortem by a meat inspector and stamped with a health mark as required under the Food (Meat Inspection) (Scotland) Regulations 1988. The health mark identifies local authority and, if required by the local authority, the inspector and/or the slaughterhouse where the inspection was carried out.

Abattoirs handling farmed venison in Scotland are detailed in Appendix 7. There is now one dedicated abattoir for killing farmed deer which was opened in Fife in 2016 (Downfield Limited[29]). A significant proportion of farmed deer produced in Scotland is slaughtered at Dovecote Park Ltd. in West Yorkshire[30].

2.5. Food safety issues relating to venison

2.5.1 Food safety weaknesses in the venison production chain

With correct recording and tagging of deer carcasses (e.g. through adherence to SQWV Stalking and Carcass Handling Assurance Scheme), it should be possible to trace any disease outbreak occurring from venison back to the retailer and place of origin of the deer carcass. This information would not be available for carcasses which are either privately consumed or supplied to local retailers in small quantities as these are exempt

[29] https://stagison.com/about/
[30] http://www.dovecotepark.com/default.aspx

from the regulations described in section 2.3.2. However, in these circumstances there should be a relatively short chain from consumer to retailer and hunter.

2.5.2 Potential impact of imports on food safety

Given the potential increase in venison imports into the UK (potentially accounting for 50% of UK venison consumption by 2021[31]), consideration should be given to how this will affect the risk of zoonotic infections arising from consuming venison. As detailed in section 2.1.1, most venison is imported from deer farms in New Zealand, with other imports from European countries. Zoonotic pathogens which have been identified in deer populations from countries exporting deer to the UK include: *Mycobacterium bovis* and other zoonotic mycobacterial species in deer from New Zealand (Buddle *et al*., 2015), the Iberian Peninsula and the Alpine range (Gortazar 2015); Hepatitis E virus from deer in Italy, Spain, The Netherlands, Belgium and Germany (Di Bartolo *et al.*, 2017; Clemente-Casares *et al.*, 2016) and Group A rotavirus in Slovenia, Jamnikar-Ciglenecki *et al.*, 2017).

The relative prevalence of these zoonotic pathogens in UK deer populations compared to deer populations from countries exporting venison to the UK is largely unknown, with the exception of *Mycobacterium bovis* which is not currently thought to be present in Scotland, but is present in farmed deer in New Zealand and in cattle bovine tuberculosis hot-spots in England (e.g. Johnson *et al.,* 2008). The impact of changes in venison imports on zoonotic disease risk is therefore unclear.

2.5.3 Consumption of raw/partially cooked venison

A number of zoonotic pathogens may be present in venison products, either through surface contamination of the meat (e.g. Shiga toxin-producing *E. coli*, Hepatitis E virus), or being present within the meat as tissue cysts (e.g. *Toxoplasma gondii* cysts, Hepatitis E virus). As venison is sometimes consumed raw (e.g. as carpaccio), undercooked or rare, there is the potential for zoonotic infection with these pathogens.

2.5.4 Impact of current cooking guidelines on zoonotic pathogen risk

Adhering to cooking guidelines provided by FSS[32] should largely mitigate these risks. These guidelines suggest minced meat products should reach a core temperature of 75˚C which should be sufficient to kill the pathogens. However, current guidelines indicate that meats such as steaks and joints can be served rare as long as the outside has been seared. This would be sufficient for most pathogens, with the exception of *T. gondii* cysts within the meat which would still be viable unless the joint was frozen prior to cooking. Freezing at -10˚C for 3 days or -20˚C for 2 days is sufficient to inactivate *T. gondii* cysts in meat from experimentally infected sheep (El-Nawawi *et al.*, 2008) but similar data is not available for venison.

[31] https://meatmanagement.com/venison-imports-could-double-by-2020/
[32] https://www.foodstandards.gov.scot/consumers/food-safety/at-home/cooking-food

2.6. Review limitations

Venison production estimates, which were calculated from Forestry Land and Scotland data, are compromised by inaccuracies as (i) cull data may not be accurate; (ii) average carcass weights used to estimate production are dead weights and not processed carcasses, leading to overestimation of production from wild Scottish deer. Furthermore, average carcass weights in this report were calculated from of all ages of deer, leading to potential underestimation of production from deer culled by non-Forestry Land and Scotland stalkers, which usually avoids culling younger, smaller deer. Uncertainties in the volume of wild venison production could compromise assessment of the risk of zoonotic disease following venison consumption if, for example, certain pathogens were more prevalent in wild vs. farmed deer.

There is limited primary data on venison consumption and quantities of imported venison into the UK. This makes assessment of trends in venison consumption, and the risk that imported venison poses to human health from countries in which zoonotic pathogens are present within deer populations.

Cull and venison return data relies on third party recording and is therefore prone to underreporting through, for instance, non-submission of returns or inaccurate returns or records. This means that it is hard to assess what proportion of deer carcasses are processed under exemptions and therefore are less traceable in the event of a foodborne disease outbreak.

3. Objective 2: A field survey to assess STEC prevalence in wild deer in Scotland

Summary

The aim of this objective was to provide information on the prevalence of STEC O157 and other STEC serotypes in Scottish wild deer destined for human consumption.

Faecal samples were collected from individual deer carcasses over a 12-month period between July 2017 and June 2018 by deer managers and the Forest Enterprise Scotland (now Forestry and Land Scotland) and assessed for presence of STEC O157 and other STEC serotypes. Levels of STEC O157 bacteria present in positive faecal samples were determined in order to identify any super-shedding deer.

Whole genome sequencing was performed on STEC O157 strains isolated from the deer faecal samples in order to compare these strains with STEC O157 strains isolated from UK human STEC cases in order to estimate their human pathogenic potential. Deer STEC O157 strains were also compared with strains isolated from UK cattle populations to determine if STEC O157 strains are likely to circulate between cattle and deer populations, as well as the STEC O157 strain isolated from the 2015 human outbreak.

Out of a total of 1087 faecal samples analysed three were positive for STEC O157. The mean estimated prevalence of STEC O157 in wild Scottish deer was 0.34% (95% Confidence Intervals = 0.02 – 6.30). Faecal samples from the three positive deer contained high levels of STEC O157 (>10^4 CFU/g faeces).

Sequence analysis indicated that all three deer STEC O157 strains were *stx2a* and *eae* positive (a known virulence factor in humans), as was the 2015 human outbreak strain. This virulence profile is associated with more severe human disease. Two of the deer STEC O157 strains were phage-type (PT) 21/28 which is also associated with more severe human disease, and both were isolated from Red deer. The third isolate was a PT8 strain from a Sika deer. Whole genome sequence comparisons of the deer strains with cattle strains and other human outbreak strains demonstrated a close genetic relationship between the deer strains and human and cattle STEC O157 isolates.

To investigate the prevalence of non-O157 STEC, all faecal samples were screened for the presence of *stx1* and *stx2* genes. A high proportion of faecal samples (69.4%) had detectable *stx1* and/or *stx2* genes, of which 25.5% were also positive for *eae*. However, this did not mean that *stx* and *eae* genes were present in the same bacteria, and therefore isolation and characterisation of STEC strains from a selection of these faecal samples was performed. Isolation of STEC strains from *stx*2+/*eae*+ faecal samples (which are present in STEC strains associated with the most severe forms of human disease) was performed on 74 samples. From these samples 68 STEC strains were isolated which were all *stx2* positive but *eae* negative.

Conclusions

These results indicate that prevalence of STEC O157 in wild Scottish deer was low. However, the levels of bacteria in positive faecal samples were high and of strain types associated with more severe human disease. Furthermore, *stx* genes were found in a high proportion of deer faeces tested, with 68 non-O157 STEC strains isolated from a subset (74) of the faecal samples representing a diverse deer species and geographical range.

It is concluded that there is a real risk of future human infections arising from consumption of venison contaminated with deer faeces. Therefore, strict hygiene precautions should be taken when processing deer carcasses to avoid faecal contamination of the carcass in order to mitigate this risk.

3.1 Introduction

Following the outbreak of human STEC O157 infections in 2015 linked to consumption of venison products, it was concluded that the likely source of infection was from a wild deer carcass heavily contaminated with STEC O157[33]. This outbreak identified knowledge gaps relating to the risk of human STEC infection associated with wild venison consumption. These gaps included (i) a lack of information relating to the prevalence of STEC O157 and other STEC serotypes in Scottish deer populations; and (ii) no information on whether deer, like cattle, can be colonised with STEC at high levels (so called 'super-shedders' (Matthews *et al.*2006). Such information would be critical to determine the potential risk of future human STEC infections as a result of consumption and/or handling of wild venison products.

The primary aim of this Objective was to provide an accurate estimate of STEC O157 prevalence in Scottish wild deer destined for the human food chain, including levels of STEC O157 in deer faeces, the presence of virulence genes such as *eae* (*E. coli* attachment and effacement gene) which encodes the adhesion factor intimin and is associated with more severe human disease outcomes (Brandal *et al*., 2015), and the relatedness of deer STEC O157 strains with those found in UK human infections and British cattle. A secondary aim was to investigate the presence of non-O157 STEC in Scottish wild deer, as currently around 24-41% of human STEC infections in Scotland are caused by non-O157 STEC (HPS STEC in Scotland 2018: Enhanced Surveillance and Reference Laboratory Data[34])

[33] http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=2987
[34] https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2847/documents/1_stec-scotland-2018.pdf

3.2 Materials and methods

3.2.1 Study design

Sample size for the study was estimated using the following formula:

$n = (z^2 \times p(1-p))/d^2$
where:

n= sample size
z=1.96 (z statistic for 95% level of Confidence)
p=prevalence estimate (here *p*=0.01)
d= precision, estimated as *p*/2 (here *d*=0.005)

A sample size of 1521 was estimated to have a 95% chance of estimating the true prevalence of the STEC O157 in Scottish Wild deer.

A sampling plan was designed to be representative of culling for a year in Scotland. In order to do this 10 years of historical data was obtained from Forestry and Land Scotland (FLS). The data represented the number of deer culled per year by species through FLS larders within Scotland. Over the 10 year time frame a mean (95% CI) of 24,674 (min, 19,059; max, 29,511) deer were culled. As the data was consistent across years, 2016/2017 was used to create the sampling design. Based on 2016/2017 the distribution of deer culled is shown in Figure 3.1 by month and in Table 3.1 by month and species. Peak culling occurred in October/November but remain relatively high throughout the winter. Approximately 52% of the deer culled were Roe deer, 38% Red deer, 9% Sika deer and less than 2% from Fallow deer.

A total of 1888 sample packs containing sterile gloves and sample pots were distributed to stalkers recruited by Deer Management Groups (DMGs) and FLS. Faecal samples from deer were collected directly from the rectum by stalkers at the time of culling using a sterile glove and transported in a sterile plastic 50 mL pot to MRI. A sampling plan was designed (see Appendix 8) to ensure a representative sample was obtained from the wild deer population, stratified for time of year, location, deer species, and sampling by either DMGs or FLS. Stalkers also completed a questionnaire form at the time of sampling detailing the location (UK National Grid Map Reference), number of the cull site, species and estimated age of the deer culled and evidence of co-grazing with other herbivores based on location of the cull site (Appendix 9).

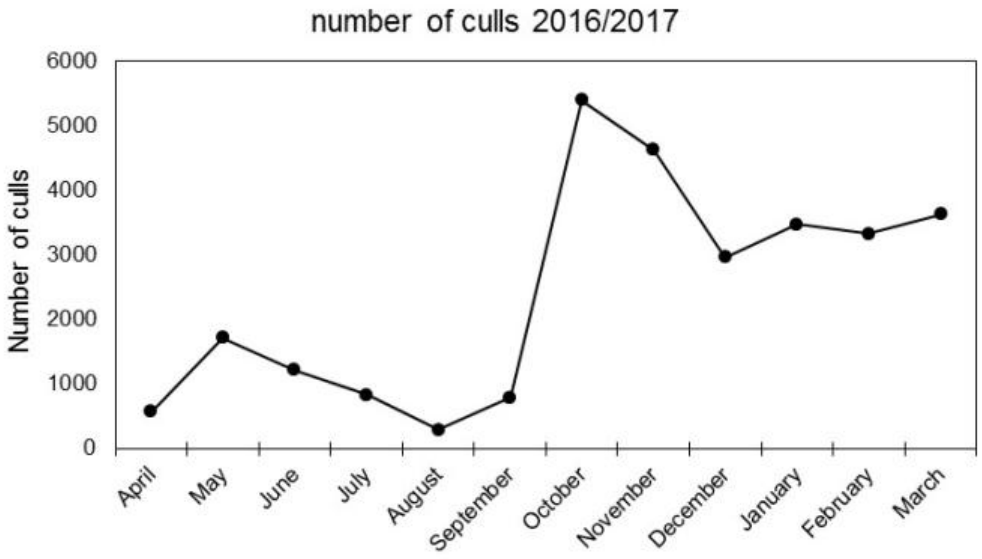


Figure 3.1 *Total number of all deer species culled by FES by month in 2016/2017 season*

Table 3.1 *Number of deer culled by FLS in 2016/2017 by month and species*

Month	Overall	Fallow Deer	Red Deer	Red/Sika Deer (Hybrid)*	Roe Deer	Sika Deer
April	567	1	194	0	333	39
May	1713	10	559	1	1051	92
June	1217	9	358	2	761	87
July	832	3	322	0	437	70
August	291	1	96	0	179	15
September	793	3	384	0	142	264
October	5403	72	2416	3	2432	480
November	4635	98	1883	1	2259	394
December	2965	57	925	1	1729	253
January	3476	64	1288	1	1873	250
February	3324	66	1248	0	1734	276
March	3634	77	1164	0	2043	350

*reported by the stalker based on deer appearance.

3.2.2 Laboratory methods

E. coli O157 were isolated from 1g of deer faecal samples by immuno-magnetic separation as previously described (Pearce *et al.,* 2004), with positive colonies confirmed by latex agglutination using an *E. coli* O157 Latex Test Kit. The number of STEC O157 in the faeces from positive samples was enumerated by culturing 10-fold

dilutions of faecal samples in PBS overnight in duplicate on CT-SMAC agar plates. Counts were expressed as colony forming units per gram of faeces (CFU/g) as previously described (McNeilly *et al.*2015) and the limit of detection was 50 CFU/g. *E. coli* O157 isolates were subjected to quantitative PCR (qPCR) analysis for the presence of *stx1, stx2* and *eae* using a method developed by the Scottish *Escherichia coli* O157/STEC Reference Laboratory (SERL).

STEC O157 isolates identified in deer faeces, plus the STEC O157 strain (SME-19-101) associated with the human disease outbreak in 2015, were subject to phage typing and whole genome sequencing (WGS) analysis by SERL on an Illumina MiSeq sequencer and using a bioinformatics pipeline recently developed by Public Health England (PHE) (Holmes *et al.*, 2018). The outputs from this analysis included serotype (O:H type), phage type (PT) and virulence gene profile (*stx* subtype, *eae*, *bfpA*, *aggR*, *ipaH* type, *aaiC*, *ltcA*, *sta1* and *stb*). Coverage of *E. coli* genome sequences ranged from 104× to 181×, which was adequate for downstream analysis (Grimstrup Joensen *et al.*, 2014).

Phylogenetic analysis of core genome sequence data was also performed as described in Mainda *et al*., (2016) to determine the relationship between deer STEC O157 isolates and those identified in UK human clinical cases and British cattle destined for the human food chain (Pearce *et al*., 2009, Henry *et al.,* 2017). Single Nucleotide Polymorphisms (SNPs) within the core sequences were identified and aligned to the reference genome *E. coli* O157 strain Sakai (ref number: GCF_000008865.2). The phylogenetic tree was constructed using FastTree software (Price et al., 2010) and visualised with ITOL (Letunic *et al*., 2007).

To provide information on the potential prevalence of non-O157 STEC, DNA extracted from broth enriched faecal samples were tested for the presence of *stx*1, *stx*2 and *eae* using a multiplex PCR (Bai *et al*., 2010). A PCR for the generic-*E.coli* gene *uid* (Heininger *et al.,* 1999) was performed on each sample as a positive control for the DNA extraction. Isolation of STEC was attempted on selected samples by plating serial dilutions of the broth enrichments onto SMAC agar plates and screening positive colonies for *stx* and *eae* genes. Samples were selected based on the presence of both *stx2* and *eae* genes, and a high level of *stx2* genes (CT value <30) by the SERL quantitative real-time PCR (Holmes et al., 2018).

3.2.3 Prevalence estimates of STEC O157 in Scottish wild deer

The mean number of deer positive for *E. coli* O157 was estimated using Generalised Linear Mixed Models (GLMMs) with binomial response terms and a logit link function using Proc Glimmix in SAS version 9.4 (SAS Institute, Cary, NC, USA). Species was included as a random effect. The Excel 2016 package (Microsoft Corporation) was used to implement a Latin hypercube sampling algorithm to convert results from the GLMMs into prevalence, taking into account the influence of random effects (Condon et al., 2004). A similar method was used to calculate the prevalence of cattle O157 (Chase-Topping et al., 2007; Pearce et al., 2009).

3.3. Results

3.3.1 Deer sampling

Of the 1888 sample packs distributed to DMGs and FES a total of 1087 samples were received, representing a return rate of 58%. The first sample was received on 31st August 2017 and the last sample collected on 21st June 2018. The distribution of deer species sampled for the survey was similar to that culled in 2016/2017 by FES although proportionally more red deer were sampled (46.0% versus 37.6%, Table 3.2). The number of samples received per month is shown in Figures 3.2 and 3.3. The distribution of samples by cull site and species is shown in Figure 3.4 which demonstrates a good distribution between cull site location and deer species.

Table 3.2 *Comparison of deer species sampled in this study with proportion culled by FES in 2016/2017*

Species	Deer species culled by FLS (2016/2017)		Deer species culled and sampled in this study (2017/2018)	
	N*	%	N*	%
Red	10,837	37.6	498	46.0
Roe	14,973	51.9	449	41.5
Fallow	461	1.6	115	1.9
Sika	2,570	8.9	21	10.6

*hybrid deer were reported in the historical data (n=9) but are not included in the table above

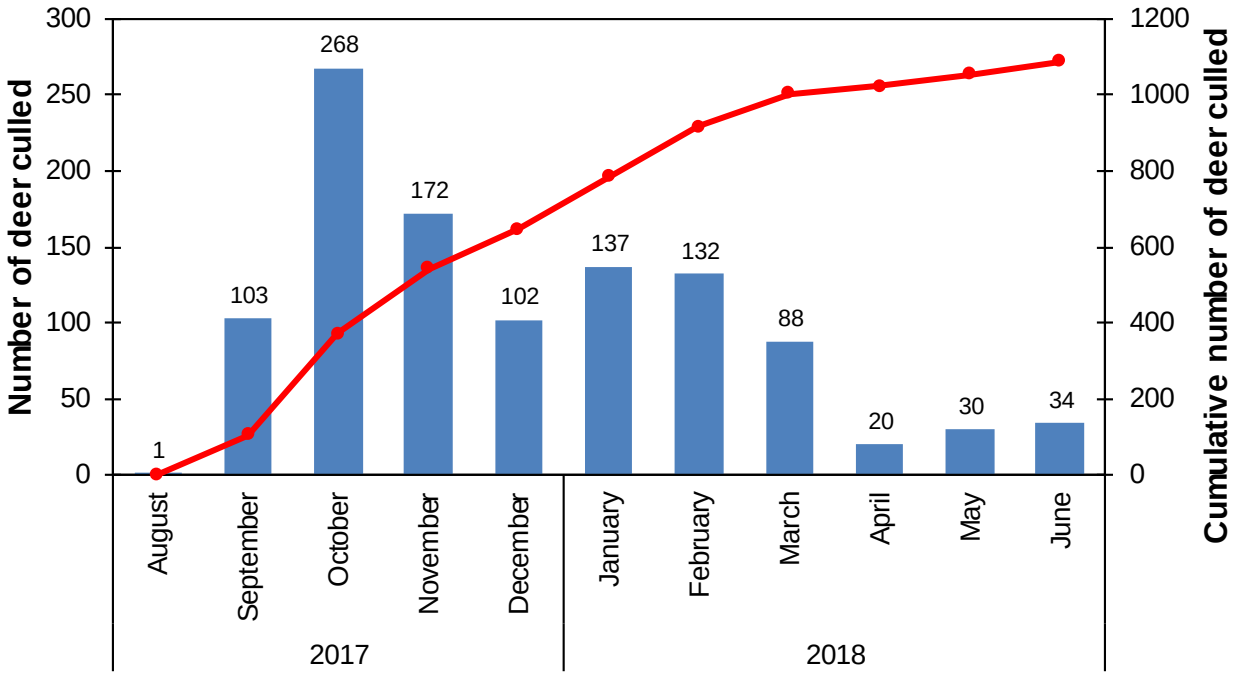


***Figure 3.2**: Details of sampling per month for STEC prevalence study. The total number of deer culled and sampled per month is indicated by the blue bars; the cumulative number of deer culled and sampled throughout the study period is indicated by the red line.*

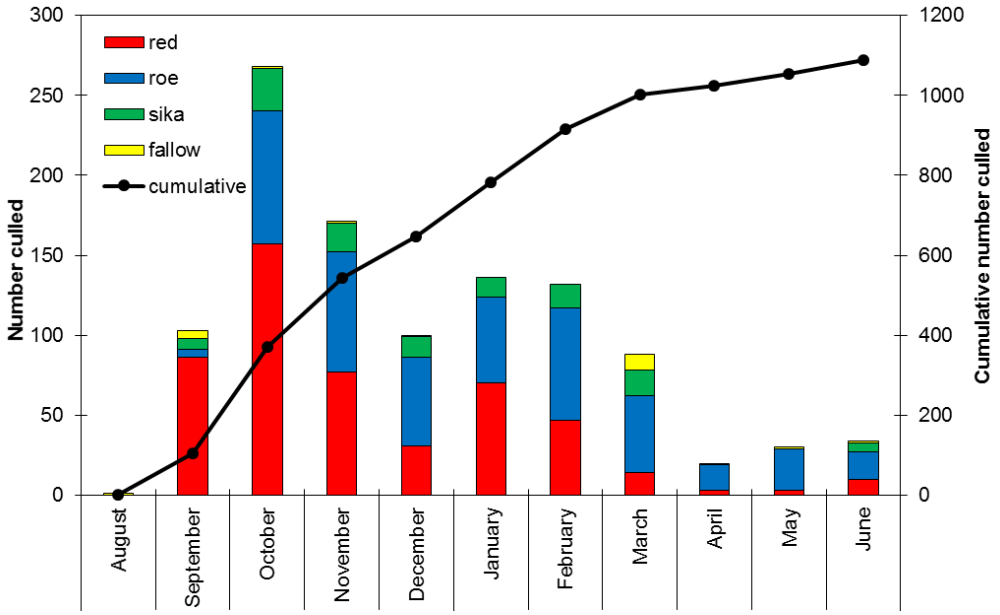


***Figure 3.3** Details of sampling per month including deer species for the STEC in venison prevalence study. The total number of deer culled including the proportion of each species culled and sampled per month is indicated by the bar chart; the cumulative number of deer culled and sampled is indicated by the black line.*

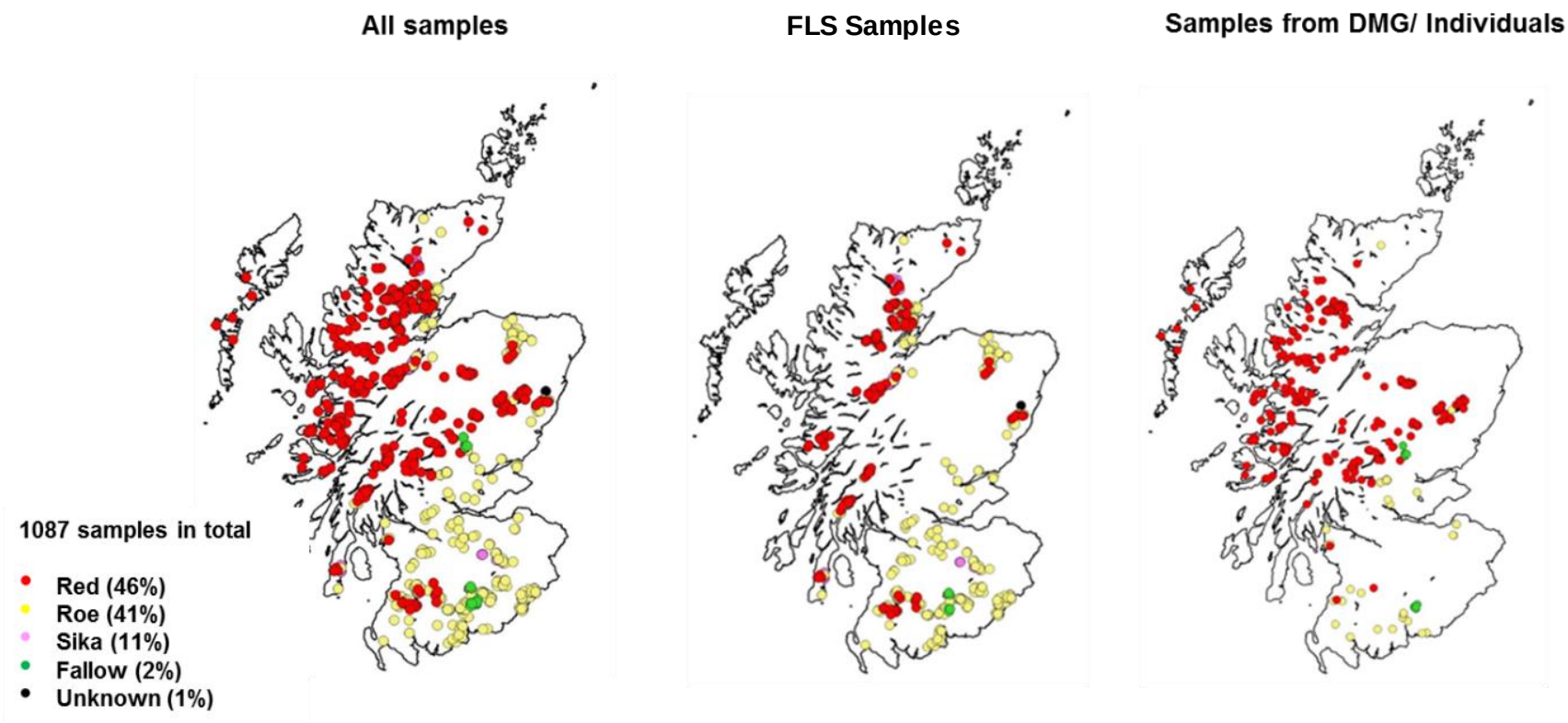


Figure 3.4 *Cull site location and species of deer sampled for the STEC prevalence study. FLS= Forestry and Land Scotland; DMG = deer management groups; individuals = not associated with a DMG or Forestry & Land Scotland; unknown = unknown deer species*

3.3.2 Prevalence of STEC O157 in Scottish wild deer

In total eight samples out of 1087 were positive for *E. coli* O157; however only 3 of the *E. coli* O157 isolates were *stx* positive. This represented an adjusted prevalence of STEC O157 in wild Scottish deer of 0.34% (CI = 0.02-6.30%) (Table 3.3).

Table 3.3 *Estimated prevalence of STEC O157 in wild Scottish deer in 2017/2018*

N	No. STEC O157	Mean prevalence	95% CI
1087	3	0.34%	0.02 - 6.30%

N = number of samples analysed
CI = Confidence Interval

3.3.3 Characterisation of STEC O157 strains isolated from Scottish wild deer

The three STEC O157 isolates identified in wild deer as well as the 2015 Scottish venison outbreak strain SME-19-101 were subjected to WGS analysis to obtain PT, H-type, *stx* subtype and selected virulence genes. These results, together with the species, age, location, co-grazing history, and faecal STEC O157 levels of positive deer are summarised in Table 3.4.

Location (by county) of the STEC O157 positive deer is also indicated in Figure 3.5. Two STEC O157 positive Red deer were from Inverness-shire and Ross & Cromarty, with the third positive deer being a Sika deer from Peebles-shire. Both red deer STEC O157 isolates were PT21/28, whereas the isolate from the Sika deer was PT8. All three isolates were positive for *stx*2a and one of the red deer isolates was also positive for *stx*2c. The isolates were all positive for *eae* but negative for other virulence factors tested. Only the PT21/28 isolate from the Red deer in the Highlands had a reported history of co-grazing land with other herbivores (in this case sheep and cattle). Faecal samples from all three positive deer contained high levels of STEC O157 (>10^4 CFU/g faeces). The 2015 venison outbreak strain SME-19-101 was PT32, and positive for *stx*2a, *stx*2c and *eae.* The H-type of all three deer isolates and strain SME-19-101 was H7.

Of the five *stx* negative *E. coli* O157 isolates, one was O157:H39, one was O157:H42 and two were O157:H43. Only the O157:H39 isolate was positive for *eae*. None of these isolates were genetically similar to any recognised pathogenic strains of *E. coli*.

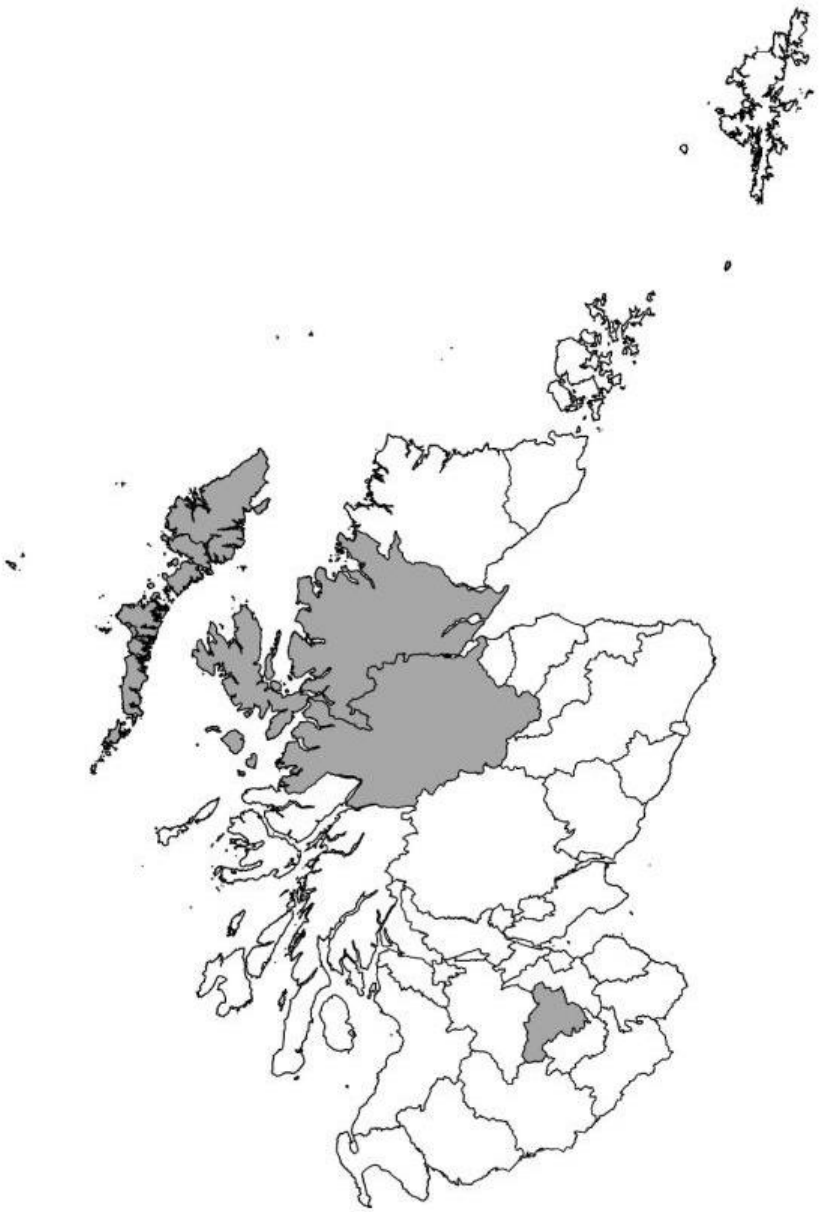


Figure 3.5 *Location of STEC O157 positive deer by county. Grey areas indicate districts where a single STEC O157 positive deer was identified.*
QGIS Development Team (2019). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org © Boundary Commission for Scotland, Local Government Boundary Commission for Scotland. Contains Ordnance Survey data © Crown copyright and database rights 2013.

***Table 3.4**: Details of STEC O157 strains isolated from wild Scottish deer in 2017/2018 and comparison with 2015 venison STEC O157 outbreak strain SME-19-101.*

Strain ID	County	Species	Sex	Age (years) *	Co-grazing history	PT	H-type	*stx* subtype	*eae*	Count (CFU/g faeces)
XH800737E	Inverness-shire	Red	F	4	None reported	21/28	7	*stx*2a / 2c	+	1.0×10^4
XH800739Y	Peebles-shire	Sika	F	5	None reported	8	7	*stx*2a	+	5.0×10^6
XH800740B	Ross & Cromarty	Red	M	1.5	Cattle/sheep	21/28	7	*stx*2a	+	7.7×10^7
SME-19-101	n/a	n/a	n/a	n/a	n/a	32	7	*stx*2a / 2c	+	n/a

ID = identity
*Age estimated by stalker
PT = phage type
n/a = not applicable

3. Objective 2: A field survey to assess STEC prevalence in wild deer in Scotland

To understand the relationship between the deer STEC O157 isolates identified in this study and UK human outbreak strains and isolates from British cattle, phylogenetic analysis was performed using core genome sequence data generated by next generation sequencing. The phylogenetic tree visualised with iTOL[35] is shown in Figure 3.6.

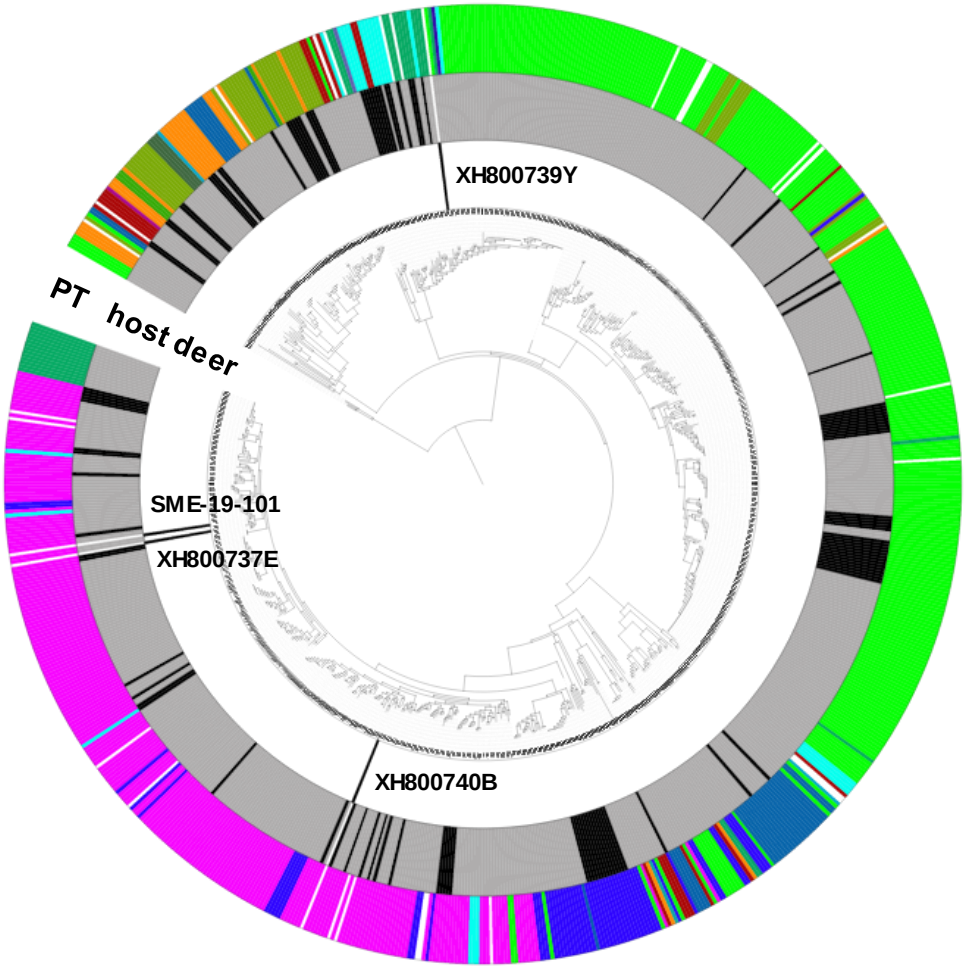


Figure 3.6 *Phylogenetic analysis of STEC O157 deer strains with UK human and bovine cattle STEC O157 strains using core genome sequence data. Outer ring = PT (Phage Type) with PT21/28 in pink, PT32 in light blue and PT8 in light green. Middle ring = host species with cattle in black and humans in grey. Inner ring = deer isolated from this study or from the 2015 venison deer outbreak. Identities of these strains are shown.*

[35] https://itol.embl.de/tree/8244209221382641561455797

The PT8 deer isolate (XH800739Y) was shown to be closely related to other PT8 isolates from UK human STEC O157 cases. PT21/28 deer isolate XH800737E was closely related to the 2015 venison deer outbreak strain SME-19-101 and both human and cattle PT21/28 isolates. PT21/28 deer isolate XH800740B was closely related to both human and cattle PT21/28 isolates but not to the other deer isolates.

3.3.4 Identification and genetic characterisation of non-O157 STEC strains isolated from Scottish wild deer

To investigate non-O157 STEC, a multiplex PCR was used to detect a generic *E. coli* gene (*uid*), *stx1*, *stx2* and *eae* within faecal broth enrichment samples. The *eae* gene encodes for Intimin, which is a key bacterial adherence factor associated with more severe human disease. In total 1082/1087 samples were tested. All samples were positive for *uid* indicating successful isolation of *E. coli* DNA. A high proportion of samples (751/1082 tested = 69.4%) were positive for *stx*1 and/or *stx*2 genes. Of these samples 192 were also positive for *eae*, representing 25.5% of the total *stx* positive samples. A summary of the multiplex PCR results is provided in Table 3.5.

Table 3.5 *PCR detection of stx1, stx2 and eae in wild Scottish deer faeces*

	***stx*1 +**	***stx*2 +**	***stx*1+ / *stx*2 +**	***stx*1- / *stx*2-**	**Total**
***eae* +**	30	93	69	70	262
***eae* -**	74	328	157	261	820
Total					1082

As the PCR testing was performed on broth enrichments containing multiple strains of *E. coli*, it was unknown whether *stx* and *eae* genes were present within the same bacteria. Isolation of STEC was therefore attempted on a sub-set of faecal samples based on the following criteria: firstly, samples were classified according to a priority list established by FAO/WHO[36] based on the human pathogenic potential of STEC strains. Samples in priority group 1 (i.e. those which may have contained the most pathogenic STEC strains) were then subjected to quantitative PCR in order to select only those with high levels of *stx2* genes (Ct < 30). This resulted in a total of 94 samples which were selected for STEC isolation. Sample selection is detailed in Table 3.6. The selected samples were from a wide geographical distribution and from all four deer species in Scotland.

From a total of 74 samples in priority group 1 selected for bacterial isolation, STEC were isolated from a total of 60 samples which were from a wide geographical range and from all Scottish deer species, representing a success rate of 81%. In 8 samples two different STEC strains were isolated from the same sample, meaning a total of

[36] http://www.fao.org/3/ca0032en/CA0032EN.pdf

68 STEC strains were isolated. All of the STEC isolates were *eae* negative. A total of 39 *eae* positive *E. coli* strains were also isolated which were *stx* negative. A summary of the *stx* profiles for the STEC isolates is shown in Table 3.7.

Table 3.6 *Selection of faecal samples for attempted STEC isolation*

Priority*	Broth enrichment PCR result	Number of samples	No. samples with *stx2* Ct <30
1	*stx*1+/*stx*2+/*eae*+ and *stx*2+/*eae*+	162	94
2	*stx*1+/*eae*+	30	n/a
3	*stx*1+/*stx*2+/*eae*-	157	n/a
4	*stx*2+/*eae*-	328	n/a
5	*stx*1+/*eae*-	74	n/a

*priority based on FAO/WHO report: Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring[37].
na = not applicable

Table 3.7 *Shiga toxin profiles of non-O157 STEC isolated from wild Scottish deer*

***stx/eae* profile of isolated STEC**	**Number of positives**
*stx*1+ only	2
*stx*2+ only	45
*stx*1+/*stx*2+	21

3.4 Conclusions

This study showed that the prevalence of STEC O157 in Scottish wild deer destined for the human food chainwas estimated to be low, with only three out of 1087 faecal samples being positive for the bacteria, representing an estimated mean prevalence of 0.0034 (0.34%), although it should be noted that there is a degree of uncertainty in the estimate meaning prevalence could be as high as 6.30%. Nevertheless, this is significantly lower than the prevalence in Scottish cattle entering the food chain, which was recently estimated in 2014/15 to be 0.106 (10.6%) (Henry *et al.,* 2017). This low prevalence is consistent with the hypothesis that unlike in cattle, STEC

[37] http://www.fao.org/3/ca0032en/CA0032EN.pdf

O157 may be unable to persist in wild deer populations, and represents 'spill-over' from cattle and/or other livestock. It was not possible to investigate the relationship between livestock and deer STEC O157 strains given the low number of deer strains identified in this study, although both PT21/28 strains were closely related to cattle strains suggesting that cross-species infections could occur between cattle and deer.

The three STEC O157 isolates were all positive for *stx*2a and *eae*, a gene profile which is associated with more severe forms of human disease such as bloody diarrhoea and HUS (Brandal *et al*., 2015; Byrne *et al.*, 2014,). Whole genome sequencing of the strains revealed a close association with strains associated with human infections and therefore these strains should be considered to have human pathogenic potential. The strains were also of two phage types, PT21/28 and PT8, which represent the two most frequently reported phage types associated with human STEC O157 infections in Scotland (HPS STEC in Scotland 2018: Enhanced Surveillance and Reference Laboratory Data[38]). Of the two phage types, PT21/28, which was isolated from two Red deer, has been previously shown to cause more severe human disease in Scottish children (Lynn *et al.,* 2005). Furthermore, all three strains were present at very high levels within the faeces (>10^4 CFU/g), which would increase the risk of carcass contamination to a level which could cause human infections. Therefore, strict hygiene precautions should be taken to avoid contamination of the carcass with faeces during the gralloching and down-stream processing of the carcass.

Finally, a significant proportion (69.4%) of deer faecal samples contained either *stx*1 or *stx*2 genes. Isolation of STEC was performed on a subset of positive samples and identified 68 non-O157 STEC strains. However, none of these strains were positive for *eae*, suggesting they may be less harmful to humans, although a significant proportion (~50%) of non-O157 STEC infections in the UK are caused by *eae* negative STEC, albeit with less severe clinical outcomes (Byrne *et al.,* 2014). Further work is required to determine the relationship between these non-O157 STEC strains isolated from deer and those which cause human clinical disease.

[38] https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2847/documents/1_stec-scotland-2018.pdf

4. Objective 3.1: Systematic review of existing literature relating to cross-contamination of venison.

Summary

The aim of this study was to conduct a systematic literature review in order to assess and identify the risk factors associated with large wild game meat contamination with Shiga toxin-producing *E. coli*, in relation to each of the steps undertaken in the production of venison in Scotland, from the hill to the end-product, ready for being sold on the market.

A systematic literature review of the scientific papers published over a period of 11 years was carried out. The selected interval (2006-2017) reflects the period from which new legislation on food hygiene entered into force.

Backwards citations of extracted publications were also carried to extend the collection of literature data to include older articles. The systematic review also included a review of the national and international guidelines available on safe production of wild venison. These concerned scientific opinions issued by official public health bodies. Finally, personal communication with stakeholders of the venison industry also took place for a better understanding of the practicalities of venison production. The risk factors that might be associated with general *E. coli* and moreover with STEC contamination of venison were extracted from each of these searches.

The results of the literature review show that under good handling procedures, meat obtained from well-dressed and processed deer carcases can present a microbial status that is safe for the consumer. Time of transfer to refrigeration facilities, storage conditions, and maintenance of the cold chain were found to be very important aspects to avoid microbiological contamination of the carcass. Unhygienic practices taking place during handling in the wild and transport to the larder may allow transfer of *E. coli* from the hide to the carcass. As *E. coli* continue to grow at temperatures as low as 7°C or survive below this limit, correct temperatures applied further down the food chain will not decrease microbial contamination of the carcass once it has been established.

This scientific literature review found limited information with regards to microbial quality of venison and deer carcasses in general and especially with regards to *E. coli* contamination and the proportion of the bacteria which carry the STEC virulence gene in these products.

Venison meat at retail was seen to harbour STEC serotypes with a prevalence ranging from 2.7% to 100%, largely depending on the processing steps the meat was subjected to and the geographical area and country investigated. The latest zoonoses report published by EFSA in 2016 showed that *E. coli* O157 was the most common STEC serotype found in human cases and food in the European Union. Cattle remain recognised as STEC reservoirs and bovine meat is considered to be a major source of food-borne infections. Deer meat is also recognised as a source of infection. A prevalence of 11.1 % (31 samples) was observed in deer, although the small sample size might skew this proportion. This literature review found that meat and meat

products from wild venison can carry a wide variety of other non-O157 STEC. Both *stx*1 and *stx*2 virulence genes have been found in retail meat.

The main risk factors identified from the literature associated with this microbial contamination essentially concern:

• The health status of the animal.
• Hygiene practices in the field from the time of killing to gralloching to transportation to the larder or AGHE.
• Maintenance of the cold chain from larder to final product.
• Handling and hygiene procedures involved in further skinning, cutting and processing.

Venison production involves a significant number of steps from shooting on the hill to the end-product. The primary processing of the carcasses is carried out near the point of despatch followed by transfer to the larder and/or to the AGHE, therefore large wild game handling requires complex operations carried out in different conditions before venison is produced. Guidelines of best hygiene practices for handling wild venison are available from each party involved, starting with hunters as primary food producers, larder operators, transporters and AGHEs. However, there is limited knowledge on how the different steps of the food chain might affect microbiological quality of wild deer meat in Scotland, with specific emphasis on STEC.

This literature review encountered a few limitations, as below:

• Lack of studies on both O157 and non-O157 STEC prevalence on deer hide.
• Very few studies of limited statistical design and representativeness were available, leading to great uncertainty on prevalence of *E. coli* O157 and non-O157 STEC in deer carcasses and venison.
• To the extent of this search, no STEC O157 targeted study has been carried out on large wild game carcasses and meat in the UK or in Scotland. However, the study carried out by (Syngh, 2006) on wild deer faeces found limited evidence of high STEC O157 shedding in Scotland .
• Lack of studies on identification of risk factors statistically associated with STEC contamination at various stages of wild game meat production along the food chain.
• The survivability characteristics of STEC serotypes of deer origin on surfaces, hands and equipment.

The importance of these data gaps on the evaluation of risk factors to STEC contamination from handling and processing of deer carcases should be considered when interpreting the findings of this literature review.

In conclusion, this study has found that peer review journals devoted more attention to identifying factors associated with hygiene procedures during the hill operations and on the implementation and maintenance of the cold chain but less data available with regards to practices that increase the risk of contamination during further handling at the larder and at AGHEs. Given that STEC has been isolated from venison collected at the retail level, there is sufficient information to indicate that STEC contamination can occur at any step of the production chain. However, assuming a general level of compliance

with regulations, guidelines and good hygiene practices, the possibility of STEC contamination in venison can be largely reduced.

4.1. Introduction

4.1.1 Background to Shiga toxin-producing *E. coli* (STEC) in Scotland

Shiga toxin-producing *Escherichia coli* (STEC) strains are important human pathogens of global public health importance, known to cause gastrointestinal disease in humans, which in severe cases can result in haemorrhagic colitis and haemolytic uremic syndrome with potentially life-threatening consequences, especially in young children and older people (WHO, 2017). Production of potent cytotoxins, called Shiga toxins (Stx) or verocytotoxins (VT), encoded on the genome of temperate lambdoid bacteriophage, is the major virulence determinant of the STEC strains. Additional virulence factors such as gene encoding the attaching function to the intestinal mucosa (*eae*) and virulence plasmid-encoding genes contribute to the pathogenicity of STEC strains (Law, 2000).

