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Microbial load of rinsed and unrinsed body cavities of roe deer (*Capreolus capreolus*) on the killing day and after cold storage: A preliminary investigation

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ABSTRACT

Ensuring good game meat hygiene is a challenge in the hunting supply chain. Game carcasses can be soiled with intestinal contents or other substances from the environment due to hunting and handling practices. This soiling can increase the microbial load (ML) of the carcass and the resulting game meat. The aim of this study was to investigate whether rinsing of soiled and unsoiled body cavities with drinking water can reduce the ML of carcasses. Carcasses of 23 roe deer (*Capreolus capreolus*) were processed, either rinsed (n = 12) or unrinsed (n = 11), and examined for ML. Swab and muscle samples were taken from the carcasses at killing day and after 3 days of cold storage. The levels of ML were comparable for the rinsed and unrinsed roe deer carcasses with an increase of *Pseudomonas* spp. during cold storage. Initial ML seems to be independent of visible soiling. Other factors affecting the initial ML should be determined in future studies.

1. Introduction

From a nutritional point of view, game meat is a valuable food with a low fat and high protein content (Hoffman & Wiklund, 2006). Game meat is gaining in popularity as consumers become search for a healthy, balanced, and regional diet that also takes into account ethical and sustainability aspects (AWA, 2021a, 2021b; IFAK Institut, 2021; Wongprawmas et al., 2021). Game meat consumption in Germany has increased by 25% from 2008 to 2015/2016 (DJV, 2017). At the same time, the number of hunting license holders in Germany increased by around 9% (DJV, 2021). These hunters are expected to place safe and hygienic game meat on the market in accordance with German and European food laws (Regulation (EC) No 178, 2002; Regulation (EC) No 853 (2004); Regulation (EC) No 852 (2004); Tier-LMHV, 2018), which require appropriate training of hunters in handling of the game meat.

Environmental and hunting conditions can hardly be standardized and pose a challenge for meat hygiene. The problem is compounded by differences in hunting and handling practices, which influence the microbial load (ML) of game meat, as well as the lack of data reporting. An example of these different hunting and handling practices is the multitude of recommended interventions following the soiling of game carcasses with intestinal contents due to an improper shot or during evisceration. Several studies have found higher bacterial counts in game killed by a shot to the abdominal region than in game killed by a proper shot to the thoracic region (Avagnina et al., 2012; Bandick & Ring, 1995; Lenze, 1977). It has been hypothesized that higher bacterial contamination, particularly with pathogens (Frank et al., 2019) follows from the presence of visible soiling with intestinal contents on the meat. Different interventions are recommended for the removal of soiling in the literature, i.e. guidelines for hunters, and books on good game meat hygiene. For example, some guidelines for hunters recommend rinsing only if there is visible soiling of the carcass (Amt für Landschaft und Natur,

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2019; Rheinisch-Westfälischer Jäger, 2017). Others recommend a general rinsing of all game carcasses (Bildungs- und Wissenszentrum Aulendorf et al., 2008; Deutz, 2012a). The rinsing process may vary depending on the device used in terms of water pressure (Anonymous, 2015). Another intervention option is the removal (trimming) of soiled parts from the carcass (Deutz, 2012b; Van Schalkwyk, 2010) as well as removal of the diaphragm together with the inner abdominal skin (serosa, *Peritoneum parietale*) of soiled body cavities (Kujawski & Heintges, 1984; Scherling, 1989). These measures, based on individual experience of the hunters, are performed with the aim of reducing the initial ML and thus of improving the shelf life of game meat. No information is available on the impact or efficiency of these measures on game meat quality. Rinsing game carcasses, as opposed to the removal of soiled parts, may improve game carcass processing since the removal of the contaminated serosa can result in an increased loss of moisture and thus reduce meat yield. However, the newly exposed inner meat surface could be re-contaminated during subsequent transport (Hadlok & Bert, 1988; Kappelhoff, 1999). This contamination with e.g. plant material or soil particles could have an additional negative influence on the game meat quality.

Recommended carcass interventions can have positive or negative effects on game meat quality and carcass yield. This depends on the initial situation, the implementation of the intervention as well as further handling of the carcass. The effect of rinsing was investigated in this study since it is more frequently discussed in the literature and guidelines for hunters in Germany than any other intervention regarding its advantages and disadvantages for game meat quality (Amt für Landwirtschaft & Havelland, 2007; Bildungs-und Wissenszentrum Aulendorf, 2008; Deutz, 2012a; Pegel & Schreiber, unknown; Rheinisch-Westfälischer Jäger, 2017).

In the present study, we tested the hypothesis of whether rinsing of the body cavity of a game carcass affects the microbial load of the carcass and/or the edible meat.

2. Materials and methods

2.1. Study design and sampling

A total of 23 hunted roe deer (*Capreolus capreolus*) carcasses were investigated between October 2020 and the end of January 2021, collected from group, stalking, or drive hunts in Brandenburg, Germany. The roe deer were shot on hunting grounds administered by the German Federal Institution for Real Estate (BImA). Hunts were organized by the German Federal Forestry Service with the intention of hunting for human consumption and wildlife management. Information about the animals and the hunting conditions was recorded. The data collected included sex, estimated age, type of hunting, shot accuracy, position of the carcass at evisceration, visible soiling (with intestinal content, plant material, blood, fur), time of killing, evisceration, transport, when the carcass was handed over to the sampling personnel, time of sampling in the field, transport to the research facility, sampling at the research facility and start of the cold storage at +4 °C for 3 days.

