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Microbial contamination of moose (*Alces alces*) and white-tailed deer (*Odocoileus virginianus*) carcasses harvested by hunters

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ABSTRACT

Hunting is currently a very popular activity, and interest in game meat is increasing. However, only limited research is available on the bacterial quality and safety of moose (*Alces alces*) and white-tailed deer (*Odocoileus virginianus*) harvested by hunters. Poor hunting hygiene can spread bacteria onto the carcasses, and inadequate chilling of the carcasses may increase the bacterial load on the carcass surface. We studied the bacterial contamination level on carcasses of 100 moose and 100 white-tailed deer shot in southern Finland. Hunters eviscerated carcasses in the field and skinned them in small slaughter facilities. During the sampling, same person visited 25 facilities located in 12 municipalities of four provinces. Moose carcasses had mean mesophilic aerobic bacteria (MAB), Enterobacteriaceae (EB) and *Escherichia coli* (EC) values of 4.2, 2.6 and 1.2 $\log_{10}$ cfu/cm^2, respectively, while deer carcass values were 4.5, 1.5 and 0.7 $\log_{10}$ cfu/cm^2, respectively. Moose carcasses were significantly more contaminated with EB and EC than deer carcasses. High bacterial counts (MAB > 5.0 $\log_{10}$ cfu/cm^2 and EB > 2.5 $\log_{10}$ cfu/cm^2) on the carcasses were associated with the smallest facilities having only one room. The outdoor temperature and days between hunting and sampling affected the bacterial counts. High EB counts on the carcasses indicated a gut hit. Male gender was significantly more contaminated by EC and meat-borne pathogenic bacteria: *Campylobacter* spp., *Salmonella* spp., enteropathogenic *Yersinia* spp., *stx*-harbouring EC (STEC) and *Listeria monocytogenes*. STEC (28/200) and *L. monocytogenes* (20/200) were the most commonly detected bacteria by PCR. *L. monocytogenes* isolates of different sequence types (ST7, 18, 29, 37, 249, 412, 451 and 611) belonged to serotypes 1/2a (seven isolates) and 4b (three isolates). The virulence gene *ail* was detected in four *Yersinia enterocolitica* biotype 1A isolates and one *Yersinia kristensenii* isolate. The bacterial counts on the moose and deer carcasses varied highly, and more attention should be paid to hunting hygiene and training of hunters. Game meat may be a source of meat-borne pathogens, and close attention should therefore be paid when handling and preparing game.

1. Introduction

Moose (*Alces alces*, European elk) and white-tailed deer (*Odocoileus virginianus*) hunting is a very popular outdoor activity and pursuit in the Nordic countries. Finnish moose and wild white-tailed deer populations are approximately 100 000 and 60 000 individuals in size, respectively. Between 35 000 and 50 000 moose are hunted yearly during the hunting period from September to December, and approximately 25 000 white-tailed deer are hunted between September and January. Moose populations, and deer populations in particular, have expanded in the Nordic countries, but also in many other parts of Europe due to regulated and selective hunting (Thulin et al., 2015). This may cause more interaction with humans and domestic animals, potentially leading to cross-contamination of pathogens.

Most game animals hunted for meat are killed in the field, where sticking, bleeding and evisceration are typically also carried out (Gill, 2007). Skinning, cooling and cutting the carcasses are usually performed in small-scale slaughterhouses or slaughter sheds. A heart-lung shot usually provides adequate bleeding, and sticking is not needed (Laaksonen and Paulsen. 2015). Evisceration of the organs and the gastrointestinal tract is usually performed in the field before transportation to a slaughter facility. Poor hunting hygiene enhances the spread

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of bacteria from the skin and digestive tract, but also from the environment onto the carcasses (Gill, 2007). Only few studies have been conducted on hunting hygiene and the microbial contamination of deer carcasses and none of moose carcasses (Paulsen and Winkelmayer, 2004; Atanassova et al., 2008; Avagnina et al., 2012; Obwegeser et al., 2012). The bacterial counts on the carcasses varied between these studies. Atanassova et al. (2008) reported very low numbers of bacteria (mean $< 3 \log_{10}$ cfu/cm^2) on deer carcasses in Germany while in Italy, 51% of the roe deer carcasses were highly ($> 2.5 \log_{10}$ cfu/cm^2) contaminated with enterobacteria (Avagnina et al., 2012). In Austria, the bacterial counts on the game carcasses were shown to be significantly higher in the summer time compared to winter time (Paulsen and Winkelmayer, 2004) and in Switzerland, the microbial contamination of deer carcasses differed between slaughterhouses by several orders of magnitude (Obwegeser et al., 2012).