E. coli O157:H7 is the most important and widely investigated STEC serotype in relation to public health, since it has been responsible for most human cases in North America and the UK (Majowicz *et al.*, 2014) but other non-O157 STEC serotypes have increasingly been involved in sporadic cases or outbreaks in recent years (Gould *et al.*, 2013). In Europe, certain STEC serogroups (namely O157, O26, O103, O111, O145 and O104:H4) are recognised to be those causing most cases of Haemolytic Uremic Syndrome (HUS) (Article 12, Commission Regulation (EC) no. 209/2013). Furthermore, serotype O104:H4 caused outbreaks of food poisoning in Germany and France in 2011, where sprouts and seeds intended for sprouting, were identified as the most likely source. Consequently, the European Commission has introduced amendment No 209/2013 of 11 March 2013 to the Regulation (EC) No. 2073/2005 to include testing requirements for ready to eat sprouts for STEC O157, O26, O111, O103, O145 and O104:H4, in order to safeguard public health. More recently there has been a move away from monitoring specific serogroups to assess human disease risk, with a new FAO proposed scheme which assesses the risk of STEC isolates according to their virulence gene profile and not serogroup[39].

Most strains of *E. coli* bacteria are harmless, being commensal to both human and animal intestines but other strains cause illness by different infective and toxin producing mechanisms. STEC strains express the various "virulence determinants" genes which determine the extra-intestinal virulence and are also viewed as intestinal colonisation and survival factors linked to commensalism as they can increase the fitness of the strains within the normal gut environment (Le Gall et al., 2007).

Cattle are considered the major reservoir of STEC and it is known that asymptomatic cattle shed the bacteria in the environment from the rectoanal site of colonisation (Chase-Topping *et al.*, 2008). Other ruminants such as sheep, goats and deer can also

[39] http://www.fao.org/3/ca0032en/CA0032EN.pdf

act as reservoirs of infection (Beutin *et al.*, 1993; Caprioli *et al.*, 2005; Rounds *et al.*, 2012). It has been shown that interspecies transmission can occur between cattle and deer by faecal contamination of farmland (Singh *et al.*, 2015). Transmission to humans can occur via environmental contamination or as a result of direct or indirect contact with STEC containing ruminant faecal material, most commonly by handling animals, through exposure to soil or vegetation contaminated by faeces, or from contaminated water or food (Chekabab *et al.*, 2013). In the case of meat and meat products, contamination can occur during the slaughter process or further up the chain via cross-contamination during cutting, or at later stage, during handling by consumers.

In Scotland, STEC poses an important public health challenge, over a decade ago recording one of the highest rates of human STEC infections in the world (Chase-Topping et al., 2008; Pearce et al., 2009). Despite best public health efforts, STEC human infections rates in Scottish patients remain one of the highest in the United Kingdom and Europe (Health Protection Scotland, 2019). Similarly to other world regions, STEC O157 is the serogroup most commonly detected in human cases, although also in Scotland a recent increase in human disease associated with non-O157 STEC has been observed (Chase-Topping *et al.*, 2012). According to Health Protection Scotland (HPS) the only serogroup of Shiga toxin-producing *E. coli* that can be routinely detected by local diagnostic laboratories in Scotland is STEC *E. coli* O157. Identification of non-O157 STEC requires submission of faecal samples to the Scottish *E. coli* O157/STEC Reference Laboratory (SERL) for further investigation. In 2015, 78 isolates of non-O157 STEC were cultured and reported, followed by 63 and 59 reports of non-O157 STEC cases in 2016 and 2017, respectively. The most common serogroups observed were O145 and O103, followed by O26 (HPS, 2018). Recent research carried by SERL showed *E. coli* O26:H11 and O103:H2 were the most common non-O157 serotypes isolated in Scotland since year 2000. These also represents the most common serotypes circulating worldwide. A third common serotype observed in Scotland was *E. coli* O145:H28[40].

From its emergence as a human pathogen in the 1980s, the number of human cases of STEC O157 in Scotland has remained consistently around 200 cases per year (HPS, 2019), amongst the highest rates of infection per 100,000 head of population in the UK (HPS, 2014). The Scottish Government worked closely with stakeholders to produce a VTEC/*E. coli* O157 Action Plan for Scotland 2013-2017[41] which aims to reduce the numbers of human infections through a series of 86 recommendations covering all aspects of STEC infection risk, including reducing environmental contamination levels from infected animals (with a major focus on cattle) as well as meat and food handling hygiene and control of human-to-human (secondary) transmission.

Despite this effort, the recent outbreak in 2015 of STEC O157 infection in humans associated with consumption and handling of products from wild venison has highlighted major gaps in our understanding of the risks of STEC infection from venison. For example, it is not fully known what risk factors are involved in the killing and

[40] https://www.foodstandards.gov.scot/publications-and-research/publications/whole-genome-sequence-typing-and-analysis-of-non-o157-stec
[41] http://www.gov.scot/Publications/2013/11/8897/3

processing of wild venison and whether the current 'Best Practice' recommendation for processing from the hill to the final product is adequate to mitigate the risk of human infections.

Such knowledge is required in order to inform policy recommendations arising from the current VTEC/*E. coli* O157 Action Plan for Scotland 2013-2017, and to inform future recommendations to local authorities from Food Standards Scotland (FSS).

The Scottish Government (SG) and FSS therefore commissioned a review of cross-contamination risks in the slaughter and processing stages of wild deer from the field to the larder. This was carried out by identifying key risk factors associated with faecal contamination of venison carcasses during processing from the hill to the final product, to provide evidence and inform recommendations to minimise STEC contamination of the final venison product.

4.1.2. Factors affecting the survivability of STEC

Growth temperature: STEC can grow in temperatures ranging from 7°C to 50°C (WHO, 2017). The mean optimum growth temperature in laboratory conditions for *E. coli* O157:H7 is 40.2°C compared with a mean of 41.7°C for non-O157:H7 and Shiga toxin is produced at all temperatures that support growth of the bacteria (James, Loessner and Golden, 2005). *E. coli* O157:H7 can persist in frozen and chilled conditions, although there is likely to be a reduction in pathogen numbers (Stringer, George and Peck, 2000).

Chilling: survival of *E. coli* O157:H7 is greatly declined by chilling temperatures below 5°C, although a number of cells will still survive (Erickson and Doyle, 2007). The greatest reduction of *E. coli* O157:H7 in beef carcasses was observed to occur at between 10 and 18 hours from the start of chilling (Mellefont and Kocharunchitt, 2015). *E. coli* bacteria respond similarly to dry chilling conditions, irrespective of the genetic diversity (Visvalingam, Liu and Yang, 2017).

Based on a comprehensive literature review, (Greig *et al.*, 2012) concluded that dry chilling for at least 24 hours is an effective step to reduce, or even inactivate the *E. coli* contamination on beef carcasses. However, such reductions following effective chilling conditions were observed to be only temporary, due to cell injury rather than cell death and after approximately 40 hours, when the temperature and water activity are stable, growth, although slow, can occur (King *et al.*, 2016) (Mellefont and Kocharunchitt, 2015). The interval when the *E. coli* counts are at their lowest, between 18-24 hours, may provide a window of opportunity in which other relevant processing methods could be applied to completely eliminate *E. coli.*

Freezing: When seeded into minced beef, *E. coli* O157:H7 had the ability to survive up to 9 months in frozen storage at -20°C (Kraft 1992). Other tested frozen meats such as pork cutlets and meat products, including burger patties, stored for over 180 days and subjected to three freeze-thaw cycles also showed no changes in the *E. coli* O157:H7 population (Ro and Ko, 2015). The same study showed that thawing, however, does not lead to *E. coli* O157:H7 multiplication.

Heat inactivation: A study looking at the heat sensitivity of *E. coli* in venison sausages has described that more than a 5 log reduction in *E. coli* levels was achieved as soon as the raw sausage samples reached a temperature of 64.4°C and further reduction was observed at 68.3°C (Roberts and Getty, 2011). Heat treating to a core temperature of 65°C, which is known to be the equivalent to medium rare cooking *may be adequate for assuring the microbiological safety of beef tissues without excessive contamination of deep tissues'*, as suggested by (Gill and McGinnis, 2004). A heat temperature of 70°C applied for 2 minutes reduces *E. coli* O157:H7 cell numbers to levels that enable safe food consumption (Stringer, George and Peck, 2000). This last cooking recommendation is also accepted in products such as burgers by the Advisory Committee on the Microbial Safety of Food (ACMSF), which advises the FSA and FSS on food safety aspects in products of animal origin[42].

Salting: Salt at concentrations relevant to meat processing (1-3%) appears to decrease numbers of *E. coli* O157:H7 but has a potentiation effect on Shiga toxin production, a process which has been linked to bacterial stress response to salt and subsequent lambdoid phase induction (Harris *et al.*, 2012). Salt concentrations of 8.5% or above do not support growth of *E. coli* O157:H7 or production of Shiga toxin (James, Loessner and Golden, 2005), although such concentration is much higher than those regularly used by the food industry.

pH: *E. coli* O157:H7 has the ability to survive in very acidic conditions. (Conner and Kotrola, 1995) observed that a pH of 4 enables survival of the bacteria for up to 56 days and that survivability is affected by type of acidulant and temperature. Growth of *E. coli* O157:H7 was inhibited at a pH of minimum 4.6 when lactic acid was used (Glass *et al.*, 1992).

Surfaces: Research has shown that *E. coli* O157:H7 can attach, grow and form network structures within the confines of a biofilm on surfaces such as stainless steel (Rivas, Fegan and Dykes, 2007) as well as on polypropylene and high density polyethylene (Simpson, Beauchamp *et al.*, 2012). The importance of biofilm formation to the survival of bacteria is explained by (Rivas, Fegan and Dykes, 2007) who observed that biofilms transferred more bacterial contamination to product than attached cells not in a biofilm, due to microbial detachment of a greater number of microbial cells present in the biofilm. Drying, pH and sudden temperature shifts of surfaces containing *E. coli* O157:H7 cells have an influence on *E. coli* survival within a biofilm by reducing bacterial population numbers (Skandamis *et al.*, 2009).

4.1.3 Aims

The aim of this study was to assess the steps of the venison food chain, including processing via Approved Game Handling Establishments (AGHEs), directly or through collecting agents, considering this is the route by which the bulk of venison product will enter the local/domestic market, with a significant part also being exported. The scope of this assessment is to understand what processes are involved in venison production,

[42] https://acmsf.food.gov.uk/sites/default/files/multimedia/pdfs/acmsfburgers0807.pdf

from ante-mortem inspection (by the hunter before shooting) to the end product. By reviewing each of the steps involved in the food chain from hill to the larder, the study draws on the factors that are likely to trigger a risk of cross-contamination of the carcass or meat with STEC.

The report includes a comprehensive review of current Wild Deer Best Practice guidance, the Scottish Quality Wild Venison Stalking and Carcass Handling Standards scheme, Regulation EC No. 2073/2005 on Microbiological Criteria for Foodstuffs and the Hygiene Regulations (EC) Nos. 852/2004, 853/2004 and 854/2004.

4.1.4 Methods

The systematic literature review consisted of several layers of search. To provide a brief description of the game harvesting process, personal communication took place with stakeholders involved in the venison industry, both deer management authorities and food business operators (FBOs).

The scope of the search also included a review of Best Practice Guidance pages generated by the Best Practice Steering Group in Scotland comprising The British Association for Shooting and Conservation (BASC), The British Deer Society (BDS), Scottish Natural Heritage (SNH), Forestry and Land Scotland (FLS), LANTRA (www.lantra.co.uk), the Association of Deer Management Groups (ADMG), and the Scottish Gamekeepers Association, were reviewed online. These recommendations were compared with the scheme on Scottish Quality Wild Venison Stalking and Carcass Handling Standards and the Hygiene Regulations (EC) No. 852/2004, 853/2004 and 854/2004 as well as the guidelines drafted by the FSA and FSS for the venison industry.

The risk factors were extracted after a systematic literature review of the scientific papers published over a period of 11 years (2006-2017), from the start of the period of entry into force of European Hygiene Regulations to the time of issuing this literature review. The search also included scrutinising the library available at Edinburgh University for books and PhD works. Keywords used to retrieve pertinent information were: game; deer; wild ruminants; wild cervids; health; infection; immunosuppression; body condition; food handling; meat hygiene; carcass; contamination; processing; venison; shiga; verotoxin; STEC; VTEC; *E. coli*.

The first step of the literature review consisted in using short string built with the Boolean operator ‘AND’ between synonym words of the species concerned in combination with one other key word added to the string with the Boolean operator ‘OR’. To optimise the findings, the search was filtered by title and abstract and the above time interval.

A total of 15 strings were constructed with this approach. For instance, the first string was built around the first key words listed after species by following the search: ((((deer[Title/Abstract]) OR (wild ruminant$[Title/Abstract])) OR (cervid[Title/Abstract])) OR (game[Title/Abstract])) AND (health[Title/Abstract]) AND ("2006/01/01"[PDAT] : "2017/11/25"[PDAT]).

A final, more complex string was constructed from synonym words reflecting the animal species connected with the Boolean operator 'OR' to which was added a combination with synonyms words of the *E. coli* outcome. The Boolean operator 'AND' was used to connect the two combinations of synonyms and the filter was based on title and period of study to narrow results. This last string consisted of the following search: ((((((((deer[Title]) OR (cervid[Title])) OR (game[Title]))OR (venison[Title])) OR (wild ruminant$[Title])) AND (shiga[Title])) OR (verotoxin[Title])) OR (E coli[Title])) OR (STEC[Title])) OR (VTEC[Title] AND ("2006/01/01"[PDAT] : "2017/11/25"[PDAT])).

The search string was applied to PubMed electronic bibliographic databases, without any geographical restrictions. All results were reviewed by title and where the title indicated the publication was relevant for the search, the abstract was also reviewed. The relevant results were added onto a table by author, year and title.

These searches yielded 371 articles, textbook chapters or papers. Duplicate citations were removed using manual de-duplication One or more criteria for relevant literature had to be satisfied and the following inclusion criteria were applied:

1) Describes either primary research studies or is a literature review of primary research studies.
2) Provides data on live deer species with regards to health status, estate management.
3) Includes data about presence and counts of *Escherichia coli* and/or Enterobacteriaceae and coliforms with reference to carcasses or meat.
4) Provides data on more than one stage of the process or data on risk factors influencing the loads of indicator bacteria on carcasses, or on the association between visual faecal contamination and indicator bacteria counts.
5) Does not have the investigation of *E. coli* prevalence in deer faeces as its primary scope (as the scope of Objective 3 was to examine the indicators for the prevalence in the venison carcasses/meat rather than the faeces).

Of the 371 papers reviewed against these criteria, 256 papers were considered irrelevant. For the remaining papers, abstracts were re-read and, where appropriate, full-text documents were obtained or accessed online for further reading. The same criteria taken as above were considered, since in some cases the abstract did not provide enough information to review against eligibility criteria. After full reading, 81 more articles were eliminated, leaving 34 to be considered. This search and inclusion strategy was followed by backwards citations review of the 34 selected papers and 21 more articles relevant for this review were identified. This last step disregarded the year of publication. Four relevant books and one PhD thesis were also found in the University of Edinburgh library.

The strategy also entailed a review of the domestic and international guidelines in terms of safe production of venison as well as the set of European Food Safety and Food Hygiene Regulations. Scientific opinions issued by official public health bodies, such as data available in the EU Summary Report on Trends and Sources of Zoonoses,

Zoonotic Agents and Food-borne Outbreaks 2017[43], were also considered for an assessment of the current situation regarding the microbial STEC status of wild game meat in the EU.

The risk factors that might be associated with subsequent *E. coli* contamination of the deer meat have been extracted from each of these searches and summarised in Appendix 10.

A separate search on the survivability factors of *E. coli* O157:H7 was carried out to extract the main characteristics of the bacteria in response to the most common food safety treatments that the meat is subjected to during production. This search included keywords such as: *E. coli* O157, STEC, chilling, temperature, freezing, salt, hands, inactivation and surface. 22 articles were included after this search.

The web-based reference management programme Mendeley Desktop was used to manage references, extract data and retail full texts of references.

4.2. Overview of the main legislation applied to the venison industry in Scotland

An overview of the legislation that is applicable at different steps of the food chain in the production of wild game meat processed via Approved Game Handling Establishments (AGHEs) directly, or through collecting agents, has been provided in section 2.3.1. Circumstances in which small scale wild game operators are exempt from certain elements of these regulations are provided in section 2.3.2.

4.2.1 The Hunter

Hunters are regarded as those individuals who shoot wild game alone or as part of a hunting party. With the exception of private domestic consumption or limited retail distribution, meat of large wild game may be placed on the market for human consumption only if the carcass is transported to an AGHE. To be able to supply game to an AGHE, hunters should show evidence of training by providing evidence of courses attended and the certificate received or other evidence that demonstrate sufficient knowledge in the pathology and ecology of deer.

Under the Deer (Scotland) Act 1996, Article 17, the hunter must be registered to shoot deer or be supervised by a registered person. For registration purposes, the individual must gain experience in both the practice of stalking and the ecology of deer. Competence can be demonstrated through the attainment of Deer Stalking Certificates 1 and 2 provided by the Deer Management Qualifications, following training by the British Association for Shooting and Conservation (BASC), the British Deer Society (BDS) or other training providers.

According to Hygiene Regulations (Annex III, Section 4, Chapter 1 of Regulation (EC) No. 853/2004), '*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, and*

[43] https://www.efsa.europa.eu/en/efsajournal/pub/5500

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'. Where game carcasses are intended for supply to an AGHE, it is required that at least one 'trained person' will be available in the hunting team to make the initial examination and to complete the Trained Hunter declaration (Appendix 6).

Deer Stalking Certificate 1 (DSC1) would offer sufficient reassurance of appropriate hygiene training. From December 2005, the requirements with regards to Trained Hunters of Regulation (EC) No. 853/2004 became an integral part of DSC1. Achieving this qualification from that date onwards thus signifies that holders have the knowledge required by Regulation (EC) No. 853/2004 for inspection of wild game. Candidates who obtained their DSC1 qualification before 2006 can upgrade their licence to include Large Wild Game Meat & Hygiene for Trained Hunter Status by attending and passing an assessment by BDS and other certified training providers[44].

Deer Stalking Certificate 2 (DSC2), or an equivalent certificate provided by a nationally recognised training and assessment centre, is a more robust way in which stalkers can show they have appropriate knowledge of deer pathology. To register for DSC2 the hunter must hold a Wild Game Meat Hygiene qualification in Wild Game. This can be obtained via three routes: 1) The hunter already holds a DSC1 obtained after 2006; 2) holds a Large or a Large and Small Game Meat Hygiene Qualification provided by a nationally recognised body, such as LANTRA; 3) Holds a Meat Hygiene Large/Large and Small Game Certification provided by the National Gamekeepers Organisation (NGO) or another certified training provider[45] (BDS, online).

In the case of hunters who do not hold a DSC 1 or 2 or equivalent certificate, and carry out the shooting and further evisceration, examination of the carcass must be fully supervised by an adequately qualified Trained Hunter who will be responsible for maintaining the carcass hygiene and will sign the Trained Hunter declaration (Appendix 6).

4.2.2 General legal aspects applicable to hunting

The deer hunting rules and offences are stipulated in the Deer (Scotland) Act 1996. A few of the principles of these regulations that might have an effect on the hygiene of the carcasses, as related to offences are, as follows:

It is an offence to:

- Discharge any firearm from any moving vehicle at any deer or to use any aircraft for the purposes of transporting any live deer other than in the interior of the aircraft or use a vehicle to drive deer.
- Taking, killing or injuring deer in a closed season, unless authorised to do so by Scottish National Heritage (SNH).

[44] https://www.bds.org.uk/index.php/training/dsc2
[45] https://www.bds.org.uk/index.php/training/dsc2

- Shoot at night (one hour after sunset to one hour before sunrise) except under licence.
- Use any firearm or any ammunition for the purpose of wilfully injuring any deer.
- Use anything except legal firearms to kill deer.
- Failure to make, or making false return of number of deer killed to SNH.
- Take or kill deer without permission from a person having such right.
- Sell, offer or expose for sale venison unless you are a licensed venison dealer or are selling to or have purchased from a licensed venison dealer.

The statutory open season periods for shooting deer in the UK are outlined in Appendix 4. These hunting seasons follow the biology of deer. For instance, in red deer which are the most common species on the hills in the northern parts of Scotland, the rut takes place between the second half of September and the first half of November, with a peak, usually in October, followed by gestation of approximately 7.5 months. Calving is between end of May and mid-July and, there is a lactation period of 3-4 months during which the calf is dependent on the mother. As reflected in Appendix 4, the hind hunting season starts on 21st October, when the majority of calves are not dependent on mothers' milk and ends on 15th February, approximately 3 months before the new birth season begins. The hunting season for stags begins in July and ends in October.

However, due to high numbers in the deer population, a number of estates and holdings undertake out-of-season culls, under licence.

In roe deer, which are distributed throughout Scotland and more commonly in wooded regions, the rut and breeding season occurs from mid-July to mid-August. Following an average of 9 months gestation (4 months of no embryonic growth followed by 5 months of foetal growth) the kids (usually two) are born May – June and are dependent on the doe's milk for approximately 4 months. The doe hunting season starts on 21st October when the kids would have been weaned or are not dependent on mother's milk and ends on 31st March, a few months before the new birth season begins. The buck hunting season (1st April to 20th October) captures the rut season.

Sika deer, which is more commonly found in wide open spaces in northern and central western areas of Scotland, follow a similar cycle to red deer.

Fallow deer have a scattered distribution in Scotland, often foraging in woodlands, moorlands but also onto agricultural land. The rut begins the second half of September to the first half of November with a peak in October, followed by 7.5 months gestation and births beginning in June until mid-July, and a lactation period that usually ends by December, although the calf is dependent on milk for 3 to 4 months.

The months for stalking female deer typically correspond to the cooler periods of the year to avoid culling the heavily pregnant or those with calves at foot, which offers some advantages in reducing carcass temperature and reduces the chances of microbial contamination (Paulsen and Winkelmayer, 2004). However, shorter days and adverse weather in winter months may increase the challenges faced by the hunter in meeting hygiene practices, yet typically lower ambient temperatures provide some latitude. The

culling of male deer in season extends into typically warmer months, and therefore offers an increased opportunity of spoilage associated with higher ambient temperature.

4.3. Supply from estates passing primarily through AGHEs

4.3.1 Overview of the process of obtaining venison

In the UK, all wild deer intended for human consumption are killed by a free bullet, under the legal prerogatives which outlined earlier. This entails a careful approach to the animal to avoid disturbance, and to increase the precision of the shot location. Weather conditions such as poor light, mist, rain or snow might make culling more difficult (Gill, 2007). However, when the stalker considers that are within range to effect a kill, they will place the bullet with the aim of achieving rapid death and to minimise the risk of gut damage and consequent contamination of the carcass cavity.

Following the shot, and once the stalker has approached the deer and confirmed it is not conscious, it is exsanguinated (bled), by a small incision in the neck. If the deer is still conscious, it should be humanely dispatched via a lawful means, such as a shot to the head. The abdominal cavity is opened with the deer lying on its back to avoid having the intestines too close to the incision line. The oesophagus is cut and knotted, the rectum emptied and tied off, removing the abdominal viscera, known as green offal (gralloching). This is followed by inspection by the Trained Hunter. The gralloching practices are prescribed in The Wild Deer Best Practice guides but may vary slightly. In larger deer, the rectum might be emptied of pellets ("milked"), sealed inside the abdominal cavity with a knot and removed later at the larder. Prompt evisceration is essential to avoid contamination, initiate the cooling process and reduce the weight, especially in larger red and sika deer species, to enable easier handling and removal from remote areas.

If good hunting practices are observed, the stomach, intestines and other body parts of wild game may be disposed of safely on the site of hunting, unless a trained hunter is not available or the organs present pathological condition(s), in which circumstances, these parts must accompany the carcass to the AGHE.

Once eviscerated, the carcass will be transported to the larder. On steep or boggy terrain it might not be possible to transfer the carcass by all-terrain vehicle (ATV) or for it to be loaded onto a pony, thus it will need to be dragged and brought to a more accessible point where it will be loaded on to mechanised transport or pony for extraction.

In more accessible areas, the carcass is loaded directly on to an ATV and transported to the larder. It is paramount to ensure that the vehicle and any gear used on the pony or the vehicle is kept clean. If transported in a wire basket type of hill trailer, it is recommended that a waterproof sheet is placed over the carcass to protect it from dirt thrown up by the vehicles' wheels.

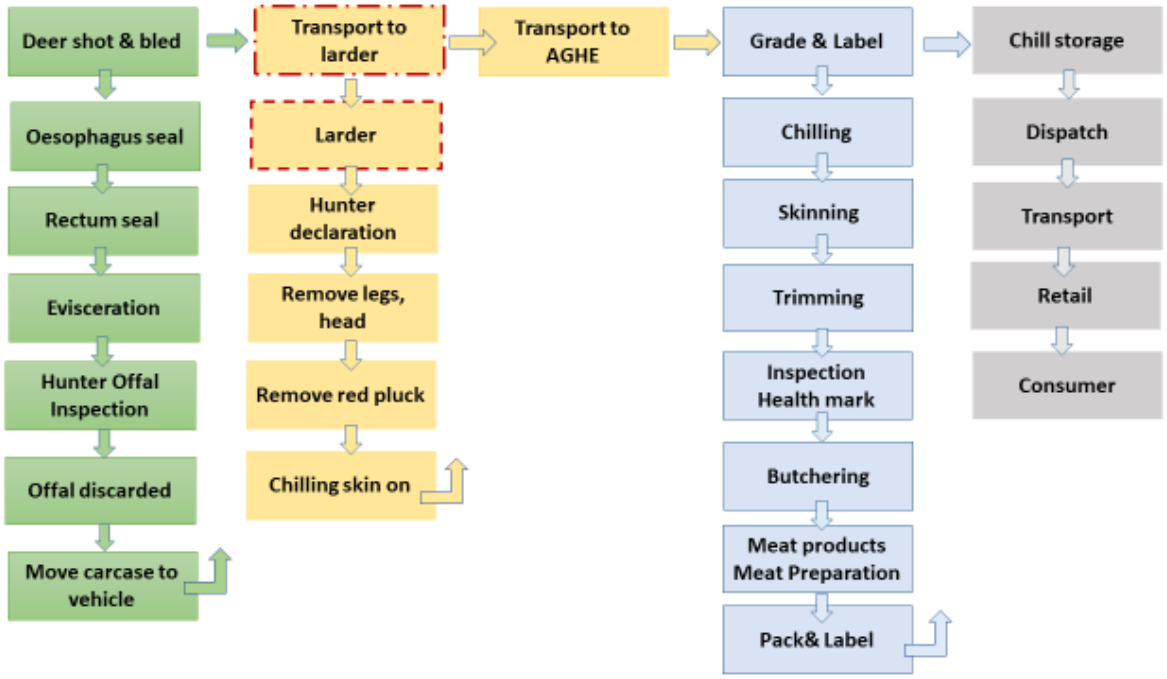
After the carcasses are collected they are stored at larders. Some are co-located to AGHEs which have been approved by the FSS, in line with the Hygiene Regulations

(EC) Nos. 852/2004 and 853/2004, or located close to hunting grounds, non-co-located to an AGHE and registered with the Local Authority where the same European hygiene controls are enforced by the authorised food safety officers of the Environmental Health Office. Larders vary in size and therefore also in throughput, but most deer will be held temporarily in estate larders before being collected for further processing at AGHEs. There are some 150 larders certified under the Scottish Quality Wild Venison scheme and follow the requirements dictated by the assurance scheme.

At the game larder, the carcass is transferred to a larder cart, the lower legs are inspected and removed, and the head is removed and discarded as animal by product (ABP). The carcass is transferred to a hanging position, and the sternum is split to enable removal of the 'red pluck' (the lungs, heart and liver), which are usually discarded as category 3 ABP. Best practice guidance sets out that the pelvis should be split and the rectum removed intact, through the cut. The carcass is then stored refrigerated until it will be transferred in a chilled vehicle to the AGHE.

At AGHEs, the carcasses are unloaded, Trained Hunter declarations (Appendix 6) are checked and the game is registered into the intake records. The carcass is graded based on weight and conformation, labelled with all traceability details and placed in a chiller designated for skin-on carcasses. After skinning and dressing, the carcass is transferred to a chiller in which only skinned carcasses are being held. This is followed by carcasses being cut into large meat cuts and subsequently into steaks or meat preparations (diced meat, raw sausages, burgers) or meat products (smoked, cured, dried venison that still requires cooking). The finished product is packed, labelled and stored in the chiller designated for wrapped products until further dispatch to commercial customers, cold stores or directly to consumers. A flow diagram of venison production from the hill to the plate is shown in Fig. 4.1

Fig. 4.1 *Flow diagram of venison production from the hill to the plate*



The red dot-dashed boxes indicates that some carcasses are not transported to the larder and do not undergo the subsequent steps in the second column, instead are transported directly to AGHEs

4.3.2 Hunting practices from the hill to the larder

This section will consider the practices that are undertaken to kill, store and transport deer from the hill to the AGHE. The techniques will be discussed in relation to specific risk factors that might trigger faecal contamination and therefore a potential contamination with STEC. Relevant sources by which STEC may be transferred to the carcass are summarised in Appendix 10 and include but are not limited to the following:

- Condition of the animal before the shot
- Type of hunting
- Location of the shot wound
- Behaviour of the animal after the shot
- Handling practices during evisceration
- Transport
- Storage

Selection of animals for the food chain: ante-mortem inspection

The hunter plays an important role in assuring the quality of game meat, not only in adopting the best hygiene practices but also in the choice of the animals that are being shot. This involves a visual assessment of the health of the animal 'on the hoof' prior to

despatch and judges whether it can be placed in the human food chain. It should be noted that carriage of STEC would not be expected to cause clinical signs of disease.

In accordance with Regulation (EC) No. 853/2004, Annex III, Sect 4, Ch. 1. (4), only animals displaying normal anatomy, physiology and behaviour can be accepted for human consumption. Therefore, those displaying abnormal behaviour or environmental contamination are not suitable. In addition, an emaciated animal would also not be fit for human consumption (Rijks *et al*., 2017).

To satisfy the requirements of the hygiene regulations, hunters undertake appropriate training (see section 2.1) to be able to recognise aspects of abnormal behaviour or contamination. The guidance given in these training courses includes aspects of deer health, best hygiene practices and welfare considerations and is now available online[46] .

It is beyond the scope of this literature review to expand on aspects of abnormal behaviour, however some traits that are key to underlying conditions that might affect the deer will be discussed since these conditions could offer an indication of the immune status of the animals.

Behavioural aspects which might indicate abnormalities have been described by the Wild Game Guide and include but are not limited to: lack of response to potential danger, isolation from the herd, light bodyweight, unusual discharges from bodily orifices, coarse coat condition or injury not provoked by hunting[47].

Immunological competence is clearly important as, generally speaking, it is associated with healthy individuals. There is growing evidence that metabolic stress-related immunosuppression predisposes animals to infection. One study has shown that wild animals in poor body condition are predisposed to infections as a result of immune depression which in turn further reduces body condition (Beldomenico *et al.*, 2008). Laboratory animal models have also been shown to be immunosuppressed due to suboptimal diet. In turn, the suppression was associated with disrupted gut microflora ecology, thus enteric bacteria, including *E coli*, were observed to spread systemically to the peritoneal cavity or adjacent tissues (Deitch *et al.*, 1993) (Berg, Wommack and Deitch, 1988). The explanation for this may be that antibodies and other key mediators of the immune response are proteins and thus protein deficiency from poor nutrition increases susceptibility to infection.

Deer are known to cope less well with cold stress than other animals of similar size and become naturally debilitated during harsh winter seasons due to the reduced food availability and also metabolic challenges. As a result of cold stress, most of the body fat is mobilised towards energy production, necessary to regulate the core temperature and heart rate (Turbill *et al.*, 2011) . Low food availability and energy reserves have been shown to have a negative impact on the optimal allocation of energy devoted to mount effective immune responses (Houston *et al.*, 2007). In turn, the effect of nutrition on the immune functions has been seen to predispose animals to health issues (Smith, 2007) (Jolles, Beechler and Dolan, 2015).Other pathological conditions, such as heavy enteric

[46] www.Bestpracticeguides.org.uk
[47] http://www.bestpracticeguides.org.uk/health/health-welfare

parasitism might lead to reduced body condition. It has been shown that although red deer might not express clinical signs of enteric parasitism (e.g. diarrhoea), some correlation between high levels of intestinal parasites and body condition does exist (Irvine *et al.*, 2006). The balance between low parasite burden and host tolerance is mediated by the immune system, and there is evidence that immunosuppression is a precursor to parasitic or general clinical disease (Jolles, Beechler and Dolan, 2015).

During ante-mortem inspection, if the body condition is appropriate, it might be difficult to determine parasitic diseases in deer. However, as a general concept and as shown by some limited peer-reviewed literature, it is believed that, similar to the effect in cattle, heavy parasitism results in a roughened, 'scruffy' coat, different from that of healthy animals [48]: Infestations with ticks and keds (biting flies), almost ubiquitous on wild deer, are usually tolerated by the animal, although hair loss and/or patchy coat might be found (De Bosschere *et al.*, 2007; Bildfell *et al.*, 2004)

To conclude, deer might become debilitated due to inadequate nutrition, adverse weather conditions, heavy enteric parasitism or a combination of these. The result is a very lean deer that might get to the point of showing behavioural changes such as isolation before dying. The assessment of bodily condition based on a 'standard' of expected bodyweight, depending on the season, as well as of back and pelvic muscle should be possible by visual inspection before culling and would be an appropriate indicator of the animal's health status. Other conditions such as a scruffy coat might also be a straightforward indicator of heavy parasite infestation.

In common with all other vertebrates, healthy deer have a finely balanced relationship with their commensal, digestive bacteria and parasites such as helminths or arthropods. Clinical infection or infestation is established when the pathogen, may it be virus, bacteria or parasite, overwhelms the defence mechanism of the immune system at either a local or systemic level (Putman, 2012). The importance of understanding the health status of the animal is that it offers an indication to the robustness of the immune system. As a general medical concept, a weaker immune system represents a lower natural defence (Kreier, 2002).

Although there is limited evidence in the peer-reviewed literature, if deer respond the same way as cattle, there is a possibility that deer with weaker immune systems might be shedding more intestinal bacteria which could allow *Enterobacteriaceae*, including STEC O157:H7, to be established in the environment, further transmitting to other deer or susceptible hosts, and increasing the likelihood of contamination of the carcass with STEC O157:H7 if good hunting and hygiene practices are not followed.

Such supposition seems to be plausible if we look at some of the risk factors that were described in the studies undertaken in cattle. Carriage of STEC O157:H7 by individual animals is typically short-lived, but some carriers, designated as super-shedders (defined as shedding $>10^4$ colony forming units per gram of faeces), may harbour high intestinal numbers of the pathogen for extended periods. The prevalence of STEC O157:H7 is higher in post-weaned calves and heifers than in younger and older animals (Ferens and Hovde, 2011). The local rectal immune responses are depressed in cattle

[48] http://www.fao.org/docrep/004/X6529E/X6529E06.htm

that are shedding *E. coli* O157 and factors such as host immunity may play a role in the interaction between the host and *E. coli* O157 (Wang et al., 2016). Stress and negative body energy, both associated with a weaker immune system, have been shown to increase the likelihood of STEC shedding (Venegas-Vargas *et al.*, 2016). Similarly, body condition score has been positively associated with both shedding and super-shedding of STEC O157:H7 (Williams *et al.*, 2015). This was further confirmed in as study which identified metabolic and emotional stress as a risk factor for super-shedding of both O157 and non-O157 serotypes of STEC (Menrath *et al.*, 2010).

Selection of deer for hunting

The hunter plays an important role in assuring the quality of game meat, not only in supplying the game meat according to best hygiene practices, but even in the choice of the hunting method. There are several different types of shooting depending on the estate and the terrain. Shooting from high seats (in woodland) is a popular management method of roe deer but the most common method for deer shot for the food chain in Scotland is stalking, where the animal is pursued by the hunter on foot (personal communication from venison stakeholders).

Best practice recommends that hunters use dogs for tracking wounded deer, or deer which have run out of sight after a shot. The dogs are trained to follow the blood trail, locate the deer and report to their owners. They can also be trained to keep a wounded deer in a particular location ('at bay') until it can be dispatched, although usually the dog would be discouraged from tackling the deer itself. If the dog has bitten the deer, then the affected tissues should be removed from the carcass as dogs' oral cavities harbour considerable quantities of bacteria (Alberto et al, 2011).

Shooting deer while they are on-the-move has been reported to increase the likelihood of abdominal shots to about 30% and the proportion of shots accurately to the chest is much improved by a still target (Deutz and Fötschl, 2014). In line with the observations from the literature and the advice from Wild Deer Best Practice Guidance, the hunter should avoid shooting at a moving/running deer unless attempting to dispatch an already wounded animal.

Deer shooting

Best Practice Guidance for culling and handling deer is available online:

1. The Deer Initiative - England and Wales Best Practice Guides; Culling Shot Placement[49]
2. The Best Practice Steering Group: Wild Deer Best Practice Guidance. Culling and Shooting[50]

These guides advise that shooting should be directed to achieve rapid death and minimise suffering. The guidelines prescribe the correct choice of ammunition and

[49] http://www.thedeerinitiative.co.uk/uploads/guides/161.pdf
[50] https://www.bestpracticeguides.org.uk/guides/

explain that shot placement is important for animal welfare, causing instantaneous death and also possibly facilitating game retrieval, early bleeding and evisceration.

The preferred area of impact is the thorax, just behind the line of the foreleg to strike the heart. If the stalker is able to approach closely, the head can also be targeted, although this is not highly encouraged. The brain is a very small target area, especially in smaller deer species and sudden head movements might trigger a misplaced head shot, which can lead to unnecessary suffering. If close enough, some stalkers might perform a neck shot, which causes less damage to the carcass (Urquhart and McKendrick, 2006), although, this is discouraged in the best practice guidelines referred above (No 2.) because these types of wounds might be non-fatal, and therefore raise welfare concerns.

Abdominal shots are discouraged by best practice guidance due to the welfare considerations related to the long interval to death, and also due to a significant risk of carcass contamination, jeopardising the carcass hygiene by the spread of endogenous gut microflora, including the potentially pathogenic microorganisms such as STEC to the muscle tissue (Bartels and Bülte., 2011).

In a survey conducted on a sample of 230 culled Scottish deer, 35 (15.21%) of these showed an abdominal shot wound and 14 (6.08%) showed a shot wound in the diaphragmatic region (Urquhart and McKendrick, 2006). This indicates that around 21% of Scottish deer received shots wounds which might result in contamination with stomach content. These data were collected in 2001, however, and it might not be representative of the current practices adopted by Scottish stalkers, showing a need to scientifically review this aspect in line with the most recent best practice guidelines.

Investigations of the microbiological conditions of carcasses indicate that there is a close relationship between a higher risk of microbiological contamination of the carcass and shot locations posterior to the diaphragm, especially in the abdominal area. Avagnina et al., (2012) and Atanassova et al., (2008), assessed freshly killed deer by measuring aerobic colony count and Enterobacteriaceae, in carcasses with abdominal shots and those expertly killed, by a shot in any area of the heart, head, neck or spine. These two studies observed that fresh deer carcasses, with no abdominal wounds presented lower microbiological counts (Table 4.1).

Table 4.1 *Median log values between microbiological contamination of carcass from two groups of deer*

Microbiological condition of carcass	Aerobic colony counts	Enterobacteriaceae	Reference
Abdominal shots (49% roe; 25% red deer)	4.0 log cfu/cm^2	No significant difference seen	Avagnina et al., (2012)
Expertly killed deer	3.6 log cfu/cm^2		
Abdominal shots roe deer (16.8%)	3.1 cfu/cm^2	2.5 cfu/cm^2	Atanassova et al., (2008)
Expertly killed roe deer	2.5 cfu/cm^2	1.9 cfu/cm^2	
Abdominal shots red deer (5.7%)	4.3 cfu/cm^2	2.3 cfu/cm^2	
Expertly killed red deer	2.8 cfu/cm^2	2.1 cfu/cm^2	

cfu = colony forming units of bacteria

It is generally accepted that muscles and deep tissue of healthy slaughter animals are sterile, and the same is expected for game (Gill and Penney, 1977; Paulsen, 2011). However, contamination of these sterile tissues might occur if large numbers of bacteria are introduced into the brain or the blood stream when animals are killed (Mackey and Derrick, 1979). Bacteria from the hide can be introduced into local tissue and a high number of counts can be found around the entry and exits wounds, introduced by the bullet itself (Dobrowolska and Melosik, 2008; Mackey and Derrick, 1979) or from the hide and hair surface (Paulsen, 2011). A second consideration in terms of shooting is that, for animals that are only injured at the first attempt and require a second shot, bacteria introduced via the wound may enter the blood flow (bacteraemia) and reach the muscular masses.

In a study of deep muscle tissues collected from fresh deer carcasses, Enterobacteriaceae were isolated in higher numbers from carcasses of killed deer requiring two or three bullet wounds (Atanassova *et al.*, 2008). These observations would suggest that fewer shot wounds contribute to less carcass contamination with enteric bacteria, thus increased safety and quality.

A UK study reported that approximately 14% of deer carcasses culled for human consumption had received more than one shot (Urquhart and McKendrick, 2003). More recent results extracted from a research project initiated by the British Deer Society, which analysed a number of 102 stalkers' reports and 2,281 animals, showed that 93% of shots hit the target animal at first attempt and resulted in an outright kill, 5.5% of deer required a second shot and 1.2% of the shot animals were lost or escaped (Aebischer, Wheatley and Rose, 2014).

(Aebischer, Wheatley and Rose, 2014) found that the following factors increased the likelihood of multiple wounding:

- Uncomfortable firing position

- Shooting in haste
- Distant target, beyond 100m (only when time was not sufficient)
- Bullet weight below 75 grains Target concealed in thicket or on the move
- Unfamiliar stalking area.

It is likely that the incidence of multiple shots and a wounded animal escaping will vary with the experience of the individual hunters (Gill, 2007) and might be increased for non-professional hunters (Ramanzin *et al.*, 2010). Best practice guidelines are comprehensive in terms of shooting technique and use of ammunition, but do not advise an appropriately safe distance to achieve an outright kill as this depends on the rifle/ammunition combination, the aspect of the land and weather conditions. It is advised to select the distance such as to produce a tight radius from the point of impact to ensure that bullets will consistently fall within a 10 cm diameter killing area, and for the hunter to be aware of their own limitations. Further factors that affect the accuracy of shooting over a range of distances are the weather conditions, particularly the effect of the wind on the path of the bullet.[51]

The points discussed above suggest that shot location and shooting conditions can influence the microbial safety of the carcasses. This is particularly true for abdominal wounds, which were associated with greater Enterobacteriaceae counts in the carcasses. STEC is part of the Enterobacteriaceae family (Paton and Paton, 1998) and therefore this observation offers indirect evidence that STEC might contaminate the carcasses via this route.

The microbial condition of the carcass might worsen with the number of bullet wounds. The possibility of STEC contamination via this route is subject to STEC being present on the hide/hair of the shot deer and penetration via the bullet wound onto the affected muscles or, in case of injured animals, into the bloodstream.

Influence of stress

It is widely recognised that wild ungulates are susceptible to stress associated with hunting (Ramanzin *et al.*, 2010), where incorrect handling or wounding before killing triggers stress in live animals, which may negatively affect the organoleptic qualities of venison but, more importantly create hygiene risks. If improperly shot, game animals can often escape and experience increased pain and stress, which may lead to migration of microorganisms and endotoxins from colonised body regions such as the gastrointestinal tract to generally sterile organs and muscles (Bartels and Bülte, 2011). Therefore, best practices identified by the venison sector such as avoiding overstressing the game[52] and also ensuring that the animals are not in high motion (running) are important preventive practices[53].

After prolonged stress, glycogen reserves in the muscles are depleted due to an excess of catecholamine release (Kenny and Tarrant, 1987). The glycogen stocked within muscles is rapidly consumed leading to a lower conversion into lactic acid which in turn

[51] http://www.bestpracticeguides.org.uk/firearms/rifles2
[52] http://www.thedeerinitiative.co.uk/uploads/guides/125.pdf
[53] https://basc.org.uk/cop/deer-stalking/

means the post mortem pH does not reach sufficiently low values, leading to quality problems such as dark, firm, dry (DFD) meat, but more importantly to decreased microbiological safety and reduced shelf life (Wiklund *et al.*, 2001). High pH and the effects of stress may lower the activity of the specific defences of muscle tissue (complement system and lysozyme), thus enhancing the risk of multiplication of microorganisms in the deep layers of meat (Casoli *et al.*, 2005).

Other stress-related factors discussed by (Bartels and Bülte, 2011) are metabolic challenges naturally occurring within the physiological system over the cold season, suggesting the hypothesis that STEC shedding in wild ruminants is more likely to occur in late winter. However, this supposition has not been confirmed by any field studies thus far. Contrarily, in domestic ruminants, a positive relationship has been observed between STEC shedding and warmer ambient temperature in both cattle (Henry *et al.*, 2017) and sheep (Evans *et al.*, 2011). Similarly, higher STEC shedding was observed in faeces of elk during warm summer months (Franklin *et al.*, 2013) and white-tailed deer (Singh *et al.*, 2015).

The association of STEC with summer season is thought to be a function of higher shedding rates and further pathogen proliferation into the environment at favourable ambient temperatures (Franklin *et al.*, 2013).

Bleeding

Technique

Bleeding is done by chest sticking with a sharp knife in front of the breast bone, pointing towards the heart and severing all the main blood vessels[54]. The blood collected in the carcass is drained by gravity, and is achieved even more efficiently if the animal is placed with the head downwards. To ensure the blood has drained, the lower foreleg should be bent back and the rib cage pressed to force out any excess blood accumulated. If the carcass is to be extracted from the hill by dragging, it is advised to ensure that any cuts to the carcass are kept to the minimum and in such cases the chest cavity could be bled out via the diaphragm when opening the abdominal cavity to perform evisceration (Laaksonen and Paulsen, 2015).

Influence of bleeding practices on carcass contamination:

- The sticking knife might act as a 'fomite' (carrier) for pathogenic or otherwise harmful microorganisms to enter the wound or the blood stream (Casoli *et al.*, 2005). Therefore, similar to techniques adopted by the livestock food industry, it would be recommended to clean and sterilise the sticking knife before and after use. Provision of an adequate number of knives and the use of 'two knives' (one knife to cut the hide to expose fresh tissue, and a different, clean knife to severe the blood vessels) would ensure the sticking is done in a hygienic way.

[54] https://www.bestpracticeguides.org.uk/carcass-preparation/grallochingl/

- Sticking and bleeding must occur immediately after killing. The shot-to-stick interval might be considerably different from that in livestock given the practicalities of reaching the shot game. It is recommended to perform sticking and bleeding within a maximum of 10 minutes after shooting, otherwise the desired effect of removing as much blood from the carcass is not obtained (Laaksonen and Paulsen, 2015). An excess of blood remaining within the muscular mass has negative effects on the hygiene, organoleptic quality and the shelf-life of meat (Casoli *et al.*, 2005).

Gralloching (evisceration)

After killing, deer must have their stomachs and intestines (green offal) removed. It is unknown whether this poses a contamination risk to other deer who may subsequently pass through the cull site. Evisceration, known as gralloching, is the process whereby these viscera are removed from carcasses. The wild deer best practice guide[55] explains that the aim of undertaking the procedure at the place of kill is to:

- Remove those parts of the carcass that may cause contamination if left within the carcass – particularly if the intestines are damaged by the shot
- Remove those parts not intended for human consumption
- Help to cool the carcass
- Reduce the weight for transportation.

The red pluck (lungs and heart) are also usually extracted at the place of cull by cutting the diaphragm and extracting these through the abdominal cavity to minimise the external cuts and exposure to contamination during transport.

