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Bacterial surface counts and visually assessed cleanliness of carcasses from hunted roe deer (*Capreolus capreolus*)

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received August 26, 2021
accepted November 8, 2021
published April 26, 2022

Keywords: roe deer, visually assessed cleanliness score, Total Aerobic Count, Enterobacteriaceae.

Schlüsselwörter: Rehwild, visuelle Sauberkeitsbewertung, Gesamtkeimzahl, Enterobacteriaceen.

Summary

We determined bacterial numbers on the surfaces of the body cavities of 352 hunted roe deer and related them to the location of shot wounds, visually assessed cleanliness of body cavities and time from evisceration to sampling, which coincided with time of carcass processing. Body cavities were swabbed with sterile sponges and Total Aerobic Counts and counts of Enterobacteriaceae determined by cultural microbiology (most-probable-number methods); the presence/absence of the pathogenic bacteria *Salmonella* sp. and *Listeria monocytogenes* was assessed by antigen tests of enrichment cultures. The Total Aerobic Counts (TAC) and counts of Enterobacteriaceae (EB) (mean ± standard deviation) in the body cavities before skinning (1–12 days after hunting) were 5.5±1.2 and 3.6±1.3 log cfu/cm^2. *Salmonella* was detected in none and *Listeria monocytogenes* in 16/352 body cavities (antigen-detection kits). In 59.9 % of the carcasses, both entry and exit wound were before the 7th rib (i.e. the abdominal organs were

Zusammenfassung

Oberflächenkeimzahlen und visuell beurteilte Sauberkeit der Leibeshöhlen von erlegtem Rehwild (*Capreolus capreolus*)

Einleitung

Wir untersuchten bei Rehwild-Tierkörpern, ob ein Zusammenhang zwischen der Lage der Schusswunde und sichtbaren Verschmutzungen der Leibeshöhlen besteht und von welchen im Rahmen der Fleischuntersuchung vorliegenden Informationen die Höhe der Oberflächenkeimzahl in den Leibeshöhlen beeinflusst wurde.

Material und Methoden

Bei 352 Rehwild-Tierkörpern wurden anlässlich der Fleischuntersuchung die Lage der Ein- und Ausschusswunden und die visuelle „Sauberkeit" protokolliert und die Oberflächenkeimzahlen der Leibeshöhlen mittels nicht-destruktiver Beprobung und einem automatisierten Most-Probable-Number Verfahren (TEMPO®; Gesamtkeimzahl und Enterobacteriaceen) bestimmt sowie auf das Vorkommen von *Salmonella* und *Listeria monocytogenes* (Antigennachweise aus Anreicherungskulturen) untersucht. Zusätzlich wurde die Zeit von der Erlegung bis zur Beprobung (in Tagen) angegeben. Der Einfluss der Lage der Schusswunden, der visuell beurteilten Sauberkeit und der Dauer von der Erlegung bis zur Untersuchung auf die (log-transformierten) Oberflächenkeimzahlen wurde mittels eines allgemeinen linearen Modells untersucht.

Ergebnisse

Die durchschnittliche Gesamtkeimzahl und die Enterobacteriaceenzahl der 352 Tierkörper betrugen 5,5±1,2 bzw. 3,6±1,3 $\log_{10}$ kbE/cm^2. *Salmonella* war auf keinem, *Listeria monocytogenes* waren auf 16/352 Tierkörpern nachweisbar.

Der Zeitraum vom Erlegen bis zur Beprobung (und damit der Fleischuntersuchung und Zerlegung) betrug 1–12 Tage. Bei 59,9 % der Tierkörper lagen Ein- und Ausschusswunde vor der 7. Rippe und damit waren keine Bauchorgane verletzt worden. Wunden an beiden Bauchseiten (nach der 13. Rippe) waren bei 3,7 % der Tierkörper nachweisbar. Die Lage der Schuss-

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not inflicted). We found perforation of both flanks in 3.7 % of the carcasses. The categorization according to the location of shot wounds was significantly associated with the level of the visually assessed cleanliness of body cavities. The TAC and counts of EB increased significantly with increasing number of days from killing to sampling. The association of TAC with visible contamination of body cavities with gastric or faecal matter was less strong. The finding confirms that the number of days between evisceration and processing of the carcass has a major impact on the bacterial contamination of roe deer carcasses.

Abbreviations: AGHE = approved game handling establishment; cfu = colony-forming-units; EB = Enterobacteriaceae; KbE = koloniebildende Einheiten; LM = General Linear Model; MKTT = Müller-Kauffmann-tetrathionate; MPN = most-probable-number; RVS = Rappaport-Vassiliadis-soy-peptone; TAC = Total Aerobic Count(s)

wunden war dabei signifikant mit der visuellen Sauberkeit der Leibeshöhlen assoziiert.

