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A review of potential risk factors linked to Shiga toxin-producing *Escherichia coli* (STEC) in wild deer populations and the practices affecting the microbial contamination of wild deer carcasses with enteric bacteria

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Abstract

In modern food industry settings, pathogenic microorganisms such as Shiga-toxin producing *Escherichia coli* (STEC) cause global public health concerns. Foodborne infections with STEC are common, and sporadically linked to venison consumption. This article reviews the scientific literature on wild deer culled for human consumption, to outline the factors that influence the carriage of STEC in the intestines of wild deer species and the practices that lead to venison contamination. It discusses the potential risk factors, from culling on the hill to final packaging, linked to this pathogen. The review found important variables influencing the presence of STEC in deer carcasses and venison. Many of these were unrelated to human intervention, being generally linked to the condition of the live animal and the probability of bacterial shedding. Other factors influencing STEC presence related to the management of the environment and dressing hygiene practices, both of which can be optimised to reduce the risk of contamination. We also highlight gaps in the current understanding of the risk related to parts of the wild venison chain that could impact on the microbial quality of food products derived from wild deer carcasses. An industry approach that considers integrating the scientific evidence collated in this review into the traditional knowledge of the hill-to-fork chain would assist in adapting the businesses' food safety management system to better mitigate the possibility of STEC contamination in venison products intended for human consumption.

Keywords

Deer, wild ruminant, venison, food safety, *E. coli*

Highlights

- Warm ambient temperatures are associated with higher STEC shedding in wild deer
- High deer population density, exposure to other ruminants increase STEC shedding
- Abdominal wounding and intestinal tears increase the risk of STEC transfer to meat
- Contamination may be more difficult to avoid in larger, male wild deer carcasses
- Contamination from the terminal rectum may have greater food safety implications

Abbreviations

AGHE: approved game-handling establishment

GI: gastrointestinal

HACCP: Hazard Analysis and Critical Control Points

STEC: Shiga toxin-producing *Escherichia coli*

Stx: Shiga toxin

1. Introduction

Escherichia coli are commensals of the intestinal microbiota of healthy animals (Tenaillon, Skurnik, Picard, & Denamur, 2010). Most of these bacteria are harmless to humans (Kaper, Nataro, & Mobley, 2004); however, some *E. coli* strains, referred as Shiga toxin-producing *E coli* (STEC), are able to produce one or multiple subtypes of Shiga-like toxins (Stx), properties that influence their ability to become hazardous to humans (Buvens et al., 2012). Clinical manifestations vary from mild diarrhoea to haemorrhagic gastrointestinal disease or life-threatening haemolytic uraemic syndrome, particularly in children and older people (Thorpe, 2004; WHO, 2017).

The STEC serogroup responsible for most human cases is O157 (Majowicz et al., 2014), although in recent years, outbreaks associated with non-O157 STEC serogroups have been increasing in North America (Gould et al., 2013). Similar trends were observed in Europe following a large outbreak of *E. coli* O104:H4 (Buchholz et al., 2011), which prompted the European Food Safety Authority (EFSA) to review the surveillance systems and their ability to detect non-O157 STEC (EFSA, 2013).

The global incidence of STEC infections is estimated to be around 2.5 million acute illnesses annually (Majowicz et al., 2014), of which around 50% are attributable to food consumption, excluding water, which causes around 10% of illnesses (Hald et al., 2016). Contaminated beef is the major source of STEC transmission globally, except for South Asia, where the meat of small ruminants is the main food source (Hoffmann et al., 2017). Cattle can be colonised at the recto-anal junction without manifesting clinical disease (Naylor et al., 2003). Small ruminants and deer may also be colonised with STEC (Ferens & Hovde, 2011).

Wild deer can excrete in faeces both O157 (Díaz-Sánchez et al., 2013; García-Sánchez et al., 2007; Synge, 2006) and non-O157 STEC types, all of which are potentially pathogenic for humans (Dias, Caetano, Torres, Fonseca, & Mendo, 2019; Díaz-Sánchez et al., 2013; Franklin et al., 2013; Mora et al., 2012; Szczerba-Turek et al., 2020). The implication is that meat of colonised deer could accidentally become contaminated with STEC during slaughter or processing. The safety of wild venison, as of other meats, therefore depends, among other factors, on control of visual contamination at harvest and during production (Heuvelink, Roessink, Bosboom, & de Boer, 2001).

