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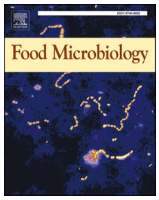

The microbial condition of Scottish wild deer carcasses collected for human consumption and the hygiene risk factors associated with *Escherichia coli* and total coliforms contamination

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ABSTRACT

Wild deer hunting is necessary in Scotland to control deer population density, with most carcasses being processed for human consumption. As limited information is available on the microbial condition of Scottish venison, we studied the variation of total coliforms and *Escherichia coli* (*E. coli*) on 214 wild deer carcasses collected from six approved establishments. Samples were collected from the hide, body cavity and external surface of each carcass and mean values were determined following bacterial plate counts. The mean $\log_{10}/cm^2$ coliforms were 5.78 (hide), 6.80 (body cavity) and 6.36 (external surface). The mean $\log_{10}/cm^2$ *E. coli* were 1.82 (hide), 2.27 (body cavity) and 2.17 (external carcass). Significantly higher coliforms counts were associated with storage-to-dressing times above 6 days and with longer transport distances. Risk factors that increased *E. coli* were red deer species, ambient temperature above 7 °C during hunting, dirty hides, faecal contamination and moisture or slimy film on the carcass. Although the bacterial counts obtained in this study indicated some hygienic processing, for around half of the carcasses, the *E. coli* counts were above 2 $\log_{10}/cm^2$. Therefore, the above risk factors suggest a few handling hygiene practices that should be further improved to enhance quality and safety.

1. Introduction

The nature of the Scottish ecosystem, particularly the lack of predators, makes wild deer culling necessary to maintain a healthy deer population at a size that ensures sufficient grazing is available for the deer and other animals, without causing land damage (Deer Working Group, 2020). In the last decade, over 100,000 deer were culled each year and over 50% of these were red deer. For instance, between 2017 and 2018, a total of 135,769 deer were culled, 58.62% being red deer, 30.10% roe deer and a small number of sika deer (5.82%) and fallow deer (2.44%) (The Deer Working Group, 2020). The vast majority of the carcasses from hunts are processed for the human food chain, supplying local farm shops, butcher shops and large retailers as well as the export market. The consumption of large wild game meat is increasing in Scotland, according to market analyst reports that showed venison sales increased throughout 2019 and the trend was expected to continue (Kantar Market research, 2019). The sales and consumption might also be supported by a recent local campaign endorsed by official bodies to promote Scottish venison for its nutritional qualities as well as the benefits of being sourced locally (Forestry and Land Scotland, 2021).

Wild deer can naturally and asymptomatically carry foodborne bacteria that can adversely affect the health of humans. Pathogenic bacteria colonise the intestinal tract of the animal or the hide/skin, and contamination from these sources can transfer to the venison product through unhygienic handling of the carcass (Gill, 2007). These organisms can subsequently affect humans through the handling of raw meat and/or consumption of undercooked meat and meat products.

The harvesting and primary processing, including bleeding and evisceration, of wild deer occur outdoors. As a result, there are multiple opportunities for microbial contamination to occur, including meat spoilage or contamination with pathogenic bacteria (Casoli et al., 2005; Ramanzin et al., 2010; Soare et al., 2021). During spoilage, bacteria

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result in undesirable changes in the fresh meat such as change of colour, odour and surface texture (i.e. slimy) (Ingram and Dainty, 1971) that are observable and can be corrected by either rejection or trimming. However, contamination with pathogenic bacteria may not have any visible signs, leading to more concerning foodborne infections in humans. The food safety risks would be low if the product were correctly handled and thoroughly cooked, although the risk from staphylococcal and clostridia enterotoxins associated with cooked meats (Jay et al., 2005) could persist. In the past 25 years, the peer review literature has reported six foodborne outbreaks that could be associated with *shiga toxin-producing Escherichia coli (STEC)* (Ahn et al., 2009; Keene et al., 1997; Ladd-Wilson et al., 2021; Rabatsky-Ehr et al., 2002; Rounds et al., 2012; Smith--Palmer et al., 2018) following consumption of venison that had been mishandled and/or undercooked. Typically, these outbreaks were in family clusters and concerned home preparation of the deer meat. However, in 2015, a larger outbreak occurred in Scotland, involving 12 people from different households who consumed venison processed by an approved game handling establishment (AGHE) and commercialised through the retail chain (Browning et al., 2016; Smith-Palmer et al., 2018). The definition of an AGHE, outlined in Annex 1 of HygieneRegulation (EC) No 853/2004, n.d., is an establishment approved by the Food Safety Authority in which game and game meat obtained after hunting are prepared and health marked for placing on the market.

Investigation of the Scottish outbreak by the authorities highlighted a number of knowledge gaps related to venison processing (Smith--Palmer et al., 2018), including a lack of information on what practices and other environmental factors attracted the greatest risks of microbial contamination, in the particular settings of the local sector. This led the official bodies, Food Standards Scotland and the Scottish Government, to commission a wider study to add to the evidence base on STEC contamination of wild venison (Food Standards Scotland, 2020). One of the research objectives, carried separately from the current study, was to determine the prevalence of STEC in the faeces of wild deer as a direct measurement of the risk that deer intended for the food chain might carry. The current study was undertaken to complement the aforementioned objective, to assess the contamination risk with index microorganisms, total coliforms and *E. coli* whilst deer were processed at AGHEs.