Die Höhe der Gesamtkeimzahl in Brust- und Bauchhöhle wurde statistisch signifikant von der Dauer vom Erlegen bis zur Untersuchung, und in geringerem Ausmaß vom Monat der Erlegung (höhere Keimzahlen im Oktober) und von der visuell beurteilten Sauberkeit der Leibeshöhlen bestimmt. Die Enterobacteriaceenzahlen wurden ebenfalls von der Dauer vom Erlegen bis zur Untersuchung, und in geringerem Ausmaß vom Monat der Erlegung beeinflusst.

Schlussfolgerung

Die Ergebnisse zeigen, dass die Höhe der bakteriellen Kontamination von Rehwild-Tierkörpern nicht nur von den Bedingungen der Primärproduktion und damit der traditionellen Wildbrethygiene abhängig ist, sondern auch von der Dauer vom Ausweiden bis zur weiteren Bearbeitung.

Introduction

Meat from wild game is traditionally processed and consumed in many countries. Besides subsistency hunting, venison production can be a sideline of the active management of wild animals that interfere with agriculture, yielding a sustainably produced food with a favourable chemical composition (Hoffman & Wiklund 2006; Hoffman & Cawthorn 2012). The European Union had no regulatory framework for placing meat from wild game on the market until 1992. In 2004, the legislation was merged with that for meat from domestic species (EC 2004a,b) and was recently updated with regard to meat inspection (EU 2019). It lays down elementary hygiene requirements and the actions and precautions to be taken by hunters and so-called "trained persons" to ensure that food safety is not compromised when the carcass is delivered to approved game-handling establishments (AGHEs).

Besides the detection of animals affected by diseases, the prevention and control of contamination of meat with foreign matter and bacteria is of paramount importance. Contamination of meat surfaces can occur at various stages of the game meat chain, for example in killing (inexpert shot causing laceration of the stomach or intestines; Winkelmayer et al. 2005; Atanassova et al. 2008; Avagina et al. 2012) and evisceration (time from killing to evisceration; perforation of the gastrointestinal tract during evisceration; washing of body cavities; see Branciari et al. 2020). There are similar issues with the slaughter and processing of cattle, with the cleanliness of hides critical for hygienic skinning (Karhan et al. 2020). The extent of bacterial multiplication on meat surfaces can be influenced by the time to the onset of cold storage (Paulsen & Winkelmayer 2004) and the temperature and duration of cold storage, as well as by intrinsic factors such as the ultimate pH of the meat (Lawrie & Ledward 2016).

Although various EU member states have Guides to Good Practice, the game meat chain is much less standardized than the production of beef, pork or poultry meat (Ramanzin et al. 2010). This should not prevent the game meat industry from establishing process hygiene criteria for game meat carcasses (Kennedy 2014) and venison cuts (Paulsen 2011) but the work requires a thorough analysis determine the steps of the game meat chain at which the microbiological quality can be significantly impaired.

We applied our previous conceptual framework (Paulsen & Schopf 2016), which is similar to that of Mirceta et al. (2017), although we did not observe the evisceration procedure. We collected information available at the point of routine meat inspection and related the data to the extent of bacterial contamination. The information included (1) data on the hunter´s declaration attached to the carcass (issued by the trained person) and (2) results of the inspection of the carcass, including visual assessment of the cleanliness of body cavities, pathology and location of shot wounds. Our aim was to elucidate the extent to which the various factors relate to the microbial contamination of the body cavities.

Materials and methods

In the period from August to October 2015, we examined 352 carcasses of hunted roe deer in an approved game-handling establishment (AGHE). Carcasses had arrived eviscerated and in-fur and had been either supplied directly by hunters or collected from cooling rooms and transported in refrigerated trucks to the AGHE. All animals had undergone an initial examina-

tion by a trained person in accordance with Reg. (EC) No. 853/2004 (EC 2004a) and the certificates were attached to the carcasses. After arrival at the AGHE, carcasses were stored in cooling rooms at 2 °C for several days. During each visit (seven in total), all carcasses available in the cold storage rooms were examined.