Each hunting party should include at least one an appropriately qualified person who is available to supervise the evisceration and handling of the carcass, and holds a Trained Hunter status to check all eviscerated carcasses to ensure the abdominal viscera removed show no abnormalities and the carcass shows no faecal or other contamination. The Trained Hunter completes the Large Wild Game Declaration form (Appendix 6) stating that "*there is no indication of environmental contamination*" of the carcass. If the Trained Hunter is not present during the hunting, and the carcass is intended for the food chain via the AGHE, all the viscera, except for green offal and antlers, must accompany the carcass to the larder for inspection (Regulation (EC) No. 853/2004, Annex I, Section IV, Paragraph 4c).

Personal hygiene considerations before evisceration

Hunter's arms and hands can act as a fomite for carcass contamination. These must therefore be clean before starting evisceration and, if necessary, should be cleaned during the procedure itself. Disposable gloves and sleeve covers should be used to reduce the risk of contamination and to prevent the risk of zoonotic infections. For the same health and safety reasons, any cuts, abrasions or sores of the hunter must be

[55] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_gralloch.pdf

kept covered with waterproof dressings. However, for food safety reasons, hunters should not handle large game if they are suffering from, or exhibiting, symptoms or conditions likely to be transmitted through food. This includes gastrointestinal symptoms, infected wounds or skin infections. These precautions are detailed in wild deer guidelines[56].

Knives and other equipment can also become a source of cross-contamination and therefore should be kept clean at all times. These must be clean before starting the operation and if they become soiled should be cleaned during gralloching. Placing knives or other tools on the ground or on the hide of the animals should be avoided. Careful attention should also be given to knife scabbards, which can become a source of contamination if not washed regularly. It is recommended to have a portable wash kit carried in the hunter's vehicle for being able to maintain standards of hygiene.

Evisceration technique

Several procedures are available to the hunters depending on deer species and the nature of the hunting ground, for example: carcass hung, laid on its back; with total removal of the red and green pluck (opening of both cavities) or with removal of the gastrointestinal mass only, leaving the rectum within the pelvic cavity tied/knotted to the loose end. For those carcasses that will be totally emptied in the wild, it is recommended that, if possible, evisceration should be done in a suspended position, for instance in wooded areas by hanging from a tree. However, irrespective of the method, careful skills and knowledge of evisceration techniques and dressing hygiene are key to reducing carcass contamination with gastrointestinal content (Bartels and Bülte, 2011).

The carcass is cut open with a single incision that typically extends between sternum and pelvis. During this operation, care should be taken not to puncture the bladder, stomach or intestines. In the case of roe deer, special care must be taken due to the looser connective tissue structure, which increases the risk of bacterial penetration in surrounding tissue during carcass cutting (Bartels and Bülte, 2011). As the incision is made, the blade of the knife is protected with the fingers of the other hand (Fig. 4.2), to prevent pinching any of the viscera. Usually, a round-tipped butcher's knife is suitable for this operation.

In their study, Avagnina *et al.*, (2012) observed an association between greater microbiological load and large openings of the body cavities on the hill. Thus it is likely that the level of contamination will be minimised if smaller gralloching cuts ensure a better microbiological condition of deer carcasses. Current Scottish venison guides advise keeping cuts to a minimum in field conditions, to minimise contamination during transport and cross-contamination between bodies.

The opening of the deer carcass typically extends from the front of the pelvis to the tip of the sternum. The advantage of this type of cut, as opposed to opening the sternum as well as the abdomen, is reduced contamination during further transport and storage. A disadvantage is, however, that small, partial opening may not cool the carcass as

[56] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf and http://www.bestpracticeguides.org.uk/carcass/gralloching

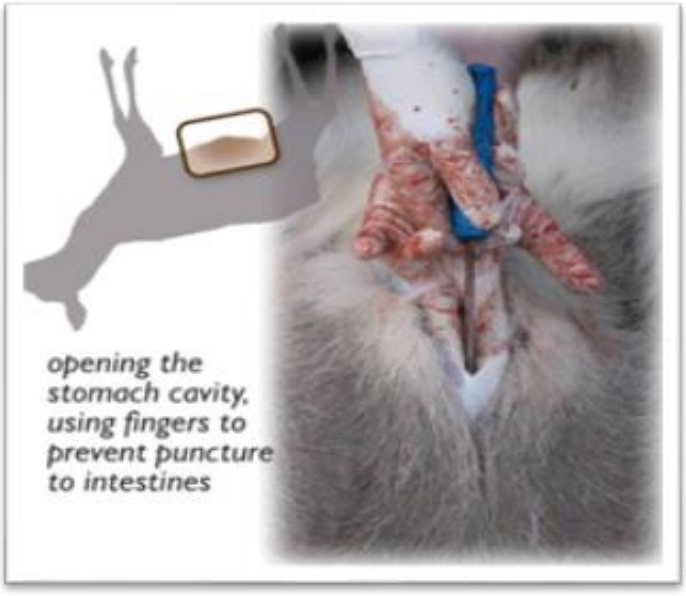
quickly or efficiently. The practical application of this literature finding is to keep cuts to the minimum to enable hygienic extractions of the gastrointestinal tract, taking in consideration the volume of ingesta as well as the outdoor conditions at the time of kill.

The carcass is protected from gastric spillage during the evisceration procedure by knotting the oesophagus (rodding). The last 10-15 cm of the large intestine is also knotted (usual practice) after it is isolated and pulled forward from the pelvic cavity. This is first manually emptied of stool by hand pressure, moving the faecal content from the anus towards the stomach (Fig. 4.3), one tie is made to seal the large intestine and a cut is made just on top of the tie, enabling removal of viscera and leaving approximately 10 cm of the large intestine (rectum) in the pelvis, which will be removed later with the anus at the larder or AGHE.

In smaller deer, the last part of the large intestine (rectum) can also be removed completely by coring it out via a circular cut inside the walls of the pelvic cavity to free up the rectum. This could be initially pulled out from the pelvic cavity, squeezed to push the pellets away from the anus and the last part of the rectum towards the stomach. The freed parts of the rectum can then be pulled out of the pelvis via the abdominal cavity.

The abdominal cavity is spread open by a person assisting and the ligaments that connect the digestive tract to the cavity should be carefully torn with clean, gloved hands (notice *Fig. 4.4*), although in older animals with tougher ligaments a clean knife might have to be used. The gastrointestinal mass is pulled out of the abdominal cavity (Fig. 4.4).

Fig. 4.2 *Incision to open the abdominal wall and hide. One of the hands keeps the tip of the knife away from the viscera to avoid faecal contamination*[57]*.*



[57] www.bestpracticeguides.org.uk/carcass/grallochin g

Fig. 4.3 *Removing faecal content from the rectum before making a knot and tying the end connecting the anus from the rest of viscera*[58].

Fig. 4.4 *Removal of the abdominal viscera with the animal on one side avoiding as much as possible to touch the hide and carcass with the abdominal viscera*. Note the lack of gloves, which poses a zoonotic disease transmission risk, such as from *E. coli,* leptospirosis, toxoplasmosis and Q fever to the hunter, as well as a contamination risk to the carcass from the hands of the operative[59].

[58] www.bestpracticeguides.org.uk/carcass/grallochingg
[59] https://www.youtube.com/watch?v=ebUK3pfL6NA

The above describes the removal of just the green offal but similar precautions can be adopted for the red pluck if the decision is made for it to also be removed as well noting that the area exposed to contamination would be much larger.

The organs are inspected visually for pathological changes by the Trained Hunter. Following inspection, the stomach, intestines and other body parts including the head of wild game may be disposed of safely on the site of hunting. This can be done by burying it far enough from the water courses (recommended distances apply- 250 m from wells, 30 m from springs and 10 m from drains but it may also be left available as a food source for wild animals and birds in areas with limited public access. However, the latter practices apply only if non-lead ammunition was used, to avoid lead poisoning of wildlife (Franson and Russell, 2014) and only if the deer is free of abnormal behaviour or any abnormal characteristics of the meat. If either of these were observed before killing, the red pluck and head must accompany the wild game body to the AGHE and viscera must be identifiable as belonging to a given carcass (Wild Game Guide[60]). Following inspection should be discarded in the appropriate animal by-product category.

Contamination factors during evisceration

The quality of wild game meat depends largely on the microbiological counts (measured as Aerobic Colony Counts and Enterobacteriaceae) of the meat surface which in turn is closely influenced by the time between killing and evisceration, the hygiene practices during the processing itself (Ramanzin *et al.*, 2010).

Delayed evisceration

Delayed evisceration is thought to increase the risk of muscle contamination as bacteria have ability to cross the intestinal barrier within a few hours from the time of kill (Ramanzin *et al.*, 2010). Additionally, the digestive flora will continue to ferment and produce gas which results in a bloated digestive tract and potential burst during gralloching, increasing the chances of contamination (Ramanzin *et al.*, 2010)

Regulation (EC) No. 853/2004 requires the wild game to be eviscerated 'as soon as possible', however there is no specific timeframe that would be considered as appropriately soon enough.

The Best Practice Guidance followed by the Scottish venison sector parallel the hygiene regulations, thus not include a time frame which would be appropriate before the evisceration operation takes place. However, according to information obtained from professionals working in the venison industry, the gralloching is encouraged immediately after the kill - up to maximum of one hour afterwards, particularly if the ambient temperatures are high. This is also in line with other venison industry guidelines, such as 'Stalking and carcass handling standards for Scottish Quality Wild Venison assurance scheme (SQWV)' which stipulates one hour is the maximum time to carry evisceration and in any case if the carcass is bloated, it is not suitable for human consumption.

[60] https://www.food.gov.uk/sites/default/files/media/document/wild-game-guide.pdf

The literature describes various intervals during which evisceration should take place without compromising the safety of the carcass. (Deutz and Fötschl, 2014) advise between 30 minutes and not later than two hours after the animal has been killed. Other authors consider evisceration should not be delayed longer than one hour (Laaksonen and Paulsen, 2015). Finally, (Atanassova *et al.*, 2008) have shown field evisceration within 90 minutes of 'expertly shot' deer resulted in carcass of superior microbiological quality and recommends periodically interrupting the hunt to carry out evisceration of deer already shot (Atanassova *et al.*, 2008), (Ramanzin *et al.*, 2010). Cumulatively these findings suggest that the time interval currently followed by the Scottish stalkers is appropriate. However, if practical circumstances allow it, the shorter the shot-evisceration interval, particularly when the ambient temperature is high, the less concerns for the safety of the carcass.

A further risk arises if the animal has been shot in the digestive tract, as discussed above in section No. 3.2.3, therefore it is important in these cases to eviscerate immediately (Laaksonen and Paulsen, 2015). There is the possibility that following gut shots the animals might not be killed outright and the hunter will have to search for the carcass. This influences the time elapsed before evisceration can take place (Gill, 2007).

The wild deer best Practice guide advises '*if gut contents have spilled into the stomach cavity, attempt to remove as quickly and as thoroughly as possible (this is best achieved by wiping out the worst of the solid content with disposable paper towels or as a last resort by using clean sphagnum moss'*[61]. Both anecdotal evidence and the scientific literature (Avagnina *et al.*, 2012) indicate that some hunters are committed to old traditions such as washing carcasses with water from streams or rivers, or wiping blood and contamination with dried moss, cloths, leaves or grass. Such practices might increase the risk of spread of non-visual contamination or can be a source of contamination, if the water or vegetation is itself contaminated with pathogens from the faeces of deer or other ruminants grazing in the area.

The latter statement is based on the observations of (Synge, 2006) who found that a human case was linked to STEC O157 water contamination from deer faeces. The author confirmed the isolates from the human patient, the water and the wild deer were indistinguishable by *Stx* type and one sample collected from deer had counts as high as $7.5x10^4$ cfu/g STEC O157 of faeces pg. 118 (Synge, 2006). To the author's knowledge, this is the only publication reporting human infections from water polluted with deer faeces in Scotland. A similar report of links between human cases and untreated water sources polluted with deer faeces has been published in US (Probert et al, 2017). In Ireland, deer faeces were identified as a source of pollution of watersheds on farm but no human cases were linked to this (Bolton, O'Neill and Fanning, 2012).

Therefore, contrary to the traditions or the above best practice guidelines, any light contamination of the carcass should always be removed promptly by trimming with a clean knife rather than washing or wiping (see below).

[61] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_gralloch.pdf

However, any carcass which becomes heavily contaminated with stomach contents at the time of kill/gralloching should not be accepted for human consumption. This is furthermore advisable where evisceration takes place in field conditions without ideal means of washing and disinfection, where the hands and equipment of the handler might become heavily contaminated. Lighter contamination with digestive spill should be removed by generous trimming, avoiding use of any environmental or tissue material which might carry contamination in itself. The importance of this step has been stressed by the FSA with regards to the application of the HACCP system in the production of venison meat. Game with contaminated abdominal or thoracic cavities due to "belly" shots or unhygienic gralloching are not to be accepted for human consumption (Food Standards Agency, 2008). The SQWV guideline also advises rejection of heavily contaminated game, although this is not quantified. Importantly, the afore mentioned venison quality scheme captures that *E coli O157* is most commonly found in the last segment of the rectum (Chase-Topping et al., 2008) and great care must be taken to minimise the contamination arisen from this area.

Even if done promptly, gralloching is a very critical step in venison production and inadequate skill and lack of hygiene can greatly influence microbial contamination of the carcass throughout the chain (Gill, 2007,Paulsen, 2011). In a study undertaken by Scotland's Rural College (SRUC) on behalf of the FSA, wild game stakeholders acknowledged the greatest risk factors involved in wild game handling relate to procedures after killing, given that hygienic standards are difficult to sustain in the wild (Lamprinopoulou *et al.*, 2012).

Environmental conditions in which hunting takes place may pose further challenges that could undermine venison quality. These include: dressing in boggy terrain or in adverse weather conditions, dressing in poor light conditions and dressing where there is restricted access to potable water (personal communication and personal observation).

Inspection after shooting

If the animal is to be supplied to an AGHE, one member of the shooting party is required to hold a recognised licence, which confirms them as a trained hunter who has knowledge on the handling of game and sufficient knowledge of pathology to carry an initial examination of the carcass on the spot and produce the hunter's declaration which confirms the game is safe for human consumption (FSS, 2015).

The inspection is an opportunity to remove any game that is obviously unfit for human consumption. This might include severely damaged/contaminated animals, underweight/emaciated animals or those displaying obvious pathological conditions.

Following assessment of the carcasses, the remaining viscera will be removed and inspected. If no abnormalities are found, the hunter discards these as well as the head and completes the 'hunter's declaration' (Appendix 6). Otherwise, in the event that the trained person is unexpectedly unavailable to complete the declaration or if abnormalities are found, the head (except for antlers and horns) and the heart, lungs and liver, but not the stomach and intestines of deer, must accompany the body to the

larder or AGHE, where inspection can take place by a trained person available at these sites (as per Regulation (EC) No. 853/2004, Annex III, Section IV, Chapter II) and (FSA, 2011)).

4.4. Transport of game carcasses to the larder or AGHE

4.4.1 Legal requirements

If wild game is intended for supply to an AGHE, the hunters, or any other operators involved in the transport, act as primary producers and thus must be registered as food business operators with the Local Authority and comply with the Hygiene requirements as laid in Annex I of Regulation (EC) No. 852/2004 as well as Regulation (EC) No. 853/2004, Annex III, section IV, which concerns the initial handling of wild game intended for subsequent supply to AGHEs.

Good hygiene practices start as soon as the animal is despatched and initial preparation (bleeding) commences. Irrespective of the method of transport (by dragging, by ponies or loading into vehicles), this operation should prevent, as far as possible, any contamination and deterioration.

When working from registered premises and supplying to AGHEs, the vehicles used to transport carcasses must be considered as part of the premises registration (FSA, 2015b).

The transport link of the food chain is overseen by local authorities up until the meat or carcasses are transported to the AGHE. After this stage the meat is under the supervision of Food Standards Scotland (FSS) and Food Standards Agency (FSA elsewhere in the UK). The control and safety practices along the transport chain are also self-regulated by the business operators, in line with the existent domestic standards advised by the competent authorities in the UK, cross-referencing the regulations. These below guidelines are available to all food operators:

1. FSS Meat industry guide[62]:
2. FSS Wild game guide, outlining the legal responsibilities applicable to the venison chain[63]:
3. FSS Wild game guide to transportation and storage with photos[64]

Recovery of wild deer from the field to the larder is not specifically addressed by the legislation but the mentioned guidelines produced by Food Standards Agency (FSA), including transport requirements applying to hunters who are regarded as primary producers. The supervision provided by the local authorities to larders and engagement with hunters is risk based and often limited. This has been discussed to be due to lack of resources at the local authority level and uncertainty over the extent of their authority (Lamprinopoulou *et al.*, 2012) which might have negative impact on the safety and

[62] https://www.foodstandards.gov.scot/business-and-industry/safety-and-regulation/approval-of-meat-plants/meat-industry-guide

[63] https://www.food.gov.uk/sites/default/files/media/document/wild-game-guide.pdf

[64] https://www.foodstandards.gov.scot/downloads/Wild_Game_Guide_Photo_Annex.pdf

quality of the meat.

According to Best Practice guidelines: '*A vehicle of suitable design should be available to ensure efficient recovery of beasts. This may include vehicles being fitted with appropriate winches or ATVs fitted with capstan winches, where the deer species require it. If ponies are the most suitable means of recovery of beasts, as is sometimes the case in Scotland, care should be taken to ensure good hygiene practices are used nonetheless'*

Any unhygienic handling that takes place during handling in the wild and transport to the larder will have consequences on the safety and quality of the final product since once present on the hide or carcass, foodborne bacteria such as *E. coli*, including STEC O157 can survive at the cold-chain temperatures as low as 5˚C (detailed in section 4.2.1)(EFSA, 2014). Therefore, key aspects of the regulations mentioned above attempt to limit the possibility of bacterial contamination of the carcass, either from the environment or between individual carcasses.

4.4.2 Potential sources of carcass contamination during transport

Frequently it is necessary to extract the red deer carcases manually (by dragging) or on a pony before it is placed in a vehicle. Smaller roe deer are more frequently carried in a roe sac.

Best Practice Guides includes advice on extraction of deer carcasses by vehicle (mechanical extraction) https://www.bestpracticeguides.org.uk/culling/mechanical-extraction/ and hygiene is enclosed in this guide. In case of extraction by pony, or if dragging is involved, no advice is given on how best to carry the carcass to prevent contamination

If dragging of the freshly gralloched carcasses is unavoidable, this will result in an almost unavoidable risk of contamination with grass, leaves, soil, pests or any other environmental factors (Laaksonen and Paulsen, 2015).

Other Best Practice Guidance available to the wild deer sector available from the deer initiative forum, http://www.thedeerinitiative.co.uk/uploads/guides/131.pdf recommends limiting any contamination by using a "drag bag", or 'sleds' into which the deer can be placed after evisceration, preventing direct contact with the ground. Whilst for roe deer this is possible, in the case of red deer this may be challenging for many deer hunters as this poses health and safety concerns when carrying a large carcass down the hill. Additionally it can be costly and few materials withstand the effect of dragging a heavy carcass over a rocky terrain. From personal communication with experienced venison stakeholders it is understood that the type of transport for deer carcasses vary, largely depending on the Scottish terrain. It is thought 95% are subject to some form of dragging – even if only to reposition the carcasses for bleeding. To further transport the carcases approximately 15% are put on a pony, 80% are carried by ATV and less than 5% lifted manually by the hunter.

If ponies are the most suitable means for extraction, good hygiene practices should still be applied in accordance with the Best Practice guideline provided online[65]. It would be almost never the case that a carcass is transported by pony before gralloching, as this would mean the time from kill to gralloching would be unacceptable, therefore the stalker and the accompanying party will load the deer on to the clean saddle – a skilled operation avoiding exposure of the abdominal cavity to the environment during transport.

Temperature controls along the food chain

Deer are transported to the field larder or directly to the larder co-located at an AGHE, as soon as the culling has finished. The use of a refrigeration unit during transport from the hill to the larder/AGHE is best practice but not compulsory, especially if ambient temperatures are low or if the journey to chilling facilities is short. Cold ambient temperature are considered to be those below 7°C, in line with the European hygiene legislation (EC) No. 853/2004 capturing the desired temperature that the carcass should achieve as soon as logistically possible. For transport from the larder to AGHE, chilling is a HACCP prerequisite to maintain the cold chain that had already been established at the larder. This is the responsibility of the transporter who can be the stalker, a game dealer or a representative of the AGHE who collects the game from remote larders.

Carcass cooling

Usually active chilling begins either at the game larder or the larder attached to AGHE. This largely depends on the remoteness of the hunting place and the proximity of the larder or AGHE.

Given Scotland's cooler ambient temperatures and that the hunting season for female deer largely coincides with colder months, when the temperature is below 7°C, the effect of high ambient temperature can be less of an issue. Regulation (EC) No. 852/2004 states: "*where climatic conditions so permit, active chilling is not necessary*."

However, in Scotland, except at the very coldest times of the year and where storage and delivery times are short, active chilling in the game larder and the use of refrigerated vehicles to transport game from the larder to the AGHE, will be necessary.

The first drop in body temperature is achieved by immediate evisceration. This cooling phase takes place at ambient temperature allowing the evaporation of moisture from freshly killed carcasses and subsequent air drying. If this natural cooling process takes place in colder temperatures, it doesn't compromise the microbial quality of the carcasses (Paulsen and Winkelmayer, 2004). It might be important to point out that once steam and moisture were reduced, prompt chilling should take to increase the quality of the carcass by preventing microbial growth (Deutz *et al.*, 2000). Exposing the carcass for long periods at ambient temperature during the natural cooling phase is likely to have negative consequences with respect to the microbial surface contamination during warmer spring or summer conditions. Paulsen and Winkelmayer,

[65] https://www.bestpracticeguides.org.uk/wp-content/downloads/culling_extractionPony.pdf

(2004) have shown that exposing of carcasses to higher ambient temperature (17.8±1.2 °C compared to 9.8±1.2 °C) influenced the surface bacterial contamination, even when the evisceration was carried out correctly. Time/temperature profile from killing to cooling had an influence on the extent of microbial contamination. Carcasses stored for 12 hours at the higher ambient temperature resulted in a higher median counts for Aerobic Colony Counts and Enterobacteriaceae (5.7 log cfu/cm^2 and 3.5 log cfu/cm^2 respectively) when compared to carcasses stored for the same length of time at the lower temperature (4.1 log cfu/cm^2 and 2.5 log cfu/cm^2, respectively). Subsequent storage of deer carcasses at 0.4˚C prevented additional bacterial growth for a further 96 hour period; However, the differences in bacterial counts persisted between carcasses initially stored at 17.8±1.2 °C and 9.8±1.2 °C (Paulsen and Winkelmayer, 2004)(Paulsen, 2011). These findings demonstrate the importance of a continuous cold chain.

Although bacterial activity on meat at chill temperatures is generally regarded as a surface phenomenon, at warmer temperatures of 20-30° C equivalating to body warmth, it has been reported that proteolytic bacteria can penetrate into meat muscle fibres to depths of 0.2-0.4 cm through the production of proteolytic enzymes, released during the bacterial growth (Gill and Penney, 1977). Once the meat surface and superficial muscle layer becomes contaminated, cross-contamination of other clean meat might be possible at any time during storage, processing and cutting.

The EU Hygiene Regulations require carcass chilling as soon as reasonably possible; however, a specific time/temperature frame from killing to cooling that would deliver a reasonably safe product is not specified. Regulations stipulating temperature and time requirements relating to carcass chilling are flexible enough to take the practicalities of the primary production process into account, mainly related to extraction and handling of carcasses during transport.

Austrian domestic guidelines indicate that kill to refrigeration time should be no longer than 12 hours (Paulsen, 2011), which in practical settings is a feasible requirement to meet. The Australia and New Zealand Food Regulation Ministerial Council Food Regulation Standing Committee (2007) specifies that a wild game animal carcass should be placed under refrigeration within two hours of being harvested. The temperature of the carcasses must be reduced to 7°C as soon as possible, but not later than 24 hours after being placed under refrigeration, unless delivered to the processing premise within this time period. The temperature of the carcass must be below 7°C on arrival at the processing premise. The Meat Safety Act No. 40 of the Republic of South Africa (2000) part V, section 11.(1)(h), part 67, requires partially dressed carcasses and offal to be chilled within 12 hours of culling to a temperature not exceeding 7°C, however, when the ambient temperature is higher than 15°C, it must be chilled within four hours (Bekker, Hoffman and Jooste, 2011).

The key factor for hygienic standards of wild game is establishing a cold-chain that is practicable and feasible but still delivers a safe product. If the cold-chain is not attained as soon as possible, this will enable bacterial growth and multiplication. Therefore, the earlier chilling commences, the more favourable microbiological counts are achieved. Once established, the cold chain must not be interrupted to prevent bacterial growth

during these intervals, as reflected by the scientific opinion on public health risks related to storage and transport of meat of domestic ungulates released by the European Food Safety Authority (EFSA, 2014). This opinion clarifies that total bacterial growth is affected by maintenance of the chilling chain during transport, in the slaughter plant, deboning, storage, retail and catering/domestic refrigeration.

A Scottish survey carried between 2011 and 2012 by Lamprinopoulou *et al* (2012) reported that carcasses might be left outside overnight in a holding area after a long day on the hill to slowly cool down at ambient temperature before being transferred to central refrigerated storage the next day. Other hunters after shooting red deer in a remote glen may be forced to leave the gralloched carcass on the hill overnight to be picked up the following day(s) (Lamprinopoulou *et al.*, 2012). From personal communications with the venison stakeholders, it is understood that although this practice is not common, sometimes hind carcases in particular may be left on the hill if there are numbers to collect, but only in very low temperatures which are more common at the time of the year when deer female hunting takes place.

Overall key risk factors widely recognised by wild game industry stakeholders in the survey as necessary to prevent proliferation of contaminating organisms to dangerous levels were: hygiene during transportation and storage, and establishment and maintenance of the cold chain (Lamprinopoulou *et al.*, 2012). Regulation (EC) No. 853/2004 requires a maximum storage temperature of 7˚C for large game. However, to keep microbial load under control, it might be necessary to store the carcass at a much lower temperature, closer to 0°C, the rationale of which has been lately confirmed on microbiological studies of carcass and meat vacuum packs (Paulsen, 2011).

Large wild game, including deer must not be frozen before skinning to avoid rapid growth of bacteria during the later thawing process (Deutz and Fötschl, 2014). Therefore, transport operators should check that game is chilled, at temperatures above 0 and below 7˚C, when collected from the primary producer and delivered to the AGHE.

Transport procedures

According to Best Practice guidelines[66], hunters must ensure that transport and associated equipment are in good working order, well maintained, and regularly serviced and cleaned. All carrying areas should be made of impermeable material which enables cleaning and disinfection before and after use with food-safe products and with facilities where bleeding knives can be cleaned and sterilised with water above 82°C or an alternative chemical sterilisation method.

Some transportation practices can increase the chance of carcass contamination, especially if the carcasses are laid in crates and not hung. Preferably carcasses will be carried in a dedicated, separate part of the vehicle, away from dogs, fuel, mud, dust, water and all other potential contaminants. The carcasses should be transported covered to prevent exposed meat from becoming contaminated, however plastic bags should not be used as this type of material creates hermetic sealing, trapping heat and

[66] www.bestpracticeguides.org.uk

moisture thus risking to compromise the maturation (Deutz and Fötschl, 2014). A close-fitting net can be useful for protection against both flies and dirt from the wheels of the vehicle (Laaksonen and Paulsen, 2015).

Heaping of deer carcasses during transport, is prohibited by the Regulation (EC) No. 853/2004, Annex III, Section, IV, Chapter II, Paragraph 6. Heaping delays carcass cooling, particularly for carcasses in the middle of the heap, due to the insulating effect from the surrounding bodies. Equally, heaping of carcasses can also allow cross-contamination between carcasses. The vehicle used would ideally be fitted with a hanging frame so the carcasses are transported in such a way that are not touching each other and so that air can freely circulate around them to enable temperature drop and moisture loss. Heavy carcasses, such as red deer should never be transported or later stored lying flat as it will prevent warmth and humidity escaping the carcass, which will affect the quality of meat maturation during storage (Deutz & Fötschl, 2014)(Laaksonen and Paulsen, 2015) as well as increasing the chance of cross-contamination. Heavy animals should be transported either suspended, or as an alternative, stored lying on washable plastic pallets to allow air circulation and body fluids to drain.

The controls necessary in transport throughout the food chain to achieve safe wild venison were described by (Bekker, Hoffman and Jooste, 2011) as adapted in Table 3. These were linked to key requirements of the European Hygiene Regulations No. 852/2004, Annex II, Chapter outlying general hygiene requirements for all food business during transport, European Hygiene Regulations No. 853/2004, Annex III, Section IV concerning meat of wild game and compared to the national guidelines, as listed below (FSA, 2008), (FSA, 2011), (FSS, 2015) to draw a parallel with the UK wild game guidelines (Table 4.2):

- Food Standards Agency (2008) *HACCP guidance for those producing wild game meat for human consumption either at an approved game handling establishment or under exemption allowed by the food hygiene regulations* https://www.foodstandards.gov.scot/downloads/HACCP_Guidance.pdf
- FSA (2011) *A guide to the hygiene regulations for people who shoot wild game and supply it in-fur or in-feather or as small quantities of wild game meat.*: http://www.food.gov.uk/foodindustry/meat/guidehygienemeat
- *The Wild Game Guide*: https://www.food.gov.uk/sites/default/files/media/document/wild-game-guide.pdf

Table 4.2 *Key aspects of hygiene controls that apply during transport along the food chain (those which apply at different steps are annotated with 'Y'). The controls are referenced with appropriate EC Hygiene Regulations No. 852/2004, 853/2004 and where mentioned by the above mentioned guidelines produced by the Food Standards Scotland they were annotated with 'Y'*

Controls	**Field to vehicle**	**Transport to larder**	**Transport to AGHE**	**Further distribution**	**Legislation**	**FSS Guidance 1-3**
If the carcass is to be transported with the hide on, it must be protected from contamination by packing or placed in suitable clean pallet or container that enables fluid drip and air flow	Y	Y	Y	NA	Regulation (EC) No. 852/2004 Annex II Ch. IV p6 Food stuffs in conveyances and/or containers are to be so placed and protected as to minimise the risk of contamination Regulation (EC) No. 852/2004 Annex II Ch. IX p3 At all stages of production, processing and distribution, food is to be protected against any contamination likely to render the food unfit for human consumption, injurious to health or contaminated in such a way that it would be unreasonable to expect it to be consumed in that state	N
Protection of the neck bleeding/rodding wound and abdomen cut where evisceration was carried on the field	Y	Y	Y	NA		N
Protection of thoracic cavity, the head and feet cut area	As applicable	Y	Y	NA		N
The hide-off carcass/meat must be packed in suitable clean, pest proof containers to protect it against contamination	NA	NA	NA	Y	Regulation (EC) No. 852/2004 Annex II Ch. IX p3 At all stages of production, processing and distribution, food is to be protected against any contamination likely to render the food unfit for human consumption, or contaminated in such a way that it would be unreasonable to expect it to be consumed in that state Regulation (EC) No. 852/2004 Annex II Ch. IV p2:	Y

					Receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs where this may result in contamination.	
Boxes used (polyethylene type or stainless steel) to transport carcasses/ meat should have a false floor to enable blood to drain and keep them above the level of the blood.	Y	Y	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. IX p2: Where conveyances and/or containers have been used for transporting anything other than foodstuffs or for transporting different foodstuffs, there is to be effective cleaning between loads to avoid the risk of contamination.	Y
Protect meat/carcasses from pests	Y	Y	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. IX p2: Adequate procedures are to be in place to control pests.	Y
Keep the time of kill to refrigeration as short as possible	Y	Y	Y	NA	Regulation (EC) No. 852/2004 Annex II Ch. IX p 5: Products likely to support the reproduction of pathogenic micro-organisms or the formation of toxins are not to be kept at temperatures that might result in a risk to health. The cold chain is not to be interrupted. However, limited periods outside temperature control are permitted, to accommodate the practicalities of handling during preparation, transport, storage, display and service of food, provided that it does not result in a risk to health.	Y
Provision of refrigerated unit able to maintain the temperature below 7°C	NA	Not for short distances	Y	Y		Not clear
Do not mix carcasses/meat at different temperatures	Y	Y	Y	Y		N
Measure and record temperature of carcass and meat during loading and off loading	NA	Y	Y	Y		Y

Equip vehicle with a temperature control device that will monitor the temperature continuously	NA	Not for short distances	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. IX p5: The cold chain is not to be interrupted. Regulation (EC) No. 852/2004 Annex II Ch. IX p 7: Where necessary, conveyances and/or containers used for transporting foodstuffs are to be capable of maintaining foodstuffs at appropriate temperatures and allow those temperatures to be monitored.	N
If the meat was chilled and is transported, it needs to be kept refrigerated	NA	Y	Y	Y		N
The vehicle should be provided with hanging rails or alternative system to avoid contamination	Y	Y	Y	NA	Regulation (EC) No. 853/2004 Annex III Sec IV Ch. II p6: During transport heaping of large wild game must be avoided if hanging is not available, it may be possible to be placed on a clean surface, but not heaped	Y
Appropriate design of the internal structure of the vehicle to enable cleaning, disinfection between loads and prevent contamination	Y	Y	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. IV p1: Conveyances and/or containers used for transporting foodstuffs are to be kept clean and maintained in good repair and condition to protect foodstuffs from contamination and are, where necessary, to be designed and constructed to permit adequate cleaning and/or disinfection.	Y
The vehicle and containers used for transport must be cleaned between loads using appropriate and approved sanitisers and kept maintained	Y	Y	Y	Y		Y

The vehicle should be provided with water and appropriate facilities to disinfect knives and other equipment	Y	Y	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. V p(1a), 2 All articles, fittings and equipment with which food comes into contact are to be effectively cleaned and, disinfected. Cleaning and disinfection are to take place at a frequency sufficient to avoid any risk of contamination	Y
Suitably manufactured polyethylene or stainless steel type box for vehicles which don't have purpose built washable vehicle liners.	Y	Y	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. IV p2: Receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs	Y
Non-edible or contaminated products shall not be transported in the same compartment with carcasses; This might include other raw materials, chemicals, feathered game, dogs, rejected products etc	Y	Y	Y	Y	Regulation (EC) No. 852/2004 Annex II Ch. IV p6: Food stuffs in conveyances and/or containers are to be so placed and protected as to minimise the risk of contamination Where conveyances and/or containers are used for transporting anything in addition to foodstuffs or for transporting different foodstuffs at the same time, there is, where necessary to be effective separation of products.	Y

4.5. Handling of carcasses at collection larders

Game larders are any premises (static or mobile) where killed wild game can be kept prior to being transported for further preparation at an AGHE or direct distribution. The primary use of a larder is to provide initial cooling of the game and to provide temporary storage to hang carcasses under hygienic, vermin and fly-proof conditions prior to despatch. As such, a larder should have sufficient capacity for throughput, protect game from contamination, be cleaned and disinfected, and maintain the cold chain.

The time period of storage can vary on the remoteness of the hunting ground and on the time of the hunting season but it is estimated that shot game will spend several days in the Scottish larders prior to dispatch to an AGHE (Radakovic and Fletcher, 2011).

Carcass preparation

Once visually assessed by the Food Business Operator (FBO) of the larder, collocated or not with AGHE, the carcasses are registered into the intake records, before undergoing further preparation. This involves removal of the head at the atlanto-occipital articulation and the distal part of the legs, with a cut at the carpus-metacarpus and tarsus-metatarsus articulation. The suitably labelled and identified head, with the tongue, must be available for inspection. The head and the distal parts of the legs are usually removed during the larder stage in adequate rooms, according to Regulation (EC) No. 853/2004. The red pluck, if it wasn't already removed on the hill, is removed at this point by cutting the brisket to open the thoracic cavity.

Opening of the thoracic cavity and head, legs removal must be carried under the supervision of the Trained Hunter. This is followed by storage in the larder together with other carcasses from other hunters, including other species, and there will have to be adequate separation between unskinned deer and other game (Regulation (EC) No. 853/2004, Annex III, Section III, Chapter II, p 8 (a)).

The factors that could influence the microbial condition of the carcasses during storage essentially concern:

Length of time spent in the larder

Some carcasses might be stored for one or more days at the larder and later transferred to an AGHE for inspection, health marking and further processing, unless they are intended for private consumption. The length of storage depends on how many carcasses are collected to make it economically feasible to send a refrigerated vehicle to the processor, on the remoteness of the larder and the proximity of AGHE.

The length of time that carcasses can be kept unskinned before further dressing and cutting is not included in the national guidelines. Form the information collected through stakeholders during the document write up, the storage interval for roe and red deer carcasses, skin on ranged from 1 day to 18 days. Whilst meat surfaces

remain protected by the hide, if stored hygienically, at below 7°C (Gill, Penney and Nottingham, 1978), putrefactive changes by spoilage bacteria can occur until desiccation of the meat surface reduces water activity (aw) to below 0.95 (Mills *et al.*, 2015). Regulation (EC) No. 854/2004 (Section IV, Chapter VIII, A, and Section IV, Chapter VIII, B) requires that a carcass showing putrefactive changes is to be declared unfit for human consumption. It is known that the ageing of small cervids takes place in approximately 10-15 days and provided that the storage hygiene condition has been rigorous, large game animals can be hung for a total of up to 20 days (Laaksonen and Paulsen, 2015).

The risk of *E. coli* (and other faecal contamination) will depend on whether or not the gut has been perforated by the shot (Atanassova *et al.*, 2008) and whether environmental soiling or faeces contaminate the carcass. It is assumed that carcass microbial content can become exponentially higher with the length of storage, especially if there are breaches in the cold chain or if trimming of such contamination will not have been carried out at the beginning of the larder stage.

Storage temperature

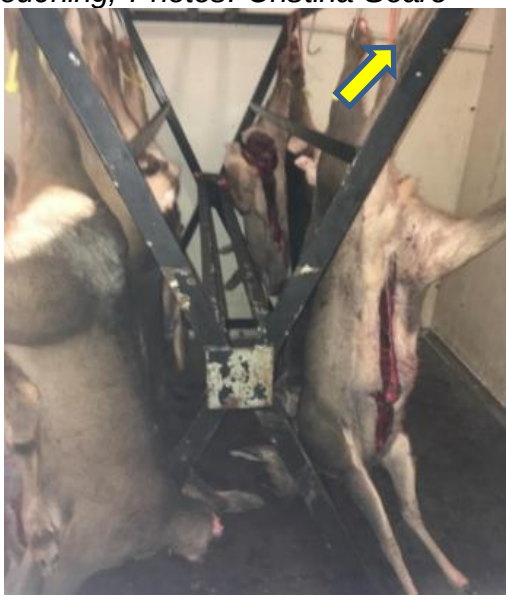
When new carcasses are brought into the larder the temperature may rise; however, any rise in temperature can be reduced if the carcasses had already cooled during transport and the chiller ventilation is efficient. A hygienic risk might arise in smaller cold storage facilities, when fresh warm game is hung close to already chilled carcasses, especially if in combination with insufficient ventilation. The damp air from the warm body could condense, increasing the moist film on the surface of the chilled carcasses, which in turn will provide good conditions for microbial growth (King *et al.*, 2016). Therefore, it is recommended to combine a short pre-cooling phase achieved by exposing the carcass to low ambient temperatures and ventilation, if the season permits with professional cooling facilities which enable appropriate ventilation and steam extraction, removing moisture effectively (Paulsen, 2011). Temperature should be checked regularly when carcasses are kept in the storage area and before they leave the larder to make sure the cold chain has not been interrupted and it's below 7°C as per Regulation (EC) No. 852/2004.

Space and handling hygiene

The carcasses should be stored in a suspended position (Fig. 4.5), enabling effective separation between hides, to avoid cross-contamination. Other good storage hygiene outlined in Regulation (EC) 852/2004 dictate avoidance of touching areas such as floors and walls (Fig 4.5 right hand side photo), not only at the larder but throughout the food chain. It is important that during preparation of the carcass, the outside of the hide, and the hands and equipment of the workers never touch the muscle surfaces on the linings of the cuts.

For adequate hygiene, in line with Regulations (EC) 852/2004, the equipment used must be of a good condition and made of a fabric that enables regular washing and disinfection. For instance, the rope used for hanging the carcasses in Fig. 4.5 (left hand side) cannot be cleaned and sterilised, and therefore might be a source of contamination (fomite) if reused for other carcasses; the rack is also not of a suitable construction to allow good cleaning between different batches of animals.

Fig 4.5 *Left: carcasses stored in a small country larder. Left: arrow indicates material which cannot be cleaned and disinfected used for hanging the carcasses and rack unsuitably maintained. Right: arrows indicate evidence of blood prints from wall touching; Photos: Cristina Soare*



4.6. Carcass processing at AGHEs

4.6.1 Skinning

Technique

For more clarity to the aspects discussed below in the skinning technique and further in the report with regards to the meat cutting at AGHE, Fig 4.6 below captures a deer diagram chosen for demonstration purposes to offer a representation of the different anatomical and meat inspection terms covered in this section.

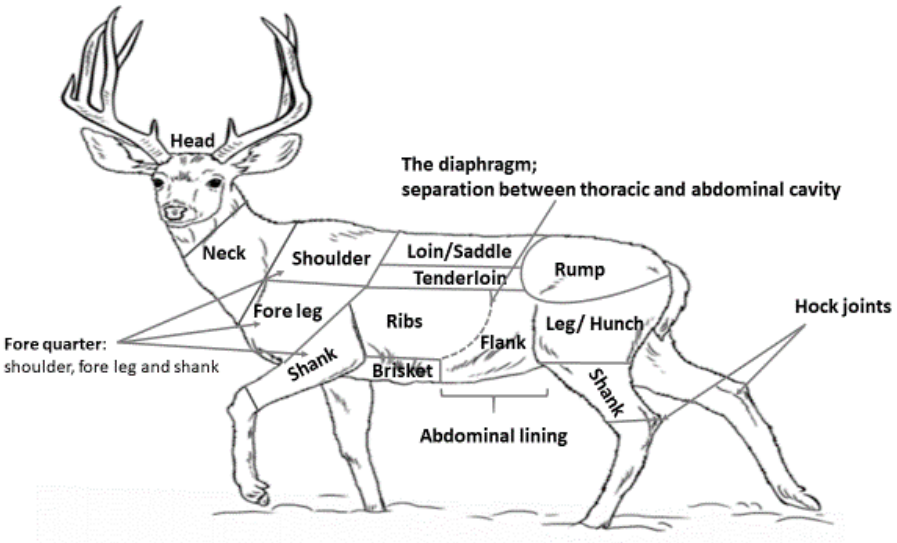


Fig. 4.6 *Anatomical body parts and meat cuts of deer carcasses*

Wild deer are skinned using the same techniques as adopted for domestic livestock. The good handling practices dictate removing hide without contact between the outer surface (e.g. hair), equipment and the freshly exposed muscle and fat on the carcasses.

Most AGHEs perform skinning of the carcasses in a vertical position, whilst suspended on two hooks from the hind legs at the hock joint. The meat is exposed by cutting a small piece of hide (fold cut) to produce a portal of entry and the cut is continued with another clean knife, from the inside toward the outside followed by manual pulling. The knife blade is positioned outward, from under the skin out, to prevent the contaminants from the hide and the hairs from getting into the meat. This prevents cutting through hair and keeps loose hairs from getting on the exposed meat surface.

The cutting of the hide occurs on the abdominal midline which had been already exposed during evisceration. The skin on the legs is opened by lengthwise, midline incisions on the inner part of the limbs. The skinning starts from the interface of the elbow and hock joints.

The hind legs and the rear part of the carcass is skinned first and the fore front of the carcass at the end, to avoid contamination of meat on the lower part of the carcass when kept in a suspended position.

The rest of the hide is removed by loosening the subcutaneous tissue with a knife and manually pulling from top to bottom. On the sides of the carcass, one hand is used to hold the skin and to apply tension and the other hand is used to break through the connective tissue between the skin and the carcass by either cutting the connective tissue or by using the fist to push the skin away from the carcass. The skinning procedure, unavoidably creates aerosols from the dried material on the skin (if any blood, dirt) and loose hair, especially if the animals had been shot during the moulting period. An alternative skinning method is by using compressed air insufflation under the hide, and subsequent pulling, either manually or with an automatic hide puller. For the second skinning technique, the automation of hide removal reduces contamination since there is less handling of the carcass and less use of knives, although the airborne contamination might still be unavoidable.

Potential risk factors

As well as gralloching, skinning is another important step in ensuring the hygiene and quality of meat. As mentioned before, it is likely that muscle tissue and offal from healthy animals will remain sterile when protected by the hide (Gill, 2007, Gill and Penney, 1977). However, it is widely known that hide itself can harbour a number of microorganisms. For livestock animals, the significance of microbial contamination from the hide has been always a matter of concern and the same will apply to game animals (van Schalkwyk, Hoffman and Laubscher, 2011).

Hide becomes contaminated when animals lie in their own or other's faeces, touch each other during allogrooming (social grooming between members of the same species which is common in ruminants) or via environmental dust (Bell, 1997). It can also be inferred that hide may become contaminated in the field conditions from the

environment (Singh *et al.*, 2015) (i.e. sharing pasture with cattle or other ruminants, during the gralloching process, or during transport (carcass touching or dragging).

Microbial contamination of the carcasses have been associated with potential risk factors such as a visually dirty hide (Obwegeser *et al.*, 2012, Avagnina *et al.*, 2012). Indeed, unhygienic skinning has been highlighted as a possible source of contamination in general, irrespective of whether the hide is visually contaminated (Casoli *et al.*, 2005, Gill, 2007, Atanassova *et al.*, 2008, Membré, Laroche and Magras, 2011).

Gouws and colleagues observed that swabs taken from the carcass in the field, following killing and evisceration were negative for *E. coli* contamination; however, later, immediately after skinning at the AGHE, up to 42% carcasses displayed *E. coli* contamination on their freshly exposed surface ranging from 2.2 log cfu/cm^2 to above 2.5 log cfu/cm^2 (Gouws, Shange and Hoffman, 2017). This was higher than the value described by Paulsen (2011) as acceptable for meat and carcases of deer. These authors postulated that presence of *E. coli* bacteria on the surface of the carcasses could have been attributed to improper execution of the skinning technique (Gouws, Shange and Hoffman, 2017).

This is based on previous observations made on cattle and recognition that faecal contamination from the hides via direct contact between animals, contact with staff hands or tools or indirect aerosol shedding is a key and likely event (Antic *et al.*, 2010, Blagojevic *et al.*, 2011).

Studies investigating the prevalence of hide contamination in deer are lacking and therefore we have considered below the data available on cattle, a similar ruminant species, although it is acknowledged by the authors of this report that deer might have a different microbial status of the hides. In a study performed at three beef plants in Midwestern part of the United States, it was found that hide prevalence of *E. coli* O157:H7 was 10 times higher than faecal prevalence (60.6% vs. 5.9%) (Barkocy-Gallagher *et al.*, 2001). This proportion seems to be in line with results of a Scottish study carried out between 2002 and 2004 which observed that 5% of the farm faecal samples were positive for *E. coli* O157, and 55% of the cattle from the same farms had contaminated hides at the time of slaughter. Interestingly, less than 1% of carcasses obtained from these animals were positive for *E. coli* O157 (Mather *et al.*, 2008). Another study suggested that between 0.003 and 1.6% of *E. coli* transfers between contaminated hide to the exposed carcass (Antic *et al.*, 2010). Other authors found that cattle carcasses positive for *E. coli* O157 were a percent of 21.8-23.1 % of animals with *E. coli* O157 on the hide transferring to the carcass, depending on the skin microflora of the animals but also on the good handling and process hygiene practices adopted by the abattoir (Blagojevic *et al.*, 2011).

Given that each animal species has a different biology and that venison production uses different practices than those involved in cattle slaughtering, it cannot be quantified what percent of positive carcasses will result from animals with E coli O157 positive skins. However, if *E coli* thrives on the skin/hide of deer, the same way as it does on cattle, the above data suggest that in principle transfer from the positive skin/hide to the carcass is possible during the skinning process.