The aim of this study was twofold. First, to determine the microbiological condition of wild deer carcasses at the time of processing for human consumption by detecting total coliforms, as indicators of both environmental and faecal contamination (National Research Council (US) Subcommittee on Microbiological Criteria., 1985). *E. coli* were used as indicators of faecal contamination (Gill et al., 1996; McEvoy et al., 2004; Sauvala et al., 2019). Second, the study aimed to determine the risk factors associated with the presence of coliforms and *E. coli* counts, to enhance the understanding of some of the hygienic procedures that might be important to improve the microbial condition during the processing stages, when storing, handling and dressing wild deer carcasses.

2. Materials and methods

2.1. Sampling

A total of 214 wild deer carcasses were sampled between October 2017 and April 2018. The samples were collected from six AGHEs, representing 35% of all processors in Scotland. Scotland was divided into four regions: North (Outer Hebrides and the Highlands), North East (Moray & Aberdeenshire, Perth, Kinross, Angus & Dundee), Central (Argyll and Bute, Stirling & Falkirk and Fife) and the South (Glasgow & Lanarkshire, Edinburgh & the Lothians, Ayrshire, Scottish Borders and Dumfries & Galloway).

The sampling took place from at least one operator in each of these regions who agreed to take part in the study. A total of six AGHEs took part – processors A and B in the North of Scotland, C in the North East, D in the Central area and E and F in the South of Scotland. A minimum of 50 carcasses were collected per region, representing all areas of Scotland from which wild deer are culled for human consumption. Of these carcasses, 132 were red deer (105 females and 27 males), 79 roe deer (51 females and 28 males) and 3 sika deer (3 females and 0 males).

Sampling took place on the day that deer were skinned and dressed, in preparation for veterinary inspection, as described in Fig. 1. After being hunted and eviscerated in the field, the carcasses were transported, either directly to the AGHE where they were stored, skin-on, refrigerated in the reception chiller, or to a refrigerated collection larder and later transferred to the AGHE. Therefore, the kill-to-sampling interval varied, ranging between 1 and 19 days, with a median of 6 days.

Samples were collected following the principles described in the standard ISO17604 for the collection of hygiene samples from red meat carcases by non-destructive method, as advised by Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. The sample surface area covered 750 cm^2 for red and sika deer carcasses and 500 cm^2 for roe deer carcasses.

The sampling was carried out with 5 × 10 cm 'TS/15-B: NaCl – sterile carcass hygiene blue sponge', provided in sterile 'Easy Open Stomacher Pouches' (Technical Service Consultants Ltd.) and came pre-moistened with 0.9% sterile saline. The areas sampled were measured with a 100 cm^2 'TS/15-T40 – sterile plastic sampling template' (Technical Service Consultants Ltd.) The technique involved holding the sterile sponge through the bag after folding the bag back over the hand to avoid contamination of the sponge, diluent, or the internal surface of the bag. The sponge was wiped with firm pressure and a slight side-to-side movement, covering the set body surface, after which the bag was refolded over the sponge and secured with a closure.

Three sample types were taken from each carcass, swabbing the same location each time between carcasses, as shown in Fig. 2. One sample was collected from the skin/hide of the animal (5 equal surfaces of 150 cm^2 for red deer and 100 cm^2 for roe deer taken from the neck, both sides of flank and rump). The second sample was collected from the cavities (5 equal surfaces from both sides of the thorax, both sides of the abdomen and from the pelvis floor). The third sample was taken from the external surface of the carcass, subsequent to skinning and dressing (5 equal surfaces from the neck, and both sides of flank and rump).

Each of the sample bags containing swabs received a sample number that corresponded to the tag number, as shown on the carcass label. The sample bags were also annotated with details of the sample type ('hide', 'cavity', 'carcass'), the AGHE where it was collected from, and the date and time of collection. Additional data were collected from the hunters' declarations, as per 'Sample and collection data sheet' provided in Appendix A (supplementary data).

2.2. Laboratory methods

All samples were transported with ice packs in insulated shipping boxes to the microbiology laboratory on the same day of collection.

On receipt at the microbiology laboratory, all samples were stored at 4 °C in the dark and processed within 48 h from collection. To detect and enumerate total numbers of *E. coli* and coliforms, the medium chromogenic agar (Merck) was used to capture the total number of viable *E. coli* and related coliforms in the sample. A volume of 10 ml 0.9% (w/v) NaCl was added in the sample bags to suspend samples thoroughly. The plating involved spreading 0.1 ml of 10-fold dilutions of sample in 0.9% (w/v) NaCl onto chromogenic medium (Sigma#81938 or Merck#110426). The plates were incubated at 37 °C overnight. Subsequently, the total colonies eliciting the salmon to red colouration for coliforms and dark blue to violet coloured colonies for *E.coli* as described by Turner et al. (2000) were counted at the highest dilution where *E. coli* or coliform colonies developed.