We examined carcasses immediately before processing (i.e. skinning, meat inspection and cutting). To determine Total Aerobic Counts (TAC) and Enterobacteriaceae (EB), an area of 300–600 cm² of the central parts of thoracic and abdominal cavities was sampled with an abrasive polyurethane sponge (EZ-DRY-PUR; World Bioproducts, Woodinville, USA) moistened with sterile 0.85 % saline prior to use. The left and right abdominal and thoracic cavity (4 sampling sites per carcass) were sampled by swabbing an area of 75 cm² each for fawns or 150 cm² each in yearlings and adults. The areas were delineated by sterile PTFE templates and a fresh sponge was used for each sampling site. Sponges were placed in sterile stomacher bags and transported under refrigeration to the laboratory. Further processing was carried out within 8 hrs of sampling. 150 or 300 ml buffered peptone water was added to the four sponges and the mixture homogenized with a Stomacher 400 lab blender (60 strokes), so that 1 ml corresponded to 2 cm² of carcass surface (primary dilution). Serial dilutions were made and TAC and EB numbers analysed with the automated Tempo® device (biomerieux, Marcy l´Etoile, F) following validated protocols (Paulsen et al. 2006, 2008). The remaining volume of the primary dilution was divided into two fractions for a qualitative ("presence/absence") test for pathogenic bacteria. The first fraction was incubated at 35 °C for 18–24 hrs, then 0.1 and 1.0 ml of the culture were transferred to 10 ml of Rappaport-Vassiliadis-soy-peptone (RVS) and Müller-Kauffmann-tetrathionate (MKTT) broth, respectively. After 6–7 hrs incubation at 42 °C (RVS) or 35 °C (MKTT), 1 ml of each culture was pipetted into 10 ml Mannose broth (biomerieux), which was then incubated at 42 °C for 18 hrs. Aliquots (1 ml) of each of the enrichments were combined and boiled at 100 °C for 15 min and the liquid was allowed to reach ambient temperature before being subjected to an immunological test to detect *Salmonella* antigens (VIDAS SLM; biomerieux). The second part of the primary dilution was used to detect *Listeria monocytogenes* (antigens). It was mixed with an equal volume of double-strength half-Fraser broth and further processed according to the manufacturer´s instructions for the VIDAS Listeria LMO2 test. Positive results were confirmed by culture according to the manufacturer´s instructions. Unless stated otherwise, bacteriological media were from Oxoid (Basingstoke, UK).

Tab. 1: Tests and inspections performed on the roe deer carcasses and accompanying information / An den Rehwild-Tierkörpern vorgenommene Untersuchungen und Begleitinformationen

Microbiological tests	Swabbing of body cavities (300–600 cm²; only visually "clean" areas swabbed)	Enumeration of Total Aerobic Count and Enterobacteriaceae; detection of *Salmonella* and *Listeria monocytogenes* antigens
Inspection of carcass	Shot wounds	Location of entry and exit wound
	Cleanliness of body cavities (thorax; abdomen and pelvic area; overall)	4-point scale: 1. clean, no dirt or faeces 2. few small green particles 3. spots of faecal matter, max 1/10th of the body cavities´ areas affected 4. larger areas affected or putrefaction
	Pathological alterations	
Information on certificate attached to carcass	Circumstances of killing	Month, day, time, region
	Abnormities	Altered behaviour, emaciation, abnormities in viscera

The locations of the entry and exit wounds were recorded and photographs of body cavities taken to classify the visually assessed cleanliness of the exposed surfaces (Table 1). The visually assessed cleanliness of the hides took into account (1) whether the hides were wet or dry and (2) whether faeces or mud were visible (a) around the ventromedian line of the body, (b) in the perianal region or (c) on other locations. Additional information was retrieved from the certificates attached to the carcasses (Table 1). Bacterial numbers were transformed to log units. For results below the limit of detection (10 cfu/cm²), 0.9 log was assumed, whereas results above the range of detection (i.e., 7.69 log cfu/cm²) were expressed as 7.70 log. The relationship between the visually assessed cleanliness scores and shot wound patterns were explored by the Fisher´s exact test, with the categories (four each) collapsed to form a 2x2 matrix.

The TAC and EB counts were log-transformed (logTAC, logEB) to assure normal distribution, which was verified by the Kolmogorov-Smirnov-test. The impact of month of killing and visually rated surface contamination and interactions of these terms on the response variables TAC and Enterobacteriaceae were analysed separately using a general linear model (LM) with days between killing and sampling as a covariate. *Post hoc* analysis was performed using Sidak's alphacorrection procedure for multiple testing. The correlation between days between killing and sampling and the logTAC was

calculated using Pearson's correlation coefficient. A multiple linear regression model was used, adding the two most important factors (from the LM). We calculated the total variance explained for both the LM and the regression model, which corresponds to the Eta² in the LM and to the R² in the regression model. We considered a p value below 5 % ($p<0.05$) as significant. All analysis was performed using IBM SPSS v24 (IBM, Armonk, NY, USA).

Results

Location of shot wounds

In 59.9 % of the carcasses, both entry and exit wounds were located in the cranial thorax, before the 7th rib (wound pattern "a"). Wound channels within the rib cage but with at least one wound behind the 7th rib were found in 103 (29.3 %) carcasses (type "b"). Perforation of both flanks was found in 13 (3.7 %) of the carcasses ("d"-type), while 25 (7.1 %) of the carcasses had one shot wound in the flank and the other in the rib cage ("c"-type) (Table 2).