Game meat is a rare delicacy, which is mostly difficult to purchase. It is often associated with sustainable meat production and it is expected to be free of meat-borne pathogens and antimicrobial resistant bacteria (Mateus-Vargas et al., 2017). In recent years, consumers are also paying more attention to nutritional and health aspects (Kwiecinska et al., 2017). Deer meat is lean, tasty and rich in proteins with a low-fat content (Kudrnácová et al., 2018). *Salmonella* spp., *stx*-harbouring *Escherichia coli* (STEC), *Yersinia enterocolitica/pseudotuberculosis* and *Listeria monocytogenes*, which are among the most common foodborne pathogens causing illness in humans, have sporadically been detected in the faeces of hunted wild ruminants (Joutsen et al., 2013; Díaz-Sánchez et al., 2013; Gnat et al., 2015). Only a few studies have dealt with the occurrence of these pathogens on game carcasses (Avagnina et al., 2012; Díaz-Sánchez et al., 2013; Bancerz-Kisiel et al., 2016). Moose meat is the most common wild game meat followed by white-tailed deer meat in Finland and so far, no studies have been conducted on the presence of meat-borne zoonotic bacteria on carcasses of wild white-tailed deer and moose.

The aims of our work are to study the bacterial contamination level and presence of zoonotic bacteria on moose and deer carcasses. We also evaluate the influence of animal age and gender, outdoor temperature during hunting, days between hunting and sampling, and slaughter facilities (number of rooms, cooling and indoor temperature) on the bacterial load of the carcasses.

2. Material and methods

2.1. Sampling and sample preparation

We studied 100 moose and 100 white-tailed deer carcasses during two hunting periods: moose during winters 2012–13 and 2013–14, and deer during winters 2013–14 and 2014–15 (Table 1). In total, 90 adults and 110 calves were studied. Slightly more females (52%) than males (48%) were studied. All animals were wild and free-living, and hunted in southern Finland. The weekly outdoor average temperatures during the hunting and sampling were obtained from the Finnish Meteorological Institute. The outdoor temperatures varied between −15.8 and 8.9 °C (Table 1). In total, 16 gut hits (8 moose and 8 deer) were recorded (Table 1).

The carcasses were eviscerated in the field by hunters and skinned in small slaughterhouses or sheds. In total, 25 small facilities located in 12 municipalities of four provinces were visited during 2012–15. Only one of the facilities was approved. Almost 50% (12/25) of the facilities had only one room and most (8/12) of the them had no cooling system. All of the two and three room slaughterhouses and sheds had cooling systems. The indoor temperatures varied between 1 and 8 °C in the facilities with a cooling system. The same person (the first author) sampled all the carcasses after skinning. The days between hunting and sampling varied between 0 and 9 days (median was one day). Only 91 out of 200 carcasses were sampled on the hunting day.

A disposable paper template was used to swab a 100-cm^2 area of each carcass using cotton pads moistened with 10 ml of buffered peptone water (BPW, LABM, Kerava, Finland). This 100-cm^2 area was divided into four swabbing sites (5 cm × 5 cm; shoulder, brisket, flank and rump). The moistened swab samples were transported to the laboratory in sterile stomacher bags (Seward stomacher 4000 classic bags, Sussex, UK) in a cooler box (4 °C). The analyses were begun within 24 h of sampling. In the laboratory, 90 ml of BPW was added to the stomacher bag and mixed by hand for 1 min.

2.2. Carcass bacterial counts

The number of mesophilic aerobic bacteria (MAB) and Enterobacteriaceae (EB) were determined using the drop plating method (DIN, 1984). The samples were diluted (1:10) in peptone saline water (distilled water with 0.85% NaCl and 0.1% peptone) to 10^{-5}. We inoculated plate count agar (PCA, Oxoid, Basingstoke, UK) for MAB and violet red bile glucose (VRBG, Labema) plates for EB with tenfold dilutions in duplicate. The PCA and VRBG plates were incubated for 24 h at 30 °C and 37 °C, respectively. *Escherichia coli* (EC) was isolated using the Chromocult plate (Merck, Darmstadt, Germany) after 100 µl of the sample (diluted in 1:10) was inoculated on the plate and incubated at 37 °C for 24 h. A detection limit of 50 cfu/cm^2 was used for the MAB count and 2 cfu/cm^2 for the EB and EC counts.