Wild deer meat is increasingly appreciated as an environmentally sustainable source of food (Serrano et al., 2020). While it is highly nutritious (Bureš, Bartoň, Kotrba, & Hakl, 2015), occasional contamination of wild venison with STEC has been observed (Díaz-Sánchez et al., 2012; Martin & Beutin, 2011; Miko et al., 2009). A significant risk for acquiring STEC infection is consumption of either raw, undercooked or cured/cold meat cuts (Mughini-Gras et al., 2018); thus, consumer habits may play a role. Several foodborne outbreaks have been linked to the handling of raw meat or the consumption of undercooked wild deer cuts contaminated with STEC O157:H7 (Ahn et al., 2009; Rabatsky-Ehr et al., 2002) or STEC non-O157 (Rounds et al., 2012). A more recent outbreak occurred in Scotland, and was linked to the consumption and handling of raw wild venison contaminated with *E coli* O157 (Smith-Palmer et al., 2018). Different form previously observed STEC outbreaks, which tended to be smaller and related to individually prepared deer carcasses, the Scottish outbreak was notable, with 12 patients affected, due to the implicated product being commercially produced and widely distributed via the retail chain.

The role of wild deer as a reservoir, and the recent outbreaks warrant public health concerns. Wild venison intended for human consumption is the product of hunting activities

and of the welfare necessity to control wild deer population density (Ramanzin et al., 2010). While conforming to hygiene requirements, the processing of deer carcasses undergoes different procedures than for livestock (Casoli, Duranti, Cambiotti, & Avellini, 2005). The differences in the wild game food chain, along with deer host biological characteristics, create some gaps in the current understanding of the factors that contribute to STEC contamination of wild venison. Further to this, the aforementioned Scottish outbreak also identified a lack of knowledge on the microbial risks for wild deer carcasses processed commercially and sold via the retail chain. This prompted a literature review to identify where the highest chance(s) of cross contamination might occur for wild deer carcasses and venison thereof. The objective of this scoping review was therefore to identify risk factors that could influence STEC shedding in live deer. A second aim was to evaluate the steps involved in wild deer processing to discuss the variables influencing microbial contamination with STEC and other enteric bacteria of carcasses and meat.

2. Method

We searched our institutional electronic library, which is linked to the scientific publication databases PubMed, Scopus, Web of Science, Directory of Open Access Journals, Science Direct and JSTOR. The search terms were: game, deer, wild ruminant*, wild cervid*, venison, food handling, processing, hygiene, carcass, contamination, shiga, verotoxin, STEC, VTEC, *E. coli*. The search strings were constructed with the Boolean operator 'OR' between synonymous terms representing the species, and connected with 'AND' between further key areas of the venison food chain. The first step of the literature review used a short string built with the Boolean operator 'AND' between the synonymous words of the species concerned, in combination with one other keyword added to the string with the Boolean operator 'OR'. The selected time interval was from January 2006, which was the date of the European Hygiene legislation entering into force, to January 2020. All results were reviewed by title, and where the title indicated the publication was relevant for the search, the abstract was also reviewed. Further review included the bibliographies of selected publications.
The findings were linked to the following areas of the venison food production chain: (1) the condition of live deer; (2) hunting practices; (3) transportation and extraction of the carcasses; and (4) hygiene practices during further processing of carcases and meat. Each of these factors will now be discussed in detail.

3. Literature review findings

3.1. Live deer

It is beyond the scope of this literature review to expand on aspects of the pathophysiology of deer; however, some key traits that might affect the health status of the animals will be discussed, since they could influence STEC colonisation of the intestinal tract and shedding of STEC in faeces.

The ability of deer to shed STEC in faeces is discussed from the perspective of transmission rates between animals due to increased environmental faecal contamination, as well as a possibility of carcass contamination during the slaughter process via accidental faecal contamination, although the latter is likely to be influenced more directly by levels of STEC within the intestinal tract.

3.1.1 Body condition and co-infection

In cattle, stress or the negative energy balance subsequent to lactation may result in higher STEC shedding (Venegas-Vargas et al., 2016). Wild deer, particularly red deer, are known to experience cold stress (Simpson, Webster, Smith, & Simpson, 1978). They enter a period of a metabolic slowdown in winter, mobilising most of the body fat towards the energy production needed to regulate core temperature (Turbill, Ruf, Mang, & Arnold, 2011). This is thought to have a negative impact on the optimal allocation of energy devoted to mount effective immune responses (Houston, McNamara, Barta, & Klasing, 2007), which can predispose animals to infections (Smith, 2007). In support of this concept, it was shown that wild bovids with inadequate diet, particularly reduced protein intake, have higher gastrointestinal (GI) parasite burdens (Ezenwa, 2004). Conversely, extensive research in a wild sheep population has found that animals in good body condition are able to invest in improved immune defence, as reflected by reduced GI parasite burdens and increased levels of circulating natural antibodies (Nussey et al., 2014).