Tab. 2: Classification of roe deer carcasses by location of shot wounds and cleanliness of body cavities / Einteilung von Rehwild-Tierkörpern nach der Lage der Schusswunden und der Sauberkeit der Leibeshöhlen

	Wound type				
	a	b	c	d	sum
Cleanliness[a] ↓	Both wounds before 7th rib*	Both wounds before the 13th rib, with one or both between 7th and 13th rib	one wound** in flank	two wounds** in flank	
1	96	36	0	0	132
2	78	37	6	2	123
3	10	10	5	3	28
4	27	20	14	8	69
Total	211	103	25	13	

[a] 1.. visually clean; 2.. few small green particles; 3.. spots of gastric or faecal matter, max 1/10th of the area of the affected body cavities; 4.. larger areas affected or putrefaction; refers to thoracic + abdominal + pelvic cavity; * cranial thorax; ** in the event of more than one hit, the most caudal wounds were considered; note that for statistical analysis, data were aggregated into four groups, as indicated by the vertical and horizontal lines in the centre of the table, and there was a statistically significant relation at $p<0.001$ / [a] 1.. mit freiem Auge sauber; 2.. vereinzelt grüne Partikel; 3.. Magen-Darm-Inhalt sichtbar, max. 1/10 der Oberflächen bedeckt; 4.. größere Bereiche betroffen oder Fäulnis; Bewertung bezieht sich auf die gesamten Körperhöhlen; * kranialer Brustraum; ** bei mehr als einer Schussverletzung wurden die am weitesten caudal gelegenen Wunden gezählt; für die statistische Auswertung wurden die Daten in vier Gruppen zusammengefasst (senk- und waagrechte Linien in der Mitte der Tabelle), es ergab sich ein statistisch signifikanter Zusammenhang, $p<0,001$.

Cleanliness of skin/fleece and body cavities

All fleeces were dry to moist, mud was present on the claws of some carcasses and faecal matter was detected around the ventromedian line of carcasses with "dirty" body cavities. As the ventromedian contamination matched with contamination of body cavities, no separate statistical analysis of the data was performed. More than 70 % of the carcasses fell in the two best categories for cleanliness of body cavity, (n=132 or 37.7 % in category 1 and n=123 or 34.6 % in category 2), with considerably fewer carcasses with "dirty" body cavities (28 or 7.9 % and 69 or 19.5 % for categories 3 and 4, respectively) (Table 2).

Additional information

No abnormal behaviour before killing or abnormities in inner organs were reported on the certificates attached to any of the carcasses. Likewise, no carcass showed signs of emaciation or had swollen joints or signs of inflammation. This suggests either that such abnormities are seldom encountered in hunted wild game or that the first examination by trained persons is effective at removing suspect animals from the food chain.

Time from killing (as given on the certificate attached to the carcass) to sampling (which coincided with skinning, veterinary inspection and cutting, followed by deep-freezing of the packaged meat cuts) ranged from 1 day to 12 days, with a median and an average of 5 days; the 95th percentile was 10 days.

Detection of *Salmonella* sp. and *Listeria monocytogenes*

Salmonella sp. (antigens) were not detected in any of the sample enrichments. *Listeria monocytogenes* (antigens) were detected in the enrichment cultures of 4.5 % (16/352) of samples.

Total Aerobic Counts and Enterobacteriaceae numbers on body cavities

Total Aerobic Counts were in the range of 2.0–7.7, with a median of 5.5 and a mean of 5.5±1.2 log cfu/cm². The corresponding numbers for Enterobacteriaceae were 1.7–6.6; 3.5 and 3.6±1.3 log cfu/cm². According to the visually assessed cleanliness scores, Total Aerobic Counts were 5.0±1.3; 5.5±1.1; 6.1±0.5 and 5.7±1.2 log cfu/cm² for categories 1, 2, 3 and 4. The corresponding figures for Enterobacteriaceae were 3.2±1.2; 3.7±1.3; 3.8±1.3 and 4.2±1.5 log cfu/cm².

Relation of location of shot wounds and cleanliness scores

Classification of the carcasses by shot wounds and cleanliness scores (thorax, abdomen and pelvic area combined) resulted in a 4x4 matrix (Table 2). We explored a possible relationship between location of shot wound and cleanliness by Fisher´s exact test, reducing data to a 2x2 matrix by combining the two "best" and the two "worst" categories each, i.e. scores (1,2) : (3,4) vs. wound patterns (a,b) : (c,d). There was a significant relation at $p<0.001$.

Significance of cleanliness scores, time from killing to testing and month of killing on Total Aerobic Counts and Enterobacteriaceae numbers

For statistical analysis, we combined data of categories 3 and 4 from the visual cleanliness evaluation to produce three groups with balanced sample sizes (Table 3). As few roe deer (n=2) had been killed in September, we considered only the carcasses from roe deer killed in August and October, i.e. 350 specimens.