2.3. Pathogen PCR screening

We screened for the presence of *Campylobacter, Salmonella*, enteropathogenic *Yersinia*, STEC and *L. monocytogenes* using real-time PCR (Nousiainen et al., 2016). DNA was extracted from the over-night (ON) enrichment (18–20 h at 37 °C) of BPW using Chelex®100 resin (BioRad, Hercules, California) (Nousiainen et al., 2016). A hundred µl of the ON enrichment was used for the extraction. Two µl of the DNA was added to 18 µl of the mastermix (iQ™ SYBRGreen Supermix, BioRad) containing primers specific for *Campylobacter* (Lund et al., 2004), *Salmonella* (Malorny et al., 2004), enteropathogenic *Yersinia* (Thisted Lambertz et al., 2008a, 2008b), STEC (Sharma and Dean-Nystrom, 2003) and *L. monocytogenes* (Scheu et al., 1999). A positive result was indicated by a threshold cycle (Ct) value below 38 and a specific melting temperature (Nousiainen et al., 2016). We additionally studied *Campylobacter* (*rrn*) -positive samples using primers specific for

Table 1
The number of moose and deer carcasses sampled during hunting periods 2012–2015.

Hunting period (Winter)	Animal	Number of						Outdoor temperature	
		Animals	Adult	Calf	Male	Female	Gut hits	Min (°C)	Max (°C)
2012–13	Moose	52	31	21	24	28	6	−9.6	5.0
2013–14	Moose	48	21	27	19	23	2	−2.2	8.9
2012–14	Moose	100	52	48	43	51	8	−9.6	8.9
2013–14	Deer	50	21	29	22	23	4	−2.2	8.9
2014–15	Deer	50	17	33	12	11	4	−15.8	−1.3
2013–15	Deer	100	38	62	34	34	8	−15.8	8.9

Campylobacter jejuni (*map*) and *Campylobacter coli* (*ceu*) (Best et al., 2003).

2.4. Salmonella, Yersinia and Listeria isolation

Salmonella, Yersinia and *Listeria* were isolated using selective agar plates after enrichment (Nousiainen et al., 2016). For *Salmonella,* we used enrichment on semisolid Rappaport-Vassiliadis (MSRV, Labema) for 24 h at 42 °C before inoculation on selective xylose-lysine-deoxycholate (XLD, Labema) plates that were incubated for 24 h at 37 °C. Cold enrichment at 6 °C for at least three weeks was used for *Yersinia* and *Listeria* before inoculation on CIN (*Yersinia*) and Oxford (*Listeria*) plates, which were incubated at 30 °C for 20–24 h and at 37 °C for 24–48 h, respectively. Up to four typical colonies were sub-cultured on blood agar plates and identified with API 20E (*Salmonella* and *Yersinia*) or API Listeria (BioMerieux, France). Serotyping was performed with commercial antisera (Denka Seikan, Japan).

2.5. Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) of *L. monocytogenes* isolates was performed according to (Ragon et al., 2008). The public database (http://bigsdb.pasteur.fr/listeria/) was used to identify sequence types (STs), clonal complex (CC) and lineages. MLST of *Yersinia* isolates was performed according to (Hall et al., 2015), and the public database (http://enterobase.warwick.ac.uk/species/index/yersinia) was used to identify the STs.

2.6. Statistical analyses

Statistical analyses were performed using the analytical software package SPSS® Statistics Version 24 (IBM Corp., Armonk, NY, USA) and R (R Core Team, 2017). All bacterial counts were log-transformed for statistical analyses. The MAB, EB and EC counts were not normally distributed (Kolmogorov-Smirnov test of normality) and non-parametric tests were therefore applied. The Kolmogorov-Smirnov test was used to analyse the significance of the difference between MAB, EB and EC mean and median values for the moose and deer samples. Half of the detection limit was used as the estimated count in cases where the bacterial count was below the detection limit. We chose to use MAB counts > 5.0, EB > 2.5 and EC > 0.1 $\log_{10}$ cfu/cm^2 to assess the acceptability of the meat for human consumption using a binomial model (0 = acceptable, 1 = not acceptable). We used both log-regression to estimate prevalence rates and a logit model by stratifying the data by hunting sheds and animal species, subsequently adding variables to the univariate and finally, multivariate model.