Specific research looking at the impact of the host's body condition, health status and immune suppression on STEC infections in deer is lacking. However, in cattle co-infection with *Fasciola hepatica*, a parasitic trematode with the capacity to modulate the host's immune response, has been linked with increased risk of shedding STEC O157 (Howell et al., 2018). All deer species, red deer in particular, are well adapted hosts for *Fasciola hepatica* throughout mainland Europe (Alasaad et al., 2007; Arias et al., 2012) and the UK (French et al., 2016), with these infections often occurring simultaneously with other helminth infections (Albery et al., 2018; O'Toole et al., 2014). Although red deer may not express overt clinical signs of parasitism, an association has been observed between poorer body conditions and relatively low levels of GI parasites (Irvine, Corbishley, Pilkington, & Albon, 2006).

While this review could not retrieve evidence from the literature of a direct link between body condition, co-infection and STEC shedding in deer, the cumulative evidence presented here indicates that further research in this area is warranted. In accordance with the European Regulation No. 853/2004, people who hunt wild game for human consumption must acquire sufficient knowledge for an initial examination by undertaking formal training that covers normal as well as abnormal anatomy, physiology, behaviour and pathological changes in wild game due to diseases. The trained hunter plays an important role by carefully selecting for human consumption only deer that display normal anatomy, physiology and behaviour. Therefore, if the health status of deer is a risk factor for STEC shedding, it may already be well controlled by the rejection of weak, emaciated or heavily parasitised animals from the food chain.

3.1.2 Shared ecosystems

Common origin or ruminant interspecies spread of genetically similar *E. coli* O157:H7 have been initially shown by Sánchez and others (2010). The transmission of STEC between domestic and wild ruminants has further been demonstrated through the isolation of STEC with highly similar genetic profiles from both cattle and deer (Mora et al., 2012; Singh et al., 2015) as well as from sheep and deer (Sánchez et al., 2012).

Interaction between domestic and wild ruminants influences the prevalence of STEC in the respective species. Faecal shedding of STEC in deer was observed to be significantly higher in areas with an overabundance of game and domestic large ruminants (Díaz-Sánchez et al., 2013). Equally, two beef herds showed higher likelihoods of STEC carriage when in frequent contact with wild deer and other wildlife (Venegas-Vargas et al., 2016), suggesting that circulation of STEC is bidirectional between ruminant species.

STEC can survive from days to months, in various substrates such as faeces, soil and pooling water (Ogden et al., 2002). Drinking contaminated water and foraging in green spaces irrigated with untreated water can thus enable transmission (Franklin et al., 2013). It is apparent from this literature search that both wild deer and domestic ruminants act as reservoir species (Díaz-Sánchez et al., 2013), enabling the maintenance of the bacteria in environments and contributing to horizontal transmission in a shared habitat via grazing territories.

3.1.3. Wild deer population density

Similar to other species, deer being in close proximity, such as may occur due to crowding at feeding sites, has been suggested to increase horizontal transmission of STEC via the oral ingestion of food contaminated with faeces. This hypothesis is supported by the isolation of identical STEC strains from the tonsils and faeces of both the same and different deer sharing feeding spaces (Eggert et al., 2013). STEC occurrence has been linked to a population density of above 15 wild deer per square kilometre (Díaz-Sánchez et al., 2013). Higher STEC intestinal colonisation due to population density pressure is also suggested by Laaksonen and colleagues (2017), who observed lower STEC prevalence in areas with low reindeer population density. These observations are likely to reflect environmental contamination with STEC.

3.1.4 Seasonality

A positive relationship between STEC shedding and warmer ambient temperature has been observed in deer, similarly to research carried out in cattle (Henry et al., 2017; Oliver, 2014) and sheep (Evans et al., 2011). Higher STEC shedding was observed in the faeces of elk during warm summer months (Franklin et al., 2013) and white-tailed deer (Singh et al., 2015).

The association with summer season is thought to be a function of higher shedding rates, likely due to changes to the diet composition (Delgado et al., 2017) and increased pathogen proliferation in the environment at warmer ambient temperatures (Franklin et al., 2013). While these findings offer some understanding of the climate and ecology of deer species in North America, it cannot assert whether roe and red deer – the main species entering the European food chain (Schulp, Thuiller, & Verburg, 2014) – follow the same ecology. Research in this area would be beneficial.