For Total Aerobic Counts, the LM resulted in a total variance explained of 32.6 %. The log TAC was most influenced by the number of days between killing and sampling (Eta^2=13.7 %; $p<0.001$), month of killing (Eta^2=2.4 %; $p<0.001$) and the overall assessment of cleanliness of body cavities (Eta^2=3.0 %; $p=0.006$). No other factor and no interaction exceeded an impact of 1 % (Table 4). The mean contamination (logTAC) in month 8 (5.10±1.21 logTAC/cm²) was significantly lower than that in month 10 (5.93±0.92 logTAC/cm²). LogTAC values increased significantly ($r=0.459$; $p<0.001$) with time from killing to sampling (Fig. 1). A multiple linear regression model using the two most important factors "days between killing and sampling" and "month of killing" as predictors showed significant results for both ($p<0.001$) with a total variance explained of

Tab. 3: Data for the LM analysis / Übersicht zu den für die LM Analyse verwendeten Werten

		Number of carcasses (total=350)
Month of killing	8	219
	10	131
Entry wound[1]	Cranial of the 7th rib	261
	≥7th rib	89
Exit wound[1]	Cranial of the 7th rib	221
	≥7th rib	129
Cleanliness of thoracic cavity	1	230
	2	95
	3	25
Cleanliness of abdominal and pelvic cavity	1	164
	2	118
	3	68
Cleanliness of body cavities[2]	1	132
	2	121
	3	97

[1] in the event of more than one hit, only the most caudal wounds were considered;
[2] highest score of the assessment of thoracal and abdominal and pelvic cavity;
note that the carcasses in the "worst" category 4 were assigned to category 3/
[1] bei mehr als einer Schusswunde pro Tier wurden die am weitesten caudal gelegenen Wunden gezählt;
[2] schlechteste Einzelbewertung der Körperhöhlen;
die Tierkörper der „schlechtesten" Kategorie 4 wurden der Kategorie 3 zugeordnet

Tab. 4: The general linear model / Ergebnisse der Analyse mittels allgemeinen linearen Modells

		LogTAC/cm²			LogEB/cm²		
Factor (source of variance)	df	F (df_{error}=338)	p-value	Eta² (%)	F (df_{error}=338)	p-value	Eta² (%)
Days from killing to sampling	1	54.64	<0.001	13.7	23.11	<0.001	6.4
Month of killing	1	8.42	<0.001	2.4	<1	0.043	1.8
Visually rated cleanliness of body cavities	2	5.25	0.006	3.0	3.18	0.955	<0.1
Entry wound	1	<1	0.403	0.2	1.32	0.251	0.4
Exit wound	1	<1	0.368	0.2	<1	0.439	0.2
Visually rated cleanliness of thoracic cavity	2	1.60	0.203	0.9	<1	0.624	0.3
Visually rated cleanliness of abdominal and pelvic cavity	2	<1	0.967	<0.1	<1	0.517	0.4