3. Results

The mean MAB, EB and EC values on the moose carcasses were 4.2, 2.6 and 1.2 $\log_{10}$ cfu/cm^2, respectively, while respective values on the deer carcasses were 4.5, 1.5 and 0.7 $\log_{10}$ cfu/cm^2, respectively (Table 2). The median values were slightly lower. The values on the moose carcasses were significantly higher for EB (p = 0.013) and EC (p = 0.001) than on the deer carcasses, but we observed no significant difference between the MAB values (p = 0.24).

Most of the moose and deer carcasses harvested by hunters were microbiologically unsatisfactory according to the process hygiene criteria for carcasses of ruminants set in EU legislation (Table 3). The MAB counts exceeded 5.0 $\log_{10}$ cfu/cm^2 on 25% of the moose and 34% of the deer carcasses. Nearly 50% of moose carcasses had EB levels over 2.5 $\log_{10}$ cfu/cm^2. EB were detected on 81% (81/100) and 65% (65/100) and EC on 71% (60/85) and 41% (41/100) of the moose and deer carcasses, respectively.

Using the univariate (Table 4) and multivariate (Table 5) analyses of the logit model (stratified by animal species and slaughter facilities) for

Table 2
Mean and median values ($\log_{10}$ cfu/cm^2) of mesophilic aerobic bacteria (MAB), Enterobacteriaeae (EB) and *Escherichia coli* (EC) found on carcasses of hunted wild moose and white-tailed deer.

Species	Sampling year (winter)	Number of samples	Mean Median Min - Max		
			MAB	EB	EC
Moose	2012–14	100	4.2	2.6	1.2[a]
			4.0	2.3	1.3[a]
			1.4–8.4	0.1–6.8	0.1–2.3
Deer	2013–15	100	4.5	1.5	0.7
			4.4	1.1	0.1
			1.4–8.1	0.1–5.0	0.1–3.3

[a] 63 samples (the first 37 were not studied for the presence of EC).

Table 3
Mesophilic aerobic bacteria (MAB) and Enterobacteriaeae (EB) counts observed on moose and white-tailed deer carcasses.

Bacteria	Limits[a]	log cfu$_{10}$/cm^2	Judgement	No. of carcasses	
				Moose n = 100	Deer n = 100
MAB	m	< 3.5	Satisfactory	39	28
		3.5–5.0	Acceptable	36	38
	M	> 5.0	Unacceptable	25	34
EB	m	< 1.5	Satisfactory	31	56
		1.5–2.5	Acceptable	22	21
	M	> 2.5	Unacceptable	47	23

[a] Process hygiene criteria for carcasses of cattle, sheep, goats and horses in Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs.

Table 4
Univariate analysis of the logit model (stratified by slaughtered animal species and slaughter facilities) for various risk factors on mesophilic aerobic bacteria (MAB), Enterobacteriaeae (EB) and *Escherichia coli* (EC) counts of moose and white-tailed deer carcasses.

Risk factors	MAB > 5.0 $\log_{10}$ cfu/cm^2 (p-value)	EB > 2.5 $\log_{10}$ cfu/cm^2 (p-value)	EC (detected) (p-value)
Animal characteristics			
Age	0.85 (0.66)	1.03 (0.94)	0.48 (0.06)[a]
Gender	2.26 (0.06)	0.50 (0.20)	1.37 (0.03)
Conditions			
Year of hunting	5.97 (< 0.001)	7.08 (< 0.001)	3.75 (0.01)
Gut hit	0.95 (0.93)	2.71 (0.12)	3.18 (0.11)
Days between hunting and sampling	1.03 (< 0.001)	1.38 (0.02)	0.86 (0.31)
Outdoor temperature	1.07 (0.12)	1.08 (0.10)	0.89 (0.005)
Indoor temperature	0.98 (0.75)	1.05 (0.35)	1.04 (0.25)
Number of rooms in facilities[b]	Singular	Singular	Singular
Cooling of facilities[b]	Singular	Singular	Singular
Pathogen			
Salmonella spp.[c]	Infinity	Infinity	Infinity
Campylobacter spp.	0.30 (0.28)	4.62 (0.19)	1.04 (0.97)
stx-positive EC (STEC)	0.38 (0.15)	0.71 (0.19)	2.21 (0.19)
ail-positive *Yersinia*	0.84 (0.80)	0.95 (0.93)	9.14 (0.01)
Listeria monocytogenes	1.09 (0.88)	1.18 (0.77)	1.02 (0.97)
Any pathogen	0.82 (0.61)	1.07 (0.86)	2.05 (0.07)

[a] p-values < 0.20 were further tested with multivariate analysis.
[b] Some variables could not be tested due to coincidence with strata variable of shed location.
[c] As only four were positive, this caused the upper limit of the confidence interval of β to go to infinity.