The microbiology laboratory provided the results expressed in counts and dilutions for each sample type, as per the traceability number annotated on the sample bag. The enumeration method had a limit of detection of <1 colony-forming unit (cfu) per 7.5 cm^2 and <1 cfu per 5

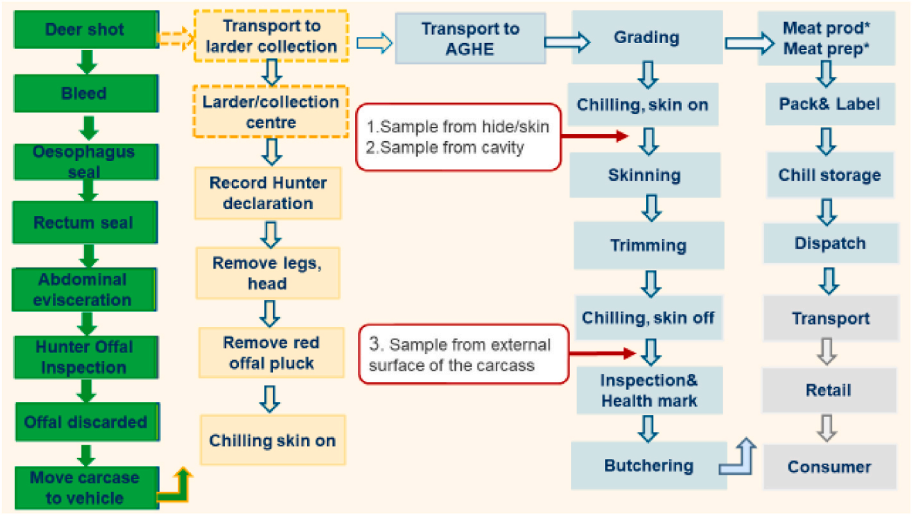

Fig. 1. Flow diagram displaying wild deer meat production from the hill to consumer and the sampling points within the process. The dot-dashed tabs are steps that might not be involved in wild deer meat production as some of the carcasses are transported directly to AGHEs and therefore the subsequent steps displayed in orange-yellow also occur at the AGHE, before grading. * Meat prod = meat products; meat prep = meat preparations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

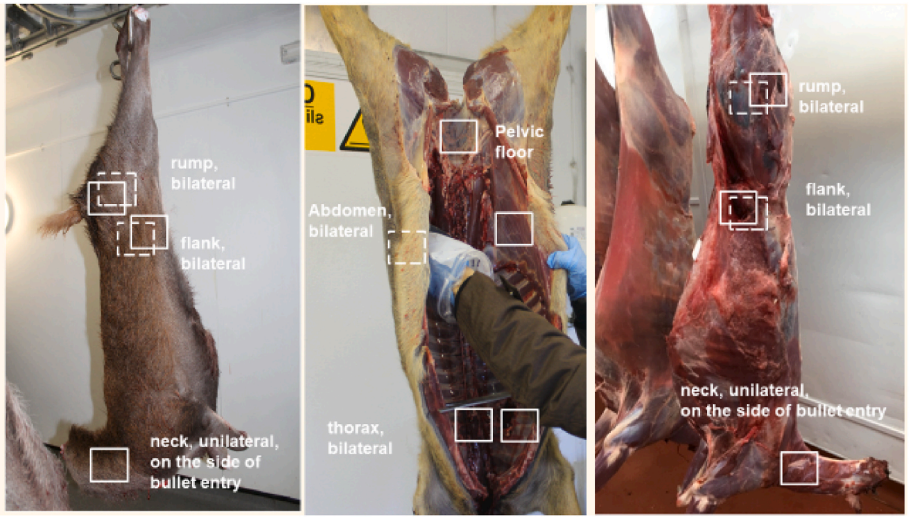

Fig. 2. Carcass swabbing locations; left depicting hide samples, middle depicting cavity samples and right depicting external surface of the carcass. The dashed squares show the sampling location for the other side of the carcass, not visible in the photo.

cm^2 sampled area for red and roe deer respectively. These values were converted into cfu per cm^2 of area sampled and transformed in log_{10}/cm^2 values. When counts were below the detection limit, a value of zero was assigned.

2.3. Database management and statistical analysis

2.3.1. Database management

All information shown in Appendix A (supplementary data) was collected on paper during the visit to the AGHE for each sample. This information and the results from the microbiology laboratory were later transferred into a data spreadsheet in Excel (Microsoft US). Data on coliform and *E. coli* cfu were provided by the lab in counts and dilution per each sample type. These values were also recorded on the data spreadsheet in Excel to allow calculation of the number of coliforms and *E. coli* per square centimetre (cm^2), which was then transformed into logarithm$_{10}$ (log_{10}/cm^2). Due to a processor misunderstanding of the logistics arrangements of sampling, 17 carcasses were skinned and the hide discarded before the sampling officer (first author) arrived on site; therefore only cavity and carcass samples were collected for 17 animals. A further carcass was rejected during veterinary inspection due to severe *Hypoderma diana* infestation. As a result, a complete set of data was available for only 197 carcasses.