df = degrees of freedom / df = Freiheitsgrade

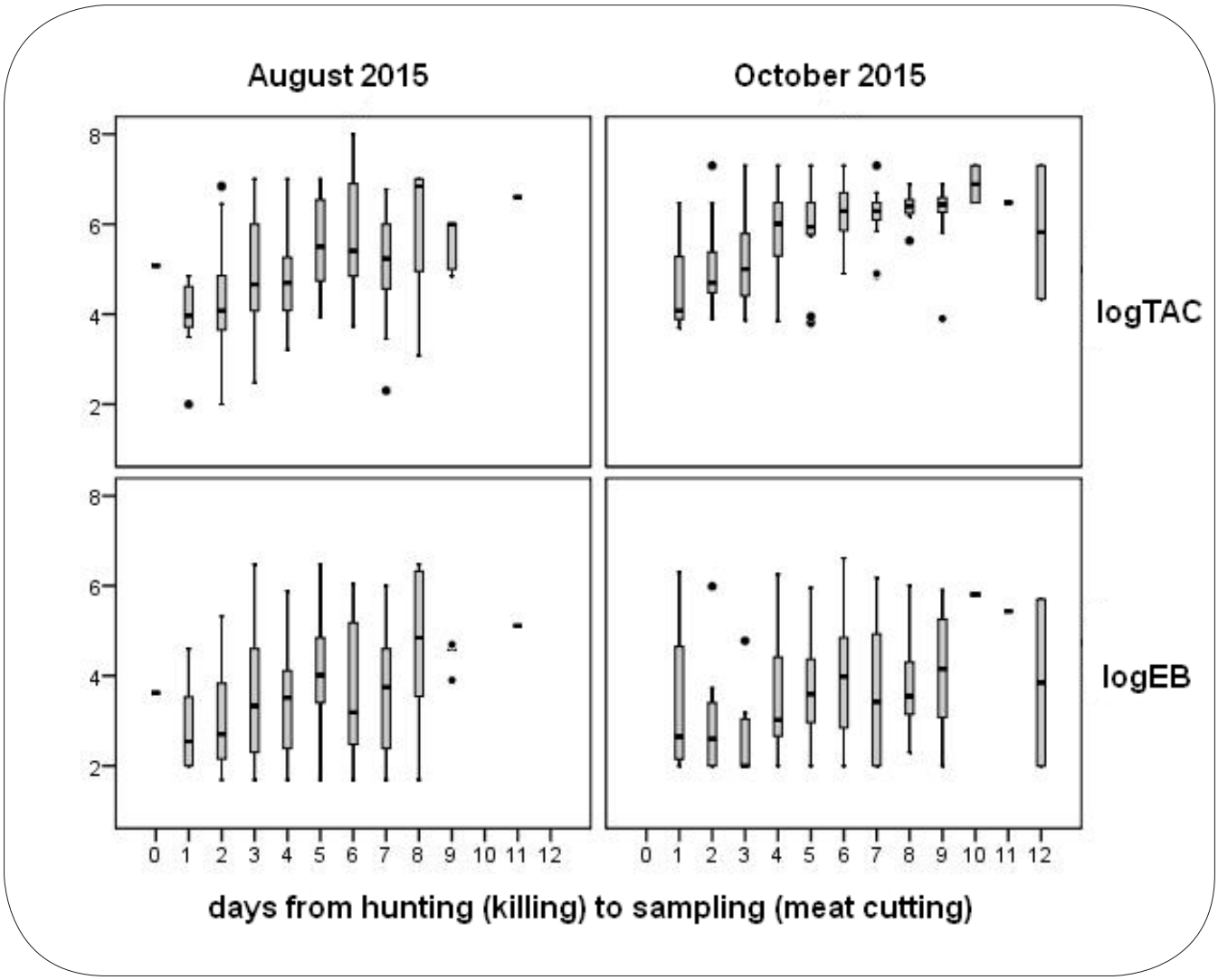

Fig. 1: Total Aerobic Counts and Enterobacteriaceae numbers (log cfu/cm²) on the surfaces of the body cavities of hunted roe deer, by month of killing and days from killing to sampling (meat cutting) (x axis) / Gesamtkeimzahlen und Enterobacteriaceen (log_{10} kbE/cm²) auf den Oberflächen der Körperhöhlen von erlegtem Rehwild, gegliedert nach Monat der Erlegung und Dauer von der Erlegung bis zur Beprobung bzw. Fleischzerlegung, in Tagen (x-Achse)

logTAC = Total Aerobic Count, logEB = Enterobacteriaceae, in log cfu/cm² / logTAC = Aerobe Mesophile Keimzahl, logEB = Enterobacteriaceen, in log_{10} kbE/cm²

27 % (a=1.77; b(days)=0.2; b(month)=0.3; r²=0.27). It indicated that logTAC increased by 0.2 log units per day.

For Enterobacteriaceae, the LM resulted in a total variance explained of 15.4 %. The logEB was most influenced by the number of days between killing and sampling (Eta²=6.4 %; p<0.001) and the month of killing (Eta²=1.8 %; p=0.043). No other factor and no interaction exceeded an impact of 1 % (Table 4).

Discussion

Location of shot wounds

In 59.9 % of the carcasses, both entry and exit wounds were located in the cranial thorax, before the 7th rib (wound pattern “a”), which means that the diaphragm or the adjacent abdominal organs were not affected by the bullet (Winkelmayer et al. 2005). Wound channels within the rib cage but with at least one wound behind the 7th rib were found in 103 (29.3 %) of the carcasses (type “b”) and perforation of both flanks in 13 (3.7 %) (“d”-type), whereas 25 (7.1 %) of the carcasses had one shot wound in the flank and the other in the rib cage (“c”-type) (Table 2).

Partial or complete abdominal shot wounds (“c” and “d” in Table 2) were recorded in 10.8 % of carcasses, which is at the lower range of results reported for various game species. In studies on carcasses from still hunts or non-specified hunts, the percentages of abdominal shots in wild boar range from 27 to 43 % (Avagnina et al. 2012; Mirceta et al. 2017; Staubmann 2017), are 39 % for chamois (Avagnina et al. 2012), are

9 to 49 % for red deer (Avagnina et al. 2012; Kennedy 2014; Staubmann 2017) and are 13.7 to 25 % for roe deer (Atanassova et al. 2008; Avagnina et al. 2012; Herbsthofer 2019).

Time from killing to sampling (cutting)

The average time from killing to sampling/cutting was 5 days, which is in good agreement with studies on various game species in the same establishment in 2017–2019 (Staubmann 2017; Herbsthofer 2019) but shorter than reported for a larger dataset on wild game, with a median of 8 days and a 95th percentile of 14 days (Paulsen et al. 2015). It is unclear whether the short period from killing to processing was due to a change in collecting and transport logistics or because of a higher seasonal demand for game meat.

Practically all carcasses had been stored in local cooling rooms, from where they were collected by refrigerated trucks from the AGHE. As we did not measure the temperatures in these local (private) cooling rooms, we cannot exclude the possibility that not only time but rather the time-temperature profiles along the game meat chain had an impact on the development of contaminant bacteria (Kennedy 2014; Paulsen et al. 2015).