Table 5
Multivariate analysis of the logit model (stratified by animal species and slaughter facilities) of those variables with a significance of $p < 0.20$ in the univariate analysis (Table 4).

Risk factors	MAB > 5.0 $\log_{10}$ cfu/cm^2 (p-value)	EB > 2.5 $\log_{10}$ cfu/cm^2 (p-value)	EC (detected) (p-value)
Animal characteristics			
Age	NT[a]	NT	NS[b]
Gender	4.53 (0.022)	NT	2.91 (0.047)
Conditions			
Year of hunting	29.87 (< 0.001)	24.91 (< 0.001)	NS
Gut hit	NT	10.25 (0.009)	NS
Days between hunting and sampling	3.08 (< 0.001)	1.62 (0.003)	NS
Outdoor temperature	1.21 (0.023)	1.27 (0.003)	0.87 (0.011)
Pathogen			
Campylobacter spp.	NT	29.30 (0.024)	NT
stx-positive EC (STEC)	NS	NS	NS
ail-positive *Yersinia*	NT	NT	16.71 (0.009)
Any pathogen	NT	NT	ND[c] [4.63 (0.007)]

[a] NT = not tested, because $p \geq 0.20$ (Table 4).
[b] NS = not significant, excluded from the final model.
[c] ND = excluded as *Yersinia* was more significant, could be used in the final model. Numbers in the brackets [] indicate the values if used instead of *Yersinia*.

MAB and EB, outdoor temperature, year of sampling and days between hunting and sampling were significant. High EB counts were also associated with a gut hit (Table 5). The EC could predict occurrence of any pathogen and male gender. The inverse significance of EC to the outdoor temperature was somewhat illogical, but may be that invert cold environments, hunting practices are less hygienic (Tables 4 and 5).

As some variables were multicollinear with the stratifying variable (slaughter facility), we tested them in the log-binomial model (Table 6). The number of rooms in the slaughter facilities was important

Table 6
Log-binomial model without stratifying for various risk factors on mesophilic aerobic bacteria (MAB), Enterobacteriaeae (EB) and *Escherichia coli* (EC) counts of moose and deer carcasses.

Risk factors[a]	MAB > 5.0 $\log_{10}$ cfu/cm^2 (p-value)	EB > 2.5 $\log_{10}$ cfu/cm^2 (p-value)	EC (detected) (p-value)
Animal characteristics			
Age (1 = adult, 0 = calf)	0.90 (0.63)	1.22 (0.30)	0.82 (0.17)
Gender (1 = male, 0 = female)	1.86 (0.02)[b]	1.39 (0.11)	1.37 (0.03)
Species (1 = moose, 0 = deer)	0.74 (0.17)	2.04 (< 0.001)	1.72 (< 0.001)
Conditions			
Year of hunting	1.61 (0.002)	0.95 (0.68)	0.89 (0.23)
Gut hit	0.84 (0.69)	1.48 (0.15)	1.49 (0.01)
Days between hunting and sampling	1.14 (< 0.001)	1.06 (0.31)	0.87 (0.02)
Outdoor temperature	1.08 (< 0.001)	1.11 (< 0.001)	0.99 (0.57)
Indoor temperature	0.98 (0.75)	1.05 (0.35)	1.04 (0.25)
Number of rooms in facilities	1.01 (0.94)	0.51 (< 0.001)	0.74 (0.01)
Cooling of facilities	1.59 (0.13)	0.71 (0.08)	0.86 (0.30)
Pathogen			
Salmonella spp.	1.72 (0.29)	2.19 (0.01)	1.87 (< 0.001)
Campylobacter spp.	0.41 (0.35)	1.85 (0.04)	1.32 (0.26)
stx-positive EC (STEC)	0.33 (0.05)	0.91 (0.74)	1.61 (< 0.001)
ail-positive *Yersinia*	1.14 (0.73)	1.37 (0.28)	1.65 (< 0.001)
Listeria monocytogenes	1.21 (0.56)	1.16 (0.61)	1.07 (0.75)
Any pathogen	0.86 (0.54)	1.26 (0.24)	1.58 (< 0.001)

p-values < 0.05 are bolded.
[a] Explanatory variables were categorised 1 = yes, 0 = no unless stated otherwise.

especially for the EB level but also for the EC level. High EB and EC levels were associated with moose carcasses.