For the statistical analysis of the risk factors, the predictor variables were selected following a systematic literature review carried out preliminary to the field study (Soare et al., 2021). A total of 24 predictors were considered, as described in Appendix B (supplementary data). Broadly, the predictors concerned the distance the carcasses were transported, the ambient temperature, the sex and breed of the animal and the condition of the carcass with regard to contamination, disease status and number of bullet wounds. The necessary information for these predictors was extracted for each carcass at the time of swabbing and annotated on the collection sheet depicted in Appendix A (supplementary data). As a result of the low sample size of Sika deer (n = 3), this species was removed from the risk factor analysis. Further variables were also considered at the time of statistical analysis, i.e. the 17 carcasses with missing skin were included in risk factor analysis.

2.3.2. Descriptive statistics

Descriptive statistics were carried out on the microbiological data using Minitab statistical software (version 20.2). Bacterial counts for the three sample types (hide, cavity and carcass) for both coliforms and *E. coli* were compared using a linear mixed model (LMM) with animal added as the random effect to account for samples from the same animal.

Bacterial counts for the three sample types (hide, cavity and carcass) for both coliforms and *E. coli* were significantly correlated (Appendix C, supplementary data). As a result, a principal component analysis (PCA) was carried out to reduce the dimensionality of the data set, while preserving as much variability as possible in the statistical information. The results of the PCA, provided in Appendix C (supplementary data), revealed one significant component for both coliforms and *E. coli* with each sample (hide, cavity and carcass) contributing approximately equally to the total variation. As such, any formal weighting of each sample would be approximately equal. Therefore, the average of the three sample types for both *E. coli* (hide, cavity and carcass) and coliforms (hide, cavity and carcass) was calculated, $\log_{10}$ transformed and then used in all subsequent analyses.

2.3.3. Multivariable risk factor analysis

Further statistical analysis was carried using R package version 4.0.5 (R Core Team, 2021). Averaged, $\log_{10}$ transformed *E. coli* and coliform counts, as described above, were used as outcome variables. Initial univariable analysis was carried out on the full set of 24 candidate predictor variables and only variables with a p-value of <0.15 were considered candidates for the multivariable model.

Multivariable LMMs were fit for *E. coli* and coliform counts respectively using AGHE as a random effect to test the effect of the dressing practices at the AGHE on the counts. The models were fit using R package nlme (Pinheiro et al., 2022; Pinheiro and Bates, 2000). Forward variable selection was used to build the final regression models, using the Akaike information criterion (AIC) to compare the models obtained and determine the one that best fitted the data. The model with the lowest AIC value was selected as the one that best predicted the outcome variables.

Because preliminary analysis revealed significant differences between species, each time a variable was added, this was assessed for interaction with species. If the added interaction decreased the AIC, it was kept in the model. The additional interactions tested included the presentation of the carcass (dry, wet, slimy) and the time it took for the carcasses to be processed; the distance between the cull site and the AGHE and the time elapsed between cull and processing; and the body condition score and any pathological condition that the carcass might have displayed (Appendix B, supplementary data).

3. Results

A summary of the mean *E. coli* and coliform counts for each of the sample types, 'hide', 'cavity' and 'carcass', collected during the study are collated in Table 1. Data are presented for all deer as well as individual deer species. Given the normal distribution of the microbiological results, the values were interpreted based on the mean.

3.1. Microbiology results for coliform counts

Coliform bacteria were recovered from all carcasses in each one of the sample types: hide N = 197; 100%; cavity N = 214; 100% and carcass N = 213; 100%. Coliform counts were significantly different across all samples (LMM: $p < 0.001$). The highest values were obtained from the samples collected from the cavity (mean 6.80 $\log_{10}$ cfu/cm^2), significantly higher than the counts obtained from the external surface of the carcass (mean 6.36 $\log_{10}$ cfu/cm^2) and the hides (mean 5.78 $\log_{10}$ cfu/cm^2) (Fig. 3).

3.2. Microbiology results for E. coli

E. coli were isolated from all carcasses, in at least one of the sample types. *E. coli* were isolated from N = 180; 91.37% hides, N = 185; 86.44% cavities and N = 203; 96.66% external surface of the carcass. Conversely, *E. coli* were not detected in 16 (8.12%) hide samples, 28 (13.08%) cavity samples and 7 (3.28%) of carcass. In 134 (68%) wild deer carcasses, *E. coli* counts were obtained from all three sample types (hide, cavity and external carcass).

There was a significant difference in *E. coli* counts (LMM: $p < 0.001$) (Fig. 4). The highest counts were obtained from the samples collected from the cavities (mean 2.27 $\log_{10}$ cfu/cm^2), followed by counts on the

Table 1
Microbiological values, expressed in $\log_{10}$ cfu/cm^2, obtained for coliforms and *E. coli* from wild deer carcass samples.