Detection of *Salmonella* sp. and *Listeria monocytogenes*

The absence of *Salmonella* sp. in roe deer samples was not unexpected, as this pathogen seems to occur rarely in wild ruminants (Gill 2007) and more recent studies failed to detect it in the faeces (0/239; Obwegeser et al. 2012) or on the muscle surfaces (0/162, Atanassova et al. 2008; 0/219, Avagnina et al. 2012; 0/64, Branciari et al. 2020) of wild ruminants. The situation in wild boars is somewhat different, as *Salmonella* was isolated from 1.4 % of skin and 1.9 % of meat samples (Mirceta et al. 2017).

The frequency of samples positive for *Listeria monocytogenes* is expected to increase along the game meat chain, from evisceration to cutting (Atanassova et al. 2008; Obwegeser et al. 2012), due to contamination events (contact with other carcasses, personnel, cooling room equipment). Although *Listeria monocytogenes* had not been detected in faecal samples (0/239; Obwegeser et al. 2012), it was expected that the prevalence on muscle surfaces ranged from 0/219 (Avagnina et al. 2012) to 4.9 % (Atanassova et al. 2008) soon after evisceration. As our samples were taken at a later processing stage, after some days of cold storage, the prevalence of 4.5 % was lower than expected. However, there was considerable variation in the frequency of detection between the seven sampling sessions (in chronological order): 6/30, 1/40, 0/40, 5/50, 1/60, 1/72 and 2/60. This might be attributable to the different origins of the carcasses (range of storage conditions provided by the different collecting points and local cooling facilities).

Total Aerobic Counts and Enterobacteriaceae numbers on body cavities

Based on previous studies on Total Aerobic Counts on surfaces of body cavities of hunted roe deer (Paulsen et al. 2003), we established the categories ≤6 and >6 log cfu/cm^2 to distinguish carcasses in hygienically acceptable condition from those in an (early-) spoilage condition. According to this categorization, 128/352 carcasses (36.4 %) were in an early-spoilage condition. TACs on 12 carcasses exceeded 7 log cfu/cm^2, indicative of apparent spoilage (limit derived from Dainty & Mackey 1992). Although the log-numbers of TAC and EB tended to be higher in samples from body cavities with visible contamination, the differences between means were small and standard deviations large, which impaired the power of visual scores to predict total aerobic bacteria counts on the surfaces. However, there is a relationship between visible contamination of venison surfaces and bacterial numbers (e.g., Deutz et al. 2000, 2006; Paulsen et al. 2003).

We observed an increase in numbers of bacteria with duration from killing to sampling (cutting), which was not unexpected during the cold storage of carcasses (Bauer & Smulders 2015). Additional factors may influence bacteria growth, such as ambient temperature and humidity and intrinsic properties such as pH and the water activity of the meat surfaces (Bauer & Smulders 2015). If the carcasses at the AGHE are not strictly processed in a first-in first-out regime, the time from killing to cutting can range from 1 to >21 days (Paulsen et al. 2015). It can be argued that carcasses are still in-fur, protecting most meat surfaces from contamination, whilst the non-covered surfaces are prone to contamination. However, some venison cuts at the retail stage show high numbers of spoilage or hygiene indicator bacteria (Membré et al. 2011) that would probably not be tolerated in beef or pork. At skinning, soiled or moist fleece constitutes a further risk for the contamination of the fresh meat surfaces. Although most the furs of the roe deer carcasses were moist, there was little to no contamination by dirt or faeces, as is not infrequent in slaughter cattle from the same regions (Karhan et al. 2020).

The higher bacterial numbers in October were somewhat unexpected as the average temperatures in this month were not unusually high, whereas the temperatures in August were considerable higher than expected (ZAMG 2015).

It is hard to compare our results with those of other studies as some authors studied the exposed adductor muscles of the hind limb (Deutz et al. 2000; Avagnina et al. 2012) or freshly exposed meat surfaces after skinning (Kennedy 2014). The size of sampling areas was comparable to that used by Kennedy (2014) for red deer and Mirceta et al. (2017) for wild boar but larger than in other studies (e.g. Atanassova et al. 2008; Avagnina et al. 2012; Obwegeser et al. 2012). If une-

ven distribution of bacteria or low levels of contamination are suspected, large sampling areas should ensure a representative sampling technique (Gill et al. 2001). Likewise, the time from killing to sampling ranged from <1 to max. 3 days in most studies, whereas it was >3 days for 69 % of the carcasses in our study; this issue was also apparent in the subsequent LM analysis. Even in a region with similar hunting traditions, the pathways from killing to cutting can show considerable variations in terms of number of transports per carcass, ranging from 1 (directly from the hunt to AGHE) to 3 (from hunt to local cooling room, then to approved collecting sites and finally to AGHE), resulting in a variety of time-temperature combinations (Paulsen et al. 2015). In some studies, the pathway from killing to cutting is well characterized (Kennedy 2014), which can help to identify the major factors contributing to the bacterial contamination of wild game carcasses. More recently, Mirceta et al. (2017) studied wild boar carcasses at AGHEs and identified factors (with a focus on evisceration and skinning) that contribute to the contamination of wild boar carcasses.