We screened all 200 swabs for *Campylobacter* spp., *Salmonella* spp., enteropathogenic *Yersinia* spp., STEC and *L. monocytogenes* using real-time PCR. STEC (14%) and *L. monocytogenes* (10%) were the most frequent findings in the swab samples (Table 7). Gene *stx*2 was more commonly detected on moose carcasses (11/16), while *stx*1 was more common on deer carcasses (9/12). We identified *Y. enterocolitica* carrying the *ail* gene on both moose (6%) and deer (9%) carcasses but found no *Y. pseudotuberculosis*. We detected *Campylobacter* sporadically in both moose (6%) and deer (2%) samples, but only detected *Salmonella* in moose samples (4%). We identified *C. jejuni* on only two moose carcasses and found no *Campylobacter coli*. In total, 64 carcasses (35% of moose carcasses and 25% of deer carcasses) were contaminated with at least one pathogen.

We additionally searched for *Y. enterocolitica*, *Y. pseudotuberculosis* and *L. monocytogenes* after cold enrichment. *L. monocytogenes* was isolated from five moose and five deer carcasses. *Y. kristensenii* was the most common finding on moose carcasses (Table 8). One *Y. kristensenii* isolate found from a moose carried the *ail* gene. *Y. enterocolitica* belonging to biotype 1A was the most common finding in deer samples. We isolated *ail*-positive biotype 1A from both moose and deer carcasses.

We characterised 10 L. *monocytogenes* isolates and five *ail*-positive *Yersinia* isolates using MLST (Table 9). We obtained eight STs from the *L. monocytogenes* isolates and two from the *Y. enterocolitica* isolates. The ST of the *Y. kristensenii* isolate was clearly different from *Y. enterocolitica* isolates.

4. Discussion

In this study, we could show that a high proportion of the harvested moose and white-tailed deer carcasses were of low hygienic quality. High bacterial counts on the carcasses were associated with primitive facilities, outdoor temperature and gut hit.

The mean MAB values ($\log_{10}$ cfu/cm^2) were high on both moose and white-tailed deer carcasses (4.2 and 4.5, respectively) in our study and we also observed a great variation between the carcasses (1.4–8.4 moose and 0.1–8.1 white-tailed deer). Obwegeser et al. (2012) also reported high mean MAB values (> 4 $\log_{10}$ cfu/cm^2) on red and roe deer carcasses, and a high variation between slaughterhouses. The sampling sites were same as in our study but the swabbing area of each site was bigger (40 cm^2 compared to our 25 cm^2). The time between shooting and sampling was not more than 3 days. In our study, the days between shooting and sampling varied between 0 and 9 days (median one day) and we showed this to have a significant influence on the bacterial counts. In the two other studies, which reported much lower MAB counts on deer carcasses, the samples were collected between 1 and 6 h after shooting demonstrating low bacterial loads on carcasses of freshly shot game (Atanassova et al., 2008; Avagnina et al., 2012).

Low MAB and EB counts on number of carcasses in our study proves that it is possible to achieve moose and deer carcasses of high hygienic level when ensuring correct evisceration in the field, and correct skinning and proper storage in the slaughter facilities. However, the MAB counts for 26% of the moose carcasses and 34% of the white-tailed deer carcasses exceeded 5.0 $\log_{10}$ cfu/cm^2, indicating low hygienic quality of game meat. The two previous studies (Atanassova et al., 2008; Avagnina et al., 2012) showed clearly fewer carcasses with MAB counts exceeding 5.0 $\log_{10}$ cfu/cm^2 than in our study. The slaughter facilities in our study had large structural differences and we showed that high bacterial counts on the carcasses were associated with facilities having only single room space. The EB counts were especially high on moose carcasses: the bacterial counts were over 2.5 $\log_{10}$ cfu/cm^2 on 47% of the carcasses. Heavier moose carcasses may be one reason for the higher EB counts detected on moose carcasses than on white-tailed deer carcasses, as greater carcass weight increases the risk of contamination during transportation to slaughter facilities and due to laborious

Table 7
Detection rates of *Salmonella* (S), *Campylobacter* (C), *stx*-positiivinen *Escherichia coli* (STEC), *Yersinia enterocolitica* (YE) and *Listeria monocytogenes* (LM) on moose and white-tailed deer carcasses by PCR.