Sample type	Species	Sample numbers	Minimum	Maximum	Mean	SD
Coliforms, hide	Red	126	3.47	7.87	5.95	0.75
	Roe	68	2.56	7.23	5.46	1.03
	Sika	3	5.23	6.43	5.99	0.66
	Red and roe	194	2.56	7.87	5.77	0.88
	All deer	197	2.56	7.78	5.78	0.88
Coliforms, cavity	Red	132	4.05	7.79	6.92	0.66
	Roe	79	4.18	7.86	6.58	0.89
	Sika	3	6.49	7.28	6.95	0.41
	Red and roe	211	4.05	7.86	6.79	0.77
	All deer	214	4.05	7.86	6.80	0.76
Coliforms, carcass	Red	131	4.03	7.81	6.49	0.64
	Roe	79	3.39	7.59	6.14	0.94
	Sika	3	6.02	6.60	6.29	0.28
	Red and roe	210	3.39	7.81	6.36	0.78
	All deer	213	3.39	7.81	6.36	0.78
E. coli, hide	Red	126	0	4.33	2.15	1.08
	Roe	68	0	3.97	1.19	1.05
	Sika	3	1.13	2.35	1.78	0.61
	Red and roe	194	0	4.33	1.82	1.16
	All deer	197	0	4.33	1.82	1.15
E. coli, cavity	Red	132	0	5.88	2.54	1.36
	Roe	79	0	6.09	1.76	1.70
	Sika	3	1.63	4.79	3.55	1.68
	Red and roe	211	0	6.09	2.25	1.54
	All deer	214	0	6.09	2.27	1.54
E. coli, carcass	Red	131	0	4.88	2.28	1.10
	Roe	79	0	4.57	1.99	1.10
	Sika	3	0.95	2.87	2.14	1.04
	Red and roe	210	0	4.88	2.17	1.10
	All deer	213	0	4.88	2.18	1.10

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.

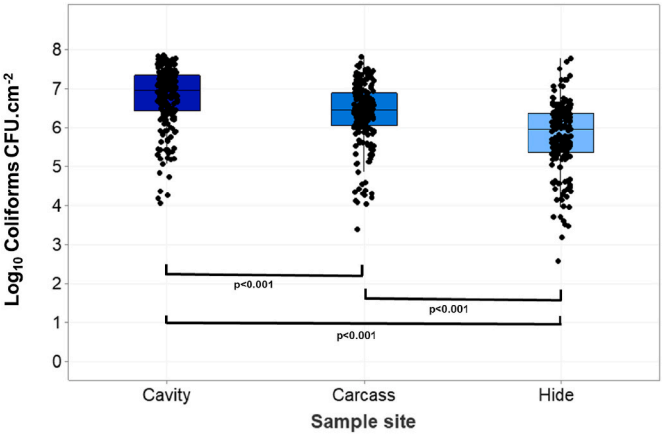

Fig. 3. Microbial variation for Log_{10} coliforms in samples collected from the hide, cavities and external surface of the deer carcasses. Data presented as decreasing mean; p-values are adjusted for multiple comparisons.

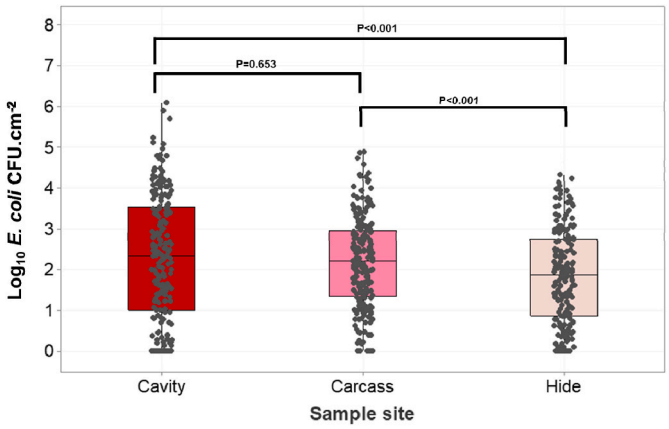

Fig. 4. Microbial variation for Log_{10} *E. coli* counts obtained from samples collected from the hide, cavities and external surface of the deer carcasses. Data presented as decreasing mean; p-values are adjusted for multiple comparisons.

external surface of the carcass (mean 2.18 log_{10} cfu/cm^2) and hides (mean 1.82 log_{10} cfu/cm^2). Differences between carcass and hide ($p < 0.001$) and cavity and hide ($p < 0.001$) were statistically significant, but there was no significant difference between carcass and cavity ($p = 0.653$).

3.3. Risk factor analysis

3.3.1. Risk factor models for coliforms

Results from screening all potential risk factors for coliforms using univariable analysis are shown in Appendix D (supplementary data). The final multivariable LMM (with mixed effects for AGHEs), is shown in Fig. 5. More time elapsed between kill and processing, and particularly storage for 6 days or more, was associated with a significant increase in coliform counts (Fig. 3). Secondly, the coliform counts were significantly higher for carcasses that were transported for distances greater than 24.7 miles and up to 111 miles. The carcasses that originated from North Scotland had significantly higher coliform counts and any animals that presented injuries such as fractures or severe bruising had lower coliform levels. The final model also indicated a significant interaction between days in storage and distance, meaning that the effect of days in storage depended on the distance travelled to the AGHE. The effect of days in storage on coliform counts is less evident if carcasses are transported only short distances to the AGHE.