3.1.5 Species

It has been unclear to date whether certain deer species are more likely to shed STEC. Several authors observed no significant differences in the faecal prevalence of STEC between red and roe deer (Bardiau et al., 2010; Eggert et al., 2013; Obwegeser, Stephan, Hofer, & Zweifel, 2012). Other studies have reported a higher prevalence of STEC in red deer than roe deer (Dias et al., 2019; Sánchez et al., 2009), although this lack of consistency could also be due to the low roe deer sample sizes used. A larger study detected STEC O157 in more rectal swabs from red deer than roe deer (Szczerba-Turek et al., 2020).

Obwegeser and colleagues (2012) observed that red deer isolates were more commonly encoding Shiga toxin type 2 (*Stx2*), which is generally more pathogenic than *Stx1* (Buvens et al., 2012). One of the *Stx2*-positive isolates was also positive for the *eae* gene, an important virulence factor involved in bacterial attachment to the intestinal epithelium (Brooks et al., 2005). *Stx2* genes were also found in all four isolates from red deer but only in one of the two isolates from roe deer, although these were subtype *stx2b*, commonly associated with mild human disease, and subtype *stx2g*, which has not been associated with human disease (Dias et al., 2019). Work by Szczerba-Turek and others (2020) found two STEC strains,

isolated from one roe and one red deer, that were positive for both *stx2a* and *eae*, a virulence profile associated with more severe forms of human disease (FAO, WHO, 2018). These findings suggest that both red deer and roe deer carry STEC strains potentially pathogenic to humans. The available research data are insufficient to conclude whether one of these species carries higher levels or more harmful STEC than the other. Few studies report isolates of STEC in the faeces of fallow deer (Sánchez et al., 2009; Szczerba-Turek et al., 2020), suggesting that this species carries STEC, but the limited sample sizes do not allow a meaningful comparison with red and roe deer.

3.1.6 Sex

Sex was suggested to be a factor affecting the intestinal microbiota of wild-tailed deer due to differences in the movement range and feeding behaviour between males and females. However, the study investigating this relationship showed that there was no significant difference in gut content associated with sex (Delgado et al., 2017). By comparing the sexes in red and roe deer, Eggert and colleagues (2013) observed that *Stx* genes were detected more frequently in *E. coli* isolated from the faecal samples of male red deer (8/10; 80%) than of females (13/20; 65%). In roe deer the *Stx* gene was detected more frequently in faeces collected from females (9/10; 90%) than males (7/10; 70%), but these sex differences were not significantly different (Eggert et al., 2013). Similarly, other studies have consistently failed to identify any differences in the proportion of *Stx*-positive faecal samples between male and female deer. Bardiau and colleagues (2010) found no significant difference in the proportion of *Stx*-positive faecal samples in male (9/58; 15.5%) and female (11/75; 14.7%) deer, irrespective of species, and Díaz-Sánchez and others (2013) also reported no statistical significance in *Stx* carriage between red male deer (50/120; 41.7%) and female red deer (32/67; 47.8%).

Cumulatively, these data suggest that there is little evidence that sex influences the shedding of STEC, although most studies used relatively small sample sizes; further research in this area is thus required.

3.2 Hunting practices

Hunting methods vary, depending on terrain, traditions and the deer species. The driven hunts, particularly those using dog packs, where wild deer are actively pursued, is discussed to increase ante-mortem stress, which may favour the translocation of STEC from the colonised gut to sterile organs and muscles. A similar translocation effect is thought to occur when deer are only wounded initially due to increased pain and stress (Bartels & Bülte, 2011).

In the European community, the same hygiene provisions as for domestic livestock regulate the culling of wild ungulates, namely Regulation (EC) No 852/2004, on general requirements for production of food of animal origin and Regulation (EC) No 853/2004 outlining specific hygiene rules for food of animal origin. There are additional guidelines produced by the industry on appropriate harvesting methods.

The small body of research that offers some understanding of the effects of hunting operations and primary dressing is synthesised below, highlighting the steps that appear more critical concerning the risk of STEC contamination of the deer carcass in field conditions.

3.2.1 Wounding accuracy

Recommended shooting procedures aim to achieve a rapid death, minimising the suffering of deer, which in turn facilitates the easy retrieval of game (Casoli et al., 2005; Laaksonen & Paulsen, 2015).

Abdominal shots are discouraged (Gill, 2007; Ramanzin et al., 2010) due to welfare concerns and a significant risk of carcass contamination. Shooting to any position posterior to the sixth rib will, in most cases, cause visible contamination of the carcass (Urquhart & McKendrick, 2006), jeopardising hygiene through the spread of the endogenous gut microflora, including potentially pathogenic microorganisms such as STEC (Bartels & Bülte, 2011).

It is generally accepted that the muscles and deep tissues of healthy animals are sterile (Gill & Penney, 1977). It is also thought that the bullet might become a fomite, introducing pathogens from the hide (Paulsen, 2011), or from the gastrointestinal tract if the shooting lacerates the intestines (Gill, 2007).