Carcass swab samples	Sampling year (winter)	Number of samples	Number of samples positive with				
			S (*ttr*)	C (*rrn*)	STEC (*stx*)	YE (*ail*)	LM (*mpl*)
Moose	2012–14	100	4 (4.0%)	6 (6.0%)	16 (16.0%)	6 (6.0%)	8 (8.0%)
Deer	2013–15	100	0	2 (2.0%)	12 (12.0%)	9 (9.0%)	12 (12.0%)
All	2012–15	200	4 (2.0%)	8 (4.0%)	28 (14.0%)	15 (7.5%)	20 (10.0%)

Table 8
Isolation rates of *Salmonella*, *Yersinia* and *Listeria* on moose and white-tailed deer carcasses.

Bacterium	Number of positive carcasses	
	Moose n = 100	Deer n = 100
Salmonella spp.	0	0
Yersinia spp.	14	12
Y. enterocolitica 1A	1[a]	8[b]
Y. kristensenii	12[a]	4
Y. intermedia	1	0
Listeria spp.	7	6
L. monocytogenes	5[c]	5[d]
L. innocua	2	1

[a] One isolate was *ail*-positive.
[b] Three isolates were *ail*-positive.
[c] All isolates were serotype 1/2a.
[d] Two isolates were serotype 1/2a and three were serotype 4b.

evisceration and dressing. High EB counts on the carcasses indicated a gut hit.

Our results showed that outdoor temperature during hunting and storing affects the bacterial load of game carcasses. The growth of most species belonging to the family Enterobacteriaceae is effectively inhibited at temperatures below 6 °C. The mean outdoor temperature during winter 2013–14 was significantly higher compared to winter 2012–13, and we observed lower EB counts on moose carcasses during the cold winter. Paulsen and Winkelmayer (2004) demonstrated that microbial surface contamination with MAB and EB was significantly higher on hunted game carcasses in June–August than in October–December, and assumed the reason to be the clearly warmer outdoor temperature in June–August (approximately 18 °C) compared to October–December (approximately 10 °C). These and our results indicate that outdoor temperature during hunting and storing affects the bacterial load of game carcasses.

The carcasses of male animals were significantly more often contaminated with EC in our study. Male carcasses are typically heavier than female carcasses, which can be one reason for the higher risk for contamination. In our study, carcasses contaminated with EC were also more likely to be contaminated with any of the tested pathogens. A positive relation between the presence of EC and *Salmonella* on pig carcasses has been observed (Biasino et al., 2018). The presence of EC may be a useful indicator for faecal contamination of game carcasses and used as a prediction of occurrence of enteropathogenic bacteria.

Salmonella and *Campylobacter*, which are the most frequent bacteria causing gastroenteritis in humans in Europe (EFSA and ECDC. 2017), were only sporadically detected by PCR in our study, especially on deer carcasses. In previous studies, *Salmonella* appears to be rarely (< 1%) found in deer faeces (Paulsen and Winkelmayer, 2004; Obwegeser et al., 2012; Díaz-Sánchez et al., 2013) or on the carcasses of wild ruminants in Europe (Paulsen et al., 2012). The most frequent finding in our study was STEC (14%); *stx*2 was the dominant type on moose carcasses and *stx*1 on deer carcasses. In a recent study, STEC, especially *stx*2-positive EC, was a common finding (36%) in semi-domesticated reindeer in northern Finland (Laaksonen et al., 2017). Deer meat has caused an EC O:157 outbreak (Keene et al., 1997). Both moose and deer carcasses were occasionally contaminated with *ail*-positive *Yersinia* and

Table 9
Sequence types (STs) and clonal complex (CC) of *Listeria monocytogenes* isolates and STs of *ail*-positive *Yersinia* isolates obtained by multi-locus sequence typing (MLST).

Code	Source	Year	*Listeria monocytogenes*									
			Sero-type	*abc*Z	MLST							
					*bgl*A	*cat*	*dap*E	*dat*	*ldh*	*lhk*A	ST	CC	Lineage
HR8	Moose	2012	1/2a	7	5	10	21	1	4	1	451	451	II
HR10	Moose	2012	1/2a	5	8	5	7	6	2	1	7	7	II
HR41	Moose	2012	1/2a	5	8	5	7	6	2	1	7	7	II
HR65	Moose	2013	1/2a	5	7	3	5	1	8	6	37	37	II
HR75	Moose	2013	1/2a	15	10	18	18	1	3	1	29	29	II
PR10	Deer	2013	4b	3	18	27	3	4	1	23	249	315	I
PR12	Deer	2013	4b	3	18	27	3	4	1	23	249	315	I
PR16	Deer	2013	1/2a	7	52	8	6	1	2	1	412	412	II
PR21	Deer	2013	1/2a	7	6	15	12	12	6	1	18	18	II
PR47	Deer	2014	4b	1	2	12	2	2	5	1	611	4	I