3.3.2. Risk factor models for E. coli

The results of the univariable analysis, shown in Appendix D (supplementary data), and the results of the multivariable LMM, presented in Fig. 6, indicated that higher *E. coli* counts were significantly associated with red deer carcases, ambient temperature of above 7 °C during the hunting, dirty, soiled skins/hides and visible faecal contamination present on the carcasses. Additionally, carcasses that displayed any surface with moist appearance or mouldy, slimy looking areas resembling colonies had significantly higher *E. coli*. The final model also indicated a significant interaction between species and season. Even though red deer have on average a higher level of *E. coli* than roe deer, this difference is less discrepant during warmer weather, above 7 °C.

4. Discussion

4.1. Coliforms

To our knowledge, this is the first study to report coliform values for carcasses of wild or domestic deer, so it is not possible provide a direct comparison with microbiological data obtained in other studies. Because coliforms represent a large sub-population of the

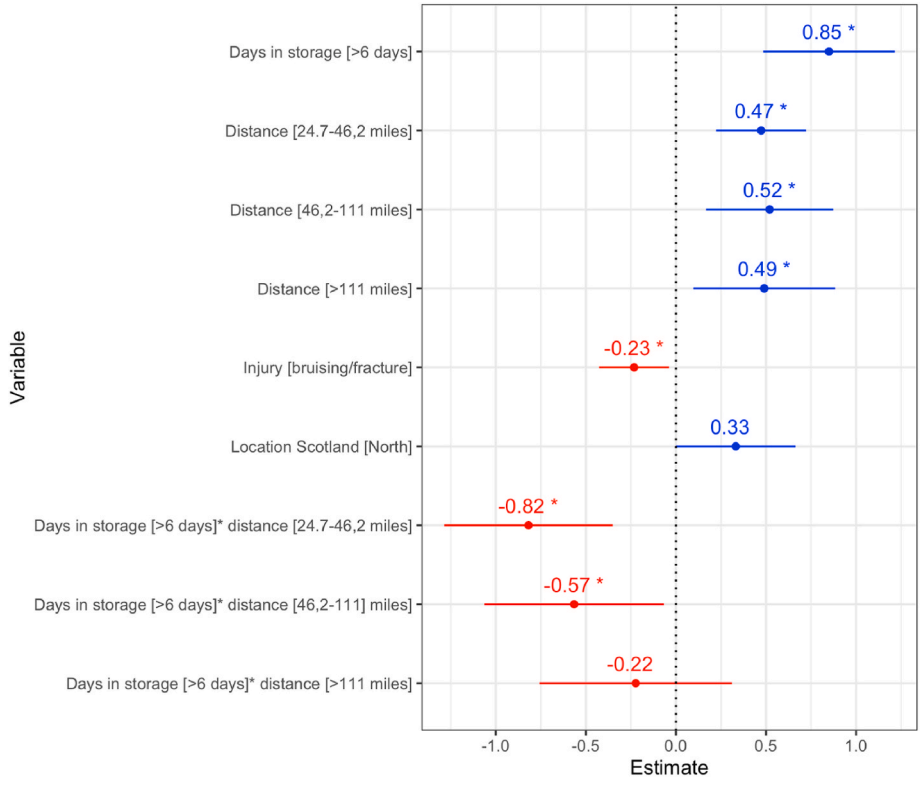

Fig. 5. Top model outputs from multivariate mixed regression analysis of risk factors associated with coliforms. The asterisk represents significant risk factors ($p < 0.05$). The red confidence intervals represent negative risk factors and the blue confidence intervals represent the positive risk factors. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