Deer carcasses with abdominal wounds were shown to have 0.2–0.6 cfu/cm^2 higher Enterobacteriaceae counts than those with no abdominal wounds (Atanassova, Apelt, Reich, & Klein, 2008). On assessing the microflora of over 300 wild deer carcasses, Obwegeser and colleagues (2012) found Enterobacteriaceae in 90% of samples. The authors suggested these results were partly linked to the difficulty of hygienic evisceration in the field, especially if the shooting affected the integrity of the gastrointestinal tract. Abdominal shooting was also recently shown to be statistically associated with high *E. coli* counts in moose and white-tailed deer (Sauvala et al., 2019). As *E. coli* and STEC are part of the Enterobacteriaceae family (Paton & Paton, 1998), these observations offer indirect evidence that STEC might contaminate deer carcasses via abdominal wounding. In a STEC outbreak linked to venison consumption, abdominal shot was thought to be the cause of wild venison contamination (Rabatsky-Ehr et al., 2002b), which further supports evidence of STEC risk.

3.2.2 Evisceration

For food safety purposes, the evisceration of deer, or gralloching, is carried out at the place of cull, with the aim of removing viscera that may cause contamination and deterioration if left within the carcass, particularly if the gastrointestinal tract was damaged, and with the scope of lowering the carcass temperature.

3.2.2.1 Delayed evisceration

Several publications describe delayed evisceration as an opportunity for increased carcass microbiological contamination (Casoli et al., 2005; Gill, 2007). One of the concerns is that digestive flora will continue to ferment, resulting in a bloated digestive tract and an increased possibility of faecal contamination during evisceration, through inadvertent puncture of the intestinal tract.

The observations of Atanassova and colleagues (2008) suggest that the evisceration of “expertly shot” deer within 90 minutes results in carcasses of superior microbiological quality. The European Hygiene Regulation (EC) No 853/2004 requires wild large game to be eviscerated “as soon as possible”, enabling member countries to establish an appropriate timeframe. Other intervals recommended for hygienic evisceration include a maximum of two hours after shooting (Deutz & Fötschl, 2014), or no longer than one hour (Laaksonen & Paulsen, 2015). The outdoor temperature during the culling process is important since mild or warm environments are likely to speed up microbial multiplication, necessitating prompt evisceration.

One of the concerns around delayed evisceration is that intestinal bacteria may penetrate the gut lining and contaminate muscle tissue after a few hours from killing (Ramanzin et al.,

2010). This concern might not be substantiated, as suggested by the study of Gill and colleagues (1978), which showed bacteria do not leave the intestinal lumen until there is a form of tissue disruption or breakdown produced by proteolytic enzymes, which occurs at least 24 hours post-mortem.

3.2.2.2 Gastrointestinal perforation

Microbiological testing of wild deer carcasses (Avagnina et al., 2012; Obwegeser et al., 2012) suggests that handling practices during harvesting play an important role in determining initial enteric microbial counts, including of *E. coli*, on carcasses, impacting on the safety of meat, a concept also reinforced by Gill (2007), and Ramanzin and colleagues (2010). The microbial contamination was significantly associated with improper evisceration procedures (Avagnina et al., 2012) that lead to faecal contamination (Obwegeser et al., 2012). Based on personal observations of multiple gralloching techniques, it can be seen that spillage of gastrointestinal contents can also occur, due to hunter-independent reasons, such as distended forestomach with excessive food content or juvenile deer having tissues that tear more easily.

In deer, *E. coli* are primarily found in the microbiota of the colon (Li et al., 2014). It is not yet known whether STEC O157 in deer, similar to cattle, mainly colonise the mucosal epithelium at the recto-anal junction (Naylor et al., 2003) and whether this is associated with high ($>10^3$ CFU/g faeces) shedding of STEC O157, as is seen in cattle (Chase-Topping, Gally, Low, Matthews, & Woolhouse, 2008). However, if colonisation of STEC O157 is similar between deer and cattle, it would be expected that the main risk of contamination came from spillage of the colon or rectal contents during gralloching. Thus, great care should be taken to minimise contamination of the carcass from gut contents arising from the lower intestinal tract.

To rectify contamination, some hunters are committed to old traditions such as washing carcasses with water from streams or canals (Avagnina et al., 2012). However, these practices might lead to further contamination spread on the carcass (Anderson, Marshall, & Dickson, 1991), or the water can become an additional source of nonvisual contamination, if polluted with pathogens from local deer or other ruminants (Probert, Miller, & Ledin, 2017).