			ail-positive *Yersinia* spp.								
			Bio-type	*aar*F	MLST						Species
					dfp	*gal*R	*gln*S	*hem*A	*rfa*E	*spe*A	ST	
HR88	Moose	2013	1A	2	4	2	79	5	11	2	UN	*Y. enterocolitica*
HR100	Moose	2013	2	10	49	80	18	72	47	11	UN	*Y. kristensenii*
PR4	Deer	2013	1A	5	4	2	2	12	11	2	UN	*Y. enterocolitica*
PR18	Deer	2013	1A	5	4	2	2	12	11	2	UN	*Y. enterocolitica*
PR20	Deer	2014	1A	2	4	2	79	5	11	2	UN	*Y. enterocolitica*

L. monocytogenes in our study. These pathogens have seldom been found in deer faeces (Joutsen et al., 2013; Gnat et al., 2015; Syczylo et al., 2018) or on deer carcasses in Europe (Paulsen and Winkelmayer, 2004; Obwegeser et al., 2012; Bancerz-Kisiel et al., 2016). One reason behind the relatively low prevalence of zoonotic meat-borne pathogens in wild deer and moose may be the infrequent contact between wild animals and intensively farmed domestic animals (Gill, 2007). In our study, 35% of the moose and 29% of deer carcasses were contaminated with at least one pathogen showing that good hunting hygiene and correct handling of game meat is essential to prevent human infections due to contaminated game meat.

L. monocytogenes serotypes 1/2a and 4b were found on the carcasses in the current study. The same serotypes were recently found in Finnish semi-domesticated reindeer (Laaksonen et al., 2017). Serotypes 1/2a and 4b are frequently reported in human clinical samples and in different food matrices (Ebner et al., 2015). Sequence types of moose and deer isolates identified in this study have been identified in human clinical isolates (http://bigsdb.pasteur.fr/listeria/), indicating that moose and deer meat may pose a human health risk if handled improperly. All *Y. enterocolitica* isolates belonged to the biotype 1A unassociated with human diseases. However, *ail*-positive *Y. enterocolitica* was isolated from one moose and three deer samples. We also isolated *ail*-positive *Y. kristensenii* from one moose sample. The three STs of the five *ail*-positive *Yersinia* isolates were unique, and thus not associated with any known source. *Y. kristensenii* was the most frequently isolated species in the moose samples. It was also the most common species reported in Finnish reindeer (Laaksonen et al., 2017). The *ail* is an important virulence gene typically found only in *Y. enterocolitica* isolates belonging to biotypes 1B, 2–5 associated with human and animal diseases (Reuter et al., 2014). This gene has quite recently been reported in *Y. enterocolitica* 1A and *Y. kristensenii* isolates from wild small mammals in Finland, supporting the hypothesis that wildlife is a reservoir of these *ail*-positive *Yersinia* isolates (Joutsen et al., 2017). However, more studies are needed to clarify the significance of these atypical *ail*-positive isolates in human and animal diseases.

5. Conclusions

A high proportion of the harvested moose and white-tailed deer carcasses were of low hygienic quality as assessed by the process hygiene criteria for carcasses of ruminants set in EU legislation. High bacterial counts (MAB > 5.0 $\log_{10}$ cfu/cm^2 and EB > 2.5 $\log_{10}$ cfu/cm^2) on the carcasses were partly due to processing in facilities having only one room with limited possibilities to separate the dirty area (skinning) from the clean are (storage of skinned carcasses). The outdoor temperature, year of sampling, gut hit and days between hunting and sampling affected the bacterial counts. EC on the carcasses predicted the presence of meat-borne pathogenic bacteria and may be used as an indicator. The most important meat-borne zoonotic bacteria, i.e. *Campylobacter, Salmonella, Y. enterocolitica*, STEC and *L. monocytogenes*, were detected on both moose and white-tailed deer carcasses. Contaminated game meat may pose a risk for humans, and therefore correct handling and preparation of this type of meat should be a priority. In human sporadic infections and small outbreaks, game meat should be considered as a potential infection source. Hunter education is also needed to improve hunting hygiene.

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