It has been observed that factors which contribute to cross-contamination from cattle hides to exposed meat are aerosolisation of particles produced by higher pressure involved in the process of skinning (from manually pulling the hide or from compressed air pressure) as well as the presence of wet hides (Antic *et al.*, 2010). Due to the practicalities of hunting and extraction, deer hides might be contaminated with dried soil, faeces, blood, all of which could produce aerosols during the skinning procedure. Immediately after skinning the exposed deer carcasses are moist and prone to catch aerosolised particles. A compounding problem is that deer hides are removed from cold carcasses which are more rigid thus more difficult to skin than warm carcasses (as is the case with cattle), and could more readily lead to cross-contamination through aerosolisation. Therefore, attention should be paid to the roughness with which hide is handled and pulled in order to limit aerosols and subsequent cross-contamination of exposed meat. The risk of contamination of the freshly exposed carcass surface with hair is also enhanced by the presence of fur, more likely at the transition between seasons when animals are changing the coats (April/May and Sept/Nov for red deer and April/May and Sept/Oct for roe deer), some of these periods coinciding with the hunting seasons.

Skinning is considered a 'dirty' process and, as in livestock species, it should be done in an isolated area or ideally in a separate room from where exposed carcasses are being handled.

Contact between carcasses, contact with hands and tools and/or airborne contamination have been identified as risk factors for microbial carcass contamination in cattle (Barkocy-Gallagher *et al.*, 2001). This suggests that hygienic storage of both hide-on carcass and hide-off carcass with sufficient space is likely important in wild species too, as well as ensuring hygienic contact with surfaces. Cleanness of work clothes, knives, hooks and working surfaces such as stands and tables is a requirement of Regulation (EC) No. 852/2004 and a prerequisite to the HACCP plan.

The FSA/FSS made available detailed guidance to the meat industry sector, including to the AGHE on cleaning procedures and how the expected hygiene standards should be met to avoid cross-contamination. This binding guidance is available in the Meat Industry Guide. Compliance with set standards are important to ensure the environment is not concussive of bacterial contamination given that *E. coli* is able to survive on meat and surfaces even at chill temperatures (King *et al.*, 2016) and incorrect practices could lead to contamination.

The hygiene Regulation (EC) No. 852/2004 gives flexibility to the food business operators to design bespoke HACCP plans and prerequisites system that meet business needs to the extent to which the food business operator is confident the hazards are under control and the final product is safe to eat. Therefore, the operator needs to demonstrate ability to control the product from the intake to dispatch. This could translate into checks to ensure the carcasses are in a good condition when delivered by performing visual assessment and taking corrective actions should any contamination be identified. This might involve liaising with hunters/larders who are regarded by legislation as primary producers and have to follow the same requirements of Regulation (EC) No. 852/2004, including HACCP controls. For

verification purposes, there are no testing requirements specified in law for wild game carcasses, however some businesses voluntarily decide to test for Aerobic Colony Counts and/or Enterobacteriaceae, especially if their customers encourage microbial verification practices.

4.6.2 Carcass dressing

The carcass is dressed by trimming the desiccated or visibly contaminated areas, which tend to affect the exposed cuts: hock, neck, the brisket and the abdominal lining (authors' personal observations). Given the scope of the process to remove most of the visual contamination which might otherwise harbour harmful foodborne pathogens, the hands must be clean, knives sterilised and the operatives must be aware of reducing contact with the carcasses to avoid blood stains or cross-contamination with the hands. The wound area may be heavily contaminated with bacteria triggered by the bullet from the hide and therefore, accurate trimming of the meat around wounds and bullet pathways removes potential microbiological and chemical lead contamination (Dobrowolska and Melosik, 2008). Trimming of the shot wounds will also remove the blood debris and/or bruised meat which is known to spoil and decompose more rapidly (Cruz-Monterrosa *et al.*, 2017).

There are publications which describe that, if necessary, water can be used to wash the abdominal and thoracic cavity but not the external surface of the carcass as this might lead to a slimy surface (Laaksonen and Paulsen, 2015). However, this seems to conflict with other recent observations where survival and growth of *E. coli* O157 has been seen to be more negatively affected by desiccation and subsequent reduction of bacterial water activity during carcass chilling than by stress conditions generated by simple cold temperature treatment (King *et al.*, 2016). In agreement with this observation, also (Visvalingam, Liu and Yang, 2017) describe that dry chilling significantly decreases *E. coli* O157:H7 which would suggest that water should never be used to clean any part of the carcass as it might affect subsequent evaporation and removal of excess moisture which in turn might prevent the reduction in water activity and subsequent cellular death of the bacteria or even contribute to its recovery.

Visual assessment of carcass cleanliness

Studies in cattle have demonstrated that higher microbial contamination of the hide-off carcass is found at sites that correspond to the hide opening cuts (Bell, 1997). This can be also true for a deer carcass, since most visible contamination is seen on the neck (hair and dried blood), brisket (desiccated meat and soil contamination) and abdominal area (faecal material) and pelvic symphysis (hair). This is based on authors' personal observations and some examples of common sites of contamination are included in Fig. 4.7. Another contamination-prone area might be the pelvic cavity, from the operation involved in rectum cutting. In cattle the terminal rectum contains the highest levels of STEC O157 (Chase-Topping et al., 2008) and it is not known whether the same is applicable to deer. However, to pre-empt the possibility of STEC O157 distribution to the carcass, it might be good practice to handle the rectal area as if it would be a major source of STEC O157 contamination.

Based on the discussions with industry stakeholders, it is known that removal of the rectum is variable between deer species. Gralloching of smaller deer is usually carried with total removal of the rectum on the hill. In larger deer, approximately 10 cm of the rectum is knotted and left in the carcass to be removed later at the larder once the pelvis symphysis is cut open. Although at the time of gralloching the rectum is emptied of stool and cut in front of a tie that has been placed to prevent content of the rectum from entering the pelvic cavity, this practice involves breaking the rectum which might still result in some faecal contamination when cutting it from the rest of the intestine or it is possible that some of the STEC O157 bacteria, if present in the remaining rectum, might cross the lining of the portion left in the pelvic cavity and transfer to the carcass, as seen possible for other enteric bacteria (Gill and Penney, 1977). Therefore, if the field conditions allow it, it might be safer to remove the entire rectum in an intact condition, without cutting it from the intestine immediately after kill.

The concerns of the Deer Initiative guidelines[67] are that pulling the cut rectum and anus '*backwards out of the pelvis not forwards through it exposes more of the carcass to potential contamination than keeping the rear end intact and dealing with it in the larder.'* To address this concern a possible technique would be to extract the intact anus and rectum through the abdominal cavity once these have been cored out and protected by a plastic bag sleeve tightened with rubber band, similar to the cattle evisceration technique. Alternatively, if the larder is in close proximity, gralloching, including rectum removal could be carried out hygienically at this facility.

Due to the slaughter conditions it is possible that deer carcasses might still show some visible faecal or other contamination at the stage of trimming and dressing at AGHE. Affected areas are removed by trimming before official inspection, according to HACCP monitoring and corrective action arrangements established by the FBO at the AGHE, in line with the guidance provided by the FSS/FSA. The FBO keeps trimmed parts in a separate tray correlated with the carcass to be presented together for inspection. Official inspection pays particular attention to contamination especially associated with gralloching (green offal removal), around the pelvis, sternum and cut flanks (FSA/FSS, Manual for Official Controls).

[67] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf

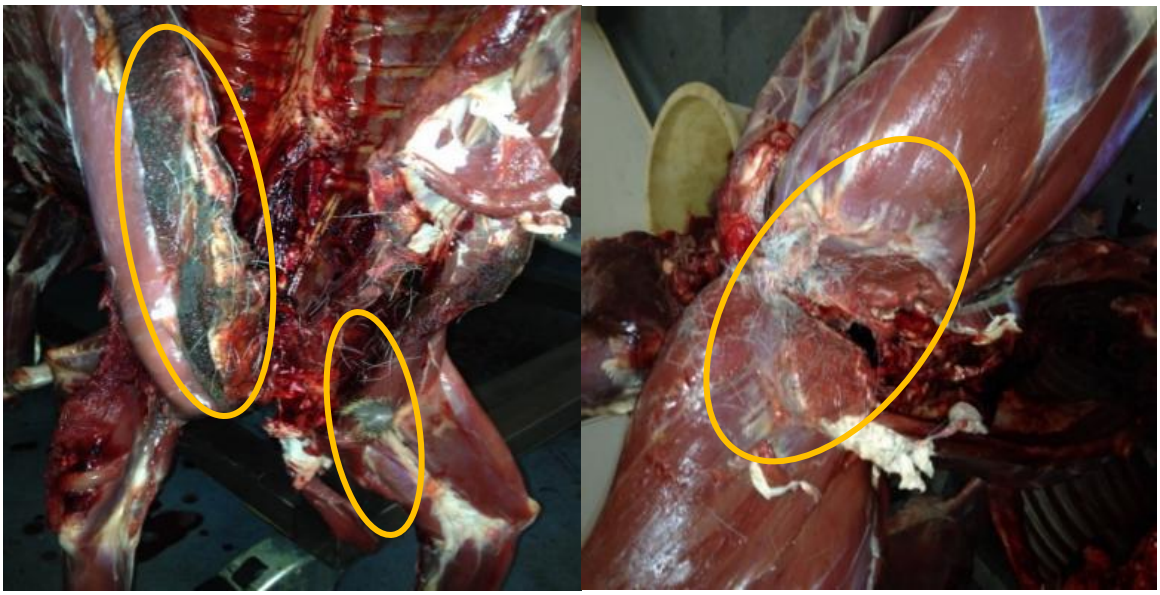


Fig. 4.7 *Carcass presented for inspection showing evidence of contamination. Left: contamination from dragging. Right: contamination from skinning. Photos: Cristina Soare, 2014*

Trimming is a valid control (Fig. 4.8) only when the carcass is not heavily contaminated with digestive content or if the meat does not show green discoloration, indicating colour tinting due to delayed evisceration or discoloration as result of putrefaction process. Any carcasses that are badly damaged and/or contaminated should be rejected as advised in HACCP guide (Food Standards Agency, 2008) since the affected areas will carry a higher microbial load. Shoulders can be opened to remove the haemorrhagic areas that might have been produced by the shot and to improve the chilling of the forequarter. However, this operation should be done up to an extent that prevents forming a pocket between the shoulder and front of carcass which creates a favourable environment for microbial growth (Fig. 4.9), as described by Laaksonen and Paulsen (2015), most likely because it enables moisture to persist in the meat 'pocket'.

In cattle there has been an increased emphasis on the importance of absence of faecal and/or environmental contamination, therefore zero-tolerance for faecal contamination of the carcass has been proposed as a tool in the control of STEC infections (Heuvelink *et al.,* 2001). Considering the type of dressing of wild venison, with some of the procedures being carried out in the wild, it might not be possible to follow the same criteria as livestock species; however, guidance could be aimed towards stricter good hygiene practices and HACCP controls to contribute to minimalistic contamination being present at time of inspection.

***Fig. 4.8** Excessive contamination and bruising rectified by substantial trimming Trimmed areas shown by yellow arrows. Photo credit: Cristina Soare*

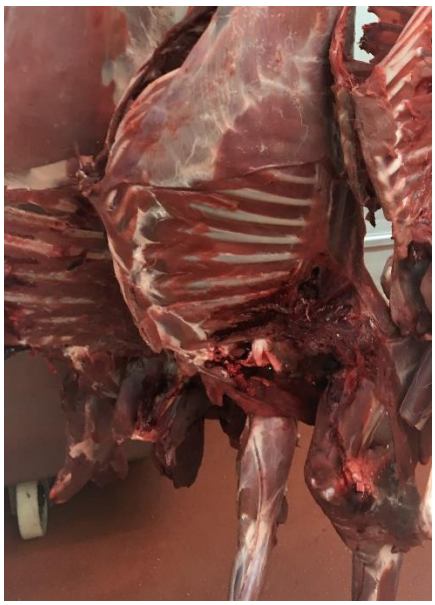


***Fig. 4.9** Loosening of carcasses shoulders. Image of a deer carcass with shoulders loosened. This should only be done partially to prevent meat pockets where moisture is maintained and bacterial growth can take place. Photo credit: Cristina Soare*

The evidence presented by the literature indicates that visible faecal or environmental contamination present on the body cavities of game is associated with statistically significant higher bacterial counts. This has been shown by Paulsen and Winkelmayer (2004) who subjected to visual examination for faecal contamination the body cavities of 47 deer and undertook microbiological testing of these surfaces to assess variations in microbial contamination. The study found that visible faecal or environmental contamination of the body cavities was associated with statistically significant higher bacterial counts. Median values for Aerobic Colony Counts (ACCs) were of 4.54 $\log_{10}$ cfu/cm^2 and of 3.53 $\log_{10}$ cfu/cm^2 for Enterobacteriaceae when compared to visually ‘‘clean’’ body cavities for which median values were lower, of 3.95 and 2.36 $\log_{10}$ cfu/cm^2 for ACCs and Enterobacteriaceae, respectively.

In a different study involving 100 roe deer, it was observed that average ACCs and Enterobacteriaceae on abdominal muscles were much higher on visually contaminated abdominal cavities (7.6 and 5.1 $\log_{10}$ cfu/cm^2 respectively), when compared to visually clean cavities (5.3 and 3.5 $\log_{10}$ cfu/cm^2, respectively). The same study isolated *E. coli* from 76 out of 100 roe deer carcasses investigated (Paulsen, 2011).

Even if presented visually clean, the carcasses or meat can become heavily contaminated if hygiene practices are not followed correctly. Paulsen (2011) showed

visibly clean deer carcasses stored at 3˚C for 3-7 days and processed hygienically had an average of 4.3 log cfu/g ACC in meat cuts and 5.1 log cfu/g in comminuted (processed) meat, whereas hygiene deficiencies resulted in an increase of microbial counts by log 2.5-3.5 cfu/g. This supports evidence that one of the sources of contamination can be contaminated surfaces, where the bacteria can survive well and can become a source of bacterial cross-contamination to the meat.

Microorganisms on wet surfaces have the ability to aggregate, grow into micro-colonies, and produce biofilm. Growth of biofilms in food processing environments is a concern as it leads to increased opportunity for microbial contamination of the processed product at the solid-liquid interface usually present on the contact surface between meats and surfaces (Chmielewski and Frank, 2003). *E. coli* O157:H7 has the potential to form biofilms and maintain acid resistance on different surfaces such as stainless steel in the presence of beef runoff fluids (washings) (Skandamis *et al.*, 2009) and survive and grow on these surfaces which enhance persistence in a food processing environment and presents a risk of contaminating the products (Simpson Beauchamp *et al.*, 2012).

The points above indicate that hygiene deficiencies, particularly on food processing surfaces where bacteria survive and form a biofilm can be a source of meat contamination.

Further to above, the environmental contamination and excessive handling is also discussed in the study of (Gouws, Shange and Hoffman, 2017). It was observed Enterobacteriaceae counts were lower on springbok carcasses at the incision area, immediately after evisceration but the counts increased as the carcasses reached the processing plant for skinning and chilling. The carcasses were swabbed on a second opportunity after skinning and again after a period of 24 hours chilling at the processing plant. It was observed that whilst in some carcasses the counts remained constant through the stages of the sampling, in some instances the Enterobacteriaceae counts gradually increased by the time the third sampling took place. This rise was associated with frequent exposure to different environments which can be encountered at the different locations through which the carcasses pass and excessive handling by the time the carcass reaches the processing point.

As *E. coli* is part of the Enterobacteriaceae family, observation of the latter can be an indirect indication that *E. coli* is part of this contamination. The statement is further supported by the observations undertaken during the FSA project FS231045 (M01049) on 'The microbiological status of wild and farmed venison' which found that there was evidence that *E. coli* counts increase during carcass processing, that this increased contamination comes from several sources and that handling practices in the AGHE could be a significant contributor to the final product flora. There was also evidence that the *E. coli* present on venison products carry pathogenicity genes and therefore represent a potential risk to food safety where *E. coli* numbers are higher.

The above data indicates the importance of visual contamination-free carcasses, clean and dry surfaces, limited exposure to different environments, avoidance of excessive handling to control occurrence and further growth of *E. coli* during processing.

4.6.3 Storage, butchering and further handling of meat during preparation

Process flow

The flow diagram of the steps involved in venison production at AGHEs is outlined in Fig 4.10, below.

After skinning and further trimming, the carcasses may be transferred within the same day to the cutting room or may be held in the chiller for a period of around three days, largely depending on the customers' orders that the FBO has to fulfil. However, generally and especially in the busiest period of the hunting season, carcass processing tends to take place in less than 3-4 days, to empty the refrigeration space and enable further intake of game.

The carcass is portioned into primal cuts which will be used subsequently to obtain steaks and further products such as diced meat, minced meat, burgers, meatballs, sausages, depending on the approval of the facility, as granted by Food Standards Scotland.

The preservation techniques also vary by processor and the meat cut involved. Whilst steaks and meat cuts tend to be preserved in vacuum packs, meat products are usually dispatched in modified atmosphere packs. This is in addition to other preservation techniques such as refrigeration or freezing.

To enable a constant supply of product, including during periods of closed hunting season, a few months per year, some of the venison is subject to freezing treatment either at a co-located cold store or partner cold stores and sold throughout the year.

The recipes of the meat preparation might also include other preservation techniques such as adding salt, preservatives and/ or antioxidants, additives (nitrites, nitrates, citric acids). Further preservation techniques could include, drying/smoking, and/or heat treatment, or fermentation.

It must be considered that survival of *E. coli* in meat products and meat preparations can be linked to the preservation techniques that the meat undergoes but this is out of the scope of this literature review. Generally, the more hurdles that are applied to the meat, the less likely *E. coli* bacteria will be able to survive or grow.

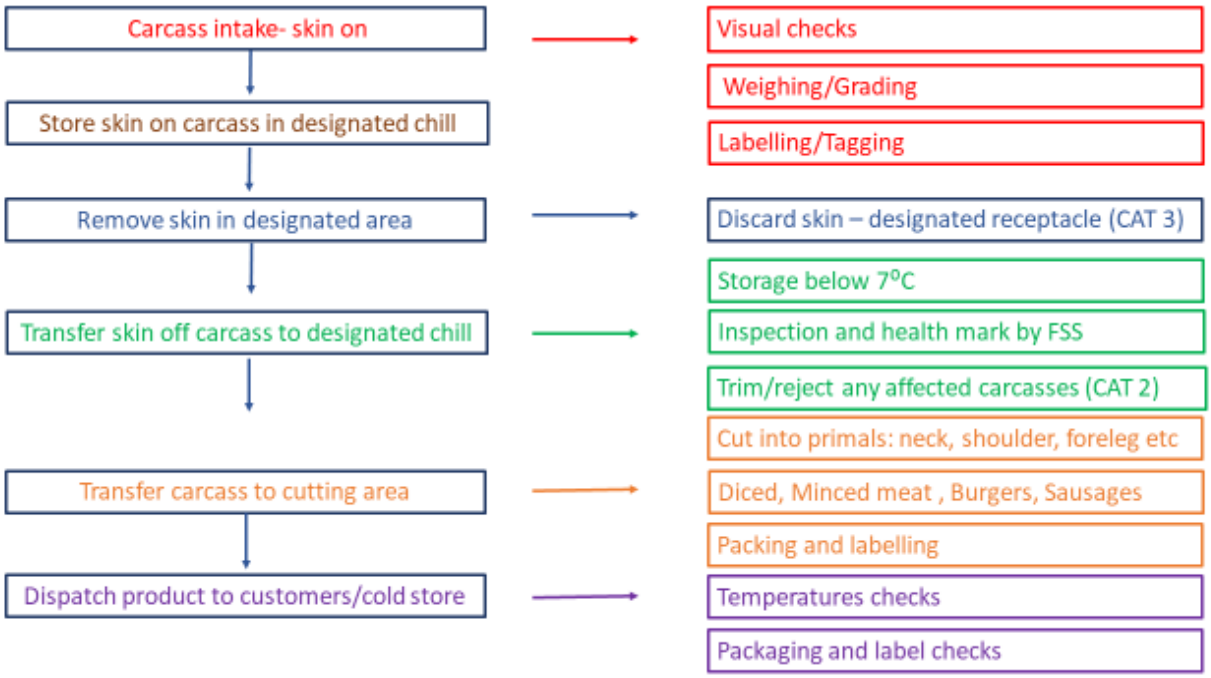


Fig. 4.10 *Flow diagram of venison production at AGHEs. CAT 2 and CAT 3 refer to category 1 and category 2 animal by-products (ABP) defined in Article 10 of Regulation (EC) No. 1069/2009. CAT 2 ABP are high risk materials which are not intended for human consumption, whereas CAT 3 is low risk material and includes parts of animals that have been passed fit for human consumption in a slaughterhouse but which are not intended for consumption.*

Hygiene of working spaces, equipment and storage areas

During skinning, working tools, hands and working clothes are exposed to bacteria that are shed from the hide and thus separation of dirty and clean operations are important considering that after skinning the carcass surfaces, and in particular moist meat surfaces, are susceptible to contamination and absorption of aerosols (Laaksonen and Paulsen, 2015). Separating the operations should concern both physical spaces and equipment (FSA, 2015a). The areas between clean and dirty operations can be separated by partition walls or plastic curtains. The spatial separation of operations should ensure this equally includes tools and operatives and that the flow continues towards clean areas and does not return carcasses or meat via the 'dirty' part of the establishment. However, where the space does not allow it, the clean and dirty operations can also be separated in time, ensuring that after skinning, the working area, equipment, hands have been washed, and overalls changed before "clean" operations commence.

Meat hygiene principles also dictate that for storage, the refrigerated area must be effective in relation to the number of carcasses held, enabling good space in between carcasses to prevent touching, enable air circulation and continuous removal of

evaporated water. This implies the ventilation system is effective and that condensation is prevented.

Pests of any type can act as vectors for pathogen transmission and therefore good pest control systems must be in place, as well as keeping the establishment well-sealed from the outside environment.

Waste management should also be functional and removal should be carried out as soon as possible. Hides should not be stored in the working spaces where the carcasses or fresh meat are being held.

All surfaces and floors must be constructed of easily cleanable material. Porous or corrugated materials such as wood or damaged hard plastic tops should be avoided as these cannot be easily cleaned and enable the proliferation of foodborne pathogens or allow bacteria to evade cleaning and disinfection. Stainless steel surfaces are suitable for heavy food industry use, however these should be cleaned at least once per day to prevent bacterial biofilm formation (Feng *et al.*, 2015). Special attention must be paid to seams, moreover to those between walls and working tables or between walls and floors or drains. Tools such as knives, metal gloves and carcass hooks that come in contact with the carcass should be cleaned after each use and sterilised regularly with hot water, at a temperature above 82˚C. Meat handling equipment such as plastic trays or boxes must never be kept in contact with the floor and should never be stacked within each other.

Contamination factors during storage and boning

The general mechanisms of meat contamination during processing operations are similar to all enteric pathogens in all meat animal species (Nørrung and Buncic, 2008). Once the processing/storage environment becomes contaminated, subsequent sources of carcass and meat contamination can contribute to spread of enteric pathogens, including *E. coli*.

A study which investigated the bacterial populations in bovine meat during processing found that higher *E. coli*, Enterobacteriaceae and ACC counts were linked to faecal contamination and poor sanitary procedures during the deboning process. *E. coli* counts were interpreted as a possible cross faecal contamination transferred by meat handlers to retail meat cuts (Nel *et al.*, 2004). Increased handling of the product, particularly if gloves were not worn or if made of a fabric material rather than rubber were also suggested as a contamination source (fomite) (Gill and Jones, 2002). An additional food safety concern is that indicators of faecal contamination such as *E. coli* can persist on contact surfaces even after routine sanitation (Yang, Devos and Klassen, 2017) The study of Yang *et al.*, (2017) showed that even though after routine sanitation, the surfaces were visibly clean, the numbers of *E. coli* organisms were not significantly impacted by the sanitation process on non-contacting meat surfaces of conveyor belts and cutting tables sides. The authors discuss that sanitation process could reduce but not eliminate *E. coli* on non-contact meat surfaces and the surviving *E. coli* likely multiplied. This is explained to be due to: difficulty of accessing such surfaces for debris removal, difficulty to apply foam

and sanitiser on narrow surfaces, reduced exposure of these surfaces to air drying and thus less bactericidal. The presence of *E coli* counts on cutting tables were thought to be a result of less cleaning effort or less drying effect. The re-growth of *E coli* on conveyors were discussed to be due to elevated temperature during the sanitation process as well as due to warmth produced during operation as a result of equipment friction.

Although *E coli* is normally non-pathogenic, the above discussion shows how small amounts of generic *E. coli* and thus faecal contamination can survive the sanitation process. This could offer some indirect evidence of how Shiga toxin-producing *E. coli* might bypass the regular cleaning and disinfection procedures.

With regards to subsequent storage of venison, studies have shown that an earlier deboning, 12 to 24 hours after kill is an appropriate interval to obtain a hygienic and better quality of meat (Konjević, 2008). In Scotland, due to the practicalities of the venison industry and the fact that most carcasses are first collected at the larder, before being transferred to AGHEs, the deboning takes place, on average, 1 week after kill.

Meat cutting, deboning and grinding operations involve relatively intensive handling of meat which infers an increased microbial risk due to: microbial cross-contamination via hands and tools (knives, saws, conveyers) and/or transfer of bacteria from the meat‘s external to internal surfaces. Cutting tools have been identified as a potential ‘fomite’ in the transmission of non-O157 STEC between venison cuts (Rounds *et al.*, 2012)

Meat processing operations, such as mincing of meat, markedly increases the risk of microbial contamination. Redistribution of surface contamination to the whole product can take place via the comminuting of the muscle tissue. Additionally, meat product contact with equipment surfaces during the mixing and forming actions, such as the grinder which was observed to be a good environment for *E. coli* survival and growth, especially around the collar (Flores, 2004).

Minced meat can be used further for meat preparations to which the same risks apply. This is also proven by the *E. coli* outbreaks being in fact connected to meat products such as venison sausages (Ahn *et al.*, 2009, Browning *et al.*, 2016).

In the outbreak reported by (Ahn *et al.*, 2009) common exposure was thought to be through deer sausages produced by an unlicensed, uninspected in-home processor. Upon environmental investigation, the facility was identified as adopting poor sanitation and refrigeration. The unapproved processor was compelled to chase operations and the remaining affected product was traced to recipient consumers, requesting destruction of suspected meat.

In Europe, all food business operators, including venison producers are responsible for making sure that the food produced by their business is safe to eat (Regulation (EC) No 178/2002 Article 14.2) and to achieve safety, Regulation (EC) No. 852/2004 Article 5 requires the operator to put in place, implement and maintain permanent procedures based on Hazard Analysis and Critical Control Points (HACCP) principles. Through the validation process the operator(s) must ensure HACCP

system is set up to deliver a safe product, in particular ensuring that all Critical Control Points (CCPs) are established correctly to identify hazards, are monitored appropriately and corrective actions are effective to rectify contamination issues. Verification measures can be set up to include microbiological tests for process hygiene criteria and for food safety criteria, according to specification of Regulation (EC) No. 2073/2005. The food business operator can decide to adopt a testing scheme above the specification of this regulation. In connection with the above paragraph which looks at, the production of minced meat, including those made of venison, the verification procedures for process hygiene criteria include microbiological testing having as indicators organisms such as ACCs and *E. coli* counts, as listed in Table 4.3, Section 4.7.1 of this report. Equally, hygiene criteria for meat preparations (such as burgers or sausages) also requires *E. coli* testing results to be within acceptable ranges (Regulation (EC) No. 2073/2005), also listed in Table 4.3 and Section 4.7.1.

As briefly discussed above, the importance of individual sources for contamination and the extent of such contamination is highly dependent on the design of facilities, the equipment available and the level of process hygiene. Differences exist between technologies and equipment available at each processing site. Further variations can occur in the process hygiene, even between operatives of the same establishment. Therefore, a mandatory food safety management system, based on Hazard Analysis and Critical Control Point (HACCP) principles, has to be designed for each establishment, with the support and the guidelines provided by the FSA (Food Standards Agency, 2008)

Even in well-designed facilities it is possible to achieve unsatisfactory hygiene, and therefore the practices and operations aim to secure hygienic end products. Appropriate self-monitoring will compliment well designed facilities. The self-monitoring must include descriptions of the handling techniques, cleaning and disinfection of facilities, use of equipment, temperature monitoring, pest control, and waste disposal. The appointment of a person in charge of operations ensures the implementation of self-monitoring is effective and appropriate records are produced for official inspection/auditing purposes.

4.6.4 Retail and storage

After the product has been dispatched for retail distribution, a period might elapse before is consumed. This introduces other opportunities, depending upon storage temperature for STEC to grow (Stringer, George and Peck, 2000). As per section 1.2 of this report, temperatures above 7°C could contribute to growth of *E. coli*, including STEC O157:H7, if this is present on the product.

Food borne infections were linked to insufficiently cooked venison steaks (Rabatsky-Ehr *et al.*, 2002), which creates a need for consumers to thermally treat the venison product for an adequate time/temperature combination.

Temperature inactivation conditions vary on the method of cooking and the thickness of the meat cut. A study carried by Gill and colleagues (2009) indicated that regular beef steak thickness (3cm thick) could achieve a reduction of at least 7 log cfu/steak

of internalised *E. coli* O157:H7 by pan-grilling to >60˚C internal temperature or more. Studies also show that roasting in the oven is less efficient to pan-grilling in killing the backeria and the thicker the meat cut, the greater the reduction in bacteria by pan-grilling (Adler *et al.*, 2012).

If venison meat and particularly steaks manifest the same cooking properties as beef steaks, a heat treatment to a core temperature of 65°C, which is known to be the equivalent to medium rare cooking, 'may be adequate for assuring the microbiological safety of tissues without excessive contamination of deep tissues', as suggested for beef (Gill and McGinnis, 2004). Furthermore, the authors showed that steaks continued to rise temperature from 63°C to 68°C after being removed from the heat treating apparatus, due the conductivity properties that muscles have.

The current guidelines for safe cooking in Scotland for both consumers and caterers indicate that meat should be brought to an internal core temperature of at least 75°C to destroy most of the common foodborne pathogens[68], above the core temperature of 70°C advised by the WHO specifically for Shiga toxin-producing *E. coli* [69].

4.7. Microbial condition of venison

The initial microbiological load can consist of both spoilage and pathogenic bacteria. Whilst spoilage bacteria can result in undesirable changes to quality of meat and meat products, such as off-flavours and odours (Casoli *et al.*, 2005), the pathogenic bacteria can result in foodborne infection in consumers, especially those with weakened immune systems.

In line with specifications of Regulation (EC) No. 2073/2005 as amended by Regulation (EC) No. 1441/2007, microbial assessment by testing of carcasses from wild or farmed game is not a legal requirement. However, as part of the HACCP system, the FBO at the AGHEs might decide to undertake regular microbial testing to verify the hygiene practices so that the safety of the product is enhanced. The drawback is that there are no explicit microbial limits considered acceptable for wild game meat with the exception of generally applicable food safety criteria (Regulation (EC) No. 2073/2005). Considering that meat from wild game will be placed on the market in the same circumstances and conditions as meat from farmed animals it would seem appropriate to adopt the same testing regimes as for livestock ruminants (Paulsen, 2011).

4.7.1 Carcasses

Although the published literature with respect to acceptance criteria on the microbiology of the carcass can be quite equivocal, most journal articles consider acceptance criteria of Regulation (EC) No. 2073/2005 which applies to cattle and other domestic ruminants (Table 4.3):

[68] Cooking temperatures advised by FSA: https://www.foodstandards.gov.scot/consumers/food-safety/at-home/cooking-food
[69] Cooking temperatures advised by WHO: https://www.who.int/news-room/fact-sheets/detail/e-coli

Table 4.3 *Process hygiene criteria (adapted from Regulation (EC) No. 2073/2005) for meat and products thereof)*

Food Category	Microorganism	Limits (m)[(a)]	Limits (M)[(a)]
Carcasses of cattle, sheep, goats	ACC	3.5 log cfu/cm^2 daily mean log	5.0 log cfu/cm^2 daily mean log
	Enterobacteriaceae	1.5 log cfu/cm^2 daily mean log	2.5 log cfu/cm^2 daily mean log
Minced meat	ACC	5x10^5 cfu/g (5.7 log$_{10}$ cfu/g)	5x10^6 cfu/g (6.7 log$_{10}$ cfu/g)
	E. coli	50 cfu/g (1.7 log$_{10}$ cfu/g)	500 cfu/g (2.7 log$_{10}$ cfu/g)
Meat preparations	*E. coli*	50 cfu/g or cm^2 (1.7 log$_{10}$cfu/g or cm^2)	5000 cfu/g or cm^2 (3.7 log$_{10}$ cfu/g or cm^2)

[(a)] The limits (m and M) are interpreted as follows: satisfactory if the daily mean log is ≤ m; acceptable if the daily mean log is between m and M; unsatisfactory if the daily mean log is > M.

With the data shown in Table 4.4, Paulsen (2011) has demonstrated that, under good handling procedures, meat obtained from well-dressed and processed deer carcasses can show a microbial limit of less than 2 log cfu/cm^2 *E. coli* and less than 6 log cfu/g ACC, therefore according to the author, a microbiological standard of ACC < 6 log cfu/cm^2, and *E. coli* < 2 log cfu/cm^2 on exposed muscle surfaces arriving at AGHE, can be considered an acceptable limit for wild venison

Table 4.4 *Microbial results on different (unspecified) venison cuts sampled in Austria* (Paulsen, 2011)

Sample	No.	Aerobic colony Count log$_{10}$ cfu/cm	Enterobacteriaceae log$_{10}$ cfu/cm^2	*E. coli* log$_{10}$ cfu/cm^2
Roe deer frozen meat cuts	44	5.7 (3.5-7.3)	3.6 (1.0-6.3)	2.3 (1.0-3.8)
Red deer frozen meat cuts	49	4.3 (1.1-6.6)	2.2 (1.0-4.2)	1.7 (1.0-3.7)
Roe deer fresh meat cuts	49	5.7	3.9	2.0
Roe deer chilled and vacuum packed meat cuts (132h post mortem)	103	5.8	4.8	Not tested

By reviewing the scientific publications in the years 2006 to 2017, as described in methods, we have found the literature is limited on *E. coli* prevalence in deer carcasses at the point of kill or along the food chain. The search also included, however, studies investigating microbial counts on wild deer carcasses for coliforms or Enterobacteriaceae. This approach was taken to extract the observations of higher bacterial counts, and the practices discussed by the authors as leading to higher enteric bacterial contamination. The approach assumes that there can be *E. coli*, including STEC, among enteric bacteria (if the animal is shedding at the time of the kill). Faecal contamination, and thus higher enteric bacterial counts, could provide indirect evidence of *E. coli* contamination and the potential risk of STEC.

A second aim of our review was to gain a baseline understanding of the previously observed microbiological status of wild deer carcasses and venison, to inform our interpretation of the microbiological results we obtained during the field study of wild game carcasses.

E. coli represents about 1% of the total 10^4–10^9 cfu/g healthy microflora of the gut content in nearly all mammals (Bartels and Bülte, 2011). According to a study carried out in New Zealand (Mills *et al.*, 2015), to be able to draw a parallel between *E. coli* and other enteric pathogens such as Enterobacteriaceae or coliforms, the following assumptions need to be taken into account:

- The sources of *E. coli* contamination and other enteric pathogens are the same;
- Considering there may be differences in distribution between individual hides (and hence carcasses), the distribution of enteric pathogens (when present) is similar to that of *E. coli*;
- The numerical ratio between *E. coli* and other pathogens (when present) is relatively constant;
- The adhesive properties of *E. coli* and other pathogens are comparable;
- The transfer of pathogens (when present) during dressing is comparable to that of *E. coli.*

In Germany, Atanassova *et al.* (2008) investigated the quality of freshly shot game to identify the microbiological quality of the meat before storage and handling, parts of the process that might otherwise alter the counts. The results (Table 4.5) show that the values obtained from freshly shot game (with sampling directly after the shot and immediate cooling) were within the legal range for slaughtered domestic cattle. The authors discussed the hygienic results were owing to the following factors:

- The animals were shot on well-organised hunts;
- There was fairly rapid opening and evisceration of the animals, within 90 minutes of death.

While the mean values of Enterobacteriaceae were low and relatively close between deer species, this group of bacteria was isolated from a higher number of roe deer carcasses (15) than red deer carcasses (10), which suggests roe deer can become more readily contaminated than red deer.

Table 4.5 *Total viable counts (TVC; equivalent to aerobic colony counts, ACC) and* Enterobacteriaceae *in wild deer carcasses* (Atanassova *et al*., 2008)

Roe deer		**Red deer**	
TVC cfu/cm^2	Enterobacteriaceae cfu/cm^2	TVC cfu/cm^2	Enterobacteriaceae cfu/cm^2
N=95 2.6 (1.0–5.7)	N=95 2.1 (1.7–2.6)	N=67 2.9 (1.1–5.3)	N= 67 2.1 (1.7–2.8)

N = number of carcasses investigated. Median values of the distribution of $\log_{10}$-levels cfu/cm^2

In Italy, Avagnina *et al.* (2012) also investigated the level of contamination (ACCs) of freshly shot carcasses related to hygienic handling and the overall indicator for faecal contamination (Enterobacteriaceae). While the ACCs were relatively similar in the two groups of animals (roe and red), the Enterobacteriaceae counts were higher in roe deer (Table 4.6). Bartels and Bülte (2011) suggest roe deer have looser connective tissue structure which increases the risk of bacterial penetration in surrounding tissue. The authors also observed higher Enterobacteriaceae counts in carcasses with visible faecal contamination suggested to be linked to improper evisceration procedures (Avagnina *et al.*, 2012). Higher ACC, above the acceptable limit of 5 log cfu/cm^2, were "highly dependent on the first hunting phases", according to the authors. The anatomical location of the shooting posterior to the diaphragm increased the ACC microbial counts of the carcasses.

In Switzerland, Obwegeser *et al.* (2012) examined 258 carcasses from hunted wild deer by swabbing these for Enterobacteriaceae. These bacteria were found in "remarkable" frequencies and counts. Enterobacteriaceae were detected on 119 out of 136 (87.5%) red deer carcasses and 109 of 122 (89.3%) roe deer carcasses. As shown in Table 4.7, the mean log cfu/cm^2 for Enterobacteriaceae counts was 2.3 for red deer and 2.6 for roe deer. These results are in accordance with above observations of Avagnina *et al.* (2012), who also discussed that Enterobacteriaceae contamination was higher in roe deer carcasses.

The mean values of Enterobacteriaceae varied in carcasses collected at different abattoirs (AGHE), as reflected in Table 4.7. This variation is discussed by Obwegeser *et al.* (2012) to be partly due to the difficulty of hygienic evisceration in the field, in particular if shooting lacerates the animal's intestines. However, this variation is also discussed to be due to visual faecal contamination as a result of unhygienic handling during the storage phase, before boning or processing, as reflected in the timing of carcass swabs (48h after hunting and another 72h after arriving at the AGHE and being subjected to chilling).

Table 4.6 *Microbiological results for freshly killed carcasses (0–6 h) eviscerated in field conditions (0–90 minutes after shooting). Adapted from* Avagnina *et al.* (2012)

Samples	**ACC**	**Enterobacteriaceae**
Total number of red deer carcasses tested	**56**	**56**
Median log cfu/cm^2	3.3	1.7
% samples below detection limit (10 cfu/cm^2)	0	20 (35.7%)
% samples ≤ 5 log cfu/cm^2 (ACC) or ≤ 2.5 log cfu/cm^2 (Enterobacteriaceae)	47 (83.9%)	23 (41.1%)
% samples > 5 log cfu/cm^2 (ACC) or > 2.5 log cfu/cm^2 (Enterobacteriaceae)	9 (16.1%)	13 (23.2%)
Total number of roe deer carcasses tested	**61**	**61**
Median log cfu/cm^2	3.46	2.47
% samples below detection limit (10 cfu/cm^2)	1 (1.6%)	16 (26.2%)
% samples ≤ 5 log cfu/cm^2 (ACC) or ≤ 2.5 log cfu/cm^2 (Enterobacteriaceae)	51 (83.6%)	14 (23.0%)
% samples > 5 log cfu/cm^2 (ACC) or > 2.5 log cfu/cm^2 (Enterobacteriaceae)	9 (14.8%)	31 (50.8%)

Threshold levels of ≤ 5 log cfu/cm^2 and ≤ 2.5 log cfu/cm^2 for ACC and Enterobacteriaceae, respectively, are taken from acceptable levels for carcasses of domestic ruminants (EC 2073/2005)

Table 4.7 *Enterobacteriaceae results (log cfu/cm^2) on carcasses from hunted wild red and roe deer sampled at 6 AGHEs in Switzerland* (Obwegeser *et al.*, 2012)

AGHE **n = total no. of sampled carcasses**	**Enterobacteriaceae results (log cfu/cm^2)**					
	Red deer carcasses			**Roe deer carcasses**		
	Number	**Mean log**	**Standard deviation**	**Number**	**Mean log**	**Standard deviation**
AGHE A	63	2.14	1.06	33	3.20	0.91
AGHE B	44	2.12	1.36	36	2.60	1.19
AGHE C	19	3.82	1.33	18	3.63	1.11
AGHE D	8	0.73	1.02	1	3.06	-
AGHE E	2	3.18	0.8	7	2.98	1.50
AGHE F	-	-	-	27	1.08	1.12
Total (n = 258)	136	2.30	1.38	122	2.60	1.40

The authors also screened the faeces of wild deer for *E. coli* harbouring Shiga toxin genes and intimin (*eae*, a key virulence gene for STEC). The *stx*-positive enrichments were confirmed biochemically as being *E. coli* and then tested by PCR for the presence of *stx1, stx2* and *eae* to characterise STEC.

The faeces were collected from wild deer hunted in the same regions in which the AGHE were located and the carcasses were tested for Enterobacteriaceae. Out of the 86 faecal samples in which *stx* genes were detected (alone or in combination with *eae*), 37 strains of STEC were isolated: 18 from red deer and 19 from roe deer (Table 4.7a). Of the 84 faecal samples collected from red deer, 36.9% tested positive for *stx* only, 6.0% for *eae* only, and 21.4% for both *stx* and *eae*. Of the 64 faecal samples collected from hunted wild roe deer, 39.1% tested positive for *stx* only, 7.8% for *eae* only, and 18.8% for both *stx* and *eae* (Table 4.7a). To assess the human pathogenicity of STECs, however, further strain characterisation is needed.

Table 4.7a *Detection of* stx *and* eae *genes in faecal samples obtained from deer hunted in the same regions as carcasses from Table 4.7. Adapted from Obwegeser* et al*. (2012)*

Deer species	**Detection of *stx* and *eae* genes in faecal samples**		
	stx+	*eae*+	*stx*+ and *eae*+
Red deer (n=84)	31 (36.9%)	5 (6.0%)	18 (21.4%)
Red juvenile (n=22)	10 (45.0%)	0 (0.0%)	5 (22.7%)
Red adult (n=62)	21 (33.9%)	5 (8.1%)	13 (21.0%)
Roe deer (n =64)	25 (39.1%)	5 (7.8%)	12 (18.8%)
Roe juvenile (n=24)	11 (45.8%)	1 (4.2%)	3 (12.5%)
Roe adult (n=40)	14 (35.0%)	4 (10.0%)	9 (22.5%)
Total red and roe (n=148)	56 (37.8%)	10 (6.8%)	30 (20.3%)

The authors discussed that *stx2* predominated in strains from red deer, and *stx*1 and *stx2* were found to an equal extent in strains from roe deer. Two of the STEC strains isolated from red deer and positive for *stx1* or *stx2* also harboured *eae.*

E. coli strains producing *stx2* are more often associated with severe symptoms of the disease than *stx1*-producing strains. In the UK, *stx2a* is the toxin most commonly associated with severe disease (Dallman *et al.*, 2015) as well as worldwide (FAO, WHO 2018). Further clinical complications, such as haemorrhagic diarrhoea or renal dysfunction, are more likely to occur if, alongside *stx2a*, the *E. coli* serotype possess additional virulence factors such as the adhesin intimin, a protein coded by the *eae* gene (Brooks *et al.*, 2005).

Although Obwegeser *et al*. (2012) have not further characterised the toxin into subtypes, their findings suggest that two of the red deer carried *stx2* as well as intimin genes that could cause some concerns in terms of the role of this specie as a

carrier of STEC. However, to confirm pathogenicity to humans, further strain characterisation would have been required.

Despite the fact that toxicity genes were not tested in the meat as well, their presence in faeces and additional high Enterobacteriaceae contamination observed on the carcasses collected from the same areas, supports evidence of risk of STEC transmission from faeces of wild ruminants' to their meat and further to humans via consumption of undercooked venison. There is also a possibility of transmission to domestic ruminants by environmental sources via shared pastures faecally-contaminated with deer STEC.

A Spanish study which looked specifically at the presence of STEC in wild red deer (Díaz-Sánchez *et al.*, 2013), found overall STEC prevalence was much higher in faeces than carcasses. The faecal prevalence for *E. coli* O157:H7 was of 1.5% (4/264) and none could be isolated from the carcasses. The faecal prevalence for non-O157 STEC was 34% (89 isolates/264 samples) and 7% in carcasses (19 isolates/271 carcasses). The most important non-O157 STEC strains identified were: O128:H2, O8:H2, O14:H-, previously associated serotypes from humans affected by haemolytic uremic syndrome (HUS). Pulsed-field gel electrophoresis of STEC showed similar patterns in faeces and carcasses and this association was suggested to occur as a result of cross-contamination during processing.

Analysis of the virulence profiles of the 89 non-O157 isolates obtained from faeces, indicated four carried the *stx1* gene alone, 78 the *stx2* gene alone and 4 carried both the *stx1* and *stx2* genes (Table 4.8). The analysis of the non-O157 isolates obtained from carcasses indicated that two carried the *stx1* gene, 16 carried the *stx2* gene and 1 isolate carried both *stx1* and *stx2* genes (Table 4.8a). Therefore, *stx2* was the most frequent Shiga toxin-encoding gene detected among the non-O157 STEC isolates obtained from carcasses and faeces.

Furthermore, the same study observed that the *stx* genes detection rate was significantly higher in the presence of livestock and with high density of more than 15 deer per square kilometre in the hunting estates (Díaz-Sánchez *et al*., 2013).

In a separate study, which concerned an analysis of 140 STEC strains isolated from game meat by Pulse-field gel electrophoresis (PFGE) genotyping of (Miko *et al.*, 2009) confirmed that certain STEC strains were distributed over most PFGE clusters from farm animals, other foodstuffs (e.g. vegetables) and human patients. This was established through dendrograms based on similarities of PGE patterns. These concerned strains O26:H11, O113:H21, O128:H8 and O146:H28. Furthermore, by cluster analysis isolates from game and cattle showed 90% or more similarity among STEC serotype O103:H2; O26:H11; and strain O113: H21, supporting evidence of transmission between these species via faecal pollution of farmland. Also by cluster analysis, over 90% similarity was shown between the same game isolates and human patients for O103:H2, O26 [H11 and O113:H21. Further multilocus sequence typing (MLST) showed that O103:H21 were the strains most responsible for human haemolytic uremic syndrome and haemorrhagic colitis and these were present amongst game isolates as well.

Table 4.8 *STEC isolation from faecal and fresh carcass samples of red deer and detection rates for Stx genes from all samples analysed. Adapted from (Díaz-Sánchez et al., 2013).*

Faecal samples at the point of kill N=264		**Carcass samples at the point of kill** N=271
E. coli O157:H7 isolates	4 (1.5%)	0
Non-O157:H7 STEC isolates	89 (34%)	19 (7%)
Stx genes detected in isolates	93 (35%)	68 (25%)

Table 4.8a *Further analysis of the virulence properties of the stx genes from non-O157 STEC in faeces and carcasses. Adapted from (Díaz-Sánchez et al., 2013)*

No of positive isolates	*stx1*	*stx2*	*stx1* & *stx2*	*eae*	*ehxA*
Faeces = 89	4	78	4	0	54
Carcasses = 19	2	16	1	0	9

The tables above (Tables 4.4-4.8) should give a broad understanding of the microbial condition of carcasses, although direct comparison is difficult due to the differences in testing methods and sampling schemes. Also for the above dataset it was not always clear if the samples were collected before, after or during chilling which could have important effects on determining the microbial status of the carcasses and more importantly the STEC prevalence. The main risk factor that could have been extracted from these studies appears to be associated with the lack of hygienic procedures during wild game processing. One author also mentioned the health status of the deer influence presence of pathogenic bacteria (Obwegeser *et al.*, 2012).