According to European Hygiene Regulation 853/2004, the carcasses of wild ungulates intended to be marketed for human consumption must be subjected to an initial examination by a person who has undergone training with regards to hygiene techniques applicable to handling, transportation, evisceration as well as training to recognise environmental contamination, and other factors that might affect human health after consumption. To this extent the trained hunter can ascertain through the provision of a ‘hunter declaration’ which accompanies the carcass of wild ungulate, that it does not present environmental contamination or abnormalities that might affect human health after consumption. Article 5 of Regulation (EC) 852/2004 on the hygiene of foodstuffs requires that food business operators of the AGHEs must identify and control food hazards, including pathogenic bacteria that might originate from faecal contamination, through the implementation of Hazard Analysis and Critical Control System (HACCP). Therefore, any light contamination with gastrointestinal contents must be removed by prompt trimming of the contaminated area with a clean knife, making sure that a wide margin of uncontaminated tissues is also removed.. The principles of the HACCP dictate that wild deer carcasses extensively contaminated with gastrointestinal contents must be rejected for human consumption (Food Standards Agency, 2008).

3.3 Extraction and transport along the food chain

Due to the terrain where wild deer thrive, it is not always possible to immediately load the eviscerated carcasses into a vehicle. In Europe, smaller roe deer can be dragged into a "drag bag", or carried in a "roe sac". Larger, heavier deer are extracted either by manual dragging, or, in rare instances, in countries such as Scotland, by loading them onto a pony.

3.3.1 The influence of extraction practices

To the extent of this literature search, there were no specific risk factors linked to STEC contamination during extraction and transport. However, dragging of freshly gralloched carcasses could present a risk of contamination with environmental materials such as soil and vegetation (Laaksonen & Paulsen, 2015). Consequently, if the environment contains ruminant faeces, dragging could result in STEC contamination.

Stacking of deer carcasses is prohibited by the European Hygiene Regulation 853/2004, given that this practice has an insulating effect, particularly for carcasses in the middle of the heap. Equally, this practice promotes cross-contamination during transport. Vehicles should therefore enable hygienic extraction such that wild deer carcasses do not touch and air can freely circulate to enable cooling (Bekker, Hoffman, & Jooste, 2011).

3.3.2 Temperature controls

In wild game settings, active chilling begins at the collection centre or approved game-handling establishment (AGHE). The time elapsed from hunting to chilling largely depends on the remoteness of the hunting place and its proximity to the collection centre or AGHE. Hygiene Regulation (EC) No. 853/2004 states: "where climatic conditions so permit, active chilling is not necessary". When the deer-hunting season coincides with colder ambient temperatures, below the recommended chilling temperature for red meat (7℃), the effect of the outdoors is less concerning. In turn, it was observed that during summer months, ambient temperatures of 17.8±1.2℃ significantly influenced the growth of Enterobacteriaceae on the surface of game carcasses lacking visual contamination (Paulsen & Winkelmayer, 2004). Subsequent cooling of carcasses at near-freezing temperatures (e.g. 0.4ºC) prevented additional bacterial growth for four days, but the counts that accompanied the carcasses going into storage persisted (Paulsen, 2011; Paulsen & Winkelmayer, 2004). Therefore, a key hygiene factor is establishing a cold chain as soon as practicably possible to maintain lower microbiological counts. Once established, an uninterrupted cold chain must be kept to prevent bacterial growth (EFSA, 2014).

Countries such as Austria have developed procedures where chilling should start a maximum 12 hours from culling (Paulsen, 2011). In South Africa, the Meat Safety Act No. 40 requires partially dressed carcasses to undergo chilling within 12 hours of hunting, to a temperature not exceeding 7°C.

Another hygiene risk might arise when new carcasses are brought into the collection centre, and warm bodies are hung close to already chilled ones, particularly in facilities with insufficient ventilation, as the dampness from the warm body increases the humidity and so moisture on the surface of the chilled carcasses. An experimental study has shown that fluctuating temperatures and fluctuating water activity result in a growth response translating into an increase in *E. coli* culturable cell numbers (Mellefont, Kocharunchitt, & Ross, 2015). An alternative to avoiding moisture on carcasses is to combine a short pre-cooling phase, at temperatures of around 10°C, with the use of professional cooling facilities, which enable effective ventilation and dehumidification (Paulsen, 2011).

Cumulatively, this information suggests that contamination of wild game carcasses with enteric bacteria is more likely to occur in summer, which is also the season associated with increased STEC faecal shedding (Franklin et al., 2013; Singh et al., 2015). Stricter hygiene and faster cooling should thus be sought to prevent contamination.