Relation of location of shot wounds and cleanliness scores

We observed a significant relation for wound channels before the 13th rib and the presence of no or minor visible contamination, although there was no perfect agreement. Even a wound channel before the 7th rib (i.e. before the most cranial part of the diaphragm) can perforate the oesophagus and result in the efflux of ruminal content. Although one could expect that wounds between the 7th and the 13th rib would inflict the liver or the *reticulum*, this most likely will happen only when the wound is in the mid to ventral part of the thorax. Due to the convex shape of the *cupula diaphragmatica*, wounds near the spine behind the 7th rib can leave the diaphragm intact (see also Winkelmayer et al. 2005). Finally, contamination can occur during evisceration, when formerly intact bowels or stomachs are inadvertently punctured. If at least one flank was wounded (38 carcasses), the odds of contamination of the body cavities (i.e. cleanliness scores of 3 and 4) were 30/38 (0.79), whereas they were 67/314 (0.21) when the flanks were not wounded. Herbsthofer (2019) reported similar findings in roe deer.

In studies of the hygienic condition of wild game carcasses, it is more common to compare the location of shot wounds and bacterial surfaces numbers. Bacterial numbers on carcasses with "abdominal shots" are higher than those on carcasses with "expert" shots. The differences between bacterial numbers according to shot wound category are sometimes small (e.g. for Total Aerobic Count 0.4–0.6 log cfu; Avagnina et al. 2012; Mirceta et al. 2017) and not always significant (Deutz et al. 2000, 2006; Mirceta et al. 2017), leading to the conclusion that other processing factors may overshadow the effect of the location of the shot wounds (Mirceta et al. 2017) or that the counts might be influenced by the interaction of two or more factors. The comparison of visually assessed cleanliness and shot wound location may help explain this finding, insofar as the literature usually does not explicitly address the combination of "expert" shot wounds and the presence of visual contamination. The carcasses in such a condition should not be disregarded: we found 37/211 carcasses with shot wounds before the 7th rib to be in contamination classes 3+4 and 30/103 carcasses with shot wounds between the 7th and the 13th rib in contamination classes 3+4 (Table 2).

Overall significance of results

This study is not the first attempt to identify the factors that significantly contribute to the microbiological condition of wild game carcasses. Unlike other works (Deutz et al. 2006; Atanassova et al. 2008; Avagnina et al. 2012; Mirceta et al. 2017), we neither collected data from questionnaires or special shooting reports nor personally observed the hunting and in-field dressing but used only the data available during meat inspection. We had less information than other studies but this exactly reflected the level of information available in game handling establishments. In addition, studies with the active participation of hunters or the observation of their work in the field can introduce bias if hunters behave differently than usual. Our results confirm the association of abdominal shot wounds with visually assessed cleanliness of body cavities of roe deer, while also demonstrating the effect of the time from killing to cutting on the bacterial numbers on the exposed muscle surfaces of the carcass. This factor can be highly variable and – together with the temperatures in cooling facilities – needs to be taken into account in studies of the importance of hygiene practices or deficiencies on the number of bacteria on exposed carcass surfaces.

Acknowledgment
The study was supported by the "Verein Grünes Kreuz", Vienna, Austria. Thanks are due to Erich Schopf for technical assistance.

Fazit für die Praxis

Bei der Untersuchung von 352 Rehwild-Tierkörpern in einem zugelassenen Wildbearbeitungsbetrieb konnte ein statistisch signifikanter Zusammenhang von abdominalen Schusswunden und der visuell beurteilten Verschmutzung von Brust- und Bauchhöhle nachgewiesen werden. Für die Höhe der Gesamtkeimzahlen und Enterobacteriaceenzahlen in den Körperhöhlen war aber vorrangig die Dauer von der Erlegung bis zur Beprobung bzw. Fleischzerlegung entscheidend. Damit ist die Höhe der bakteriellen Kontamination von Rehwild-Tierkörpern nicht nur von den Bedingungen der Primärproduktion und damit der traditionellen Wildbrethygiene abhängig, sondern auch von der Dauer der nachfolgenden Kühlung. Dass *Salmonella* (-Antigen) in keiner der Proben nachgewiesen wurde, deckt sich mit Angaben aus der Literatur, nachdem dieses Pathogen bei (Wild-)Wiederkäuern selten auftritt. Das Vorkommen von *Listeria monocytogenes* kann durch Kontaminationsereignisse während Transport und Kühllagerung bedingt sein, was in weiteren Studien abgeklärt werden könnte.

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Please cite as:

Paulsen P, Smulders FJM, Kukleci E, Tichy A. Bacterial surface counts and visually assessed cleanliness of carcasses from hunted roe deer (*Capreolus capreolus*). Wien Tierarztl Monat – Vet Med Austria. 2022;109:Doc3.
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