At present, very little data has been published on the prevalence of pathogenic *E. coli* in wild deer carcasses and meat in the UK; the only references found on *E. coli* occurrence in the UK were (i) a qualitative risk assessment commissioned by the FSA in 2005 which concluded that at that time the risk to human health for *E. coli* O157:H7 from the consumption of wild deer meat in the UK was low and the risk from handling carcasses was very low (Coburn *et al.*, 2005), (ii) a second study commissioned by the FSA in 2010 concerning the microbiological status of wild venison reported evidence of *E. coli* presence on venison products with increased *E. coli* counts during carcass processing (FSA project FS231045 (M01049) on 'The microbiological status of wild and farmed venison'), (iii) finally, a survey carried by (Synge, 2006) included a sample size of more than 784 faeces from Scottish wild deer, collected between 2003-2004, but all were negative for STEC O157. These faecal samples, however, were collected using a convenience sampling method and

therefore may not be representative of the total Scottish wild deer population. A follow-up study (Synge, 2006) on a human clinical case involved testing a further 15 wild deer faeces and 4 of these yielded positive to STEC O157. Farms with larger number of animals had lower mean O157 prevalence, unhoused animals with water supplied from a natural source had lower prevalences than unhoused animals supplied from mains or private supplies. Other risk factors included presence of deer which were also associated with higher prevalence.

In conclusion, an evaluation of presence and carriage of *E. coli* in carcasses and meat can be a valuable tool in assessing the overall hygienic conditions during food processing. Acceptable microbiological limits could be adopted from the guidelines available for domestic ruminants or as applicable directly to wild ruminants from previous studies (Paulsen, 2011). Similar to domestic livestock species, process hygiene criteria, as laid out in Table 4.3, could serve as guidance values, indicating that exceeding limits should trigger improvements to the hygienic handling.

4.7.2 Retail meat

A summary of results from studies evaluating the presence of STEC or STEC-associated genes from various venison retail products is shown in Table 4.9. The zoonoses report of EFSA and the European Centre for Disease Prevention and Control (EFSA, 2016), includes testing results for STEC at appropriate stages of the food chain from across member states as part of reporting obligations under Directive 2003/99/EC. The above report summarises data following investigation of a total of 2560 cattle, 528 sheep and 31 deer meat samples, as per Appendix 11. Samples were collected for monitoring purposes in a variety of settings, such as slaughterhouses, cutting plants, wholesalers and at retail level, although the report does not mention what type of meat samples were investigated and only two countries contributed deer meat samples (Austria and the Netherlands). The report shows that the most common food source for STEC was meat from ruminants with the highest proportion in sheep (12.2%), followed by deer (11.1%) and by cattle (1.6%). However, these percentages might be biased given the low sample size from sheep and deer in comparison to cattle. None of the STEC isolated were O157, two of the isolates were O146 and the rest O110 and O108. In 2016, 8 of the 48 (16.6%) deer meet samples were STEC positive and the serogroups reported were O146 (57.1%), O6, O8 and O53 (EFSA., 2017)

According to EFSA (2016), STEC O157 remains the most frequent serogroup reported in European food chain, although, as shown above, a wide range of other serotypes can be present in deer meat. The EFSA (2016) data collection reflects the surveillance and monitoring programmes in member states which are still focused on the O157 serogroup due to difficulties in isolating non-O157 STEC. However, as reflected by the zoonoses report (EFSA, 2016), there is an increasing trend of STEC O26 reporting in food samples, explained by the adoption of a non-biased ISO method (ISO TS 13136:2012).

Magwedere *et al.* (2013) carried out a limited investigation on STEC prevalence in different types of venison retail meat samples in the USA using a multiplex PCR. They found that three out three minced meat samples collected from white tailed deer were positive for *E. coli* O45, O103 or both. Red deer samples exhibited the

presence of O103 in 10% (2/20) of meat products. Further PCR testing showed that only one of the meat samples (O45 positive meat samples from white tailed deer) was positive for the *stx*1 gene, with the other deer meat samples being negative for *stx*1 and *stx*2.

In a Spanish study, Díaz-Sánchez *et al.* (2012) PCR analysis found Shiga toxin encoding genes in 5.4% (2/37) of the chilled products and 45.8% of the frozen products (22/48). STEC was isolated from 2.7% (1/37) of chilled products and a higher proportion of STEC were isolated from 8.3 % (4/48) frozen deer meat products (Table 4.10). The serotype isolated from frozen products was O27:H30 which was associated to human infection in the country. In agreement with other genetic studies carried out in Germany (Miko *et al.*, 2009), the Spanish study found that the predominant gene was *stx2,* but the STEC isolates lacked *eae* genes which have been shown to increase the likelihood for haemolytic uremic syndrome in humans (Brooks *et al.*, 2005). Serotypes O146:H- and O27:H30 were the most frequently found in deer meat (Díaz-Sánchez *et al.*, 2012), in correlation with findings of another Spanish study concerning red deer carcasses (Díaz-Sánchez *et al.*, 2013).

A study into the prevalence of STEC in game meat samples conducted by the German Federal Institute of Risk Assessment (BfR) between 2002 and 2006 showed that the contamination of raw game meat with STEC strains was 3% in 2002 and later data for the year 2006 revealed a contamination rate of up to 14.8%. This was higher than the prevalence reported for beef at the time (Bandick and Hensel, 2011) and included serotypes O26:H11, O103:H2 and O128:H2, all of which had been associated with human disease in Germany (Bartels and Bülte, 2011).

Table 4.9 *Detection of STEC (or STEC associated genes) from various venison retail products worldwide in the interval 2005–2017*

Deer meat at the retail point	No of samples	Samples positive for STEC by isolation	Samples positive for *stx* and O-type genes	Prevalence	Reference
Deer meet	48	O146		8 (16.6)	(EFSA, 2017)
Deer meets	45	O146 O110 O108	-	5 (11.1%)	(EFSA, 2016)
Minced white tailed deer meat	3	-	O45 O103	3 (100%)	(Magwedere *et al.*, 2013)*
Minced red deer meat	20	-	O103	2 (10%)	
Frozen deer meat	48	-	O27:H30 O146:H-	4 (8.3%)	(Díaz-Sánchez *et al.*, 2012)*
Meat products (cured sausage, dry cured meat)	37	-	O11:H5	1 (2.7%)	
Deer Meat samples collected 2002-2006 (types of meat samples not specified)	Not specified	O26:H11, O103:H2 O128:H2	-	3-14.8%	(Bandick and Hensel, 2011) (Bartels A and Bülte M., 2011)

(Martin and Beutin, 2011) investigated 593 STEC isolates derived from food, including deer meat to determine the relationship between STEC present in food and the animal food sources as well as the proportion of the strains which show virulence properties. The samples were provided by public health laboratories from Germany, Switzerland and France between 2005 and 2009. From 117 isolates extracted from deer meat, 46 (39%) were STEC isolates red deer meat and 65 (55%) STEC isolates from roe deer meat, suggesting that roe deer meat is carrying STEC more often.

Following lab investigation, the serotypes more frequently found amongst deer isolates, were O21:H21, O146:H28, O128:H2 and O146:H21. The genes *stx1c* (24.8%) and *stx2b* (55.6%) were significantly more frequently carried by STEC isolated from deer meat, in line with a previous study carried by Miko *et al.* (2009). Importantly, the study found similarities between the virulence profiles and serotypes of STEC derived from the same animal species regardless of whether they were from faeces or from food product (Martin and Beutin, 2011). Virulence profiles and serotypes of STEC from food showed remarkable similarities to those of faecal STEC that were from the same animal species. These findings are supportive of the source of STEC in meat being from faecal contamination, although this was not demonstrated. The authors suggest sound hygiene measures implemented during food production, especially during the steps which increase the likelihood of faecal

contamination (shooting, evisceration, skinning) should be most effective in reducing the frequency of STEC contamination of food derived from wild deer.

In line with an EFSA opinion (EFSA, 2009), each member country which carries risk-specific surveys in food products should focus on the foodstuffs that are perceived to be the most important sources of human STEC. The choice of STEC serogroups to be monitored should also reflect the human STEC serogroups causing disease at any given time. In Scotland, according to the data provided by the Health Protection Scotland[70], between years 2014-2018 there have been 156 human cases of STEC O157 and 91 cases of non-O157 STEC infections. The general reported trends over this period indicate a slight reduction in STEC O157 infections and a stable number of non-O157 STEC infections (HPS, 2018). Therefore it would seem appropriate to test meat samples for generic *E. coli*, followed by serotyping of positive samples, indicating values of above 2 log cfu/cm^2 *E. coli* as estimated in Paulsen (2011) as the average limit for wild deer meat, and finally by determination of virulence for the *E. coli* isolates and association of these factors with the human clinical cases of O157 and non-O157 STEC foodborne infections.

In terms of the microbial quality of game meat after processing, Membré, Laroche and Magras, (2011) observed that the level of contamination due to coliforms was higher than the level of *E. coli* (Table 4.10), but in line with the observation made also by Mills *et al.*, (2015) found the levels of contamination between coliforms and *E. coli* were correlated (Pearson's correlation coefficient (ρ) = 0.95).

The same study also observed high variations in the contamination levels of wild game meat which showed different values albeit not statistically significant between the countries where the meat was traded from. This was discussed to be related to variations in forest management although it was believed it was more likely that the variation was due to shooting practices and non-expertly killed game. The authors suggest in some countries animal shooting is reserved for professional hunters, while in others non-shooting experts are allowed to shoot game under the supervision of a trained hunter leading to possible cross-contamination from the gastro-intestinal tract to muscles (Gill, 2007)(Membré, Laroche and Magras, 2011)(Atanassova *et al.*, 2008).

Table 4.10 *Testing results obtained from frozen and chilled meat cuts traded in France (Adapted from Membré, Laroche and Magras (2011)*

Samples 1120 frozen and chilled meat cuts	***E. coli*** Log cfu/cm^2 (5% and 95% confidence intervals)	**Coliforms** Log cfu/g (5% and 95% confidence intervals)
Red deer 2005-2006	1.62 (1.01, 2.21)	1.98 (1.35, 2.58)
Roe deer 2005-2006	2.03 (1.43, 2.62)	2.37 (1.75, 2.97)
Red and roe deer 2006-2007	2.78 (1.90, 3.61)	

[70] https://www.hps.scot.nhs.uk/web-resources-container/stec-in-scotland-2018-enhanced-surveillance-and-reference-laboratory-data/

In summary the data with regards to the microbiological quality of game meat at retail point found in the literature is quite limited. Based on the available evidence although it is difficult to compare between the results presented in this section, overall this data shows that deer meat can carry a significant range of serotypes at retail stage depending on specie and product concerned and none of the observed serotypes were O157:H7.

4.8 Conclusions

The main findings of this report concerning STEC microbial contamination of wild game meat are summarised in the following paragraphs:

Hides

No estimates of the prevalence of STEC on hides of deer could be found in the scientific literature. Based on the literature data available from cattle, the microbiological condition of the hide appears to play an important role in the microbiological condition of the carcasses and the meat. Transfer of *E. coli*, including O157 can take place from the contaminated hide to the carcass, in a manner dictated by process hygiene practices adopted. If *E. coli* thrive on the skin/hide of deer, the same way as it does on cattle, the above data suggest that, in principle, transfer from the positive skin/hide to the carcass is possible during the skinning process and dressing.

Carcasses

Since the data are limited on STEC contamination in the carcass and the meat of wild deer, this study also considered publications that investigated Enterobacteriaceae and *E. coli*. The findings of contamination with these bacteria may provide indirect evidence of STEC contamination. Two of the studies reported that Enterobacteriaceae counts on wild deer carcasses were within the acceptable limit set for domestic livestock of maximum 2.5 $\log_{10}$ cfu daily mean log, as per regulation EC 2073/2005 as amended. One study reported that in some instances, Enterobacteriaceae exceeded the acceptable limits, and this variation was discussed to be associated with gut shot and with the hygiene practices adopted by the processing site.

One of the studies in the search interval 2006-2017 was identified as reporting on *E. coli* counts in wild game carcasses. This study suggests that 2 $\log_{10}$ cfu is the average *E. coli* value expected to be achieved for game processed under good hygiene practices by both destructive or non-destructive sampling methods. However, the values found did not distinguish any differences between the game species studied.

Only one study was found to have reported the prevalence of STEC in deer carcasses. This study, carried out in Spain, reported that carcasses were negative for O157:H7 but there was a 7% prevalence of non-O157 STEC The same study found similar STEC serotypes and virulence factors between carcasses from deer and from

livestock animals, indicating that there is the potential of transmission between wild deer and livestock hosts during shared grazing.

Meat and meat products

The literature review also observed that STEC present in deer meat is not limited to *E. coli* O157. In fact, many other STEC serogroups were reported, including O146, O76, O45, O103, O27, O11, O26 and O128. The prevalence of STEC and serogroups/serotypes reported differ from one study to another, depending mainly on the geographical location from where the venison was sourced, and largely on the product investigated and the processing or preservation techniques to which the meat was subjected.

The European zoonoses reports indicate that prevalence in deer meat ranged between 11.11% and 16.6% in 2015 and 2016 and involved only non-O157 isolates.

The prevalence studies identified in the current literature review were used to inform the risks and to identify practices that could be improved to reduce these risks. The conclusion to draw from the close scrutiny of these studies is that food may become contaminated with STEC at any phase of the processing and retail and that, in all cases, the source of STEC in wild deer meat has remained unknown. However, some findings indicate that STEC found as a contaminant in food can be traced to the food-producing animal as a source rather than the environmental or human sources along the food chain (Martin and Beutin, 2011).

Only a limited number of studies concerning large wild game meat in Scotland or the UK could be retrieved, and two of these were historic. To the extent of this literature review, therefore, the prevalence of STEC in Scottish venison or serotypes present is not known.

Risk factors

This review identified the main risk factors leading to microbial contamination of wild game meat at each of the steps involved in venison production.

These factors are, as detailed in Appendix 10: the health status of the live animals; and the environmental conditions such as shared grazing, deer stocking density and other environmental components such as seasonality; the hygiene practices in the field from the time of killing to gralloching, to transportation to the larder or AGHE; the maintenance of the cold chain from larder to final product; hide contamination; and the handling and hygiene procedures involved in further skinning, cutting and processing.

Health status of the deer

Based on the findings of the literature reviewed, the immunity of the deer can become challenged if they show heavy parasitism, and during the winter season when the animals experience stress due to food scarcity and cold temperatures, which could contribute to increased shedding of enteric bacteria, including *E. coli*. These conditions may lead to contamination of the hide when animals lie in their own faeces or when they touch each other during allogrooming, which is common in ruminants.

Species

The data found in the literature review cumulatively indicate that roe deer carcasses present faecal contamination more commonly and often with higher Enterobacteriaceae counts. However, two studies reported *stx2* was the most frequent virulence gene detected in red deer, and red deer were also positive in a few instances for the *eae* gene responsible for the more severe human infections. To fully understand the pathogenicity of isolated STECs for humans, further strain characterisation would be required.

Seasonality

One author discussed the hypothesis that STEC shedding in wild ruminants is more likely to occur in late winter, due to nutritional and cold stress (Bartels and Bülte, 2011). Contrary to this, however, other studies observed higher STEC shedding in warm summer months; the association with warmth is thought to be a function of favourable ambient temperatures producing higher shedding rates and further pathogen proliferation into the environment.

Environmental contamination

There is evidence that interspecies transmission between cattle and deer can occur during shared grazing. Overabundance of game and/or farm animals has been suggested to increase horizontal transmission. The specific environmental risk factor for STEC discussed in the literature is deer stocking density. These findings are summarised in Appendix 10.

Hygiene practices in the field

A greater number of the publications described the practices that lead to the microbial contamination of deer carcasses during primary production (i.e. from culling to arrival at AGHEs); fewer studies addressed the hygiene of the 'clean' operations carried out at AGHEs. It is not clear from the total body of literature whether STEC contamination of large wild game meat is primarily linked a to lack of hygiene during killing or otherwise during meat processing, and whether any of the steps involved in venison production have higher impacts on the microbial quality of deer carcasses.

Several studies discussed field hygiene during gralloching. These suggested that the time elapsing before evisceration is a factor behind the possibility of enteric bacteria and STEC, when present, crossing the intestinal lining and contaminating otherwise sterile muscles.

Although there were no studies investigating the possibility of STEC contamination via primary handling, high Enterobacteriaceae counts were associated with visible contamination of the carcass with soil and gut content, which offers indirect evidence that STEC contamination can occur via this route.

Multiple studies have indicated that shots in the abdomen result in faecal spoiling of the carcasses. The literature also describes faecal contamination also being partly due to the difficulty of undertaking hygienic evisceration in the field. The latter may concern delayed evisceration or intestinal perforation and spillage due to factors that may or may not be dependent on the experience of the hunter.

Hygiene practices during further handling

Faecal matter can be transferred to the carcass via cross-contamination during skinning and handling practices, resulting in pathogenic *E. coli* being present in game meat.

The microbiological condition of red and roe deer carcasses was seen to be directly associated with the AGHE where carcasses were processed.

High Enterobacteriaceae counts were suggested to occur during processing due to faecal contamination resulting from the unhygienic handling of carcasses during the storage phase or during processing. Increased handling of the product, particularly if gloves are not worn or if they are made of a fabric material rather than rubber, may serve as the contamination source. An additional food safety concern is that pathogens such as STEC O157 can persist on non-contact surfaces even after routine sanitation, although levels of *E. coli* can be reduced on meat-contacting surfaces during routine sanitation. Two key aspects that prevent or minimise contamination are the cleaning of all meat contact surfaces, including difficult-to-clean spaces (e.g. gaps in the conveyor belts, sieves of the meat grinders), and enabling surfaces to dry before use.

Cold chain

The data extracted from the literature indicate that exposing deer carcasses at ambient temperature from the time of shooting until reaching the larder directly influences Enterobacteriaceae growth. This is largely influenced by the climatic conditions. Further growth is not promoted during exposure to cooling temperatures in larders or the AGHE close to 0°C, but the microbial load remains high once it has been established, supporting the need for a prompt and continuous cold chain.

Further conclusions

The hygienic conditions of deer carcasses at the primary production level were variable in the studies; however, results indicating low *E. coli* and Enterobacteriaceae on some of the carcasses sampled in Europe demonstrate that wild deer that are hunted can be of good hygienic status after kill if considerations are taken at the harvesting stage and along the food chain.

The results of this literature review indicate that the factors that affect carcass and meat contamination with STEC are multifactorial. Therefore, strict compliance with good hygiene practices at every step of the product flow is important, starting with shooting, through evisceration in the field and skinning, to chilling and processing. Such measures have an importance that goes beyond STEC occurrence in meat and prevent most zoonotic foodborne pathogens from entering the food chain, ensuring the overall safety of venison.

UK guides to good practice at different levels of the food chain from forest to fork have been developed by the appropriate organisations, in alignment with the relevant European legislation.

Access to comprehensive HACCP guidance and to guidelines on the control of *E. coli* cross-contamination is available via the FSS to all FBOs producing large wild game

meat for human consumption. Adherence to these guidelines mitigates risks linked to hygiene practices during skinning and processing and ensures the maintenance of the cold chain.

Reduction of the risks posed by the health status of the animals, the hygiene practices during primary production, and the time to appropriate cooling of carcasses can all be expected to be mitigated by ensuring the competence of hunters, who should receive the appropriate training after registration with nationally recognised bodies offering continuous professional development.

Risks may also be reduced by the access consumers have to the public health information generated by FSS on the prevention of STEC, and on safe handling and cooking practices in the home.

5. Objective 3.2. Field studies to identify risk factors associated with *E. coli* and coliform levels in wild venison

Summary

The aim of objective 3.2 was to conduct field sampling of wild deer carcasses at all stages of production, to assess the microbiological condition and related factors that might influence the hygiene and quality of the carcasses. Risk factors related to each of the steps undertaken in the production of venison in Scotland, from the hill to the end-product, were investigated for association with the microbiological condition of wild deer carcasses. The response variables for the risk factor analysis were counts of coliforms and generic *E. coli* obtained from wild deer carcasses processed at Scottish approved game handling establishments (AGHEs). The counts of these bacteria are proxies for faecal contamination, and thus potential STEC contamination, of the carcasses.

Microbiological results of carcass samples collected on the field and at larders

The bacterial counts obtained at primary sources were generally very low. For the samples collected on the hill, the mean counts were 2.33 $\log_{10}$ cfu/cm^2 in the case of coliforms and 0.43 $\log_{10}$ cfu/cm^2 in the case of *E. coli*. The samples collected at the larder, two to four hours from the time the carcasses were culled and sampled on the field, gave mean values of 2.62 $\log_{10}$/cm^2 and 0.83 $\log_{10}$ cfu/cm^2 for coliforms and *E. coli* respectively, marginally higher than the values obtained from hill samples. This indicates a small amount of bacterial growth in the interval between kill and transfer to the larder. A previous study observed that hygienically dressed wild deer carcasses generated Enterobacteriaceae counts (consisting of both coliforms and *E. coli*) of median 2.1 $\log_{10}$ cfu/cm^2 for both red and roe deer (Avagnina *et al.*, 2012). The observations for coliforms and *E. coli* in the present study were in line with the recommendations of regulation (EC) No 2073/2005 as amended by (EC) No 1441/2007 on microbiological criteria for domestic ruminant carcasses after dressing but before chilling. The results show that, overall, freshly killed carcasses were dressed hygienically to a standard suitable for human consumption

Microbiological results for meat contact surfaces in the field, larder and AGHE

Except for two of 28 environmental swabs at AGHE and one of seven environmental swabs collected in the field or larders, all meat contact surface swabs were positive for coliforms, with values ranging from 1.07–7.31 $\log_{10}$ cfu/cm^2. The counts observed were higher from the environmental samples collected at the AGHE, with a mean of 5.46 $\log_{10}$ cfu/cm^2, compared with those collected from meat contact equipment used in the field or at the larder, with a mean of 2.89 $\log_{10}$ cfu/cm^2.

For *E. coli*, 9 out of 35 meat contact surface samples were positive, with values ranging from 0.69–4.88 $\log_{10}$ cfu/cm^2. At AGHEs, environmental samples were more often positive (8 out of 28) when compared with field or larder samples (1 out of 7). The *E. coli* counts observed on meat contact surfaces at AGHEs were in higher

ranges, with mean 0.76 log_{10} cfu/cm^2, compared with field or larder samples, with mean 0.15 log_{10} cfu/cm^2.

Microbiological results of carcases collected at AGHE

Samples were taken from the hides and internal cavities of the carcass prior to skinning, and the external surface of the carcass after skinning. Carcass geometric means for the coliform counts, irrespective of the deer species were:

• **Coliforms**: 5.78 log_{10} cfu/cm^2 on the hide, 6.80 log_{10} cfu/cm^2 in the cavity and 6.36 log_{10} cfu/cm^2 on the external surface of the carcass. The comparison of the combined mean values of log coliforms obtained from each sample type from all the three deer species indicate the coliform counts were the highest from the samples collected from the cavity and the lowest from the samples collected from the hides.

• ***E. coli***: 1.82 log_{10} cfu/cm^2 on the hide, 2.27 log_{10} cfu/cm^2 in the cavity and 2.17 log cfu/cm^2 on the external surface of the carcass. Comparing the mean values obtained on the three types of sample, the *E. coli* counts were marginally higher in the cavity samples when compared with the carcass samples, and much higher than in the hide samples.

The mean values of both log_{10} coliforms and log_{10} *E. coli* were higher for red compared with roe deer in all three types of carcass sample. This differs from the scientific literature where higher levels enteric bacteria have been found on red compared to roe deer carcasses.

When following the guidelines of the process hygiene criteria outlined in the EU legislation 2073/2005 as amended by (EC) No 1441/2007, and the literature on wild game carcasses, the mean coliform results were higher in the cavity and external carcass sample types, for both red and roe deer, than expected limits for similar bacterial groups in domestic ruminants. An analysis of the *E. coli* results shows that the mean bacterial counts were just below the described upper limits for similar bacterial groups for domestic ruminants. However, 55% of the cavity and 57% external carcass samples exceeded the more stringent 2 log_{10} cfu/cm^2 recommended maximum value expected for well-dressed wild deer carcasses (Paulsen, 2011).

Microbiological results of meat samples collected at AGHE

• **Coliforms** on meat preparation samples (burger meat, diced meat and trimmings) ranged from 5.92- 7.60 log_{10} cfu/g (mean 7.01, 95% CI:6.52- 7.50). These values for coliforms are higher than previously described in the literature for coliforms in deer meat, which at a mean of 2.56 log_{10} cfu/g (95% CI: 2.00–3.10) were around 4 log_{10} cfu/g lower than our findings.

• ***E. coli*** on meat preparation samples ranged from 1.14- 4.77 log_{10} cfu/g (mean 3.30, CI: 2.26-4.34). According to hygiene criteria of Regulation (EC) No. 2073/2005 as amended, the acceptable limit for *E. coli* in meat preparations from livestock is 5,000 cfu/g (3.7 log_{10} cfu/g). Except for two burger samples, the *E. coli* meat counts obtained in this study were within the limits considered acceptable for livestock.

Statistical analysis to identify risk factors for bacterial contamination

Twenty-three variables were analysed statistically for their influence on the microbiological condition of the carcasses in terms of coliform and *E. coli* counts averaged across samples from the hide, cavity and carcass for each deer. Four statistical models were created, for:

- Coliform counts on roe deer carcasses;
- *E. coli* counts on roe deer carcasses;
- Coliform counts on red deer carcasses;
- *E. coli* counts on red deer carcasses.

Results of the multivariable analysis for average log coliforms in roe deer

The factors that were associated with higher average log coliform counts in roe deer were:

- Time in storage of 6 days or more (p=0.019);
- Distance (in miles) between cull location and AGHE (p=0.009);

The increase in coliforms with time in storage would explain the increasing coliform levels on carcasses that were observed from swabs taken in the field (lowest levels), through marginally higher levels at the larder, to much higher levels for carcasses swabbed at the AGHE.

Results of the multivariable analysis for average log *E. coli* in roe deer

The factors that were associated with higher average log *E. coli* counts on roe deer carcasses were:

- Warmer temperature, above 7ºC (p=0.015);
- High levels of faecal contamination, marked in this study as level 3 (1–2cm) and level 4 (>2cm) (p=0.003 and 0.028 respectively);
- Dryness, where wet and slimy carcasses were significantly associated with higher average log *E. coli* counts (p=0.011);
- Males (p=0.037).

Results of the multivariable analysis for average log coliforms on red deer carcasses

There were no factors that were significantly associated with higher average log coliform counts on red deer carcasses.

Results of the multivariable analysis for average log *E. coli* on red deer carcasses

The factors that were associated with higher average log *E. coli* counts on red deer carcasses were:

- Dirty skin condition (p<0.001);
- High levels of faecal contamination (>2cm) (p=0.001).

5 Objective 3.2: Field studies to identify risk factors associated with E. coli and coliforms in wild venison

5.1. Introduction

The wild venison sector has evolved from what has been viewed historically as a minority sport to a well-developed food industry. Wild deer carcasses that are culled for both sporting and population control are now commercialised as meat for human consumption. Although this sector functions quite differently from the livestock sector, the venison industry is highly regulated by governmental bodies as well as by non-governmental, professional organisations that develop and advise on best practices for culling and processing deer for human consumption.

Wild deer, like other domestic ruminant species, are known to carry pathogens that can adversely affect the health of humans. In October 2015, an outbreak of *Escherichia coli* O157 Phage Type 32 took place in Scotland and this has been linked to the consumption of venison product (Browning *et al.*, 2016). This has led to the need to understand the key factors that influence the microbiota of wild deer as well as to determine the risk factors that affect the microbiological condition of carcasses and meat that reach the commercial food chain in Scotland.

In response, the Scottish Government and Food Standards Scotland (FSS) commissioned a quantitative risk assessment study to determine the risk factors that are associated with generic *E. coli* and coliform bacteria in wild deer carcasses and meat. The study involved visually assessing the condition of deer carcasses and determining the microbiology of samples collected all stages of production from the field to the AGHE, followed by a statistical analysis of the findings to determine the risk factors associated with the two groups of bacteria.

The scope of the project was to identify key risk factors associated with the microbiological condition of venison carcasses during processing from the hill to the final product, and to provide the findings that can inform recommendations to support the venison sector in producing safe and quality wild deer food products.

5 Objective 3.2: Field studies to identify risk factors associated with E. coli and coliforms in wild venison

5.2. Field and laboratory study

5.2.1 Methods

This study included the following types of sample:

- Carcasses collected at AGHE (n=214);
- Meat samples (n=7);
- Carcasses collected on the field and at the larder (n=14);
- Swabs of meat contact surfaces (environmental swabs) collected at AGHE (n=28) and on the field and at larder (n=7).

5.2.1.1 Sample collection

Locations for sampling

According to the Food Standards Scotland (FSS) database of approved establishments[71], as of September 2017 there were 11 approved game handling establishments (AGHEs) that handle venison (see Appendix 5). All AGHEs were contacted by email prior to the start of this study to explain the aims of the project and assure them of the anonymity of the outputs in order to discuss interest in collaboration for data collection.

Six venison food business operators of AGHEs (35% of all in Scotland) of a range of sizes and locations agreed to participate in the study. Three were located in the North, 1 Central and 2 in the South of Scotland. There are no AGHEs on the west coast of Scotland, yet deer are culled from this region and sent to establishments located either in the north-east or the central part of the country. Sampling was carried out to ensure the study represented all areas of Scotland from which deer are culled for human consumption with the exception of the Islands.

[71] https://www.foodstandards.gov.scot/publications-and-research/publications/approved-premises-register

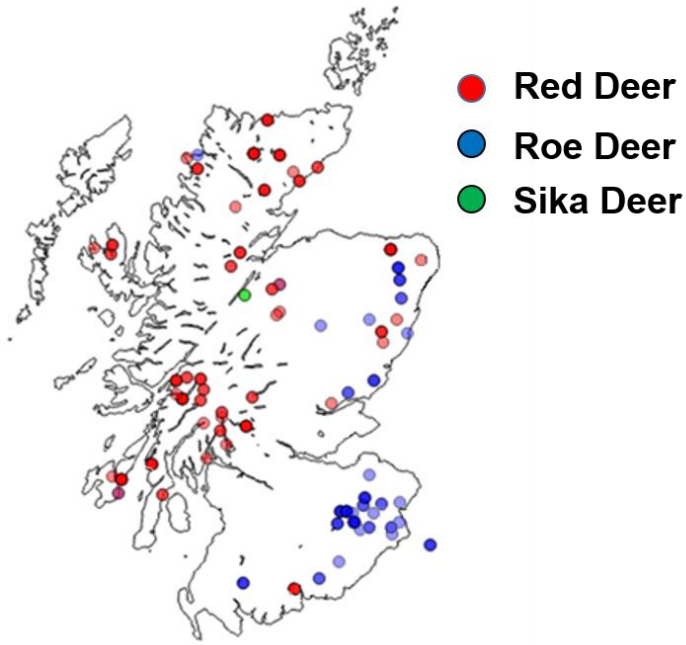


***Figure 5.1** Location of deer cull sites included in the study. Red, blue and green dots indicate the cull sites for the red, roe and sika deer carcasses sampled respectively. Darker shades of red and blue indicate locations where more than one red or roe deer was culled; lighter red and blue dots indicate locations where only one red or roe deer was culled. There were no cull locations where more than one Sika deer was culled.*

This study focused on mainland Scotland. Establishments that operate on the islands (Mull, Islay, and North Uist) were excluded because it was not feasible to transport the samples in a timely manner back to the microbiology lab. Some of the carcasses that are processed on the mainland do come from the islands so deer from some of those islands are represented in this study.

As Table 5.1 below shows the number of wild deer samples (total and by species) that were collected from the different AGHEs in this study.

Selection of deer carcasses for sampling

The number of deer carcases selected for the study depended on the size of the AGHE. For small-scale operators, all deer carcasses available onsite on the sampling date were included in the study. This ranged from 4 to 26 deer, whether red, roe or a mixture of both species, depending on the type of wild deer that the operator was processing on the sampling day. In the case of medium- or large-scale operators, carcass selection was carried out to represent as many locations of Scotland as possible. Whenever possible, we have attempted to capture as many species as possible and both sexes.

Over the course of the sampling timeframe, from October 2017 to April 2018, around 50 deer per region were sampled. However, the samples were not necessarily taken in the same region where the AGHE was located, because AGHEs often receive carcasses from outside their region.

Table 5.1 *The size of the different AGHEs that participated in the study and the total number of deer of each species collected.*

	Size of AGHE*	AGHE	Number of carcasses			
			Total	**Red**	**Roe**	**Sika**
	Medium	A	31	25	6	0
	Large	B	50	46	2	2
	Medium	C	57	2	54	1
	Large	D	54	53	1	0
	Small	E	17	6	11	0
	Small	F	5	0	5	0
Totals			**214**	**132**	**79**	**3**

*Small, <50 carcasses; medium, 50-200 carcasses; large, >200 carcasses

Sample types at approved game handling establishments (AGHEs)

Carcasses: In total, 214 carcasses were collected, which included red deer (n=132; 105 females and 27 males), roe deer (n=79; 51 females and 28 males) and sika deer (n=3; 3 females and 0 males). Due to the low sample size for sika deer it was not possible to perform statistical analysis on this species and therefore only the microbiology of the carcasses was assessed. The spatial distribution of red and roe deer in Scotland is not even (Figure 5.1). Red deer thrive at higher altitude, and roe deer on low hills or flatter terrain). As a result, the distribution of samples from different species is not similar across the AGHEs. Red deer species were more commonly processed in the northern and central parts of Scotland, whereas roe deer were more readily available from the operators in the south of Scotland.

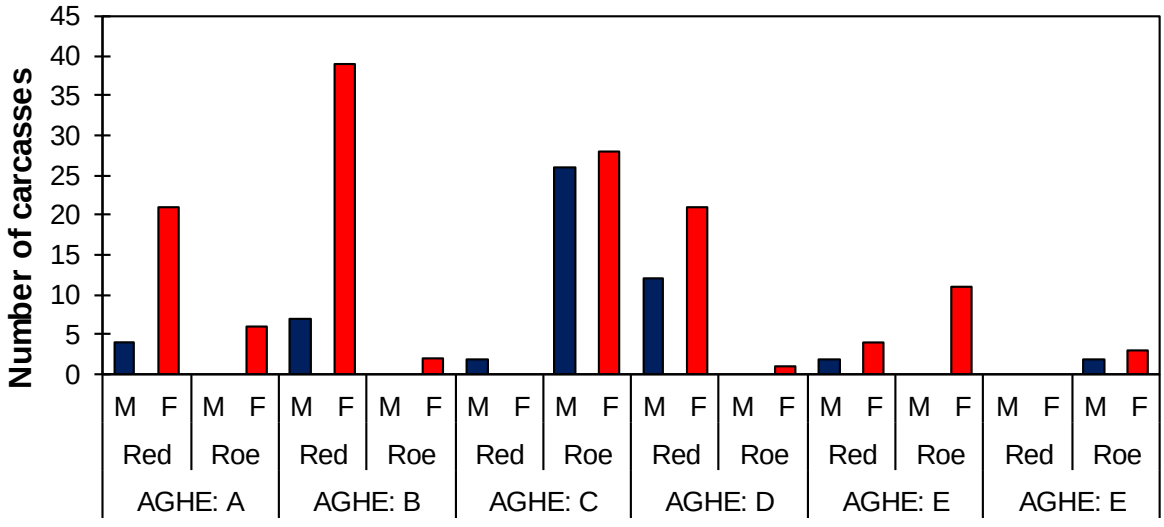


Figure 5.2 *Numbers of Red and Roe deer by sex collected from each of the six AGHEs sampled. M = male; F = female.*

Both sexes were represented in the overall sample size, however, the number of male carcasses were generally lower than female carcasses (Figure 5.2). This is partially due to the time of the year when operators were available to participate in the study but also because the numbers of culled females is generally higher than males, especially in red deer species.

The information collected at the time of sampling from the AGHEs included: deer species, sex, time and place of kill, time and date when the sample was collected. For each deer carcass three types of swabs were collected, as per below:

1. **The hide ('hide')**: these were collected before skinning to evaluate any contamination on the hide that might have occurred in the field or during transport of carcases.
2. **The internal surface of cavities** (abdomen, pelvis and thorax) (**'cavity'**): these were collected before skinning, to have a baseline evaluation of hygiene practices during gralloching and to understand whether microbial contamination takes place via the shot wound.
3. **The external surface of the carcasses ('external carcass')**: these were swabbed immediately after skinning to evaluate the microbial contamination of the external carcass surface in relation to the cleanliness of the skin, the hygiene of the skinning technique including operator's hand/tool hygiene.

Environmental swabs: In addition to the carcass samples, 35 environmental swabs were collected from a variety of meat contact surfaces: knives, cutting tables, carcass hooks, holding racks, chiller walls The purpose of assessing the microbiology of the environmental samples was to have a baseline understanding of hygiene practices via measurements of coliforms and to see whether *E. coli* survives on contact surfaces at cold temperature.

Meat samples: In total, 7 samples of diced meat (n=4), burger meat (n=2) and meat trimmings (n=1) intended for human consumption were analysed. These samples were collected from site A: 1 burger sample and 1 diced meat sample; site B:3 diced meat samples; site C: 1sample consisting of meat trimmings; site E: 1 burger sample. Although data collection was carried over 13 sampling sessions, meat samples weren't collected on each opportunity as one of the large operators from which we sampled does not carry cutting operations and the rest of the establishments did not always make meat preparations on the same day when skinning and dressing of carcases take place, thus when sampling was carried.

The total number of carcasses were collected between 16th Oct 2017 and 23rd April 2018. Specific times of the sessions, locations and species collected are outlined below in Table 5.2a. The days of collection were agreed with the AGHE operators who informed us when skinning and final dressing of deer carcases took place. We were not able to organise collection of samples during late spring and summertime as the AGHEs were either not operational or they were processing sporadically a very small number of carcasses that would have made the collection of samples very difficult and of small statistical significance. However, within the sampling period there were periods of milder weather (October, November and April) as well as very

cold weather (December to February) and therefore we were able to assess the influence of outdoor temperature on the microbiological condition of carcasses. Most of the sampling sessions took place during the culling of female (hinds/doe); therefore, a greater proportion of females compared with males (stags/bucks) are represented in the study.

Table 5.2a *The times and deer sexes included in the study*

Collection dates	AGHE	Sex collection	Hunt season
16.10.2017	C	Roe bucks	Roe buck
23.10.2017	C	Bucks, doe	Transition buck to doe
	C	Hind sika	Sika and red hinds
01.11.2017	A	Doe	Doe
	A	Stags and Hinds	Transition stag to hind
08.11.2017	B	Hind and Stags	Transition stag to hind
29.01.2018	D	Hinds and stags	Hind
		Doe	Doe
30.01.2018	B	Sika and red hinds	Sika and red hinds
		Red stags	Out of season
		Doe	Doe
06.02.2018	A	Hinds and Stag	Hind
		Doe	Doe
07.02.2018	E	Hind and Stags	Hind
		Doe	Doe
12.02.2018	D	Hinds and Stag	Hind
12.03.2018	C	Stags	Out of season
		Doe	Doe
19.03.2018	C	Doe and buck	Doe
27.03.2018	F	Doe and buck	Doe
23.04.2018	C	Bucks	Buck

Sample types at larder and hill

Additionally, to the samples collected at AGHEs, a pilot study was conducted at the place of cull where a total of 14 deer carcasses were collected. Table 5.2b provides details of the concerning these sampling sessions.

Table 5.2b *Field and larder sampling sessions*

Sampling date	Area	Deer specie	Number females	Number males
13.09.2018	Strathaven	Deer not found	0	0
03.10.2018	Aberfoyle	Roe	0	1 Roe
05.10.2018	Aberfeldy	Roe	2 Roe	1 Roe
10.10.2018	Galloway	Red and Roe	1 Red 1 Roe	4 Red 1 Roe
29.10.2018	Kelty	Roe	0	2 Roe
28.11.2018	Dumbar	Roe	0	1 Roe

Stalkers were contacted during May 2018 and again in July 2018 with a view to assist in facilitating access to carcass swabbing during the stalking sessions. Due to the practicalities of deer culling with vegetation being high during summer and poor visibility for hunting as well as busier tourist season, the sampling was not possible during summer. We have been successful in arranging sampling dates in autumn 2018, as per Table 5.2a. However, each sampling session was generally limited to one or two deer carcasses, if any, depending on how successful the stalker was. On one opportunity the sampling session was more productive and 7 carcasses were collected as the sampling operator accompanied three deer stalkers who hunted the same day in nearby locations. The sampling sessions required full day allocation including early travel or overnight stay in a nearby location, stalking for the entire morning and travel back on the afternoon to dispatch the samples to the laboratory. The sampling sessions were organised based on the limited response of the deer stalkers and were suspended in early December 2018 to be able to process the data and collate the report due in January 2019.

The method was the same as at AGHE, and the hide and carcass cavity samples were taken in field condition, immediately after deer were culled to assess the microbiology of freshly shot deer and to determine whether significant contamination occurs at the time of kill. The same sampling procedure was repeated on the same deer carcasses at the larder, 90 minutes to 4 hours after the time of kill. The sampling carried at the larder had the scope to determine the microbiology of carcasses after transport and to assess if the dressing of carcasses reduced any contamination that might have occurred in the field.

5.2.1.2 Sampling technique

The sample tools are shown in Figure 5.3. The hides and carcasses were swabbed with 5 x 10cm 'TS/15-B: NaCl – sterile carcass hygiene blue sponge' in 'Easy Open

Stomacher Pouches' dosed with 0.9% saline. The areas swabbed were measured with a 10cm² 'TS/15-T40 – sterile plastic sampling template'. Carcasses were sampled in the internal cavity ('cavity sample') and the carcass surface after skinning ('carcass sample').

Figure 5.3 *Sterile sponge, pre-soaked with saline solution (left) and sterile frame used for measuring the swabbed surface (right)*

For hide samples, the surface area swabbed was 750cm^2 in red and sika deer and 500cm^2 in roe deer. One sponge was used for each of the areas: hide, cavity and external carcass resulting in three sponges for each carcass. Both sides of the sponge were used. If the sponge became visually contaminated, it was turned on the other sterile side. To cover the desired surface, the sterile plastic frame was moved to different locations of the body until the set surface was covered. The sampling areas were selected from both sides of the body, including the area around the tail and anus, rump, flank, thorax and neck. The sample was collected by swabbing with 20 strokes (up-and-down or side-to-side movements were counted as single strokes) using enough pressure to remove any dried debris.

For the cavity and carcass samples, the swabbing followed the requirement of Regulation (EC) 2073/2005 Annex I Chapter 3 (as amended by 1441/2007) for the collection of hygiene samples from red meat carcases. This non-destructive method covered 750cm^2 in red and sika deer and 500cm^2 in roe deer, from the thoracic, abdominal and pelvic cavities before the skin was removed. The external surface of the carcass after skinning was also swabbed, by following a continuous swab taking an 'S' shape running from the back leg, through the flank and thorax, to the front leg and neck. One side of the sponge was used for each side of the carcass, totalling to the same surface areas of 750cm^2 for red deer and 500cm^2 for roe deer.

The sponge was held through the sterile bag, folding the bag back over the hand (Figure 5.4) and was wiped with firm pressure and a slight side-to-side movement. The bag was refolded over the sponge and secured with the seal provided.

Each of the three swabs belonging to one carcass received a sample number that corresponded to the tag number of the animal, as shown on the carcass label. Each

of the sample bags were annotated to include details such as the sample type ('hide', 'cavity', 'external carcass') date, time and sample location.

All samples were immediately cooled and stored at 4°C, and transported with ice packs in insulated shipping boxes to reach the microbiology laboratory at the Roslin Institute within 24 hours from sample collection.

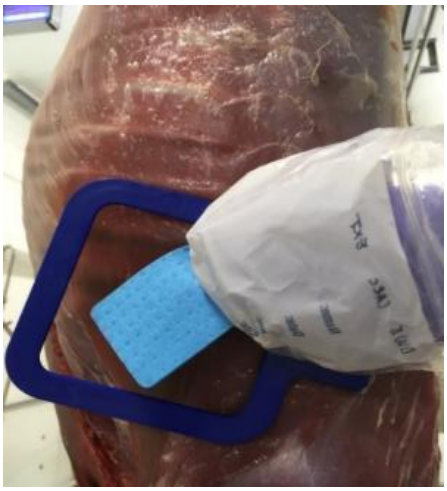


Figure 5.4 *Sampling technique*

The sample numbers enabled them to be cross-referenced with the **data collection sheet** (see Appendix 12), that included a visual assessment from each carcass sampled and other data variables to be included in the statistical analysis of risk factors associated with the bacterial counts obtained from the laboratory results.

5.2.1.3 Laboratory microbiology

The following method was used to identify and enumerate the generic *E. coli* and coliform bacteria from the carcass, environmental and meat samples.

Upon receipt to Roslin microbiology laboratory, swab samples in bags (pouches) were stored at 4°C in the dark for a maximum of 1 week. 10ml 0.9% (w/v) NaCl was added to the samples in bags (pouches) and suspended thoroughly. The plating involved spreading 0.1ml of 10-fold dilutions of sample in 0.9% (w/v) NaCl onto chromogenic medium (Sigma #81938 or Merck #110426) at 37°C overnight following by counting of the highest dilution where at least 10 *E. coli* and coliform colonies developed.

Broth enrichment in Oxoid #CM1049 buffered peptone water was carried out for later use of the samples collected. The sample in the sterile tube was inoculated directly into 5ml buffered peptone water and was left at 37°C overnight on a shaking incubation bed. Following incubation, 1ml of enrichment was removed from the sterile tube, centrifuged and after the supernatant was discarded the precipitate pellet was stored at -20°C for future InstaGene DNA extraction (Bio-Rad #7326030). The rest of the enrichment was also centrifuged and after the supernatant was discarded the

precipitate pellet was re-suspended in 1ml 50% (v/v) glycerol and stored at -75°C for future *E. coli* isolation.

The microbiology laboratory provided the results expressed into counts and dilutions for each sample type, as per traceability annotated on the sample bag to give a three values per carcass, one for each sample type: 'hide','cavity' and 'external carcass'. The counts, dilution counts and dilution factors were multiplied to obtain total colony forming units (cfu) per sample, which were further calculated per unit area of meat sampled and transformed into logarithm$_{10}$ per square centimetre (cm^2). Prior to log$_{10}$ transformations a value of one was added to each indicator (cfu/cm^2) to avoid obtaining negative logarithm results in instances when counts and dilutions readings were very low.

5.2.1.4 Risk factor variables

The risk factors considered in this study were selected following a systematic literature review carried out in Section 4: Objective 3.1 of this report. These risk factors were included in our data collection sheet (Appendix 12), and embedded into the risk factor analysis.

Response variables

The response variables included the level (log$_{10}$/cm^2) of coliform bacteria and generic *E. coli* as general indicators of faecal contamination of carcases.

Predictor variables

A detailed description of the predictor variables considered during the statistical analysis is provided below as extrapolated from the systematic literature review. A summary of the predictors is also provided in Appendix 13 (variables for statistical analysis).

1. Distance from cull

Based on the postcode of the cull location we have calculated the distances that the carcasses were transported before they reached the AGHE. These distances (in km) represent the exact road distance between the two coordinates, calculated using Google Maps[72] . However, it also possible that some of the carcasses were uplifted by an approved game dealer who travelled between larders to collect more carcasses in one given day. In our model, we had assumed these were transported straight to the AGHE by the fastest route.

2. Seasonality

Evidence in the scientific literature suggests that deer might be shedding more *E. coli* at the end of the winter (Bartels A and Bülte M., 2011). There are other studies that observed higher STEC contamination of carcases in the warmer summer months compared with the colder winter months (Franklin et al., 2013).

An ambient temperature threshold was used in this study to capture seasonality. The threshold between warm and cold outdoor temperature was set at 7ºC, which is the

[72] www.google.co.uk/maps

legal required storage temperature for large wild game carcasses, as per Regulation (EC) 853/2004, Annex III, Section IV-wild game meat, Chapter 2, Paragraph 5. Any carcasses that were killed on days when the outdoor temperature was below 7ºC received the category 'cold' and animals that were culled when temperatures were of 7ºC or above received the category 'warm'.