3.4 Hygiene practices along the food chain

The procedures applicable to venison differ depending on whether the carcasses are intended for higher-throughput facilities or are sold on the small-throughput, local market, which is exempt from the European Hygiene Regulations, allowing the resulting small quantities of wild deer carcasses and venison to be sold locally without passing through AGHEs (FSA & FSS, 2018). In line with the aims of this review, this section refers to the wild deer food chain that falls under the European legislation since this represents the wild game meat sold widely for human consumption via large retailers or the export market.

3.4.1 Length of time for primary storage of carcasses

The storage of skin-on carcasses is an accepted practice, as part of the maturation and the development of characteristic game flavour (Soriano et al., 2016). The time in storage of unskinned carcasses may vary. While storage intervals of 1–4 days for maturation purposes have been described (Soriano et al., 2016), up to 20-day intervals have also been observed (Gill, 2007; Laaksonen & Paulsen, 2015). Recently, Sauvala and colleagues (2019) showed that the storage time (0–9 days) was statistically associated with *E. coli* counts on white tailed deer carcasses.

It is generally accepted that the muscle surface remains protected by the skin, if stored hygienically, but it should be considered that STEC are able to withstand chilling (Mann & Brashears, 2006). Evidence from studies of *E. coli* on beef carcasses suggests that the reductions of *E. coli* growth following effective chilling conditions can be temporary and that after around 40 hours, bacterial growth can start to increase as the bacteria adapt to the new environmental conditions (King, Kocharunchitt, Gobius, Bowman, & Ross, 2016; Mellefont et al., 2015). Therefore, it is expected that some STEC will survive during storage, more so if not all the contamination is rectified by generous trimming on arrival and if there are breaches in the cold chain.

3.4.2 Skinning

Removal of the skin occurs at the AGHE before the carcass undergoes further trimming and final veterinary inspection. Skinning has been discussed repeatedly as an important step in ensuring the hygiene of wild game carcasses (Atanassova et al., 2008; Avagnina et al., 2012; Casoli et al., 2005; Gill, 2007; van Schalkwyk, Hoffman, & Laubscher, 2011). Some evidence that skinning can result in *E. coli* contamination of game carcasses comes from the study of Gouws and colleagues (2017). Swabs taken from the incision line immediately after evisceration were negative for *E. coli* but, following skinning, up to 42% of carcasses displayed *E. coli* contamination, suggesting that improper execution of the skinning can result in carcass contamination with *E. coli.*

In cattle, a number of studies have examined the influence of visual cleanliness on carcass microbial load, including Enterobacteriaceae, generic *E. coli* and STEC O157 (Antic et al., 2010; Blagojevic, Antic, Ducic, & Buncic, 2011, 2012). Using a four-category visual cleanliness scoring scheme, based on an adaptation of the UK Clean Livestock Policy, Blagojevic and colleagues (2012) showed that category 4 (very dirty) cattle presented significantly higher Enterobacteriaceae compared with cleaner categories (1–3), and a greater proportion of the category 4 carcasses were *E. coli* O157-positive. The observations on slaughtering of cattle suggest that soiling or dry debris on the hide increase the risk of presence of STEC on the carcass (Blagojevic et al., 2012).

Another study exploring the ability of contaminated cattle hides to transfer bacteria to meat *in vivo* found hide-to-meat transmission of generic *E. coli* occurred in 10% of contacts,

suggesting the transfer of about 1% of the total *E. coli* population on the hide to the meat (Antic et al., 2010).

Specific procedures that have been described as influencing the transfer of pathogens from the hide to the carcass include the pressure with which the hide is pulled during skinning (Antic et al., 2010). Sudden pressure may produce aerosols that are likely to be taken up by the freshly exposed, moist meat. A compounding problem is that deer skins are removed from cold, set carcasses, which makes the process more forceful compared with working on warm carcasses, due to rigor mortis and harder subcutaneous fat as a result of chilling. Depending on the hunting season, which might overlap with moulting, skin removal can lead more readily to hair contamination (Casoli et al., 2005).

3.4.3 Further dressing and processing

In other species, there has been an emphasis on the role of faecal contamination, and zero tolerance has been proposed as a tool in the control of STEC (Heuvelink et al., 2001). Considering the type of dressing that wild venison undergo, it might not be possible to achieve the same criteria as for livestock, yet minimal contamination should be aimed for. Microbiological analysis in game carcasses, including roe and red deer, confirms that visible faecal contamination on the body cavities was associated with significantly higher (median 0.97 $\log_{10}$ cfu/cm^2) counts of Enterobacteriaceae, compared with visually clean body cavities (Paulsen & Winkelmayer, 2004). Similarly, Paulsen (2011) reports that Enterobacteriaceae counts on visually contaminated abdominal cavities were an average of 1.6 $\log_{10}$ cfu/cm^2 higher in roe deer carcasses.