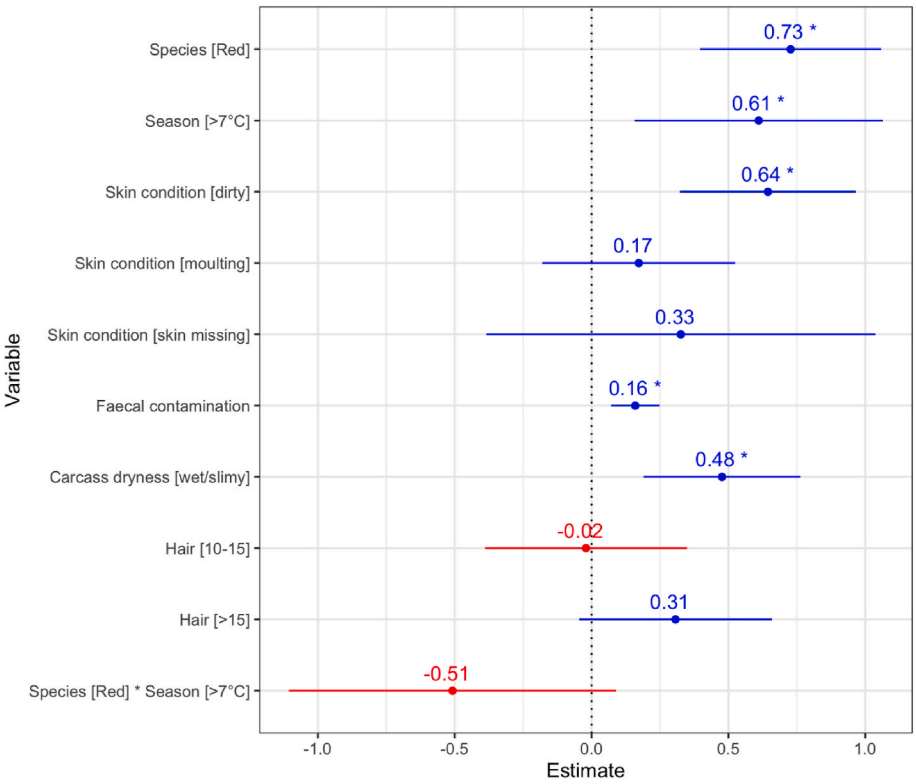

Fig. 6. Top model outputs from multivariate mixed regression analysis of risk factors associated with *E. coli*. The asterisk represents significant risk factors ($p < 0.05$). The red confidence intervals represent negative risk factors and the blue confidence intervals represent the positive risk factors. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Enterobacteriaceae family (Halkman and Halkman, 2014; Leclerc et al., 2003), it is expected that coliform counts obtained in the study should be lower or marginally lower than 2.5 $\log_{10}$ cfu/cm^2 *Enterobacteriaceae,* recommended as the maximum value for hygienic criteria on fresh carcasses by Commission Regulation (EC) No 2073/2005 and subsequent amendments. Our results, presented in Table 1, show that minimum coliform counts were higher on all cavity and external carcass samples than the upper acceptable limit for *Enterobacteriaceae* in fresh domestic ruminant carcasses.

The hygiene criteria recommended by the EU legislation apply to fresh carcasses, prior to chilling. Our samples originated from animals killed 1–19 days prior and subjected to chilling, which creates a limitation when attempting to compare with expected microbiological values on freshly killed livestock carcasses. However, a study carried out in Switzerland, which examined carcasses of wild deer 48 h after being hunted and another 72 h after arriving at the AGHE, and subjected to chilling and skin removal, reported mean *Enterobacteriaceae* counts of 2.6 $\log_{10}$ cfu/cm^2 (0–5.2) for roe deer and 2.3 $\log_{10}$ cfu/cm^2 (0–5.1) for red deer (Obwegeser et al., 2012). Our results were approximately 3.7 logs higher on the external surface of the carccasses than the enteric bacteria ranges described by Obwegeser et al. (2012) and suggest high bacterial load during later stages of processing. Although the external surface of the carcass is expected to be sterile, contamination could be introduced during the process of removing the skin/hide (Lawrie et al., 2006), and therefore the counts will reflect the care taken during this procedure and subsequent dressing steps.

Other studies assessed *Enterobacteriaceae* on freshly killed deer carcasses (1–6 h after shooting) and reported lower mean values, within the expected legal hygiene criteria for domestic ruminants (Atanassova et al., 2008; Avagnina et al., 2012), which demonstrates that low bacterial loads on freshly shot deer carcasses are achievable.

Wild deer carcasses are processed partly outdoors and transferred through several types of environment, which increases the chance of cross-contamination and might allow unintended opportunities for delays or breaks in the cold chain. It has been described previously that the extent of *Enterobacteriaceae* multiplication on wild deer carcasses can be influenced by the time to the onset of cold storage (Paulsen and Winkelmayer, 2004). Other authors found that the duration between hunting and sampling was statistically associated with *Enterobacteriaceae* (Sauvala et al., 2019) and our results also confirm that total coliforms, part of the *Enterobacteriaceae* family, were significantly higher for carcasses that were stored for longer than 6 days at the AGHEs. Although our study did not assess further parameters related to storage conditions, such as the time to onset of chilling, the temperature regimes, below 7 °C to minimise the growth of mesophilic bacteria, and coliforms (Smith, 1985) as well as air humidity, which can be between 80% and 95% (Liu et al., 2016), can influence the survival or growth of undesirable bacteria (Lawrie et al., 2006; Liu et al., 2016). Conversely, it has been demonstrated on beef carcasses that strict dry chilling regimes at 0 °C and 88% humidity result in significant reduction of coliforms after 67 h (Liu et al., 2016). The result in this study concerning higher trends of coliform bacteria on carcass surface and body cavities might thus warrant further investigation of possible causes linked to the conditions deployed during the storage interval as the factors that might contribute to survival or growth of coliforms.

The higher coliform counts obtained from cavity samples suggest that contamination could be arising during the evisceration procedures. These results could be linked to difficulty in carrying out hygienic evisceration in field conditions (Mirceta et al., 2017; Obwegeser et al., 2012). There is a possibility that bacteria from the cavity could be transferred onto the external surface of the carcass during the skinning and dressing procedures via the hands or the equipment of plant operatives (Nørrung and Buncic, 2008).

The modelling concerned the road distance for the transport of the carcasses and the location from where these originated, with both being statistically associated with higher coliform counts. Significantly lower

counts were obtained from carcasses that displayed injuries such as old calluses or fresh fractures. The data collected did not allow further investigation as to why these associations were found but given that no other study described it, it was felt important to highlight these, to inspire further review that might establish causation.