The date and location given on the hunter's declaration was used to determine the temperature at the time and place of culling, by cross-reference[73] where temperatures can be checked historically for all regions of Scotland, providing both hourly and daily average temperatures. The average temperatures on the days cross-referenced did not differ significantly from the early morning and evening temperatures – the times when culling generally takes place. These daily averages were therefore used to decide whether deer were culled on a cold or warm day.

3–5. Days in storage

Information such as the date when the animal was killed and the date when the sample was collected helped to calculate the time difference between the day of the cull and the day of the sampling. This was used to assess whether the storage interval had any influence on the microbiological quality of the carcass. In the statistical analysis, days in storage was treated as both a continuous variable and a categorical variable. The length in time (in days) (Variable 3) between the date of the cull and the date of the sample was the interval that the carcasses spent in storage, hanging with skin on, ranged from 1 to 19 days, with a median of 6 days (Interquartile range (IQR: 6). Two categorical variables were created around the median of 6 days (Time category 1: less than 6 days (variable 4); Time category 2: less than 7 days (variable 5)).

6. Species

Based on the available evidence, it is inconclusive whether Red deer are likely to shed more STEC than Roe deer. Based on a sample size of 30 Red and Roe deer (Eggert *et al.* (2013) observed 93% of Red deer samples were positive for Shiga toxin, compared to 73% of Roe. However, Bardiau *et al.*, (2010a), (Obwegeser *et al.*, 2012) observed marginally higher prevalence of STEC in Roe deer. Therefore, species was considered as a variable in the statistical analysis, however, only Roe and Red deer were included due to the very low sample size for Sika deer (n=3). This reflects the main deer species handled in Scottish AGHEs and consequently, the majority of venison product available for human consumption.

7. Sex

The study carried by Bardiau *et al.* (2010) did not find a statistically significant difference between deer sexes with regards to carriage of STEC, however these observations are based on a very small sample size and the current study aimed to assess whether there is a difference between sexes in STEC carriage. This variable included a total of 55 males: (n=27 Red deer and n=28 Roe deer) and 159 females (n=105 Red deer, n=51 Roe and 3 Sika) were collected.

[73] www.timeanddate.com

8. Body condition

The body condition scores were adapted from the Scottish Natural Heritage report 'Welfare indicators for deer' (Scottish National Heritage, 2016). Fat was an indicator of the carcass body condition, looking specifically at the quantity of the fat distributed across the external surface of the carcass, as well as inside of the abdominal and the pelvic cavity, particularly around the kidneys (Figure 5.5 below).

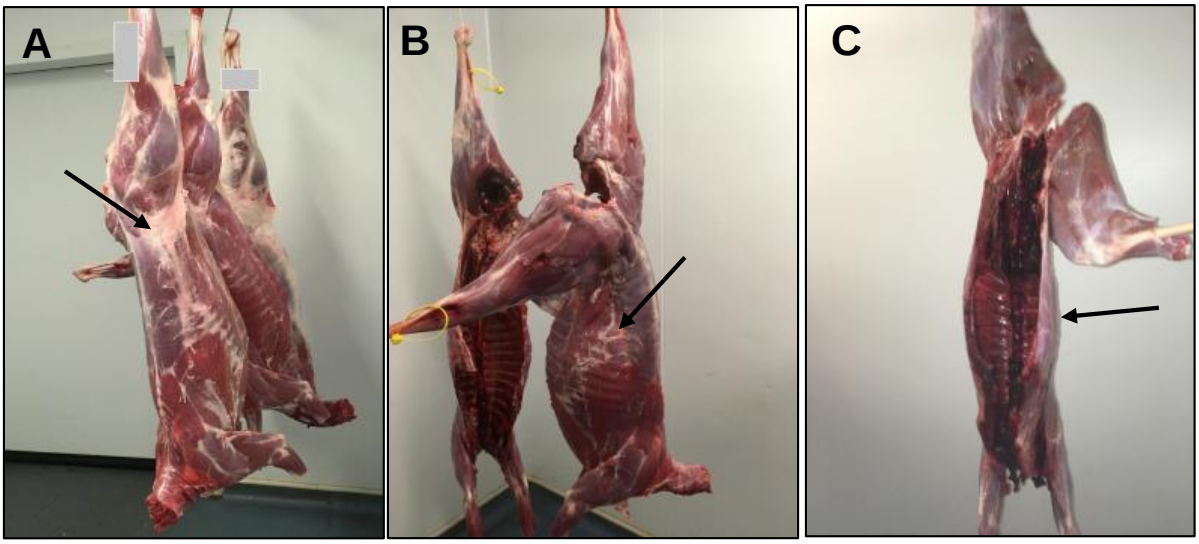


Figure 5.5 *Credit photos: C. Soare Body condition scoring: (A) condition 1, fat; (B) 2, lean; (C) 3, very lean. Note the subcutaneous fat (indicated by the arrows) is abundant in (A), less but still present in (B) and scarce in (C).*

Body condition 'Fat'=1 was allocated to those carcasses that were well covered with muscle and fat. This body score was allocated if the bone landmarks were well covered by muscles presenting a round outline, the spine and ribs could not be appreciated from under the muscle and the subcutaneous fat and the kidney fat was well represented.

Body condition 'Lean'=2 was allocated to carcasses where the bone landmarks of the pelvis were not obvious from under the muscles, had a flat contour of the rump and leg muscles, the ribs, neck were covered in thinner layer of muscles and the fat over the body and abdominal cavity was less represented but still well present.

Body condition 'Very lean'=3 was allocated to carcasses that presented a pelvic cavity with a concave contour and with the landmarks of the pelvis clearly visible whilst hanging. Other characteristics that were considered were the outlines of the thorax, with ribs clearly visible, and the neck and shoulder bones well shaping from underneath the muscles. The carcass surface fat and the cavity fat had to be scarce to be categorised as very lean.

9. Skin condition 1 (3 levels)

The hides/skins of the carcasses were visually assessed for post mortem condition. If presenting a shiny appearance, with a thick strong hair, the hide was categorised as '1' or very good. Skins that looked relative healthy but not with a shiny appearance

were categorised 2, or good. Finally, hides that had dull appearance and patches of missing fur, were categorised as 3 or coarse.

10. Skin condition 2 (4 levels)

The hides were also assessed visually for hygiene. Those that were very clean and dry were marked as Category 1. Skins that were presented dirty (blood, soil,) or wet (from the environment) were marked as Category 2. Skins that showed evidence of moulting were marked as category 3 and a final category (4) was created for when skin condition was not determined as the operator accidentally had removed the skin before sampling took place.

11–13. Disease

The main reason for assessing the diseases status of the deer was to test the hypothesis whether deer in poor condition are more likely to have a higher *E. coli* counts, in comparison to healthy deer due to the underlined stress that the body is experiencing to sustain its functions. The carcass was inspected for any abnormalities and the main condition criteria for the deer, as recorded in the data collection sheet (Appendix 12). For carcases that showed any pathological abnormalities such as old wounds, anaemia, abscesses, warble, ticks (*11. Disease, any (Any health issues)*), these were marked with 1 as opposed to healthy carcasses that were marked with '0'. The most common conditions observed were skin parasitic diseases, therefore two further variables were created to represent these observations (*12. Disease, warbles*; *13. Disease, ticks*).

14. Injury

Injuries in this category were considered fresh fractures as a result of the fall immediately after the shot, excessive fresh bruising generated during the cull process, as well as older bruising, older fractures and calluses due to old injuries. All carcasses that presented these lesions were marked as '1'; those that did not present injuries were marked as '0'.

15. Bullet wounds

The number of entry bullet wounds, not including the exit bullet wounds were counted and deer that were killed with one shot were marked as '1' as opposed to those that required two or more attempts, which were marked as '2'.

16. Bullet wound contamination

The wounds left by the bullet were assessed to determine the extension of the damage (bruising, haemorrhage) and level of contamination (hair, faeces). Particular attention has been paid to bullet wounds closer to the diaphragm or the abdomen due to their higher potential to be contaminated. Carcasses that presented contamination of the bullet wound with hair, stomach content were marked '1' and those which had clean bullet wounds were marked '0'.

17–20. Carcass contamination

This data was collected once the skin was removed and the carcass was dressed to remove dried debris or any contamination in preparation for health marking, each

carcass was visually inspected on the external surface and cavities for faecal, hair, blood and environmental contamination. If any contamination was present, this was categorised as seen in Table 5.3, and the location affected was recorded on the data collection sheet (Appendix 12). These contamination categories are based on an adaptation of guidelines for livestock carcasses captured in the manual of official controls available at the FSS website (Food Standards Scotland, 2018).

Table 5.3 *Visual carcass contamination scores*

17. Faecal contamination (One or more locations cumulatively)	No faecal contamination	0
	<0.5cm^2	1
	0.5-1cm^2	2
	1-2cm^2	3
	>2cm^2	4
18. Hair contamination (One or more locations cumulatively)	0-10 hairs	1
	10-15 hairs	2
	>15 hairs	3
19. Blood contamination	When excessive dried blood was observed in the carcass, either in the abdominal or thoracic cavity, this was recorded to determine whether insufficient or delayed bleeding could have had any implications on the microbiological condition of the carcases	1: Blood in excess 0: No blood in excess, no cavity staining
20. Environmental contamination (Soil, leaves, grass)	No contamination	0
	Soil or foreign bodies	1

21. Dryness of carcass

This variable was extracted from the literature review carried out as part of the first objective of this research project. Carcasses were visually assessed to determine their dampness/dryness on the cavities to determine whether any slimy film was present. The external surface of the carcass was often damp as the samples were collected very shortly after the skin was removed, however cavities had been exposed to air from the time of evisceration. If the ventilation and velocity in the air in the chiller is not adapted to the quantities of carcasses held, is likely that for a few days after the time of kill carcasses maintain a damp/ wet appearance on the cavity, particularly in the abdomen. If this wet appearance persisted for a longer interval, the

carcasses can sometime develop a slimy, jelly-looking appearance or slightly mouldy appearance, same as in case of beef that is kept for a long in interval in the chiller for maturation purposes. Based on their moisture condition, which could also be due to a larger storage interval, the carcasses were classified as either 'dry'=1, 'wet'=2 or 'slimy'=3.

22. Dryness/wet or slimy category

A further category was created to differentiate the dry carcasses from those that were either wet or slimy to assess the influence of dampness on bacterial growth. Carcasses were either categorised as 'dry' (0) or 'wet or slimy' (1).

5.2.2 Results

5.2.2.1 Lab results from carcasses collected at AGHEs

A summary of the mean and median values for each of the sample types: '**hide**', internal cavity ('**cavity**') and carcass surface after skinning ('external **carcass**') collected during the study are collated in Table 5.4.

Coliforms: The geometric means for log coliform counts, irrespective of the species, were of 5.78 log_{10} cfu/cm^2 on the hide, 6.80 log_{10} cfu/cm^2 on the cavity and 5.32 log_{10} cfu/cm^2 on the external surface of the carcass. Comparison of the mean values of log coliforms obtained from each sample type from all three deer species indicates the coliform counts were the highest from the samples collected from the cavity and the lowest from the samples collected from the hides.

***E. coli**:* Mean logarithmic values of *E. coli* from the three sample types, in all the carcasses swabbed, indicate the highest value was obtained from the samples collected from the abdominal cavity (mean 2.27 log_{10} log cfu/cm^2), followed by the samples collected from the external surface of the carcass (mean 2.17 log_{10} cfu/cm^2) and the lowest counts from the hide (mean 1.82 log_{10} cfu/cm^2).

In most cases, *E. coli* counts were obtained from all three types of sponges (hide, cavity and external surface of the carcass). In few instances *E. coli* was not isolated from sponges collected from the hide and we propose this could be due to the fact that *E. coli* bacteria survives for a limited time on the skin and hair surface, which may be an unfavourable environment for enteric bacteria. The carcasses that did not display *E. coli* on the sponges taken from the hide, always had *E. coli* bacteria on the sponges taken from abdominal cavity or from external surface of the carcass or both.

Comparison or red vs. roe deer: Statistical comparisons of mean logarithmic coliforms and *E. coli* levels in red vs. roe deer carcasses for 'hide', 'cavity' and 'carcass' samples, were performed using a General Linear model (GLM) with log count as the response variable and species (Red, Roe) and location (hide, cavity, carcass) as the predictor variables. The interaction was tested and removed if it was not significant ($p>0.05$). Coliform counts were significantly higher in Red deer than Roe deer (GLM coliforms species: $F=34.39$, $df=1,611$, $p<0.001$) and for both species the location was significant (GLM coliforms location: $F=85.11$, $df=2,611$, $p<0.001$) with coliforms higest

on the cavity and lowest on the hide (Table 5.4); *E. coli* counts within a species depended on the location (GLM *E. coli* species*location: F=85.11, df=2,615, p=0.026), although red deer were always higher than roe deer and the *E. coli* counts were always the lowest on the hide (Table 5.4).

5.2.2.2 Lab results from meat samples collected at AGHEs

The values obtained from meat samples collected from four of the six processors that participated in the study are reported in Table 5.5. Overall, the results obtained from the meat were mean 7.01 (95% CI:6.52, 7.50) $\log_{10}$ cfu/g coliforms and mean 3.30 (95% CI: 2.26, 4.34) $\log_{10}$ cfu/g *E. coli*. Previous literature (Membré, Laroche and Magras, 2011) describe coliforms in venison meat resulting in a mean 2.56 $\log_{10}$ cfu/g (95% CI: 2.00, 3.10) and *E. coli* in mean 2.20 $\log_{10}$ cfu/g (95% CI: 1.65, 2.76). According to process hygiene criteria (Regulation (EC) No. 2073/2005) for meat and products thereof, the acceptable range for *E. coli* in meat preparations is less than 5000 cfu/g (3.7 cfu/g) (see Table 4.3). Two samples (burger samples from AGHE A and AGHE E) exceeded these ranges, with levels of *E. coli* = 4.77 $\log_{10}$ cfu/g and 3.96 $\log_{10}$ cfu/g respectively.

5.2.2.3 Lab results from carcasses at field and larder

Table 5.6 provides a summary of the results obtained in the field study, from all 14 carcasses sampled. The results reported include minimum and maximum values observed and the mean of all 14 values. Generally, the bacterial counts obtained at primary sources were very low. The colony counts and the dilution obtained from the laboratory were in the lowest ranges, which led to negative logarithmic values when the mathematical calculations were applied (dividing the counts and dilution by the surfaces tested and further converting these values into logarithms). Thus, a value of one was added to to each indicator bacteria (cfu/cm^2) to correct negative logarithm results.

Results for Coliforms collected on the field, generated mean 2.34 $\log_{10}$ cfu/cm^2 for skin and 2.42 for cavities. These means were lower than those for Coliform skin samples collected at the larder which resulted in mean 2.83 $\log_{10}$ cfu/cm^2 and equal to the mean of 2.42 $\log_{10}$ cfu/cm^2 for cavities sampled at the larder.

Mean *E. coli* results from the field samples were marginally lower in both the skin (0.30 $\log_{10}$ cfu/cm^2) and cavity (0.48 $\log_{10}$ cfu/cm^2) samples when compared to mean values for the skin and cavity samples collected at the larder which were 0.63 and 1.03 $\log_{10}$ cfu/cm^2, respectively as shown in Table 5.6.

The average time between shooting and gralloching was 43 minutes. The average time elapsing between shooting and dressing of carcasses at the larder was 3.3 hours.

Table 5.4 *Microbial count results for all carcass sample types collected at AGHEs.*

Sample type	Species	No. of samples	Min,*	Max.*	Mean*	SD	Median*
Coliforms, hide	Red	126	3.47	7.87	5.95	0.75	6.04
	Roe	68	2.56	7.23	5.46	1.03	5.54
	Sika	3	5.23	6.43	5.99	0.66	6.32
	Red and roe	194	2.56	7.87	5.77	0.88	5.96
	All species	197	2.56	7.78	**5.78**	0.88	5.96
Coliforms, cavity	Red	132	4.05	7.79	6.92	0.66	7.00
	Roe	79	4.18	7.86	6.58	0.89	6.63
	Sika	3	6.49	7.28	6.95	0.41	7.09
	Red and roe	211	4.05	7.86	6.79	0.77	6.96
	All species	214	4.05	7.86	**6.80**	0.76	6.96
Coliforms, carcass	Red	131	4.03	7.81	6.49	0.64	6.47
	Roe	79	3.39	7.59	6.14	0.94	6.34
	Sika	3	6.02	6.60	6.29	0.28	6.27
	Red and roe	210	3.39	7.81	6.36	0.78	6.46
	All species	213	3.39	7.81	**6.36**	0.78	6.46
E. coli, hide	Red	126	0	4.33	2.15	1.08	2.12
	Roe	68	0	3.97	1.19	1.05	1.06
	Sika	3	1.13	2.35	1.78	0.61	1.88
	Red and roe	194	0	4.33	1.82	1.16	1.85
	All species	197	0	4.33	**1.82**	1.15	1.86
E. coli, cavity	Red	132	0	5.88	2.54	1.36	2.61
	Roe	79	0	6.09	1.76	1.70	1.46
	Sika	3	1.63	4.79	3.55	1.68	4.23
	Red and roe	211	0	6.09	2.25	1.54	2.33
	All species	214	0	6.09	**2.27**	1.54	2.33
E. coli, carcass	Red	131	0	4.88	2.28	1.10	2.27
	Roe	79	0	4.57	1.99	1.10	1.93
	Sika	3	0.95	2.87	2.14	1.04	2.61
	Red and roe	210	0	4.88	2.17	1.10	2.19
	All species	213	0	4.88	**2.17**	1.10	2.20

** Values = log_{10} cfu/cm^2; SD = standard deviation. Hide = swab of external hide before skinning; Cavity = swab of internal cavity of carcass before skinning; Carcass = swab of external surface of carcass after skinning. No. = number; Min. = minimum value; Max. = maximum value.*

***Table 5.5** Microbial count results for meat samples collected at AGHE*

Sample type	**AGHE**	**Date collected**	**Coliforms log10 cfu/g**	***E. coli* log$_{10}$ cfu/g**
Diced Meat	B	November 2017	5.92	3.38
Diced Meat	B	January 2018	7.16	3.51
Diced Meat	B	January 2018	7.60	2.84
Burger sample	A	February 2018	7.12	4.77*
Diced meat	A	February 2018	7.01	3.53
Burger sample	E	February 2018	6.93	3.96*
Trimmings	C	April 2018	7.34	1.14
All samples mean and(95% CI)			7.01 (6.52- 7.50)	3.30 (2.26-4.34)

** indicates only sample which exceeds acceptable levels of E. coli for ruminants as defined by Regulation (EC) No. 2073/2005.*

***Table 5.6** Microbial count results for all 14 carcases collected on the field or at the larder*

Sample type		**Coliform log$_{10}$ cfu/cm^2 Minimum and maximum**	**Coliform log$_{10}$ cfu/cm^2 Mean**	**Coliform log$_{10}$ cfu/cm^2 *SD***	***E. coli* log$_{10}$ cfu/cm^2 Minimum and Maximum**	***E. coli* log$_{10}$ cfu/cm^2 Mean**	***E. coli* log$_{10}$ cfu/cm^2 *SD***
Field	Skin	0.95 - 4.39	2.34	1.17	0 - 1.20	0.30	0.44
	Cavity	1.15 - 3.96	2.42	0.97	0 - 2.2	0.48	0.61
	All field samples	0.95 - 4.39	2.33	1.06	0 - 2.20	0.43	0.54
Larder	Skin	0.98 - 4.79	2.83	1.14	0 - 2.30	0.63	0.70
	Cavity	0.5 - 4.5	2.42	1.25	0.50 - 4.50	1.03	0.52
	All larder samples	0.50 - 4.79	2.62	1.19	0 - 2.30	0.83	0.64

SD = standard deviation.

5.2.2.4 Lab results from environmental samples at AGHE, larder and hill

The results of the samples collected from different surfaces that came in direct contact with carcasses or the meat are found in Table 5.7 below.

Coliforms: Except for three environmental swabs collected from chiller wall and a knife at AGHE and one knife used in the field for gralloching, all other samples were positive for coliforms, as outlined in Table 5.7. The coliform values ranged from 1.07-7.31 log_{10}/cm^2, and the values observed were higher from the environmental samples collected from the AGHEs compared with those collected from the field or at larders.

E. coli: 8 out of 28 samples collected at AGHE contained *E. coli* with values ranging from 0.69-4.88 log_{10}/cm^2. *E coli* was isolated from surfaces such as carcass hook, chiller wall which demonstrates the bacteria can withstand well cold temperature and suggest faecal contamination of the surfaces swabbed. For the samples collected in the field and at the larder, only 1 out of 7 was positive for *E. coli*. This sample was collected from a cutting saw and generated 1.07 log_{10}/cm^2 counts

Table 5.7 *Microbial count results for environmental samples collected at AGHEs, larders or in the field*

Sample type	AGHE	Date collected	Coliform log_{10} cfu/cm^2	*E. coli* log_{10} cfu/cm^2
AGHE				
Chiller wall	C	October 2017	5.12	0
Knife	C	October 2017	6.41	0
Carcass hook	C	October 2017	7.14	2.69
Knife	C	October 2017	5.74	0
Hook	A	November 2017	6.17	3.90
Chiller wall	A	November 2017	0	0
Cutting table	A	November 2017	5.70	0
Knife	A	November 2017	5.74	0
Cutting table	B	November 2017	5.38	2.23
Knife	B	November 2017	6.36	4.13
Carcass hook	B	November 2017	6.91	0
Chill wall	B	November 2018	5.35	2.20
Carcass hook	B	November 2018	7.31	0
Cutting table	B	January 2018	6.73	0
Knife	B	January 2018	7.08	4.88
Knife	A	February 2018	6.69	0
Cutting table	A	February 2018	5.51	0
Knife	E	January 2018	1.98	0
Carcass hook	E	January 2018	4.93	0
Carcass rack	C	March 2018	6.84	0
Knife	C	March 2018	2.26	0
Cutting table	C	March 2018	4.59	0

Table 5.7 continued				
Knife	C	April 2018	5.50	0.82
Carcass hook	C	April 2018	3.65	0
Carcass rack	C	April 2018	4.07	0
Cutting table	C	April 2018	5.12	0.69
Carcass hook	F	April 2018	3.74	0
Knife	F	April 2018	0	0
Mean value for all AGHE samples			5.46 (SD=1.41)	0.76 (SD 1.46)
Field samples				
Knife	hill	October 2018	3.93	0
Larder table	larder	October 2018	2.83	0
Knife	Larder	October 2018	0	0
Knife	Hill	October 2018	3.68	0
Knife	Larder	October 2018	1.07	0
Knife	Hill	October 2018	1.34	0
Saw	Larder	October 2018	3.49	1.07
Mean value for all field and larder samples			2.89 (SD 1.21)	0.15 (SD 0.40)

0 = none detected.

5.3. Risk factor analysis

5.3.1 Statistical methodology

5.3.1.1 Database description and modelling approach

A total number of 214 carcasses, 79 Roe deer, 132 Red deer and 3 Sika deer were collected, totalling to 642 carcass sponges from each of the area swabbed. From each swab, *E. coli* and coliform counts were obtained, resulting in 642 coliform and 642 *E. coli* counts for statistical analysis. The response variables for both *E. coli* (hide, cavity, external carcass) and coliforms (hide, cavity and external carcass) were combined to provide an average *E. coli* or coliform value for each animal.

Separate analyses were run for Red and Roe deer because preliminary exploratory data analysis revealed many of the predictor variables were different between the species. For example, distance between cull and AGHE and coliform and *E. coli* counts. Red deer having significantly higher coliform and *E. coli* counts compared to Roe deer (see section 5.2.2.1). Furthermore, these two deer species originated from different regions within Scotland, were of different body size and samples were often collected from different processors. Analysis of sika deer (n=3) was not possible due to low numbers of sika deer sampled.

5.3.1.2 Method for multivariate analysis of risk factors

All analyses were carried out within the R statistical software environment version 3.6.1 (R Core Team (2019)). Analysis was carried out separately for the four combinations of deer species (red and roe) and bacterial group (coliform and *E. coli*). Prior to analysis all continuous variables were checked for normality. Only coliforms and *E. coli* counts were log transformed. Initial univariate analysis was carried out using on all predictor variables (see Appendix 14) and only variables with a p-value of < 0.15 were considered for the multivariate model. Pairwise comparison of variables was performed to identify highly correlated variables (correlation > 0.70). If variables were highly correlated, only the variable which gave the best model fit (based on a lower Akaike information criterion (AIC)) was included in the multivariable model.

Multivariable general linear regression analysis was used to identify variables that explained a significant amount of variation in average bacterial load. The data set was analysed in four models which were built to predict average $\log_{10}$ *E. coli* and coliform load in red and roe deer respectively. The AGHE was included as a random effect in the model.

Models with all possible explanatory variable combinations, including interactions, were considered for the final model, using the dredge function from MuMIn: Multi-Model Inference package in R (Bartoń, 2019). Variables remaining in the final model are those that significantly improve the fit of the model as opposed to p values of <0.05 (Appendix 15). Model averaging was used, and the top models (delta AIC ≤ 2) were chosen (Appendix 16), to extract the final multivariable regression model. Overall summary of variables included in the Univariable and Multivariable models are shown in Appendix 14 and Appendix 15 respectively. The final models presented in the report are based on model averaging. A summary of all variables considered (univariable and multivariable) are in Appendix 17.

5.3.2 Results of the multivariate risk factor analysis

5.3.2.1 Multivariable roe deer results

Coliforms: As seen in Figure 5.6 and Appendix 15 the variables that were associated with higher average $\log_{10}$ coliform counts for roe deer were:

- Time in storage ≥6 days (p=0.019);
- Distance (in miles) between cull location and the AGHE (p=0.009).

Samples which had been storage for longer the 6 days were more likely to have higher average $\log_{10}$ coliform counts then those which were stored for for less then 6 days. The longer distance between cull location and the AGHE was associated with a higher $\log_{10}$ coloiforms counts.

E. coli: As shown in Figure 5.7 and Appendix 15, the variables that were associated with higher average $\log_{10}$ *E. coli* counts were:

- Warmer temperature, above 7ºC (p=0.016);

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- High levels of faecal contamination, at levels 3 (1-2cm^2) and 4 (>2cm^2) (p=0.003 and 0.028 respectively);
- Dryness; wet and slimy carcasses (p=0.011);
- Sex; male (p=0.037).

Male carcasses sampled in warmer temperatures (above 7ºC) had higher average log$_{10}$ *E. coli* counts. In addition, carcasses with high levels of faecal contamination that were wet or slimy had higher average log$_{10}$ *E. coli* counts.

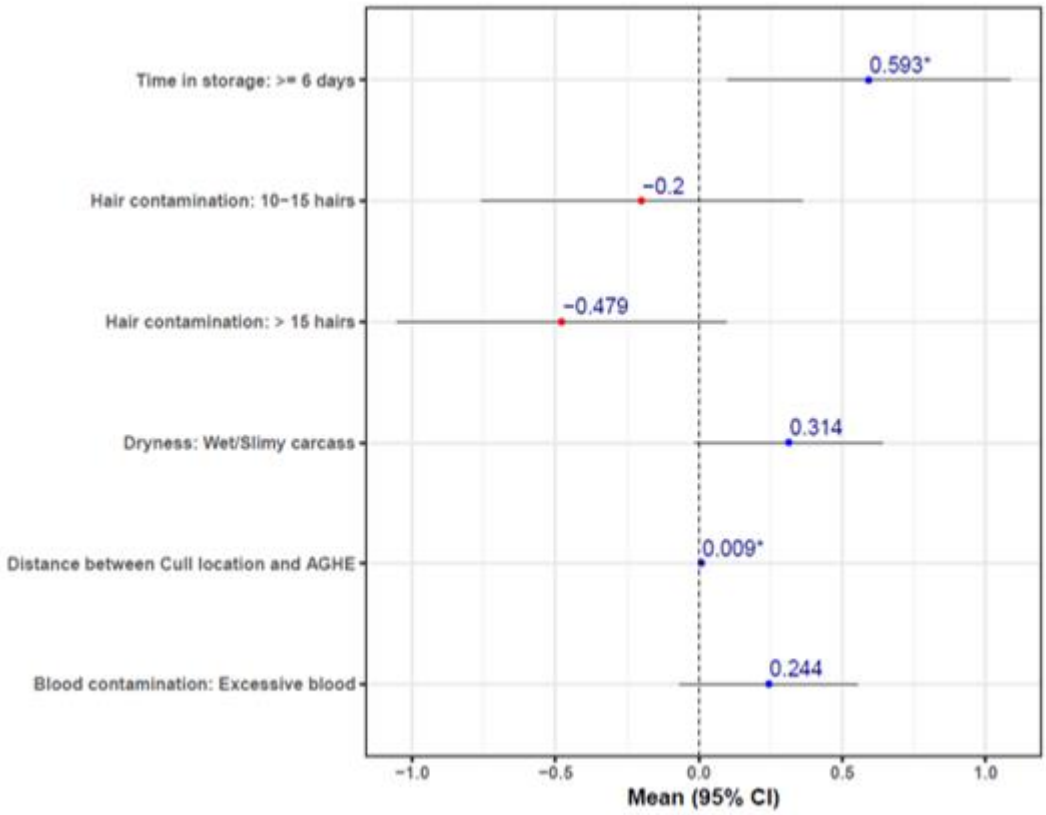


Figure 5.6 *The final multivariable model of the mean coefficients and 95% confidence intervals for Roe deer for coliforms after model averaging. Red dot, variable is associated with lower coliform counts. Blue dot, variable isassociated with higher coliform counts. *, statistically significant (p<0.05). Table of results in Appendix 15.*

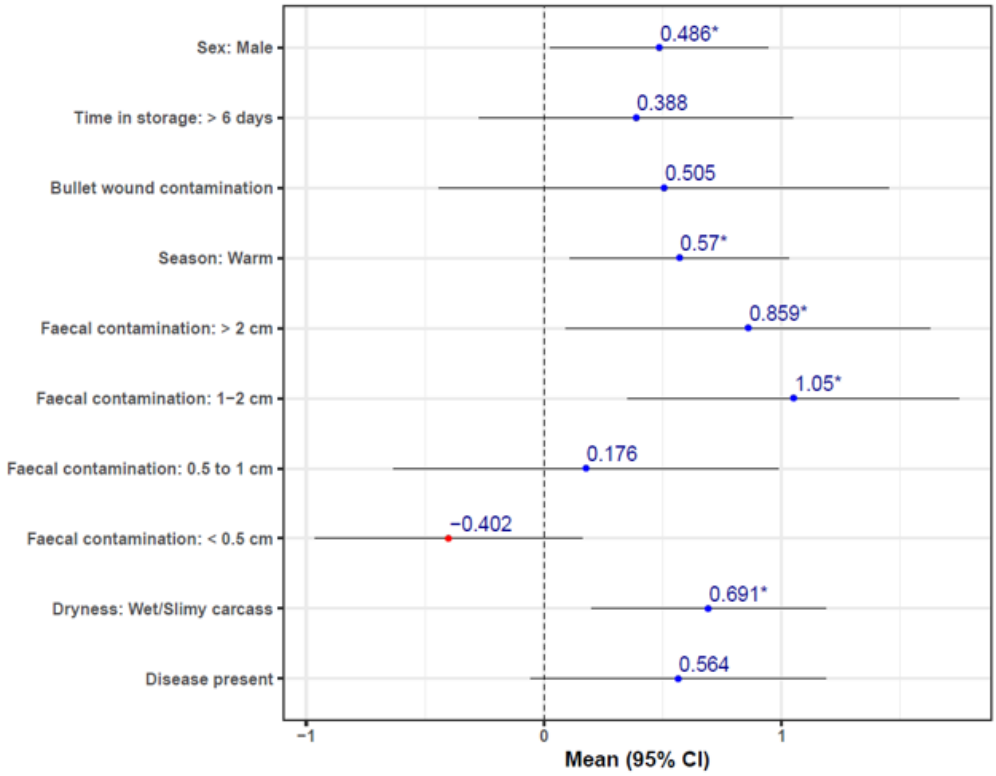


Figure 5.7 *The final multivariable model of the mean coefficients and 95% confidence intervals for Roe deer for coliforms after model averaging. Red dot, variable is associated with lower E. coli counts. Blue dot, variable is associated with higher E. coli counts. *, statistically significant (p<0.05). Table of results in Appendix 15.*

5.3.2.2 Multivariable red deer results

Coliforms: There were no statistically significant factors associated with average $\log_{10}$ coliform counts on red deer carcasses, (Figure 5.8, Appendix 15).

E. coli: As shown in Figure 5.9 and Appendix 15, the statistically significant factors associated with average $\log_{10}$ *E. coli* counts on red deer carcasses were:

- Dirty skin condition (p<0.001);
- High levels of faecal contamination, >2cm (p=0.001);

Carcasses with high levels of contamination had higher average $\log_{10}$ *E. coli* counts.

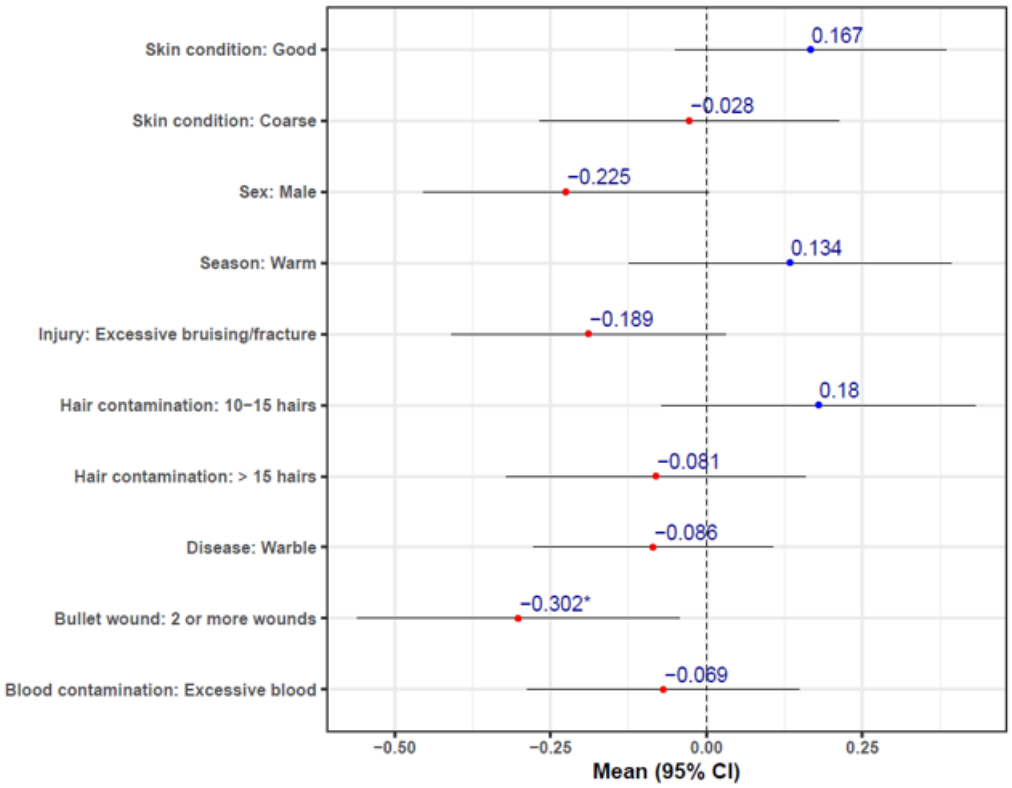


Figure 5.8 *The final multivariable model of the mean coefficients and 95% confidence intervals for Red deer for coliforms after model averaging. Red dot, variable is associated with lower coliform counts. Blue dot, variable is associated with higher coliform counts. *, statistically significant (p<0.05). Table of results in Appendix 15.*

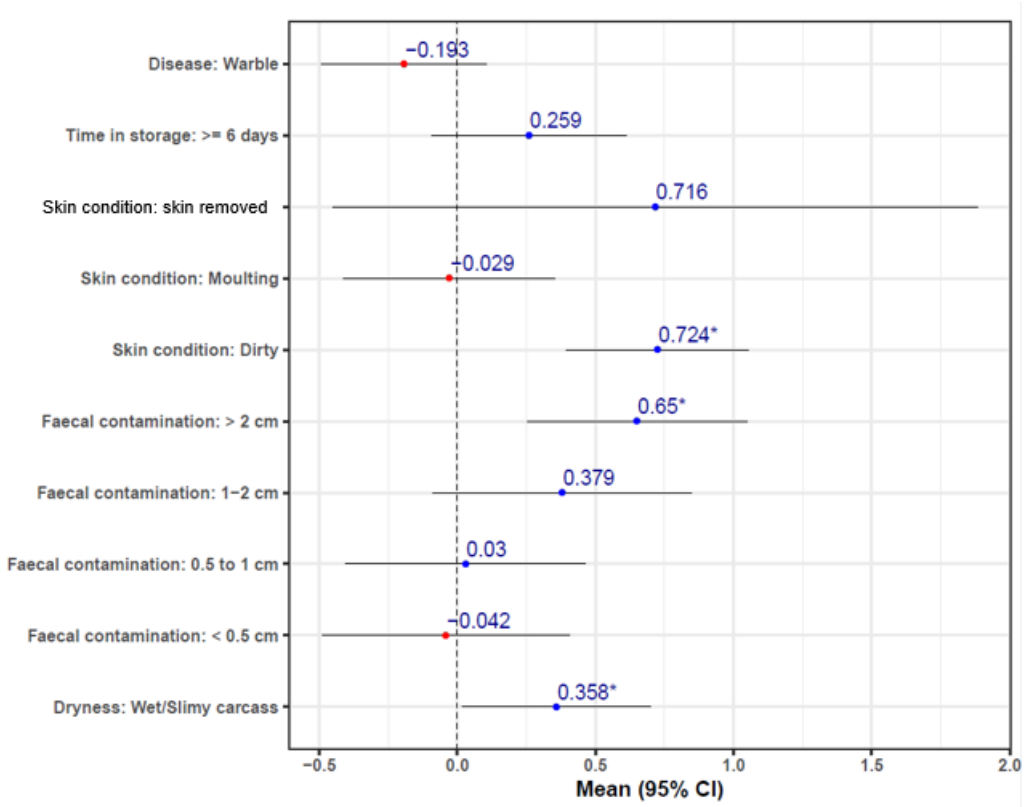


Figure 5.9 *The final multivariable model of the mean coefficients and 95% confidence intervals for Red deer for E. coli after model averaging. Red dot, variable is associated with lower E. coli counts. Blue dot, variable is associated with higher E. coli counts. *, statistically significant (p<0.05). Table of results in Appendix 15.*

5.4. Discussion

One of the main findings of this study was that red deer had statistically significant higher coliforms and *E. coli* compared with roe deer.

Overall, when we compared the mean values of log coliforms and log *E. coli* in hides, cavities and external surfaces of the carcass, similarly higher counts were seen for the larger deer species (red) for all three sample types, and this difference was statistically significant between red and roe deer.

The difference between red and roe deer influenced the method approached for the statistical analysis to identify risk factors associated with bacterial contamination of the carcass. The data set was analysed separately for red and roe deer and also with separate consideration for each bacterial group, coliforms and *E. coli*.

Coliforms – carcass samples collected at AGHEs

To our knowledge, this is the first study to report coliform values in wild or domestic deer carcasses; we cannot therefore provide a comparison with other data from wild deer. The EU legislation on microbiological criteria for foodstuffs, regulation (EC) No. 2073/2005 as amended by CE 1441/2007, includes guidelines on Enterobacteriaceae as a predictive indicator for process hygiene criteria and the assessment of faecal contamination. Similarly, other wild deer studies that have investigated the microbiological condition of carcasses have used Enterobacteriaceae as predictors of faecal contamination. The presence of *E. coli* in samples is best predicted, however, by the density of coliforms, followed by the density of Enterobacteriaceae (Jordan *et al.*, 2007), which has informed the rationale of the current testing approach.

The Enterobacteriaceae daily mean log on ruminant carcasses is expected to be between 1.5 $\log_{10}$ cfu/cm² and a maximum of 2.5 $\log_{10}$ cfu/cm², according to EU legislation on microbiological criteria for foodstuffs (Regulation (EC) No. 2073/2005 as amended by CE 1441/2007). In Germany, Atanassova *et al*. (2008) investigated the quality of freshly shot deer carcasses to assess the microbiological quality, and the results indicated that Enterobacteriaceae were within the legal range advised by EU legislation for domestic ruminants, with values of 2.1 $\log_{10}$ cfu/cm² (1.7–2.6) in roe deer and 2.1 $\log_{10}$ cfu/cm² (1.7–2.8) in red deer. In Switzerland, Obwegeser *et al*. (2012) examined 258 carcasses from hunted wild deer 48 hours after being hunted and another 72 hours after arriving at the AGHE, and found Enterobacteriaceae at a mean of 2.3 $\log_{10}$ cfu/cm² for red deer and 2.6 $\log_{10}$ cfu/cm² for roe deer.

Because coliforms represent a large proportion of the Enterobacteriaceae population, it is expected that, depending on the culture medium used, the coliform and Enterobacteriaceae counts from meat and carcasses are either very similar or coliform counts are marginally lower. The results on carcass samples outlined in the microbiological results section indicate that coliform counts in Scottish wild deer carcasses are higher than the acceptable limit for enteric bacteria on domestic ruminant carcasses and higher than the enteric bacteria level described by other studies on wild deer.

The microbiology regulation for foodstuffs, EC No. 2073/2005 as amended by CE 1441/2007, recommends in the case of domestic ruminant carcases that a satisfactory daily mean log ACC should lie below 3.5 log cfu/cm², and an acceptable daily mean log for aerobic colony count is above 3.5 but below 5.0 log cfu/cm² for results by the destructive sampling method, which typically generates higher counts than the swabbing method. More recently, Paulsen (2011) concluded that, following good hygienic practice, skin-on large game carcasses can be expected to generate a

total aerobic count/aerobic colony count not exceeding 6 log cfu/cm^2 by swabbing method. Coliforms represent only a proportion of aerobic colony counts, although this proportion is not always linearly correlated. In cattle, the number of coliforms recovered from carcasses before chilling, by swabbing method, was a mean of 1.95±0.77 log cfu/cm^2 – 3.38 log cfu/cm^2 lower than the number of aerobes observed (Liu, Youssef and Yang, 2016). The acceptable values for coliform counts in deer are thus expected, as in cattle, to be lower than the indicated values for ACC. Based on the median counts our microbiological results show, however, that more than 50% of the coliform values obtained for cavities and external carcasses exceed the expected values for ACC in domestic ruminants. These observations suggest that coliforms in wild deer carcasses reach higher values than in cattle. This is to be expected given the very different processing, with cattle being processed in a well-controlled indoor environment while deer are processed partly outdoors and transferred through several types of environment, increasing the chance of cross-contamination and allowing opportunities for breaks in the cold chain.

E. coli – carcass samples collected at AGHE

For both types of bacteria, the counts were the lowest on the hides, which could be due to the possibility of limited survival time of the bacteria on the skin/hide, particularly if this is dry, creating an unfavourable environment for the metabolic needs of the bacteria. The lower counts could also be the reflection of a limitation in the sampling technique. If the hide was very dry and the sponge was not sufficiently pre-soaked, it is possible that bacteria were captured less.

E. coli counts were in the higher ranges in samples collected from cavities, when the carcasses were in storage, with the skin on. This sampling was used to measure the bacterial counts on the carcasses when they were received at the AGHE. These laboratory results are consistent with the contamination that was observed at the time of the sample collections in the cavities (mainly pelvic and abdominal, and to a lesser extent, carcass cuts). Given that evisceration takes place in the field, carcass cavity contamination may be assumed to occur before carcasses are transferred to the AGHE. This contamination can transfer to the external surface of the carcass during skinning and dressing, via staff hands and equipment. This could explain why the external surfaces of the carcasses had bacterial counts with a similar distribution to those observed in the cavities but with slightly lower values.

The median values and the distribution of results indicate that, overall, *E. coli* counts were within the acceptability limits described for wild game carcasses by Paulsen (2011), who from the collation of all the published microbiology results for wild deer as well as his own results, advises a maximum acceptable limit for generic *E. coli* of 2 log$_{10}$ cfu/cm^2. Our data set indicates that a great proportion of the *E. coli* values were within the acceptable limits for all three types of sample. As shown in the results section, the highest mean values were observed in the cavities, and expressed in proportions, 55% of these values were above the 2 log$_{10}$/cm^2 acceptable limit. The samples collected from the external surface of the carcasses generated lower mean counts, however 57% of these counts exceeded the

recommended 2 log_{10}/cm^2 limit. For both roe and red deer species, the higher *E. coli* counts stemmed, as expected, from faecal contamination, but wet or slimy carcasses also positively influenced the *E. coli* microbiological results.

Meat samples collected at AGHEs

The results obtained from the seven meat samples were a mean of 7.016 (95% CI: 6.52 – 7.50 –) log_{10} cfu/g for coliforms and a mean of 3.30 (95% CI: 2.26-4.34) log_{10} cfu/g for *E. coli*. For coliforms, these values are higher than those previously described by Membré, Laroche and Magras (2011), who observed, in a sample size of more than 1,000 wild deer fresh and frozen meat cuts, coliforms at a mean of 2.56 log_{10} cfu/cm^2 (95% CI: 2.00–3.10) and *E. coli* at 2.20 log_{10} cfu/cm^2 (95% CI1.65–2.76). This difference between studies might be due to sample size, but genuine contamination may have led to our higher coliform counts, which could be due to additional handling and processing through different environments. For *E. coli*, the values found in this study are only marginally higher than those described by Membré, Laroche and Magras (2011).

According to process hygiene criteria (EC Regulation 2073/2005) for meat and meat products, the acceptable limit for *E. coli* in livestock meat preparations is a mean of less than 5,000 cfu/g (<3.7 log_{10} cfu/g). The *E. coli* results from meat preparations in this study are generally within acceptable limits, except for two of the meat preparations, both of which being burger samples that were collected from separate operators. This represents a breach of process hygiene criteria and indicates more hygienic practices should be adopted to reduce faecal contamination in the meat product.

Three of the meat samples were obtained entirely from carcasses that had also been tested during the study, and these carcasses showed high values for both coliforms and *E. coli* in cavities and on the external surface. These high values could explain the higher coliform results in these three meat samples when contrasted by findings in the literature (Membré, Laroche and Magras, 2011).

Coliforms – carcasses collected in the field and at larders

For both types of bacteria, the median values were higher in larder samples. For the carcasses that showed higher counts, these involved either coliforms or *E. coli*, and not in the same carcass for any of the observations, showing that these two groups of bacteria can be influenced by different factors.

Two of the carcasses had coliforms above 4 log_{10}/cm^2 on the skin at the time of culling and, although it is not possible to demonstrate this statistically due to the small sample size, the information collected for these carcasses at the time of sampling indicates that the ambient temperature was above 7ºC on the day, both animals had ticks and the hides were wet and dirty at the time of collection.

When the skins of deer were sampled again at the larder, the coliform count values increased slightly.

Cavities sampled in the field generally had low coliforms, with a mean of 2.42 $\log_{10}$ cfu/cm^2, and a highest value of 3.96 $\log_{10}$ cfu/cm^2. For the cavities where higher coliform counts were observed, the information collected at the time of sampling indicates a warm outdoor temperature (well above 7ºC) on the day, and that both these animals had ticks and came from the same culling site. When resampled at the larder, the coliform values in the cavities of these same carcases increased slightly.

E. coli – carcasses collected in the field and at larders

The skin counts for generic *E. coli* in field samples were very low, below the limit of detection in 6 out of 14 animals and the highest count was 1.20 $\log_{10}$ cfu/cm^2. The low *E. coli* values were consistently maintained in the results obtained from the larder resampling. It is likely that the skin and fur is not a very favourable environment for the survival or growth of *E. coli* bacteria, or that additional fluid has to be added to be able to retrieve the bacterial cells, although in some instances the hides were already humid due to environmental conditions and in these instances the counts remained low.