Regarding STEC, there is very limited evidence on its prevalence in deer carcasses. STEC O157 was found in the faeces of red deer (4/264, 1.5%); however, no STEC O157 was isolated from the carcasses sampled (Díaz-Sánchez et al., 2013). In contrast, this same study found a higher faecal prevalence of non-O157 STEC (89/264, 34%) and a correspondingly higher carcass prevalence of non-O157 STEC (19/271, 7%). The authors concluded that the higher detection rate of non-O157 STEC on carcasses was a result of faecal cross-contamination during carcass processing (Díaz-Sánchez et al., 2013). The mechanisms of meat contamination during processing operations are similar for enteric pathogens in all meat animal species (Nørrung & Buncic, 2008). Once operatives' hands, the processing environment or the equipment become contaminated, this can contribute to the spread of enteric pathogens, including STEC, during storage, boning or processing.

To the authors' knowledge, no studies have assessed in detail the hygiene practices during venison dressing and cutting. Work in Switzerland identified that the processing establishment was associated with Enterobacteriaceae contamination (Obwegeser et al., 2012). A Finnish study confirmed that Enterobacteriaceae on the carcasses were associated with the smaller facilities having only one room, although this was not statistically significant for *E. coli* (Sauvala et al., 2019). Sauvala and colleagues (2019) also found that male wild deer carcases were significantly more often contaminated with *E. coli* than female carcasses, which could be a consequence of the more difficult hygiene handling of male carcasses, which are typically larger and heavier than females. In cattle, higher *E. coli* counts have been interpreted as possibly resulting from cross-contamination of meat cuts by meat handlers (Nel, Lues, Buys, & Venter, 2004). Increased handling of the beef product, particularly if gloves are not worn or are made of fabric (Gill & Jones, 2002) may be a contamination risk. An additional food safety concern is that naturally occurring pathogens such as *E. coli* can persist on contact surfaces even after routine sanitation, particularly on surfaces that are more difficult to access for debris removal and are less exposed to air drying (Yang, Wang, He, & Tran, 2017).

4. Conclusions

This review highlights a complex journey between the hill source and the consumer's plate, where food safety risks may exist at every step. The risk factors for STEC contamination in venison are directly linked to the ability of the live animal to harbour these pathogens and, further, to multifactorial steps related to dressing practices in field conditions, handling of the venison during extraction, chilling and further cutting, as well as to the environment through which wild deer carcasses pass. The available evidence suggests that STEC risk can be most efficiently managed through interventions applied at the primary production combined with later optimisation of the dressing and butchering hygiene. To efficiently control foodborne pathogens such as STEC, a robust, continuous hill-to-fork food safety management system should be in place. The risk consideration should not stop at the hygiene possibilities along the food chain covered by this work, but should ultimately consider consumers' attitudes and behaviours, which were not included in this review.

Although delivering pathogen-free carcasses to AGHEs might not be a realistic target under the wild conditions of deer hunting, there are many factors that could be managed to deliver minimally contaminated game to the game processors. The findings in the literature reinforce the importance of accurate shooting, prompt evisceration, generous trimming of lightly contaminated parts if contamination accidentally occurs, avoidance of excessive handling, strict adherence to the cold chain, fast processing of venison carcasses and clean and dry processing environments to control the occurrence of STEC during culling and processing.

Gaps identified in the literature include the lack of information on the role of extraction practices on the microbiology of the carcasses. Further gaps result from a scarcity of data regarding STEC carriage on deer carcasses, which would have offered a better baseline understanding of the risks related to handling procedures influencing the STEC contamination specifically. The great majority of the studies retrieved assessed the microbial condition of deer carcases by looking at other enteric bacteria, and focused more on the impact of primary production in field conditions, thus offering less opportunity for discussion of the factors linked to good manufacturing practices at collection centres and AGHEs.

In cattle, several studies have shown that the condition of the hide influences the condition of the carcass. There are no studies to date, however, reporting the prevalence of STEC on deer skins, and fieldwork in this area would be necessary to better understand whether skinning processes have a role in contaminating deer carcasses with STEC. Following up from these findings, a holistic research study that considers the risk of STEC contamination from the primary source, and examines the handling practices along the food chain, from hill to fork, would promote a better understanding of which of the above factors have a significant impact on the STEC contamination of wild deer carcasses. These risk factors should be considered in local circumstances, as applicable to the ecology and the species of deer that are harvested for human consumption. This would assist the research community and venison sector to work towards adapting food safety management systems to contemporary changes, and improving consumer protection.

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