4.2. *E. coli*

A recent study tested generic *E. coli* on white tailed deer carcasses reported median values of 0.7 (range 0.1–3.3) $\log_{10}/cm^2$ (Sauvala et al., 2019). A review collating published microbiology results for wild deer meat and carcasses suggests it is possible to produce skin-on wild deer carcasses with *E. coli* not exceeding 2 $\log_{10}$ cfu/cm^2 under good handling practices (Paulsen, 2011). In our study, the deer carcasses where minimal visual contamination was observed at the time of sampling, namely no visual contamination or faecal contamination below 0.5 cm in one body part, generated *E. coli* counts at a mean value of 1.79 $\log_{10}$ cfu/cm^2 (95% CI 1.36–2.23) in cavity samples and a mean of 1.99 $\log_{10}$ cfu/cm^2 (CI 95% 1.62–2.35) in carcass samples, as per appendix E (supplementary data). As these results were below the 2 $\log_{10}$ cfu/cm^2 value recommended by Paulsen (2011), we interpreted the counts below this threshold as deriving from hygienically handled carcasses.

In the current data set, *E. coli* were below 2 $\log_{10}$ cfu/cm^2 in 108 (54.82%) hide samples, 95 (44.39%) cavity samples and 92 (43.19%) samples from the external surface of the carcass, showing overall that, for about half of the carcasses, the handling hygiene should be improved (Appendix E, supplementary data).

The results of the multivariable analysis indicated that red deer had significantly higher *E. coli* counts than roe deer. Variation in *E. coli* counts between cervid species has also been observed by Sauvala et al. (2019) and this is likely to be attributed to difficulties in hygienically handling larger deer carcasses. Although we did not assess the pathogeny of *E. coli* isolated from the carcasses, it is important to highlight that red deer, as well as being more commonly contaminated with *E. coli*, have been reported to carry Shiga toxin genes in faeces more often than roe deer. The faecal prevalence observed was 3/500 (0.6%) for red deer; 0/445 (0%) for roe deer (McNeilly et al., 2020), 31/84 (36.9%) for red deer, 25/64 (39.1%) for roe deer (Obwegeser et al., 2012), 4/97 (4.1%) for red deer and 1/134 (0.75%) for roe deer (Szczerba-Turek et al., 2020). Thus, the hygiene quality of red deer carcasses is of additional public health significance.

Warm ambient temperatures, above 7 °C, during the hunting led to significantly higher *E. coli* counts on wild deer carcasses, results that are consistent with the findings of Sauvala et al. (2019), who also found outdoor temperature during hunting to be a significant risk factor for detection of *E. coli*, stratified by deer and moose species ($p = 0.011$).

An additional risk factor was visible faecal contamination, which was associated with higher *E. coli* counts. This is an intuitive finding, given that *E. coli* is present in the faeces of livestock. Although some literature discusses the existence of variation to *E. coli* concentration present in faeces between individual ruminant animals (Oliver, 2014), the modelling performed on the current data set showed an increasing trend in the *E. coli* counts, consistent with the amount of visual faecal contamination observed on the carcass.

The hygiene of the hides/skins also had a significant impact on the *E. coli* counts, with higher counts associated with dirty skins/hides. The role of contaminated hides has been discussed in domestic ruminant species by Antic et al. (2010), who found that hide-to-meat transmission of *E. coli* occurred in 10% of contacts and estimated a transfer of about 1% of the total *E. coli* population from the hide to the meat (Antic et al., 2010). This study did not attempt to quantify the amount of contamination transferred, but the results highlight the importance of maintaining the skin/hide unsoiled and dry during the extraction from the hill.

5. Conclusions

Our sanitary assessment of the carcasses based on coliforms counts has been challenging due to limited the ability to perform a direct comparison given that other similar studies on deer have tested for *Enterobacteriaceae*. The aims of the current study were informed by local research needs, but there is scope for further data to be retrieved, by screening the preserved samples for the presence of toxigenic *E. coli* to determine their pathogenicity to humans.

The microbiological data discussed cumulatively indicate that the process hygiene should be improved to enhance the microbial condition of deer carcasses intended for the food chain. Contaminated meat, particularly with *E. coli,* increases the possibility of foodborne bacteria, posing a public health risk if the product is insufficiently cooked; this makes the hygienic handling of this type of meat a priority. Whilst associations of higher counts with species, ambient temperature during hunting, and hide hygiene are informative, other issues with transportation, dressing and storage conditions need to be revised to determine the corrective actions warranted.

This study did not test for all practices and procedures that could have contributed to high counts, so a future study concerning the hygiene conditions deployed during processing, including primary stages, would further contribute to the knowledge base for improving hygiene practices. It might also be useful for AGHE operators to assess the microbial condition of carcasses and therefore the effectiveness of the good handling practices at their premises, as part of the Hazard Analysis and Critical Control Programme. This would help to establish whether corrective actions are necessary and, if so, to determine appropriate means of reducing contamination, in line with the results found.

Baseline studies such as the current one can provide a reference to benchmarking the levels of commensal or enteric bacteria on wild deer carcasses processed in AGHEs. A further significance is that the current results could contribute to informing some of the hygiene procedures advised in the venison best practice guides.

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Appendix A. Supplementary data

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