When resampled at the larder, the skins maintained low *E. coli* counts in almost all cases. Three of the skin samples collected at the larder yielded no *E. coli* counts. The highest skin value ovserved at the larder was 2.30 $\log_{10}$ cfu/cm^2 but on one occasion the count increased from 0 in the field to 2.30 $\log_{10}$ cfu/cm^2 at the larder. This deer was recovered by partial dragging and was loaded onto an all-road vehicle together with four other deer, some of which had minor contamination occurring during gralloching. Without conclusions being possible from a single observation, it is possible that the skin of this deer could have become contaminated in transport by contact with other deer carcasses.

In the field samples, 11 out of 14 cavities were positive for generic *E. coli*, although the colony counts obtained from the swabs were very low. The mean *E. coli* result for field cavity samples was 0.48 $\log_{10}$ cfu/cm^2. The highest counts among field cavity samples were 1.17 and 2.20 $\log_{10}$ cfu/cm^2 *E. coli*, and both these animals were male. When resampled at the larder three hours later, these two cavities generated lower counts, respectively, of 0.84 and 1.0 $\log_{10}$ cfu/cm^2 *E. coli*.This could be explained by the fact that both carcasses showed visual faecal contamination in the field, which was rectified by trimming at the larder before resampling took place.

All 14 samples collected at the larder produced low *E. coli* counts, although the mean value when compared with the samples collected in the field had slightly higher values. This could be due to the fact that the transfer to the larder for some of those animals was between three and four hours when outdoor temperatures exceeded 7°C, whereas other carcasses with lower *E. coli* counts were transferred to the larder within about two hours. Yet an acceptable time frame, according to all the Austrian and South Africa venison guidelines discussed in the cooling section, is up to four hours when the temperature is above 15°C and up to 12 hours when the temperature

is below that – given the practicalities of transferring deer carcasses from remote areas to larders.

Environmental samples collected at AGHE, field and larder

Higher counts were observed on meat contact surfaces for both coliforms and *E. coli* at AGHE compared with larder and field samples. This could be explained by a larger throughput handled by approved meat operators and the fact that the sampling sessions took place in the middle of the processing, when deep cleaning and disinfection are more difficult than at the start of the day.

Overall, these microbiological results show there is both environmental and faecal contamination on meat contact surfaces at AGHEs and larders as the primary production sites. In some instances, this contamination was exceedingly high, for both coliforms and *E. coli*. The contaminated surfaces can be a source of cross-contamination to other food that might subsequently come into contact with unhygienic surfaces.

Risk factor analysis

Species (Red versus Roe) explained the majority of the difference in the average $\log_{10}$ coliform cfu/cm^2 and average $\log_{10}$ *E. coli* cfu/cm^2 observed in this study. Given the differences in the ecology of Red and Roe deer in Scotland, "species" could be a marker for such variables as temperature and habitat. As a result, Red and Roe deer were analysed separately within this study in order to examine risk factors related to the deer management that might be useful for policy building. Although using this approach has the disadvantage of producing more models and hence increasing the chance of obtaining spurious results we felt it was necessary. The goal of the risk factor study is to determine factors associated with higher coliforms and *E. coli*. Obviously any results will need to be investigated further before making any policy recommendations. The outputs of the multivariable analyses offered an understanding of the factors associated with the coliform and *E. coli* microbiological results obtained from the carcasses collected at AGHEs.

For roe deer carcasses, the factors associated with increased coliform counts were time spent in storage (more than 6 days) and the distance deer were transported. Ultimately, these factors confirm that coliform counts on the carcasses become increasingly higher with longer storage intervals. This explains why the results were lowest for the carcasses swabbed in the field, marginally higher at the larder and much higher for the carcasses swabbed at the AGHE.

Factors for *E. coli* observed in roe deer carcasses were warmer outdoor temperatures at the time of culling (above 7ºC), male carcasses, visual faecal contamination and carcasses that were wet or slimy. Findings related to warmer outdoor temperature reinforce the importance of carrying out evisceration and transporting the carcass to a chilling facility as soon as possible when the ambient temperature is milder. With regard to faecal contamination, given that *E. coli* is a part

of the intestinal microflora, it is expected that faecal contamination was a significant factor for *E. coli* in the carcass. However, an important finding was the level of contamination that became significant – this was any faecal contamination above 1cm^2. This finding should be used with caution as *E. coli* counts were observed even in visually uncontaminated carcasses. Nonetheless, overall, this finding suggests that faecal contamination above 1cm^2 results in a significant risk of increased higher *E. coli* count.

The multivariable models for red deer carcasses resulted in different variables associated with each bacterial population. In the case of *E. coli* isolated from red deer, higher bacterial counts were associated with hides with faecal contamination above 2cm^2. This association was also seen for *E. coli* counts in roe deer, reinforcing the importance of hygienically dressing the carcasses and preventing faecal contamination of the carcass. Higher *E. coli* counts were also found on the carcasses obtained from deer with dirty hides. This result is in agreement with the findings of Blagojevic *et al*. (2012), who observed that cattle with very dirty and wet hides (cleanliness category 4) had significantly higher Enterobacteriaceae counts when compared with lower categories and therefore carcases obtained from the dressing of animals within category 4 also resulted in significantly higher counts compared with carcasses of bovines from other cleanliness categories (1, 2, 3). Regulation (EC) No. 853/2004 requires that animals accepted for slaughter should be visually clean to prevent cross-contamination of the carcasses and to maintain the environmental hygiene of the abattoir. Accordingly, European countries including the UK have developed clean livestock policies.[74]

5.5 Conclusions

One of the main findings of this study was that red deer had statistically significant higher coliform and *E. coli* counts compared with roe deer. The the risk factors associated with either coliform or *E. coli* contamination were different for the two deer species. Analysis of data from Sika deer was not possible due to the low number of samples collected.

The microbiological results obtained from the carcasses collected from AGHEs indicate that the *E. coli* counts were within expected ranges for 45% of the cavity samples and 43% of the external carcass samples. Coliforms exceeded the values expected for domestic ruminants by the EU microbiology criteria for foodstuffs as detailed in regulation (EC) No. 2073/2005. The current study's results were on average 3.8-4.3 log coliform/cm^2 higher than the values required for domestic ruminants, although this was dependent on the sample type. The higher coliform counts observed in this study could be due to repeated carcass transfer through several facilities/locations, creating additional handling and storage in different environments. Minimising carcass handling and transfers, and providing sufficiently large spaces to

[74] Red Meat Safety and Clean Livestock
https://www.foodstandards.gov.scot/downloads/Red_meat_safety_and_clean_livestock.pdf

carry out operations and allow a separation of procedures may enable a reduction of the coliform counts.
For a baseline understanding of hygiene criteria, meat samples from AGHEs were also tested. The microbiological results obtained for generic *E. coli* from carcasses and meat collected at AGHE indicate that levels of *E. coli* in meat were generally within the limits previously described by Paulsen (2011). However, coliform counts in meat were around 4 $\log_{10}$ cfu/g higher than identified previously in the literature by Membré, Laroche and Magras (2011). The limited sample size, however, means a conclusion on expected coliform and *E. coli* values in deer meat cannot be asserted, and further research is warranted for a better understanding of the microbiological condition of deer meat intended for human consumption.

The microbiological results obtained from the carcasses collected on the hill and at the larder were in very low ranges for both coliforms and *E. coli* – lower than previous results described in the literature or required in the EU legislation for livestock carcasses. However, a slight increase in the counts was observed in the microbiological results of the carcasses swabbed at the larder compared with those swabbed on the field, even if the time interval was only two to four hours between sample sessions. The carcass results obtained from the AGHE gave higher coliform and *E. coli* counts compared with those obtained from the carcasses collected in the field immediately after culling and at the larder two to four hours from culling. More than half of the carcasses collected at the AGHE were hung for 6 days or longer, preserved at legally required temperature. Overall, these results suggest that coliform and *E. coli* counts rise as a result of bacterial multiplication during storage, even if the carcasses are subjected to chilling.

To verify hygiene criteria, swabs of meat contact surfaces were collected from all the locations visited. The environmental swabs were positive more often, and the counts were higher for both coliforms and *E. coli*, in the samples collected from the AGHEs than in the field and larder samples. These more frequent positive results and higher counts could be explained by the larger throughput handled by these operators and the fact that swabbing took place at the peak of the processing, when it is more difficult to carry out deep cleaning. Nonetheless, it remains noteworthy that contaminated surfaces can become a source of cross-contamination to other carcasses and meat.

The results of the statistical analysis using the multivariable models indicate that the risk factors appear different for each deer species and for both bacterial populations. Faecal contamination, however, was a significant risk factor for *E. coli* levels in both red and roe deer, indicating that particular care should be taken when processing carcasses with visible faecal contamination, and that care should be taken to avoid such contamination if possible.

References

Acoura - https://www.acoura.com/

Adler, J.M., Geornaras, I., Belk, K.E., Smith, G.C., Sofos, J.N. (2012) Thermal Inactivation of *Escherichia coli* O157:H7 Inoculated at Different Depths of Non-Intact Blade-Tenderized Beef Steaks. J Food Sci. 77(2):M108-14.

Aebischer, N. J., Wheatley, C. J. and Rose, H. R. (2014) 'Factors associated with shooting accuracy and wounding rate of four managed wild deer species in the UK, based on anonymous field records from deer stalkers.', PloS one. Public Library of Science, 9(10), p. e109698.

Ahn, C. K. *et al.* (2009) 'Deer Sausage: A Newly Identified Vehicle of Transmission of *Escherichia coli* O157:H7', The Journal of Pediatrics, 155(4), pp. 587–589.

Alberto, J.R., Serejo, J.P., Viera-Pinto, M. (2011) 'Dog bites in hunted large game: a hygienic and economical problem for game meat production', in Game meat hygiene in focus. Wageningen: Wageningen Academic Publishers, pp. 101-105.

Antic, D. *et al.* (2010) 'Distribution of microflora on cattle hides and its transmission to meat via direct contact', Food Control. Elsevier, 21(7), pp. 1025–1029.

Association of Deer Management Groups (ADMG) - http://www.deer-management.co.uk/

Atanassova, V. *et al.* (2008) 'Microbiological quality of freshly shot game in Germany', Meat Science, 78(4), pp. 414–419.

Australia and New Zealand Food Regulation Ministerial Council. Food Regulation Standing Committee. (2007) Australian standard for the hygienic production of wild game meat for human consumption. CSIRO Publishing. Available at: http://www.publish.csiro.au/book/5697/

Avagnina, A. *et al.* (2012) 'The microbiological conditions of carcasses from large game animals in Italy', Meat Science, 91(3), pp. 266–271.

Bai J, Paddock ZD, Shi X, Li S, An B, Nagaraja TG (2010) Applicability of a multiplex PCR to detect the seven major Shiga toxin-producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. Foodborne Pathog Dis. 9(6):51-8.

Bandick, N. and Hensel, A. (2011) 'Zoonotic diseases and direct marketing of game meat: aspects of consumer safety in Germany', in Game meat hygiene in focus. Wageningen: Wageningen Academic Publishers, pp. 93–100.

Bardiau, M. *et al.* (2010) 'Enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) Escherichia coli in wild cervids', Journal of Applied Microbiology, 109(6), pp. 2214–2222.

Barkocy-Gallagher, G. A. *et al.* (2001) 'Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States.', Applied and environmental microbiology. American Society for Microbiology (ASM), 67(9), pp. 3810–8.

Bartels A, C. and Bülte M. (2011) 'Verotoxigenic *Escherichia coli* (VTEC) in wild ruminants in Germany', in Paulsen Peter., Bauer A., Vodnansky M., Winkelmeier R., A. S. F. J. . (ed.) Game meat hygiene in focus – microbiology, epidemiology, risk analysis and quality assurance. Wageningen, the Netherlands, pp. 107–110.

Bartoń, K. (2019) CRAN - Package MuMIn, MuMIn: Multi-Model Inference. R package version 1.42.1. 2018. Available at: https://cran.r-project.org/web/packages/MuMIn/index.html

Basic Payment Scheme - https://www.gov.uk/government/collections/basic-payment-scheme

Bekker, J. L., Hoffman, L. C. and Jooste, P. J. (2011) 'Essential food safety management points in the supply chain of game meat in South Africa', in Game meat hygiene in focus. Wageningen: Wageningen Academic Publishers, pp. 39–65.

Beldomenico, P. M. *et al.*(2008) 'Poor condition and infection: a vicious circle in natural populations', Proceedings. Biological sciences. The Royal Society, 275(1644), pp. 1753–9.

Bell, R. G. (1997) 'Distribution and sources of microbial contamination on beef carcasses', Journal of applied microbiology, 82(3), pp. 292–300.

Berg, R. D., Wommack, E. and Deitch, E. A. (1988) 'Immunosuppression and Intestinal Bacterial Overgrowth Synergistically Promote Bacterial Translocation', Archives of Surgery. American Medical Association, 123(11), p. 1359.

Best practice guides - https://www.bestpracticeguides.org.uk/guides/

Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F. (1993) Prevalence and some properties of verotoxin (Shiga-like toxin) - producing *Escherichia coli* in seven different species of healthy domestic animals. J Clin Microbiol 31:2483-2488.

Beyond the Glen strategy for wild and farmed venison - https://scotlandfoodanddrink.blob.core.windows.net//media/1555/venison-strategy-brochure.pdf

Bildfell, R. J. *et al.*(2004) 'Hair-loss syndrome in black-tailed deer of the pacific northwest', Journal of Wildlife Diseases, 40(4), pp. 670–681.

Blagojevic, B. *et al.*(2011) 'Ratio between carcass-and hide-microflora as an abattoir process hygiene indicator', Food Control. Elsevier, 22(2), pp. 186–190.

Blagojevich, B. *et al.*(2012) 'Visual cleanliness scores of cattle at slaughter and microbial loads on the hides and the carcases', Veterinary Record, (170(22)), p. 563.

Bolton DJ, O'Neill CJ, Fanning S.(2012) A preliminary study of Salmonella, verocytotoxigenic *Escherichia coli*/*Escherichia coli* O157 and Campylobacter on four mixed farms. Zoonoses Public Health. 2012 May;59(3):217-28.

Brandal LT, Wester AL, Lange H, Løbersli I, Lindstedt BA, Vold L, Kapperud G (2015) Shiga toxin-producing *Escherichia coli* infections in Norway, 1992-2012: characterization of isolates and identification of risk factors for haemolytic uremic syndrome. BMC Infectious Diseases 15:32

British Association for Shooting and Conservation (BASC) deer stalking - https://basc.org.uk/cop/deer-stalking

Browning, L. *et al.*(2016) National Outbreak of *Escherichia coli* O157 Phage Type 32 in Scotland. Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=2987

Brooks, J. T. *et al.*(2005) 'Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections in the United States, 1983–2002', The Journal of Infectious Diseases, 192(8), pp. 1422–1429. doi: 10.1086/466536.

Buddle BW, de Lisle GW, Griffin JFT, Hutchings SA (2015) Epidemiology, diagnostics, and management of tuberculosis in domestic cattle and deer in New Zealand in the face of a wildlife reservoir. New Zealand Veterinary Journal 63:19–27.

Buvens G, De Gheldre Y, Dediste A, de Moreau AI, Mascart G, Simon A, Allemeersch D, Scheutz F, Lauwers S, Piérard D (2012) Incidence and virulence determinants of verocytotoxin-producing *Escherichia coli* infections in the Brussels-Capital Region, Belgium, in 2008-2010. J Clin Microbiol. 2012 50:1336-45.

Byrne L, Vanstone GL, Perry NT, Launders N, Adak GK1, Godbole G, Grant KA, Smith R, Jenkins C. Epidemiology and microbiology of Shiga toxin-producing *Escherichia coli* other than serogroup O157 in England, 2009-2013. J Med Microbiol. 63:1181-8.

Caprioli A, Morabito S, Brugère H, Oswald E. (2005). Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Veterinary Research 36:289–311

Casoli, C. *et al.*(2005) Wild ungulate slaughtering and meat inspection. Veterinary research communications 29, pp 89–95.

Chase-Topping ME, McKendrick IJ, Pearce MC, MacDonald P, Matthews L, *et al.*(2007) Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. J Clin Microbiol. 45:1594-603.

Chase-Topping, M. E. *et al.*(2012) 'Pathogenic potential to humans of bovine *Escherichia coli* O26, Scotland.', Emerging infectious diseases, 18(3), pp. 439–48.

Chase-Topping, M. *et al.*(2008) 'Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157', Nature Reviews Microbiology, 6(12), pp. 904–912.

Chekabab, S. M. *et al.*(2013) 'The ecological habitat and transmission of *Escherichia coli* O157:H7', FEMS Microbiology Letters, 341(1), pp. 1–12.

Chmielewski, R. A. N. and Frank, J. F. (2003) 'Biofilm Formation and Control in Food Processing Facilities', Comprehensive Reviews in Food Science and Food Safety, 2(1), pp. 22–32.

Clemente-Casares P, Ramos-Romero C, Ramirez-Gonzalez E, Mas A (2016) Hepatitis E Virus in Industrialized Countries: The Silent Threat. Biomed Res Int. 2016:9838041.

Coburn, H. L. *et al.*(2005) 'Qualitative risk assessment of the hazards and risks from wild game', Veterinary Record, 157(11), pp. 321–322.

Condon J, Kelly G, Bradshaw B and N Leonard (2004). Estimation of infection prevalence from correlated binomial samples. Preventative Veterinary Medicine, 64(1):1-14.

Conner, D. E. and Kotrola, J. S. (1995) 'Growth and survival *of Escherichia coli* O157:H7 under acidic conditions', Applied and environmental microbiology. 61(1), pp. 382–5.

Cruz-Monterrosa, R. G. *et al.*(2017) 'Bruises in beef cattle at slaughter in Mexico: implications on quality, safety and shelf life of the meat', Tropical Animal Health and Production. Springer Netherlands, 49(1), pp. 145–152.

Dallman TJ, Ashton PM, Byrne L, Perry NT, Petrovska L, Ellis R, Allison L, Hanson M, Holmes A, Gunn GJ, Chase-Topping ME, Woolhouse MEJ, Grant KA, Gally DL, Wain J, Jenkins C (2015) Applying phylogenomics to understand the emergence of Shiga-toxin-producing *Escherichia coli* O157:H7 strains causing severe human disease in the UK. Microb Genom. 1(3):e000029.

De Bosschere, H. *et al.*(2007) 'Severe alopecia due to demodicosis in roe deer (*Capreolus capreolus*) in Belgium', The Veterinary Journal, 174(3), pp. 665–668.

Deer Health (no date) Deer Health | bestpracticeguides, online. Available at: http://www.bestpracticeguides.org.uk/health/health-welfare

Deer Industry New Zealand - https://deernz.org/deerhub/deer

Deer management in Scotland: Report to the Scottish Government 2016 - http://www.parliament.scot/parliamentarybusiness/CurrentCommittees/102641.aspx

Deitch, E. A. *et al.*(1993) 'Elemental Diet-Induced Immune Suppression Is Caused by Both Bacterial and Dietary Factors', Journal of Parenteral and Enteral Nutrition, 17(4), pp. 332–336.

Deutz, A. and Fötschl, H. (2014) '18. Game meat hygiene under Alpine conditions', in P., P., A., B., and F.J.M., S. (eds) Trends in game meat hygiene. The Netherlands: Wageningen Academic Publishers, pp. 211–222.

Deutz, A. *et al.*(2000) 'Hygiene risks in venison and their microbial load and human pathogens' Fleischwirtschaft -Frankfurt- 80 pp. 106-108

Di Bartolo I, Ponterio E, Angeloni G, Morandi F, Ostanello F, Nicoloso S, Ruggeri FM (2017) Presence of Hepatitis E Virus in a red Deer (*Cervus elaphus*) Population in Central Italy. Transbound Emerg Dis. 64:137-143.

Díaz-Sánchez, S. *et al.* (2012) 'Detection and characterization of Shiga toxin-producing *Escherichia coli* in game meat and ready-to-eat meat products', International Journal of Food Microbiology. Elsevier, 160(2), pp. 179–182.

Díaz-Sánchez, S. *et al.* (2013) 'Prevalence of Shiga toxin-producing *Escherichia coli*, Salmonella spp. and Campylobacter spp. in large game animals intended for consumption: Relationship with management practices and livestock influence', Veterinary Microbiology, 163(3–4), pp. 274–281.

Dobrowolska, A. and Melosik, M. (2008) 'Bullet-derived lead in tissues of the wild boar (Sus scrofa) and red deer (*Cervus elaphus*)', European Journal of Wildlife Research, 54(2), pp. 231–235

Dovecote Park Ltd. - http://www.dovecotepark.com/default.aspx

EFSA (2009) 'Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food)', EFSA Journal, 7(11), p. 1366.

EFSA (2014) 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat from all species)', EFSA Journal, 12(7), p. 3783.

EFSA (2016) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015, EFSA Journal. doi: 10.2903/j.efsa.2016.4634.

EFSA (2017) 'The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016 Acknowledgements: EFSA and the ECDC wish to thank the members of the Scientific Network for Zoonoses Monitoring Data and the Food and Wat', EFSA Journal, 15(12), p. 5077. doi: 10.2903/j.efsa.2017.5077.

Eggert, M. *et al.* (2013) 'Detection and characterization of Shiga toxin-producing Escherichia coli in faeces and lymphatic tissue of free-ranging deer', Epidemiology and Infection, 141(2), pp. 251–259.

El-Nawawi FA, Tawfik MA, Shaapan RM (2008) Methods for inactivation of Toxoplasma gondii cysts in meat and tissues of experimentally infected sheep. Foodborne Pathog Dis. 5:687-90.

Erickson, M. C. and Doyle, M. P. (2007) 'Food as a vehicle for transmission of Shiga toxin-producing Escherichia coli', Journal of food protection, 70(10), pp. 2426–49.

Evans, J., Knight, H., McKendrick, I. J., Stevenson, H., Varo Barbudo, A., Gunn, G. J., & Low, J. C. (2011). Prevalence of Escherichia coli O157 : H7 and serogroups O26, O103, O111 and O145 in sheep presented for slaughter in Scotland. Journal of Medical Microbiology, 60:653–660.

FAO (no date) deer farming, online. Available at: http://www.fao.org/docrep/004/X6529E/X6529E06.htm

FAO, W. (2018) Shiga toxin-producing Escherichia coli (STEC) and food: attribution, characterization, and monitoring MICROBIOLOGICAL RISK ASSESSMENT SERIES 31 REPORT Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterisation, and monitoring.- http://www.fao.org/3/ca0032en/CA0032EN.pdf

Feng, G. *et al.* (2015) 'Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small is small enough?' NPJ biofilms and microbiomes, 1(1), p. 15022.

Ferens WA, Hovde CJ (2011) *Escherichia coli* O157:H7: Animal Reservoir and Sources of Human Infection. Foodborne Pathog Dis. 8: 465–487.

Flores, R. A. (2004) 'Distribution of *Escherichia coli* O157:H7 in beef processed in a table-top bowl cutter.', Journal of food protection, 67(2), pp. 246–51.

Food Standards Agency (2008) HACCP guidance for those producing wild game meat for human consumption either at an approved game handling establishment or under exemption allowed by the food hygiene regulations HACCP. Available at: https://www.food.gov.uk/sites/default/files/media/document/wild-game-guide.pdf

Food Standards Scotland (2018) 'Post-mortem, health and identification marking' (Chapter 2.4). In: Manual for Official Controls | Amendment 12. Available at: https://www.foodstandards.gov.scot/downloads/Chapter_2.4.pdf

Food Standards Scotland Approved Premises Register http://www.foodstandards.gov.scot/publications-and-research/approved-premises-register

Food Standards Scotland Cooking Food guidelines - https://www.foodstandards.gov.scot/consumers/food-safety/at-home/cooking-food

Franklin, A. B. *et al.*(2013) 'Wild ungulates as disseminators of Shiga toxin-producing *Escherichia coli* in urban areas.', PloS one, 8(12), p. e81512.

Franson, C., Russell, R.E. (2014) Lead and Eagles: Demographic and Pathological Characteristics of Poisoning, and Exposure Levels Associated With Other Causes of Mortality. Ecotoxicology 23(9):1722-31.

FSA (2008) HACCP guidance for those producing wild game meat for human consumption either at an approved game handling establishment or under exemption allowed by the food hygiene regulations. Available at: https://www.foodstandards.gov.scot/downloads/HACCP_Guidance.pdf

FSA (2011) A guide to the hygiene regulations for people who shoot wild game and supply it in-fur or in-feather or as small quantities of wild game meat. This guide to the hygiene regulations is for people who shoot wild game and supply it either in-fur or in-feather, online. Available at: http://www.food.gov.uk/foodindustry/meat/guidehygienemeat

FSA (2015) the wild Game Guide. Available at: https://www.food.gov.uk/sites/default/files/media/document/wild-game-guide.pdf

FSA (2015a) '*E. coli* O157 Control of Cross-contamination'. Available at: https://www.food.gov.uk/sites/default/files/media/document/ecoli-cross-contamination-guidance.pdf

FSA Industry guidance on EU Hygiene Regulations relating to the supply of wild game for human consumption (outside approved premises) the wild game handling guide. Available at: http://www.reading.ac.uk/foodlaw/pdf/uk-05048-wild-game-guidance.pdf

FSA (no date b) 'Manual for Official Controls | Amendment 80', Manual for Official Controls, Chapter 2 (80), pp. 60–73. Available at: https://www.food.gov.uk/sites/default/files/media/document/chapter-2.4-post-mortem-health-and-identification-marking_7.pdf

FSIS (2014) FSIS Notice 40-12, FSIS Verification Testing for Non-O157 Shiga toxin-producing *Escherichia coli* (Non-O157 STEC) under MT60, MT52 and MT53 Sampling Programs. http://www.usda.gov/wps/portal/usda/usdahome

FSIS (2016) Raw beef product sampling. Available at: https://www.fsis.usda.gov/wps/wcm/connect/50c9fb74-c0db-48cd-a682-b399ed6b70c0/29_IM_Raw_Beef_Prod_Sampling.pdf?MOD=AJPERES

Gill, C. O. (2007) 'Microbiological conditions of meats from large game animals and birds', Meat science, 77(2), pp. 149–60.

Gill, C. O. and Jones, T. (2002) 'Effects of wearing knitted or rubber gloves on the transfer of *Escherichia coli* between hands and meat', Journal of food protection, 65(6), pp. 1045–8.

Gill, C. O. and McGinnis, J. C. (2004) 'Microbiological conditions of mechanically tenderized beef cuts prepared at four retail stores' International journal of food microbiology, 95(1), pp. 95–102.

Gill, C. O. and Penney, N. (1977) 'Penetration of bacteria into meat', Applied and environmental microbiology. 33(6), pp. 1264–6.

Gill, C. O., Penney, N. and Nottingham, P. M. (1978) 'Tissue sterility in uneviscerated carcasses', Applied and environmental microbiology. 36(2), pp. 356–9.

Gill, C.O, Devos, J., Youssef, M.K., Yang, X. (2014). Effects of Selected Cooking Procedures on the Survival of *Escherichia coli* O157:H7 in Inoculated Steaks Cooked on a Hot Plate or Gas Barbecue Grill. J Food Prot. 77(6):919-26.

Glass, K. A. *et al.*(1992) 'Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage.', Applied and environmental microbiology, 58(8), pp. 2513–6.

Gortazar C (2015) Open questions and recent advances in the control of a multi-host infectious disease: animal tuberculosis. Mammal Review 45:160–175

Gould LH, Mody RK, Ong KL, Clogher P, Cronquist AB, Garman KN, *et al.*. (2013). Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: epidemiologic features and comparison with *E. coli* O157 infections. Foodborne Pathog. Dis. 10, 453–460.

Gouws, P. A., Shange, N. and Hoffman, L. C. (2017) '14. Microbial quality of springbok (*Antidorcas marsupialis*) meat in relation to harvesting and production process', in Game meat hygiene. The Netherlands: Wageningen Academic Publishers, pp. 223–228.

Greig, J. D. *et al.*(2012) 'The efficacy of interventions applied during primary processing on contamination of beef carcasses with *Escherichia coli:* A systematic review-meta-analysis of the published research'. Food Control, 27, pp. 385-397.

Grimstrup Joensen K , Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM (2014) Real-time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic *Escherichia coli*. J Clin Microbiol 52(5):1501-10.

Harris, S. M. *et al.*(2012) 'Salt at concentrations relevant to meat processing enhances Shiga toxin 2 production in *Escherichia coli* O157:H7', International Journal of Food Microbiology, 159(3), pp. 186–192.

Hara-Kudo Y, Takatori K. (2011) Contamination level and ingestion dose of foodborne pathogens associated with infections. *Epidemiol Infect.* 139:1505-10.

Health Protection Scotland: National Outbreak of *Escherichia coli* O157 Phage Type 32 in Scotland http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=2987

Heininger A, Binder M, Schmidt S, Unertl K,, Botzenhart K, Döring G (1999) PCR and blood culture for detection of *Escherichia coli* bacteraemia in rats. J Clin Microbiol. 37:2479-2482.

Henry MK, Tongue SC, Evans J, Webster C, McKendrick IJ, Morgan M, Willett A, Reeves A, Humphry RW, Gally DL, Gunn GJ, Chase-Topping ME (2017) British *Escherichia coli* O157 in Cattle Study (BECS): to determine the prevalence of *E. coli* O157 in herds with cattle destined for the food chain. Epidemiol Infect. 145:3168-3179.

Heuvelink, A. E. *et al.*(2001) Zero-tolerance for faecal contamination of carcasses as a tool in the control of O157 VTEC infections, International journal of food microbiology. Int J Food Microbiol. 66:13-20.

Holmes A, Dallman TJ, Shabaan S, Hanson M, Allison L. (2018) Validation of Whole-Genome Sequencing for Identification and Characterization of Shiga Toxin-Producing *Escherichia coli* To Produce Standardized Data To Enable Data Sharing. J Clin Microbiol. 22:56(3).

Houston, A. I., McNamara, J. M., Barta, Z., & Klasing, K. C. (2007). The effect of energy reserves and food availability on optimal immune defence. Proceedings of the Royal Society B: Biological Sciences, 274(1627), 2835–2842.

HPS (2014) E. coli O157: Culture positive cases, 1984-2013. Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?resourceid=1543

HPS (2018) STEC in Scotland 2017: Enhanced Surveillance and Reference Laboratory Data . Available at: https://www.hps.scot.nhs.uk/web-resources-container/stec-in-scotland-2017-enhanced-surveillance-and-reference-laboratory-data/

HPS (2019) HPS Surveillance Report. STEC in Scotland 2018: Enhanced Surveillance and Reference Laboratory Data - https://www.hps.scot.nhs.uk/web-resources-container/stec-in-scotland-2018-enhanced-surveillance-and-reference-laboratory-data/

Irivine RJ. *et al.*(2006) 'Low-level parasitic worm burdens may reduce body condition in free-ranging red deer (Cervus elaphus)', Parasitology, 133(4), p. 465.

James M, J., Loessner, M. J. and Golden, D. A. (2005) 'Foodborne Gastroenteritis Caused by *Escherichia coli*', in Modern Food Microbiology. Second. Boston, MA: Springer US, pp. 637–655.

Jamnikar-Ciglenecki U, Kuhar U, Steyer A, Kirbis A (2017) Whole genome sequence and a phylogenetic analysis of the G8P[14] group A rotavirus strain from roe deer. BMC Vet Res. 13:353.

Johnson LK, Liebana E, Nunez A, Spencer Y, Clifton-Hadley R, Jahans K, Ward A, Barlow A, Delahay R. (2008) Histological observations of bovine tuberculosis in lung and lymph node tissues from British deer. *Vet J.* 175(3):409-12.

Jolles, A. E., Beechler, B. R. and Dolan, B. P. (2015) 'Beyond mice and men: environmental change, immunity and infections in wild ungulates', Parasite immunology. 37(5), pp. 255–66.

Jordan, D. *et al.*(2007) 'Relationships between the density of different indicator organisms on sheep and beef carcasses and in frozen beef and sheep meat', Journal of Applied Microbiology. 102(1), pp. 57–64.

Kenny, F. J. and Tarrant, P. V. (1987) 'The reaction of young bulls to short-haul road transport', Applied Animal Behaviour Science. 17(3–4), pp. 209–227.

King, T. *et al.*(2016) 'Physiological Response of *Escherichia coli* O157:H7 Sakai to Dynamic Changes in Temperature and Water Activity as Experienced during Carcass Chilling.', Molecular & cellular proteomics 15(11), pp. 3331–3347.

Konjević, D. (2008) The roe deer (*Capreolus capreolus*) - from breeding to highly valuable food. The first Croatian meat journal. https://hrcak.srce.hr/21205

Kraft, A.A. (1992). Psychrotrophic bacteria in foods : disease and spoilage. Boca Raton (Fla.): CRC press.

Kreier, J. P. (2002) Infection, Resistance and Immunity, 2nd Edition. Taylor & Francis, USA.

Laaksonen, S. and Paulsen, P. (2015) Hunting Hygiene. The Netherlands: Wageningen Academic Publishers. doi:10.3920/978-90-8686-249-8.

Lamprinopoulou, C. *et al.*(2012) Food Hygiene Regulation in the Scottish Wild Game Sector. Available at: http://www.foodstandards.gov.scot/downloads/Final_Report_Part_1.pdf

Law, D. (2000) 'Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing E. coli', Journal of Applied Microbiology. 88(5), pp. 729–745.

Le Gall, T., Clermont, O., Gouriou, S., Picard, B., Nassif, X., Denamur, E., Tenaillon, O. (2007) Extraintestinal Virulence Is a Coincidental By-Product of Commensalism in B2 Phylogenetic Group *Escherichia coli* Strains. Molecular Biology and Evolution, 24: 2373–2384.

Letunic I, Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23:127–8.

Liu, Y., Youssef, M. K. and Yang, X. (2016) 'Effects of Dry Chilling on the Microflora on Beef Carcasses at a Canadian Beef Packing Plant', Journal of Food Protection, 79(4), pp. 538–543.

Lowland Deer Network Scotland (LDNS) - http://www.ldns.org.uk

Lynn RM, O'Brien SJ, Taylor CM, Adak GK, Chart H, Cheasty T, Coia JE, Gillespie IA, Locking ME, Reilly WJ, Smith HR, Waters A, Willshaw GA (2005) Childhood hemolytic uremic syndrome, United Kingdom and Ireland. Emerg Infect Dis. 11:590-6.

Mackey, B. M. and Derrick, C. M. (1979) 'Contamination of the Deep Tissues of Carcasses by Bacteria Present on the Slaughter Instruments or in the Gut', Journal of Applied Bacteriology. 46(2), pp. 355–366.

Magwedere, K. *et al.* (2013) 'Incidence of Shiga toxin–producing *Escherichia coli* strains in beef, pork, chicken, deer, boar, bison, and rabbit retail meat', Journal of Veterinary Diagnostic Investigation. 25(2), pp. 254–258.

Mainda G, Lupolova N, Sikakwa L, Bessell PR, Muma JB, Hoyle DV, McAteer SP, Gibbs K, Williams NJ, Sheppard SK, La Ragione RM, Cordoni G, Argyle SA, Wagner S, Chase-Topping ME, Dallman TJ, Stevens MP, Bronsvoort BM, Gally DL (2016) Phylogenomic approaches to determine the zoonotic potential of Shiga toxin-producing *Escherichia coli* (STEC) isolated from Zambian dairy cattle. Sci Rep. 6:26589.

Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, *et al.* (2014) Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne pathogens and disease. 11:447-55.

Martin, A. and Beutin, L. (2011) Characteristics of Shiga toxin-producing *Escherichia coli* from meat and milk products of different origins and association with food producing animals as main contamination sources, International Journal of Food Microbiology, 146(1), pp. 99–104.

Mather, A. E. *et al.* (2008) Factors associated with cross-contamination of hides of Scottish cattle by *Escherichia coli* O157, Applied and environmental microbiology, 74(20), pp. 6313–9.

Matthews L, Low JC, Gally DL, Pearce MC, Mellor DJ, Heesterbeek JA, Chase-Topping M, Naylor SW, Shaw DJ, Reid SW, Gunn GJ, Woolhouse ME (2006) Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. Proc Natl Acad Sci U S A. 103:547-52.

Meat Management. Com. Meat industry enjoys a successful festive season - https://meatmanagement.com/meat-industry-enjoys-a-successful-festive-season/

Mellefont L.A., Kocharunchitt, C., R. T. (2015) 'Combined effect of chilling and desiccation on survival of *Escherichia coli* suggests a transient loss of culturability', International Journal of Food Microbiology. 208, pp. 1–10.

Melton-Celsa A, Mohawk K, Teel L, O'Brien A. (2012) Pathogenesis of Shiga-toxin producing *Escherichia coli*. Current topics in microbiology and immunology. 357:67-103.

Membré, J.-M., Laroche, M. and Magras, C. (2011) 'Assessment of levels of bacterial contamination of large wild game meat in Europe', Food Microbiology, 28(5), pp. 1072–1079.

Menrath, A. *et al.* (2010) 'Shiga toxin producing *Escherichia coli*: identification of non-O157:H7-Super-Shedding cows and related risk factors', Gut pathogens. BioMed Central, 2(1), p. 7.

Miko A, Pries K, Haby S, Steege K, Albrecht N, Krause G, et al (2009) Assessment of Shiga toxin-producing *Escherichia coli* isolates from wildlife meat as potential pathogens for humans. Appl Environ Microbiol. 75:6462-70

Mills, J. *et al.*(2015) Assessment of cooling practises applied during harvesting of New Zealand feral venison : final report to Ministry of Primary Industries. Available at: https://books.google.co.uk/books/about/Assessment_of_Cooling_Practises_Applied.html?id=iDc_nQAACAAJ&redir_esc=y

Nel, S. *et al.*(2004) 'The personal and general hygiene practices in the deboning room of a high throughput red meat abattoir', Food Control. Elsevier, 15(7), pp. 571–578.

Nørrung, B. and Buncic, S. (2008) 'Microbial safety of meat in the European Union', Meat Science, 78(1–2), pp. 14–24. doi: 10.1016/j.meatsci.2007.07.032.

Obwegeser, T. *et al.*(2012) 'Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants', Veterinary Microbiology. Elsevier, 159(1–2), pp. 149–154.

Paton, J. C. and Paton, A. W. (1998) 'Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections', Clinical microbiology reviews. 11(3), pp. 450–79.

Paulsen, P. and Winkelmayer, R. (2004) 'Seasonal variation in the microbial contamination of game carcasses in an Austrian hunting area', European Journal of Wildlife Research. 50(3), pp. 157–159.

Paulsen, P. (2011) 'Hygiene and microbiology of meat from wild game: an Austrian view', in Game meat hygiene in focus. Wageningen: Wageningen Academic Publishers, pp. 19–37.

Pearce MC, Chase-Topping ME, McKendrick IJ, Mellor DJ, Locking ME, Allison L, Ternent HE, Matthews L, Knight HI, Smith AW, Synge BA, Reilly W, Low JC, Reid SW, Gunn GJ, Woolhouse ME. (2009) Temporal and spatial patterns of bovine *Escherichia coli* O157 prevalence and comparison of temporal changes in the patterns of phage types associated with bovine shedding and human E. coli O157 cases in Scotland between 1998-2000 and 2002-2004. BMC Microbiology 9:276

Price MN, Dehal PS, Arkin AP (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. PLoS One 5(3):e990.

Probert, W. S., Miller, G. M., & Ledin, K. E. (2017, July 1). Contaminated stream water as source for *Escherichia coli* O157 illness in children. *Emerging Infectious Diseases*, Vol. 23, pp. 1216–1218.

Public Services (Reform) (Scotland) Act 2010 - https://www.legislation.gov.uk/asp/2010/8/contents

Putman (2012) 'Scottish Natural Heritage Scoping the economic benefits and costs of wild deer and their management in Scotland Scoping the economic benefits and costs of wild deer and their management in Scotland'. Available at: https://media.nature.scot/record/~f6377f7895

Rabatsky-Ehr, T. *et al.*(2002) 'Deer Meat as the Source for a Sporadic Case of *Escherichia coli* O157:H7 Infection, Connecticut1', Emerging Infectious Diseases, 8(5), pp. 525–527.

Radakovic, M. and Fletcher, J. (2011) 'Risk Management of game: from theory to practice', in Game meat hygiene in focus. Wageningen: Wageningen Academic Publishers, pp. 209–221.

Ramanzin, M. *et al.*(2010) 'Meat from wild ungulates: ensuring quality and hygiene of an increasing resource'. Italian Journal Of Animal Science. 9 (e16).

Richards PJ, Wu S, Tinker DB, Howell MV, Dodd CER. (2011) Microbial quality of venison meat at retail in the UK in relation to production practices and processes. P. Paulsen *et al.*(eds.), Game meat hygiene in focus. Wageningen Academic Publishers. doi:10.3920/978-90-8686-723-3_7

Rijks J.M., Montizaan M. G.E., Dannenberg H., Algra-Verkerk L..A. ,. Nourisso A, G. A. and . H. M. (2017) 'European Community food safety regulations taking effect in the hunted game food chain: an assessment with stakeholders in the Netherlands', in Paulsen P., Bauer A., S. F. J. M. (ed.) Game meat hygiene Food safety and security. Wageningen Academic Publishers, pp. 153–171.

Rivas, L., Dykes, G. A. and Fegan, N. (2007) 'A comparative study of biofilm formation by Shiga toxigenic Escherichia coli using epifluorescence microscopy on stainless steel and a microtitre plate method', Journal of microbiological methods, 69(1), pp. 44–51.

Rivas, L., Fegan, N. and Dykes, G. A. (2007) 'Attachment of Shiga toxigenic *Escherichia coli* to stainless steel', International Journal of Food Microbiology, 115(1), pp. 89–94. doi: 10.1016/j.ijfoodmicro.2006.10.027.

Ro Y.E., Ko Y. M., Y. K. S. (2015) 'Survival of pathogenic enterohemorrhagic Escherichia coli (EHEC) and control with calcium oxide in frozen meat products', Food Microbiology. 49, pp. 203–210.

Roberts, M. N. and Getty, K. J. K. (2011) 'Validation of heating conditions in production of direct acidified venison with beef fat summer sausage for elimination of *Escherichia coli* O157:H7', Journal of Food Safety. 31(4), pp. 480–486.

Rounds, JM, Rigdon CE, Muhl LJ, Forstner M, Danzeisen GT, Koziol BS *et al.*(2012) Non-O157 Shiga Toxin–producing *Escherichia coli* Associated with Venison. Emerg Infect Dis. 18: 279–282.

Scheutz F, Teel LD, Beutin L, Pierard D, Buvens G, Karch H, *et al.*(2012) Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. Journal of clinical microbiology. 50:2951-63.

Scottish Government (no date) VTEC/E. coli O157- action plan for Scotland 2013-2017. Available at: http://www.gov.scot/Publications/2013/11/8897

Scottish Government livestock statistics: https://www2.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/agritopics/alllivestock

Scottish National Heritage (2016) Practical indicators to assess the welfare of wild deer in Scotland. Commissioned Report No. 944. - https://www.nature.scot/snh-commissioned-report-944-practical-indicators-assess-welfare-wild-deer-scotland

Scottish Quality Wild Venison Ltd - SQWV, http://www.sqwv.co.uk/

Scottish Venison Partnership - http://scottish-venison.info/

Scottish Venison Partnership application to register "Scottish Wild Venison" as a Protected Geographical Indication. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/775607/pfn-scottish-wild-venison-spec.pdf

Scottish Venison website - http://www.scottish-venison.info/index.php?page=Deer-farming-in-Scotland

Scottish Venison, an industry review 2010. McKellar K and McKellar, Ashwood Management Ashwood Management, Troon, South Ayrshire, UK

SFLEC guidance (Guidance for Local Authority Enforcement Officers on the Safe Service of Less Than Thoroughly Cooked Beef Burgers- https://www.foodstandards.gov.scot/downloads/A14659647.pdf

Simpson Beauchamp, C. *et al.*(2012) 'Transfer, Attachment, and Formation of Biofilms by *Escherichia coli* O157:H7 on Meat-Contact Surface Materials', Journal of Food Science, 77(6), pp. M343–M347.

Singh, P. *et al.*(2015) Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem, Frontiers in Cellular and Infection Microbiology, 5, p. 29.

Skandamis, P. *et al.*(2009) *Escherichia coli* O157:H7 survival, biofilm formation and acid tolerance under simulated slaughter plant moist and dry conditions', Food Microbiology, 26(1), pp. 112–119.

Smith, V. (2007). Host resource supplies influence the dynamics and outcome of infectious disease. Integrative and Comparative Biology, *47*(2), 310–316.

Smith-Palmer A, Hawkins G, Browning L, Allison L, Hanson M, Bruce R, McElhiney J, Horne J. (2018) Outbreak of *Escherichia coli* O157 Phage Type 32 linked to the consumption of venison products. Epidemiol Infect. 146:1922-1927.

6. References

Stagiston website - https://stagison.com/about/

Stalking and Carcass Handling Standards SQWV Assurance Scheme - https://services.acoura.com/media/doc_65738073/SQWV%20Producer%20Standards%20-%20Issue%2010%20%2005%20May%202017%20V5.pdf?pbc=1010947

Stringer, S. C., George, S. M. and Peck, M. W. (2000) 'Thermal inactivation of *Escherichia coli* O157:H7', Journal of Applied Microbiology. 88(S1), p. 79S–89S.

Synge, B. (2006) Epidemiological studies of Verocytotoxin-producing *Escherichia coli* infections in animals in Scotland. PhD, University of Edinburgh. doi: 103-104.

The Food (Meat Inspection) (Scotland) Regulations (1988) - http://www.legislation.gov.uk/uksi/1988/1484/introduction/made

The Welfare of Animals at the Time of Killing (Scotland) Regulations 2012- http://www.legislation.gov.uk/ssi/2012/321/introduction/made

Turbill, C. *et al.* (2011) 'Regulation of heart rate and rumen temperature in red deer: effects of season and food intake', Journal of Experimental Biology, 214(6), pp. 963–970.

Urquhart, K. A. and McKendrick, I. J. (2003) 'Survey of permanent wound tracts in the carcases of culled wild red deer in Scotland', The Veterinary record. British Medical Journal Publishing Group, 152(16), pp. 497–501.

Urquhart, K. A. and McKendrick, I. J. (2006) 'Prevalence of "head shooting" and the characteristics of the wounds in culled wild Scottish red deer.', The Veterinary record, 159(3), pp. 75–9.

van Schalkwyk, D. L., Hoffman, L. C. and Laubscher, L. A. (2011) 'Game harvesting procedures and their effect on meat quality: the Africa experience', in Game meat hygiene in focus. Wageningen: Wageningen Academic Publishers, pp. 67–92.

Venegas-Vargas, C. *et al.* (2016) 'Factors Associated with Shiga Toxin-Producing *Escherichia coli* Shedding by Dairy and Beef Cattle', Applied and Environmental Microbiology. Edited by J. Björkroth, 82(16), pp. 5049–5056.

Venison Advisory Service Ltd - http://venisonadvisory.co.uk/

Venison dealer licence (Scotland) - https://www.gov.uk/venison-dealer-licence-scotland

Visvalingam, J., Liu, Y. and Yang, X. (2017) 'Impact of dry chilling on the genetic diversity of *Escherichia coli* on beef carcasses and on the survival of *E. coli* and *E. coli* O157', International Journal of Food Microbiology, 244, pp. 62–66.

VTEC/E. coli O157 Action Plan for Scotland 2013 2017 - http://www.gov.scot/Publications/2013/11/8897

Wang, O., McAllister, T. A., Plastow, G., Selinger, B., Stanford, K., & Guan, L. L. (2016). 0478 Transcriptome analysis of the intestinal tissues of cattle suggests an association among host immune responses, lipid metabolism and the super-shedding of E. coli O157. *Journal of Animal Science*, 94:228–229.

WHO (2017) WHO | E. coli, WHO. World Health Organization. Available at: http://www.who.int/mediacentre/factsheets/fs125/en/

Wiklund, E. *et al.* (2001) 'Electrical stimulation of red deer (*Cervus elaphus*) carcasses - effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity', Meat science, 59(2), pp. 211–20.

Williams, K. J. *et al.* (2015) 'Risk factors for *Escherichia coli* O157 shedding and super-shedding by dairy heifers at pasture', Epidemiology and Infection, 143(5), pp. 1004–1015.

Yang, X. *et al.*(2017) 'Microbial efficacy and impact on the population of *Escherichia coli* of a routine sanitation process for the fabrication facility of a beef packing plant', Food Control. Elsevier, 71, pp. 353–357.

Appendix 1. Estimated Average Weight (at the Larder) by Deer Species

Deer species	Average dead weight (kg)
Red	41 (47)
Roe	12 (12)
Sika	21 (24)
Fallow	24 (22)

The above is based on the mean weights supplied by Forestry Commission Scotland for each season from 2007-08 to 2016-17. Weights represent the combined average value for the combined stag and hind of each species. Weights in brackets represent average weights from previous report in 2010.

Appendix 2. Proportion of deer culled by Forestry and Land Scotland between 2001-2016

Season	Red (%)*	Roe (%)*	Sika (%)*	Fallow (%)*	Total (%)†
2007-2008	14.4	35.8	51.6	20.2	23.3
2008-2009	15.0	37.1	48.8	18.2	24.2
2009-2010	20.3	44.0	75.2	18.2	29.7
2010-2011	20.5	39.4	49.5	22.2	28.6
2011-2012	20.6	41.7	52.8	20.3	30.0
2012-2013	21.2	42.7	53.7	25.5	30.6
2013-2014	19.5	41.4	47.8	31.9	28.9
2014-2015	19.7	41.1	48.1	24.0	28.6
2015-2016	19.7	39.8	47.4	22.9	28.8

** % values represent the proportion of the total deer culled for each species per annum that are culled by Forestry and Land Scotland (formerly Forest Enterprise).*
† values represent the proportion of the total annual cull (all deer species) which is culled by Forestry and Land Scotland.
Source: Scottish Natural Heritage and Forestry and Land Scotland.

Appendix 3. Proportion of deer culled out of season 2007-2016

	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16
Red	11%	12%	7%	12%	13%	12%	13%	13%	14%
Roe	9%	9%	4%	9%	10%	11%	11%	11%	12%
Sika	30%	29%	9%	28%	25%	27%	30%	27%	31%
Fallow	9%	8%	8%	11%	18%	7%	6%	6%	6%
Total	11%	12%	6%	12%	13%	12%	136%	13%	14%

% values indicate the proportion of deer which are culled out of season for each species, and as a proportion of the total annual cull.
Source: Scottish Natural Heritage

Appendix 4: Deer hunting seasons in the UK

Species/sex	Scotland	England and Wales	Northern Ireland
Red, including hybrids with sika			
Stag	1st July-20th Oct	1st Aug-30th Apr	1st Aug-30th Apr
Hind	21st Oct-15th Feb	1st Nov-31st March	1st Nov-31st March
Roe			
Buck	1st Apr-20th Oct	1st Apr- 31st Oct	-
Doe	21st Oct- 31st March	1st Nov-31st March	-
Sika			
Stag	1st July-20th Oct	1st Aug-30th Apr	1st Aug-30th Apr
Hind	21st Oct-15th Feb	1st Nov-31st March	1st Nov-31st March
Fallow			
Buck	1st Aug-30th Apr	1st Aug-30th Apr	1st Aug-30th Apr
Doe	21st Oct-15th Feb	1st Nov-31st March	1st Nov-31st March

Source: The Deer Act 1991

Appendix 5. Approved Game Handling Establishments (AGHEs) in Scotland for deer

Approval Number	Premises Name	Local Authority
1125	John M Munro Ltd	Dingwall / Highland
1178	Highland Game Limited	Dundee / Dundee City
1184	The Ardgay Game Factory Ltd	Sutherland / Highland
1190	Simpson Game Ltd., Trading as Falconer Game	Newtonmore / Highland
1585	Mull Slaughterhouse Ltd	Isle of Mull / Argyll & Bute
1641	Richard Carmichael (Carmichael Estate Farm Meats)	Biggar / South Lanarkshire
1685	Charmaine Bain and John Andrew Bain (Aberdeenshire Larder)	Ellon / Aberdeenshire
1699	Richard Pickup and Celia Pickup (Craigadam Country Larder)	Castle Douglas / Dumfries & Galloway
1701	Hubertus Game Ltd	Pitlochry / Perth & Kinross
1704	J Rutherford T/A Burnside Farm Foods	Kelso / Scottish Borders
1742	David Killoh Meat Co. Ltd	Peterhead / Aberdeenshire

Source: Food Standards Scotland approved premises register (correct as of September, 2017)

Appendix 6. Format suggested by the FSS and FSA for large wild game hunter's declaration – source FSA (2015)

LARGE WILD GAME DECLARATION

Tag Number: ..... **Species: ROE FALLOW RED MUNTJAC**

Date/Time of Kill: ...../..... **SIKA CHINESE**

Location/Estate:..... **OTHER**.....

Sex: **M** **F** **Weight:** ..... **(KGs)**

I declare in accordance with EU Regulation 853/2004 that no abnormal behaviour was observed before killing and there is no indication of environmental contamination. I have inspected the head, pluck and viscera without observing abnormalities*.

Notes:.....

Trained person qualification:..... **Contact details** .....

Name:.....

.....

Signature:.....

.....

Appendix 7. Approved abattoirs for farmed deer in Scotland

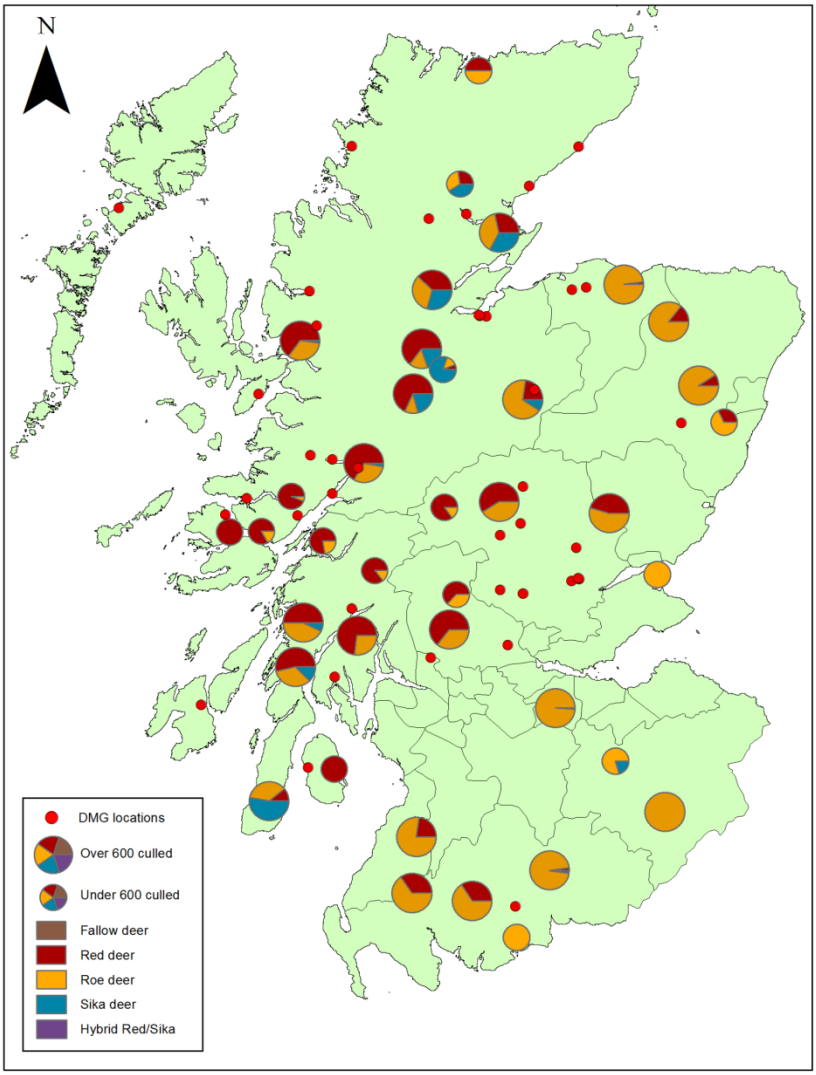
Approval Number	Premises Name	Local Authority
1125	John M Munro Ltd	Dingwall / Highland
1201	Downfield Limited*	Cupar / Fife
1517	Wishaw Abattoir Ltd	Wishaw / North Lanarkshire
1635	Barony Agricultural College	Lockerbie / Dumfries & Galloway
161	Carmichael Estate Farm Meats	Biggar / South Lanarkshire
1705	Hazel Weaver (T/A Northfield Farm)	Holm / Orkney
1712	Ali Loder & Sandra Loder (T/A Culquoich Estate/Strathdon Venison)	Alford / Aberdeenshire
1753	Robert Webster (T/A Northwood Wild Boar)	Aberlady/East Lothian

*Also an AGHE as handles wild deer

Source: Food Standards Scotland approved premises register (correct as of September 2017)

Appendix 8. Sampling strategy for STEC prevalence study

Sample packs (800) were distributed to all Scottish deer management groups (ADMG, LDNS) indicated by the closed red dots in the figure below. In addition 1088 sample packs were distributed to 18 Forestry Commission larders (Scottish Forestry). Sampling from Forestry commission larders was weighted for species, sex, month, larder size (3 large larders handling >600 carcasses/year and 3 small larders handling >600 carcasses/year) for the north, central and southern forestry regions). Forestry Commission larders are indicated by the pie charts in the figure below which indicate the deer species and size of the larders. Those chosen for the study are circled in red. Figure curtosy of G. Robertson.



Appendix 9. Questionnaire completed by deer stalkers at time of sample collection

Scottish Deer Health Survey 2017-2019

Moredun

QUESTIONNAIRE

Date	
Time	
Tag number (if available)	
OS Reference of cull site	OS Sheet:_____ 6-digit grid reference:_____
Larder Address	
Deer species	Red ☐ Roe ☐ Sika ☐ Other ☐ If other provide details:_____
Gender	Male ☐ Female ☐
Condition Score	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 1 = very poor condition; 5 = 'very good condition
Estimated age	_____years
Shared range with other livestock/ wild herbivores	Cattle ☐ Sheep ☐ Wild herbivores ☐ If wild herbivores provide details below: _____
Other comments	

THANK-YOU FOR PARTICIPATING IN THIS SURVEY

Appendix 10. Potential sources of *E. coli* contamination in wild deer carcasses and meat

Risk Factors	Sources of contamination	Reference	Suggested corrective actions	Included in best practices guides (1* and 2*), wild game guide or legislation
Risk of infection in live animal	The types of microorganism present in the intestine/hide; higher proportion of animals colonised with STEC strains	(Obwegeser *et al.*, 2012) (Miko *et al.*, 2009)	Observe abnormal behaviour, scouring, body condition and hide condition of the deer	1- guide on disease assessment [75] 2- Guide on health-welfare[76] Regulation 853/200, Annex III, Section IV, Chapter I, P
	Co-grazing with other livestock	(Miko *et al.*, 2009) (Díaz-Sánchez *et al.*, 2013) (Bardiau *et al.*, 2010b)	Prevent shared grazing between wild and domestic ruminants	Not stipulated in guidelines nor legislation
	Stress in live animal	(Obwegeser *et al.*, 2012) (Ramanzin *et al.*, 2010) (Casoli *et al.*, 2005)	Avoid injuries, escaped deer, chasing the deer	2- Guide on health-welfare [77]
	High density of deer	(Díaz-Sánchez *et al.*, 2013)	Reduce deer stocking density to below 15	1- guides on population dynamics [78]and cull planning [79]

[75] http://www.thedeerinitiative.co.uk/uploads/guides/117.pdf
[76] https://www.bestpracticeguides.org.uk/health-welfare/
[77] https://www.bestpracticeguides.org.uk/health-welfare/
[78] http://www.thedeerinitiative.co.uk/uploads/guides/113.pdf
[79] http://www.thedeerinitiative.co.uk/uploads/guides/115.pdf

		(Eggert *et al.*, 2013)	deer/square km described as high stocking by (Díaz-Sánchez *et al.*, 2013)	2- Guide on setting cull targets [80]
Risk of carcass contamination with STEC	Cold Chain	(Paulsen and Winkelmayer, 200) (Ramanzin *et al.*,2010)	Prompt evisceration, chilling, and transfer to chilled facilities in warmer months	-guide on carcass gralloching[81] 2- carcass gralloching [82] and gralloching part II [83] EC 853/200, Sect IV, Ch II, P5
	Wound location Abdominal wounds	(Deutz *et al.*, 2000) (Casoli *et al.*, 2005) (Gill, 2007) (Atanassova *et al.*, 2008) (Membré, Laroche and Magras, 2011) (Paulsen, 2011) (Obwegeser *et al.*, 2012) (Ramanzin *et al.*, 2010) (Avagnina *et al.*, 2012)	Ideal shot location-thorax in the heart area, good bleeding and instantaneous death Avoid circumstances that increase the risk of shots shots r to the abdomen	Guide on culling and shot placement[84] Guide on culling/shot placemen t[85]
	Bleeding	(Casoli *et al.*, 2005)	Clean knives	Only clean knives stipulated Guide on carcass hygiene [86]

[80] https://www.bestpracticeguides.org.uk/planning/setting-cull-targets/
[81] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[82] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_gralloch.pdf
[83] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_grallochTwo.pdf
[84] http://www.thedeerinitiative.co.uk/uploads/guides/161.pdf
[85] https://www.bestpracticeguides.org.uk/culling/shot-placement/
[86] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf

			Two knives technique to access blood vessels Rapid bleeding Protect the bleeding wound from cross-contamination	Guide on carcass/hygiene [87]
	Large openings of the body cavities	(Avagnina *et al.*, 2012)	Small incisions, just to remove abdominal viscera	1- Guide on gralloching [88] 2- Gralloching [89]
			Protect cuts from cross-contamination	1- Guide on extraction and transport [90]
	Evisceration	(Deutz *et al.*, 2000) (Casoli *et al.*, 2005) (Ramanzin *et al.*, 2010)	Evisceration to be carried as soon as possible	1- Guide on gralloching [91] 1 hour 2- Guide-carcass/bulk-handling [92] - gralloching to be carried within 30 minutes, Regulation (EC) No. 853/200 Annex III, Section IV, Ch.2, P1 and 2 as amended by Commission Regulation 150/2011
			Avoid lacerating intestines	1- Guide on gralloching [93] 2-Guide on gralloching [94] EC 852/2004, Annex , Chapter IX, Par. 3
			Clean hands and tools	1- guide on carcass hygiene [95] 2- guide on hygiene principles[96]

[87] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/
[88] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[89] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_gralloch.pdf
[90] http://www.thedeerinitiative.co.uk/uploads/guides/131.pdf
[91] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[92] https://www.bestpracticeguides.org.uk/carcass-preparation/bulk-handling/
[93] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[94] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_grallochTwo.pdf
[95] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[96] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/

			Avoid contact between hides and exposed meat	1- Guide on gralloching [97] 2- Guide on gralloching[98] EC 852/200, Annex , Chapter IX, Par. 3
	Breaking the digestive lining	(Obwegeser *et al.*, 2012) (Ramanzin *et al.*, 2010) (Miko *et al.*, 2009)	Avoid lacerating intestines	1- Guide on gralloching [99]2--Guide on gralloching (2)[100]
			Reject carcasses with heavy intestinal content soiling	1- Guide on Carcass Inspection [101] 2- Unclear. Carcass inspection (1 and 2) [102]
			Rodding of oesophagus	1- Guide on gralloching [103] 2- Guide on gralloching (2)[104]
			Seal rectum	1- Guide on gralloching [105]
	Use of potentially contaminated water (river, streams) to wash cavities	(Avagnina *et al.*, 2012)	Avoid washing carcass to prevent further spread of contamination and spoilage	1- Guide on carcass hygiene, in notes, carcass washing is not recommended [106] 2- Carcass inspection [107] washing **is** advised : '*Remove any contamination by washing and/or cutting back* '-
			Contamination removed by trimming	1- not specified 2- Guide on carcass inspection [108]
	Cross-contamination between carcasses of	(Gill, 2007)	See Table 4.3 of this report	Table 4.3 of this report

[97] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[98] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_gralloch.pdf
[99] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[100] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_grallochTwo.pdf
[101] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_grallochTwo.pdf
[102] https://www.bestpracticeguides.org.uk/carcass-preparation/carcass-inspection/
[103] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[104] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_grallochTwo.pdf
[105] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[106] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[107] https://www.bestpracticeguides.org.uk/carcass-preparation/carcass-inspection/
[108] https://www.bestpracticeguides.org.uk/carcass-preparation/carcass-inspection/

	same or different species	(Atanassova *et al.*, 2008) (Ramanzin *et al.*, 2010) (Avagnina *et al.*, 2012)		1- Guide on extraction and transport [109] 2- Mechanical and manual extraction (1 and 2)[110] and extraction by pony (1 and 2) [111]
	Practice of hanging unskinned carcasses	(Obwegeser *et al.*, 2012)	Avoid contact between carcasses during storage	1- Butchering [112] 2- Guides: carcass/bulk-handling [113], carcass/venison-supply [114] and butchering [115] EC 852/200 Annex, II, Food Premises: Ch 1, P2 HACCP Guidance-Step -Chilling and chilled storage - Visual check of carcass spacing during cooling[116]
			Avoid contact with surfaces	1- Guide on Carcass basic hygiene [117]
			Use clean equipment to store carcasses	1- Guide on Carcass basic hygiene [118]2 **Not found** in Hygiene principles [119] EC 853/200, Annex 1, Part A, II, P. 3 and (a and b)
			Do not delay storage, no prescribed interval described in the legislation or the national	1- guide 133, up to 10 days 2- **No specification**

[109] http://www.thedeerinitiative.co.uk/uploads/guides/131.pdf
[110] https://www.bestpracticeguides.org.uk/culling/mechanical-extraction/
[111] https://www.bestpracticeguides.org.uk/culling/pony-extraction/
[112] http://www.thedeerinitiative.co.uk/uploads/guides/133.pdf
[113] https://www.bestpracticeguides.org.uk/carcass-preparation/bulk-handling/
[114] https://www.bestpracticeguides.org.uk/carcass-preparation/venison-supply/
[115] https://www.bestpracticeguides.org.uk/carcass-preparation/butchering/
[116] https://www.foodstandards.gov.scot/downloads/HACCP_Guidance.pdf
[117] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[118] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[119] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/

			guidelines. 2 h is described as appropriate storage time by (Mills *et al.*, 2015)	
	Storage conditions: fluctuations in storage temperature, moist conditions	(Obwegeser *et al.*, 2012) (Ramanzin *et al.*, 2010) (Rabatsky-Ehr *et al.*, 2002)	Adequate, uninterrupted chill temperature	1- Guide on Carcass butchering [120] 2- Carcass/venison-supply [121] EC 853/200 Annex III, Section IV, Chapter II, P5, as amended by Commission Regulation 633/201 Wild Game Guide P50 and 51 (FSS, 2015)
			Sufficient ventilation to prevent condensation	1- Guide on larder design[122] larder hygiene and safety [123] 2- Larder design [124] EC 852/200, Annex II, Chapter IX, P3 Wild Game Guide, P9 and 52 ((FSS, 2015)
			Avoid storing meat or carcasses for prolonged periods	1- Guide on carcass butchering[125], recommends up to 10 days 2- No time specified on the guides Wild game Guide, P 68 '*Meat of wild game may be placed on the market only if the carcases have been transported to an AGHE as soon as possible after the examination performed by a trained person'.*

[120] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[121] https://www.bestpracticeguides.org.uk/carcass-preparation/venison-supply/
[122] http://www.thedeerinitiative.co.uk/uploads/guides/139.pdf
[123] http://www.thedeerinitiative.co.uk/uploads/guides/140.pdf
[124] https://www.bestpracticeguides.org.uk/carcass-preparation/larder-design/
[125] http://www.thedeerinitiative.co.uk/uploads/guides/133.pdf

			Do not accept heavily contaminated carcasses into larder	1- Guide on carcass inspection[126] 2- carcass inspection(2) [127]- **Not clearly specified** what is the necessary action in case of heavy contamination HACCP Guidance, Process Step 1- Acceptance of carcasses ((Food Standards Agency, 2008)
	Warmer months of the year	(Paulsen and Winkelmayer, 200) (Ramanzin *et al.*, 2010) (Rabatsky-Ehr *et al.*, 2002)	Prompt evisceration	1- Guide on Gralloching[128] recommends 1 hour 2- Guide-carcass/bulk [129]-recommends handling within 30 minutes, EC 853/200 Annex III, Section IV, Ch.2, P1 and 2 as amended by EC Regulation 150/2011
			Prompt chilling in warmer months	1- Guide Carcass Butchering [130]- cool without chilling for 6 hours and chill at 1°C after; Guide Gralloching explains warm temperature compromises the preservation, Guide on Lardering [131]-recommends as soon as possible and if extraction is delayed ensure carcass will cool 2- Larder design[132]- carcass temperature is brought below 7°C as soon as possible, (suggested

[126] http://www.thedeerinitiative.co.uk/uploads/guides/159.pdf
[127] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_inspectTwo.pdf
[128] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[129] https://www.bestpracticeguides.org.uk/carcass-preparation/bulk-handling/
[130] http://www.thedeerinitiative.co.uk/uploads/guides/133.pdf
[131] http://www.thedeerinitiative.co.uk/uploads/guides/160.pdf
[132] https://www.bestpracticeguides.org.uk/carcass-preparation/larder-design/

				temperature 1-3°C); Gralloching 1 [133]-explains warmer temperature accelerate the activity of bacteria EC 853/200 Annex III, Section IV, Chapter II, P5, as amended by Commission Regulation 633/201 Wild Game Guide P50 and 51(FSS, 2015)
	Delayed cooling	(Deutz *et al.*, 2000) (Paulsen and Winkelmayer, 200) (Casoli *et al.*, 2005) (Membré, Laroche and Magras, 2011) (Paulsen, 2011)	Cooling as soon as practicable after shooting. No prescribed time in the legislation. 3 hours has been suggested as a reasonable time (Paulsen, 2011)	1 and 2- no time prescribed 1- Guide Carcass butchering [134]- cool without chilling for 6 hours and chill at 1°C after; Guide Gralloching [135]explains warm temperature compromises the preservation, Guide Lardering [136]- as soon as possible and if pick up is delayed ensure carcass will cool 2- Guidearder design[137] carcass temperature is brought below 7°C as soon as possible, (suggested temperature 1-3°C); gralloching 1-explains warmer temperature accelerate the activity of bacteria Wild Game Guide P50 and 51
	Temperature applied to the meat	(Paulsen and Winkelmayer, 200)	Temperatures below 7°C closer to 0°C achieve better microbial quality, however freezing of carcass should be avoided, in line with	1- Guideon venison supply[138] explains temperature controls 2- Guides where temperature control is covered:, venison supply[139], Lardering 3 [140],

[133] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_gralloch.pdf
[134] http://www.thedeerinitiative.co.uk/uploads/guides/133.pdf
[135] http://www.thedeerinitiative.co.uk/uploads/guides/157.pdf
[136] http://www.thedeerinitiative.co.uk/uploads/guides/160.pdf
[137] https://www.bestpracticeguides.org.uk/carcass-preparation/larder-design/
[138] http://www.thedeerinitiative.co.uk/uploads/guides/137.pdf
[139] https://www.bestpracticeguides.org.uk/carcass-preparation/venison-supply/
[140] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_larderingThree.pdf

		(Membré, Laroche and Magras, 2011)	requirement of EC hygiene regulations	
	Breaking the cold chain	Ramanzin *et al.*, 2010) (Avagnina *et al.*, 2012) (EFSA, 201)	Uninterrupted cold chain once the carcass has reached below 7°C	1- Guide 136 skinning [141]and guide venison processing/supply [142] 2- gralloching(2)[143] and venison supply (1) [144] HACCP Guidance, acceptance of carcasses (Food Standards Agency, 2008) EC 853/200 Annex III, Section IV, Chapter II, P5, as amended by Commission Regulation 633/201 Wild Game Guide P50 and 51(FSS, 2015)
	Visible contamination on the hide	Avagnina et al., 2012 (Obwegeser *et al.*, 2012)	Avoid fouling or soiling of the hide during extraction and visual check of the hide	1 and 2- Carcass extraction and Transport **No stipulation of hide soiling** (EC) No 852/200 Article 5 HACCP Guide-Process Step 2-skinning (Food Standards Agency, 2008)
			Reject heavily contaminated carcasses	1- GuideCarcass inspection[145] 2- **Not fully in line with the suggested corrective action**; Lardering 3:[146]*'The recommended way to remove any contamination is trimming it with a clean knife…Washing down with low pressure might be used before trimming'*; Carcass inspection guide repeats the above

[141] http://www.thedeerinitiative.co.uk/uploads/guides/136.pdf
[142] http://www.thedeerinitiative.co.uk/uploads/guides/137.pdf
[143] http://www.thedeerinitiative.co.uk/uploads/guides/137.pdf
[144] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_venisonSupplyTwo.pdf
[145] http://www.thedeerinitiative.co.uk/uploads/guides/159.pdf
[146] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_larderingThree.pdf

				(EC) No 852/200 Article 5 HACCP Guidance, acceptance of carcasses (Food Standards Agency, 2008)
	Hide might be apparently clean but transfer from the hide to the carcass	(Casoli *et al.*, 2005) (Gill, 2007) (Atanassova *et al.*, 2008) (Membré,Laroche and Magras, 2011) (Avagnina *et al.*, 2012)	Perform skinning in a separate area from clean carcasses or separation of operations in time and space	1- Guide Skinning [147]- Not specified 2- Skinning [148]-Not specified EC 852/200, Annex II Food Premises, Chapter 1, P.2
			Clean tools, hands	1- Guide basic hygiene [149] 2- Hygiene principles [150] EC 852/200, Annex II Personal Hygiene, Chapter VIII, P. 1
			Avoid aerosols generated by rough pulling	1- Guide on skinning [151]- separating hide and flesh 2- Guide on skinning[152] -Not specified (skinning)
			Avoid in in-rolling of hide towards exposed meat	1- Guide skinning-separating hide and flesh[153] 2- Not specified -Carcass skinning (cuts) [154]

[147] http://www.thedeerinitiative.co.uk/uploads/guides/136.pdf
[148] https://www.bestpracticeguides.org.uk/carcass-preparation/skinning/
[149] https://www.bestpracticeguides.org.uk/carcass-preparation/skinning/
[150] https://www.bestpracticeguides.org.uk/carcass-preparation/skinning/
[151] http://www.thedeerinitiative.co.uk/uploads/guides/136.pdf
[152] https://www.bestpracticeguides.org.uk/carcass-preparation/skinning/
[153] http://www.thedeerinitiative.co.uk/uploads/guides/136.pdf
[154] https://www.bestpracticeguides.org.uk/carcass-preparation/skinning/

	Wet hides (based on cattle observation)	(Antic *et al.*, 2010)	Avoid washing carcasses at any stage but especially with hide on	1- Guide Larder hygiene and safety (hygiene and cleaning) [155]; **Not in line with the suggested corrective actions**: 'Dirty carcasses should have the hide washed before being brought into the larder , taking care not to contaminate breaks in the hide) 2- Carcass inspection[156] - **Not fully in line with the suggested corrective actions:** '*Remove any contamination by washing and/or cutting back'*
	Hands, tools, airborne contamination (based on cattle observation)	(Barkocy-Gallagher *et al.*, 2001)	Hygienic handling to prevent cross-contamination	1- Guide -Basic Hygiene [157] 2- Hygiene principles [158] EC 852/200, Annex II Training, Chapter XII, P. 1 EC 852/200, Annex II Personal Hygiene, Chapter VIII, P.1
	Visible contamination on the carcass	(Avagnina *et al.*, 2012) (Miko *et al.*, 2009)	Trimming light contamination	1- Guide carcass inspection[159] 2- Guide on lardering [160] (EC) No 852/200 Article 5 HACCP Guides-Process Step 5-Inspection, cutting, trimming (Food Standards Agency, 2008)
			Avoid aerosols	1 and 2- **Not specified**

[155] http://www.thedeerinitiative.co.uk/uploads/guides/140.pdf
[156] https://www.bestpracticeguides.org.uk/carcass-preparation/carcass-inspection/
[157] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[158] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/
[159] http://www.thedeerinitiative.co.uk/uploads/guides/159.pdf
[160] https://www.bestpracticeguides.org.uk/carcass-preparation/lardering/

				EC 852/200 Annex I A, Chapter II Hygiene Provisions, P 2 and 3(a)
			Avoid cross-contamination	1- Guide Basic Hygiene[161] 2- Hygiene principles[162] EC 852/200, Annex II Training, Chapter XII, P. 1 EC 852/200, Annex II Personal Hygiene, Chapter VIII, P.1
			Reject grossly contaminated carcasses	1- Guide Carcass inspection [163] 2- Carcass Inspection 2- [164] **Not clear** (EC) No 852/200 Article 5 HACCP Guidance, Process Step 1-Acceptance of carcasses (Food Standards Agency, 2008)
	Insufficient wound trimming	(Dobrowolska and Melosik, 2008)	Trim and discard wound areas and associate bruising	1- Guide Butchering (1)[165] - **Not fully in line with suggested corrective action:'** *Trim out any bullet damage…Any trim can be used for mince'*. Guide Carcass inspection [166] in line with suggested corrective action 2- Carcass inspection (2)[167] in line with suggested corrective action

[161] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[162] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/
[163] http://www.thedeerinitiative.co.uk/uploads/guides/159.pdf
[164] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_inspectTwo.pdf
[165] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_inspectTwo.pdf
[166] http://www.thedeerinitiative.co.uk/uploads/guides/159.pdf
[167] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_inspectTwo.pdf

				HACCP Guide and Wild Game guide **– Not specified**
	Unhygienic handling and butchering	(Miko *et al.*, 2009) (Ramanzin *et al.*, 2010) (Avagnina *et al.*, 2012) (Obwegeser *et al.*, 2012)	Storage of skinned deer and meat must take place separately from other game species	1- Guide Venison processing/supply[168] 2- Guide Venison supply (2)[169], Guide carcass inspection (2) [170], HACCP Guide-Process Step 5 (Food Standards Agency, 2008) EC 852/200 Annex II Food Premises, Chapter 1, P2- adequate working space EC 853/200 Annex III, Section I, Slaughter Hygiene, Chapter IV, P 3 and P19-separation on either time or space of operations carried out on different wild game species
			Hygienic hands, tools and surfaces	1- GuideBasic Hygiene [171] 2- Hygiene principles [172] EC 852/200, Annex II Training, Chapter XII, P. 1 EC 852/200, Annex II Personal Hygiene, Chapter VIII, P.1 EC 852/200 Annex II Chapter I, P1- '*Food premises are to be kept clean and maintained in good repair and condition'*

[168] http://www.thedeerinitiative.co.uk/uploads/guides/137.pdf
[169] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_venisonSupplyTwo.pdf
[170] https://www.bestpracticeguides.org.uk/wp-content/downloads/carcass_inspectTwo.pdf
[171] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[172] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf

	Contamination between carcasses during dressing and cutting	(Díaz-Sánchez *et al.*, 2013)	Adequate space between carcases	1- Guide Carcass: supply of venison [173]- **Not fully in line with recommended corrective action** as only separation between meat and unskinned carcasses is covered 2- Venison supply (2) [174]-**Not fully in line with suggested corrective action**.
			Adequate HACCP checks to ensure no contamination takes place during carcass or meat handling	HACCP Guidance-Process Step 5- Visual check of product to agreed specifications before cutting (Food Standards Agency, 2008)
	Cross-contamination during cutting from equipment and surfaces	(Casoli *et al.*, 2005) (Ramanzin *et al.*, 2010) (Obwegeser *et al.*, 2012) Data from cattle: (Nel *et al.*, 200); (Flores, 200)	Adequate maintenance and sanitation	1- Guide-Basic Hygiene [175] 2- Guide on hygiene principles [176] EC 852/200 Annex II Chapter I, P1- '*Food premises are to be kept clean and maintained in good repair and condition'*
	Unwashed hands	(Rounds *et al.*, 2012)	Provision of sufficient washing facilities	1- Guide -Basic Hygiene[177] 2- Hygiene principles [178] EC 852/200 Annex II , Chapter I General Hygiene Requirements, P.
			Adequate supervision	1 and 2- Not specified

[173] http://www.thedeerinitiative.co.uk/uploads/guides/137.pdf
[174] http://www.thedeerinitiative.co.uk/uploads/guides/137.pdf
[175] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[176] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/
[177] http://www.thedeerinitiative.co.uk/uploads/guides/138.pdf
[178] https://www.bestpracticeguides.org.uk/carcass-preparation/hygiene-principles/

				EC 852/200 Annex II Training, Chapter 12, P. 1: supervision and instruction commensurate to the work activity
Risk of human exposure to viable STEC	Consuming raw or undercooked venison	(Obwegeser *et al.*, 2012) (Rounds *et al.*, 2012) (Ahn *et al.*, 2009)	Safe cooking at home with steak meat to be sealed and other meat to reach a core of 75°C and no pink middle	FSS guide on safe cooking of food: http://www.foodstandards.gov.scot/consumers/food-safety/at-home/cooking-food
	Cross-contamination of raw venison and ready to eat products		Adequate separation between raw and cooked food during storage and cooking Don't wash meat before cooking it	FSS guide on cross-contamination: http://www.foodstandards.gov.scot/consumers/food-safety/at-home/washing-and-preparing-food-1

1* Best practice guides | The Deer Initiative (England and Wales) - http://www.thedeerinitiative.co.uk/best_practice/
2*. SNH, ADMG, BASC, BDS, FLS, LANTRA and SGA. Best practice guidance (Scotland): http://www.bestpracticeguides.org.uk/

Appendix 11. Proportion of meat positive samples for any STEC and STEC O157 in EU Member States, 2015

Proportion of positive samples for STEC serogroups in food of animal origin in Member States and Non-Member States, 2015

Food category	Samples tested for STEC by any method	positive (any STEC)		positive for STEC O157	
	no meat samples	n	%	n	%
Bovine meat	2,560	41	1.60	5	0.24
Sheep meat	528	64	12.20	8	1.52
Other ruminants [(a)]	31	5	11.11	0	0.00
Pig meat	308	15	4.87	9	2.92
Other meat [(b)]	355	4	1.12	0	0.00
Total	**3,782**	**129**	**3.41**	**22**	**0.58**

Note: data originating from any analytical method are included.

(a): Includes only deer;

(b): Includes poultry, horse, rabbits, wild boar, meat from other animal species or not specified

Appendix 12. Data collection sheet used for all carcases swabbed at AGHE

AGHE	**Collection date**	
ID Number	**Estate address**	
Date/Time of kill	**Species, Sex**	
Body condition and body condition scoring (fatness or leanness)	Fat (1) Lean (2) Very Lean (3) Emaciated ()	
Condition of the coat and fur	Very good (1) Good (2) Coarse (3)	Visually clean (1) Visually dirty (2) Moulting (3)
Injury other than bullet wound	Type: Location: Extension:	
Disease	Abnormal colour Abscess Other (specify) Peritonitis Pleurisy Warble	
Bullet wounds	Number: Localisation: Extension damage:	
Carcass visual contamination Faecal / gut content (0-) Hair (0-3) Environmental (Y/N) Excessive blood (Y/N)	Type of contamination: Extension: Location:	
Carcass dryness	Dry Wet Slimy	
Other comments		

Appendix 13. Variables included in statistical analysis

	C/Q	Description
	C=categorical variable; Q=quantitative/continuous variable	
Response variables		
Log average coliforms/cm^2	Q	Mean of the coliforms/cm^2 from the hide, cavity and carcass
Log average *E. coli*/cm^2	Q	Mean of the *E. coli* /cm^2 from the hide, cavity and carcass
Predictor variables		
1. Distance from cull	Q	Miles from cull location to AGHE
2. Seasonality (average air temperature on day of cull)	C	Warm (>7º C); Cold (≤7º C)
3. Days in storage	Q	Time days from cull to collect
4. Time from kill to processing category 1	C	Level 1 <6 days; level 2 ≥6 days
5. Time from kill to processing category 2	C	Level 1 ≤6 days; level 2 >6 days
6. Species	C	Roe, Red
7. Sex	C	Male/Female
8. Body condition	C	1. Fat 2. Lean 3. Very lean
9. Skin condition (3 levels)	C	1. Very good 2. Good 3. Coarse
10. Skin condition (levels)	C	1. Clean 2. Dirty 3. Moulting 4. Skin removed
11. Disease, any	C	0. No disease 1. Disease, any
12. Disease, warble	C	0. No warble 1. Warble observed
13. Disease, ticks	C	0. No ticks 1. Ticks observed
14. Injury	C	0. No injury 1. Excessive bruising, fracture
15. Bullet wounds	C	1. One bullet wound 2. Two or more wounds

16. Bullet wound contamination	C	0. No contamination 1. Contamination
17. Faecal contamination	C	0. No faecal contamination 1. Less than 0.5cm^2 2. 0.5-1cm^2 3. 1–2cm^2 4. > 2cm^2
18. Hair contamination	C	1. 0–10 hairs 2. 10–15 hairs 3. > 15 hairs
19. Blood contamination	C	0. Appropriate bleeding 1. Excessive blood cavities
20. Environmental contamination	C	0. No soil or foreign bodies 1. Any of the above
21. Dryness	C	1. Dry carcass 2. Wet carcass 3. Slimy carcass
22. Dryness category wet	C	0. Dry carcass 1. Wet or slimy carcass
Random variable		
Site (AGHE)	C	Sites A to E

Appendix 14. Results of the Univariable analysis

Results of univariable analysis for Red and Roe deer for coliforms and *E. coli*. Variables in bold are those that were carried forward to the multivariable analysis italicized variables were significant (p<0.05).

	Red deer		Roe deer	
Variable	**Coliformes**	***E. coli***	**Coliformes**	***E. coli***
Distance cull AGHE	0.699	0.865	***0.0299***	**0.0913**
Season	***0.011***	0.238	0.515	***0.0173***
Site (AGHE)*	<0.001	0.7421	<0.001	0.1710
Time (days)	0.499	**0.124**	***0.007***	0.790
Time1	0.713	**0.081**	***0.004***	**0.148**
Time2	0.508	0.772	***0.014***	***0.031***
Sex	***0.0343***	0.192	0.987	**0.099**
Body condition	0.977	0.391	0.443	0.549
Skin condition 1	**0.141**	0.270	0.399	0.346
Skin condition 2	0.196	***0.030***	0.762	0.235
Conditions.any	0.166	0.890	0.985	***0.042***
Conditions.warble	**0.0804**	**0.073**	0.600	0.324
Conditions.ticks	0.653	0.377	0.211	0.324
Injury	***0.0302***	0.525	0.792	**0.151**
Number of bullet wounds	***0.010***	0.824	0.537	0.362
Bullet wound contamination	0.350	0.393	***0.070***	***0.026***
Faecal contamination	0.3596	***<0.001***	***0.011***	***0.0078***
Hair contamination	***0.0131***	0.169	**0.052**	0.176
Blood contamination	**0.0808**	0.223	**0.091**	***0.044***
Environmental contamination	0.8513	0.822	0.592	0.816
Dryness	0.186	***0.001***	0.256	***0.077***
Dryness.wet	0.827	***0.007***	**0.098**	***0.024***

*, treated as a random variable in the multivariable models

Appendix 15. Tables of final Multivariable model results

The following tables represent the data behind Figure 5.6 – Figure 5.9 in the main text. They contain the output from the average of all models with delta AIC ≤ 2 (Appendix 16 contains the number of models used for each average model).

Multivariable model outputs for average log_{10} coliforms on roe deer carcasses (Figure 5.6)

Variable	Estimate	P-value	Lower 95% CI	Upper 95% CI
Time in storage: ≥6 days	0.593	0.019	0.099	1.087
Distance to AGHE (miles)	0.009	0.009	0.002	0.016
Dryness: wet/slimy carcass	0.314	0.061	-0.051	0.642
Hair contamination: 10-15 hairs	-0.200	0.484	-0.760	0.360
Hair contamination: >15 hairs	-0.479	0.104	-1.055	0.098
Blood contamination	0.244	0.123	-0.066	0.555

Multivariable model outputs for log_{10} average E. coli in roe deer (Figure 5.7)

Variable	Estimate	P-value	Lower 95% CI	Upper 95% CI
Disease: present	0.564	0.075	-0.057	1.186
Dryness: wet/slimy carcass	0.691	0.006	0.198	1.184
Faecal contamination: <0.5cm (level 1)	-0.402	0.161	-0.962	0.159
Faecal contamination: 0.5-1cm (level 2)	0.176	0.669	-0.634	0.987
Faecal contamination: 1-2cm (level 3)	1.050	0.003	0.354	1.747
Faecal contamination: >2cm (level 4)	0.859	0.028	0.093	1.626
Season: warm	0.570	0.015	0.110	1.031
Blood contamination	0.505	0.295	-0.440	1.451
Time in storage: >6 days	0.388	0.249	-0.272	1.047
Sex: Male	0.486	0.037	0.029	0.943

Multivariable model outputs for log average coliforms on red deer carcasses (Figure 5.8)

Variable	Estimate	P-value	Lower 95% CI	Upper 95% CI
Bullet wounds: 2 or more	-0.302	0.022	-0.559	-0.044
Injury: excessive bruising/fracture	-0.189	0.092	-0.379	0.016
Sex: Male	-0.225	0.054	-0.457	0.045
Disease: Warble	-0.086	0.381	-0.121	0.089
Skin condition: good	0.167	0.131	-0.103	0.384
Skin condition: coarse	-0.028	0.822	-0.080	0.075
Season: warm (>7ºC)	0.134	0.309	-0.125	0.174
Blood contamination: excessive blood	-0.069	0.534	-0.080	0.068
Hair contamination: 10-15 hairs	0.180	0.161	-0.107	0.137
Hair contamination: >15 hairs	-0.081	0.507	-0.089	0.075

Summary of multivariable model results for log average E. coli in red deer (Figure 5.9)

Variable	Estimate	P-value	Lower 95% CI	Upper 95% CI
Dryness: wet/slimy carcass	0.311	0.123	-0.085	0.707
Faecal contamination: <0.5cm^2	-0.042	0.854	-0.490	0.405
Faecal contamination: 0.5-1cm^2	0.030	0.893	-0.404	0.463
Faecal contamination: 1-2cm^2	0.379	0.112	-0.088	0.846
Faecal contamination: >2cm^2	0.650	0.001	0.252	1.048
Skin condition: dirty	0.724	<0.001	0.394	1.054
Skin condition: moulting	-0.029	0.881	-0.413	0.354
Skin condition: skin removed	0.716	0.229	-0.452	1.885
Time in storage: ≥ 6 days	0.131	0.473	-0.226	0.487
Disease: warble fly	-0.089	0.528	-0.367	0.188

Appendix 16. Models used for model averaging

The following tables are the models with change in AIC ≤ 2 that were used in the model averaging. The columns contain the variables that appeared across all models and whether they were included (+) or not included (NA) in a specific model.

Coliforms in Roe deer :

	(Intercept)	Blood.contam	Bullet.wound.contamination	Category.time1	Distance.Cull.AGHE	Dryness.category.wet	Faecal.contam	Hair.new.cat2	Category.time1:Faecal.contam	df	logLik	AICc	delta	weight
93	4.849191	NA	NA	+	0.0088962	+	NA	+	NA	8	-79.86238	161.6271	0.0000000	0.2172467
29	4.620415	NA	NA	+	0.0083701	+	NA	NA	NA	6	-80.89734	161.9357	0.3085733	0.1861862
30	4.564705	+	NA	+	0.0092088	+	NA	NA	NA	7	-80.76375	162.0229	0.3957510	0.1782449
94	4.846590	+	NA	+	0.0094779	+	NA	+	NA	9	-79.85159	162.0468	0.4197039	0.1761228
14	4.655611	+	NA	+	0.0097409	NA	NA	NA	NA	6	-81.33523	162.6733	1.0461397	0.1287618
78	4.954857	+	NA	+	0.0099581	NA	NA	+	NA	8	-80.52043	162.9267	1.2995603	0.1134377

E. coli in Roe deer:

	(Intercept)	Blood.contam	Bullet.wound.contamination	Category.time2	Conditions.any	Distance.Cull.AGHE	Dryness.category.wet	Faecal.contam	Season.2level	Sex	Blood.contam:Dryness.category.wet	Category.time2:Faecal.contam	df	logLik	AICc	delta	weight
233	0.4404433	NA	NA	NA	+	NA	+	+	+	NA	NA	NA	10	-105.5713	227.0147	0.000000	0.2944133
225	0.5096992	NA	NA	NA	NA	NA	+	+	+	NA	NA	NA	9	-107.0056	228.1197	1.104966	0.1694404
235	0.4411925	NA	+	NA	+	NA	+	+	+	NA	NA	NA	11	-104.8303	228.4444	1.429680	0.1440477
229	0.5281416	NA	NA	+	NA	NA	+	+	+	NA	NA	NA	10	-106.2465	228.5975	1.582768	0.1334332
237	0.4630918	NA	NA	+	+	NA	+	+	+	NA	NA	NA	11	-105.2747	228.6156	1.600862	0.1322314
361	0.3639254	NA	NA	NA	+	NA	+	+	NA	+	NA	NA	10	-106.0339	228.7052	1.690527	0.1264340

Coliforms in Red deer:

	(Intercept)	Blood.contam	Bullet.wounds.cat	Disease.warble	Hair.new.cat2	Injury.other	Season.2level	Sex	Skin.condition.comment.3level	Disease.warble:Sex	df	logLik	AICc	delta	weight
83	5.787427	NA	+	NA	NA	+	NA	+	NA	NA	6	-107.3675	217.5016	0.0000000	0.21192949
67	5.767353	NA	+	NA	NA	NA	NA	+	NA	NA	5	-107.5332	218.1793	0.6777038	0.15101840
87	5.821320	NA	+	+	NA	+	NA	+	NA	NA	7	-108.4447	218.9804	1.4787904	0.10117568
211	5.749823	NA	+	NA	NA	+	NA	+	+	NA	8	-108.4705	219.0177	1.5160842	0.09930655
115	5.774849	NA	+	NA	NA	+	+	+	NA	NA	7	-108.3260	219.0214	1.5198265	0.09912090
84	5.799696	+	+	NA	NA	+	NA	+	NA	NA	7	-108.4591	219.3088	1.8072344	0.08585299
19	5.713010	NA	+	NA	NA	+	NA	NA	NA	NA	5	-107.9661	219.3317	1.8300940	0.08487730
107	5.723365	NA	+	NA	+	NA	+	+	NA	NA	8	-108.9554	219.3546	1.8529726	0.08391189
71	5.808219	NA	+	+	NA	NA	NA	+	NA	NA	6	-108.4945	219.3811	1.8794871	0.08280679

E. coli in Red deer:

	(Intercept)	Category.time1	Disease.warble	Dryness.category.wet	Faecal.contam	Skin.condition.comment.4level	Category.time1:Faecal.contam	df	logLik	AICc	delta	weight
29	1.170177	NA	NA	+	+	+	NA	11	-155.5101	319.8889	0.000000	0.3370270
30	1.076186	+	NA	+	+	+	NA	12	-155.7423	320.9374	1.048512	0.1995185
32	1.159516	+	+	+	+	+	NA	13	-155.7802	321.2265	1.337671	0.1726605
31	1.244869	NA	+	+	+	+	NA	12	-156.1428	321.3841	1.495293	0.1595754
28	1.258327	+	+	NA	+	+	NA	12	-156.3364	321.7754	1.886597	0.1312186

Appendix 17. Overall summary of variables included in the Univariable and Multivariable model analyses

U* = statistically significant variable in the univariable model analysis
M+ = Variable in the multivariable model analysis, positively influencing counts
M- = Variable in the multivariable model analysis, negatively influencing counts
Empty cells denote variables not significant in either the univariate or multivariate model.

Predictors	Roe deer		Red deer	
	Coliforms	*E. coli*	Coliforms	*E. coli*
Distance cull AGHE	U* M+			
Seasonality (warm)		U* M+		
Time. Days (cull-processing)	U*			
Category.time1	U*			
Category.time2	U* M+	U*		
Species				
Sex		U* M+	U* M-	
Body condition				
Skin condition				
Skin condition.4level				U* M+
Disease. any		U*		
Disease. warble				
Disease ticks				
Injury			U*	
Bullet wounds category			U* M-	
Bullet wound contamination		U*		
Faecal contamination	U*	U* M+		U* M+
Hair contamination			U*	
Blood Contamination			U*	
Environmental contamination				
Dryness				U*
Dryness category wet	M+	U* M+		U*
Site collected	U*		U*	