Prior to the hunts, a randomized list was prepared according to which the roe deer carcasses were to be either rinsed or unrinsed to prevent sampling bias. After the end of the hunt, samples were taken from different areas of the roe deer carcasses after being hung headlong on a game gallows (Fig. 1).

The meat surface of the belly flaps (*M. obliquus internus abdominis*) and the fillets (*M. psoas major*) were sampled in the center of the mentioned body cavity part three times for rinsed carcasses and two times for unrinsed carcasses. The first sampling of the meat surface of belly flap and fillet (sampling point I) on one half of the carcass was only executed of roe deer to be rinsed in the field. The entire inner surface of the body cavity was then rinsed and the carcass left hanging for 20 min to allow the rinsing water to drain more easily over the head of the roe deer carcass. The rinsing was performed with water of drinking quality from a low-pressure outdoor cleaner (Fontus, Bosch, Gerlingen, Germany). The water spray pressure from the head nozzle resembled a weak spray from a showerhead (pressure setting 1, 1460 ml/min). Each roe deer carcass was rinsed until all visible soiling (intestinal content, blood, fur, plant material) was removed, as is common practice. The amount of water used varied from 730 to 2400 ml (calculated by multiplying the rinse time and the water flow rate). Samples were again taken from rinsed carcasses from the other half of the body cavity (sampling point II), to avoid repeated sampling of the same location, and for the first time from unrinsed carcasses. The carcasses were then transported to the research facility, where meat surface swabs and muscle samples of the leg (*M. adductor longus*) and back area (*M. longissimus thoracis*) were

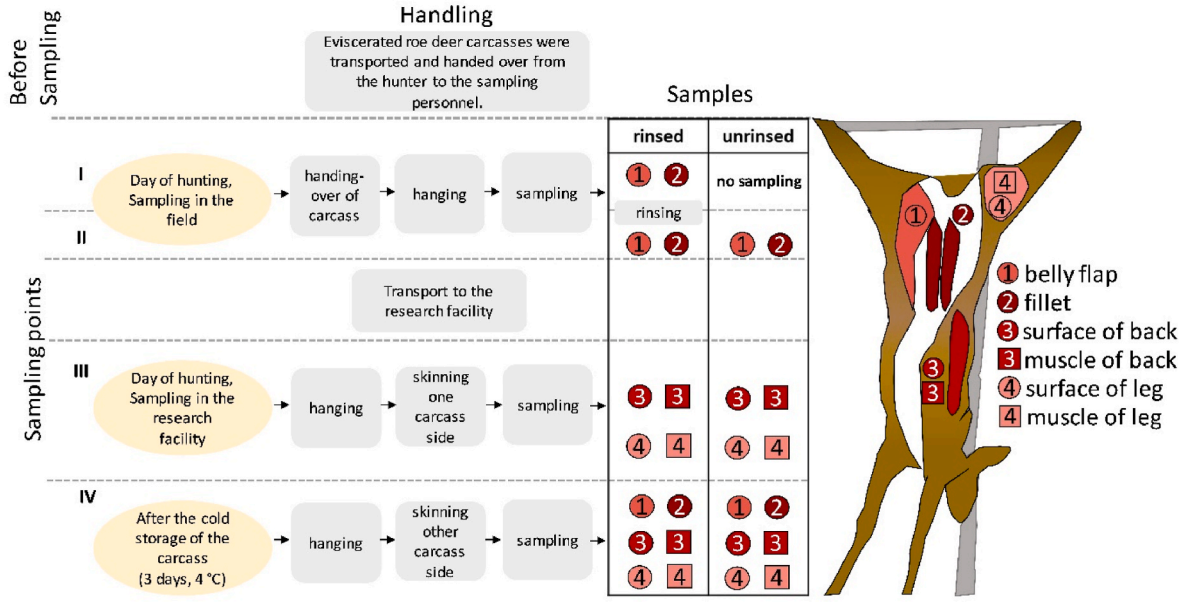

Fig. 1. Study design and sampling of rinsed and unrinsed roe deer carcasses. Gray fields indicate the handling process. The yellow ovals mark the sampling points. The sampled areas of the roe deer carcasses were numbered (1 – 4, meat surface samples were arranged in circular fields and muscle samples in square fields) and assigned to sampling points (I – IV) of the rinsed and unrinsed carcasses.

collected after manual skinning of one half of the carcass for both the rinsed and the unrinsed group (sampling point III). The skin remained on the other half of the carcasses during cold storage. After 3- days of cold storage at +4 °C (meat maturation), samples of belly flaps and fillets were taken from rinsed and unrinsed carcasses (sampling point IV), alternating the body half used. Meat surface swabs and muscle samples of the leg and back were taken from the other carcass half after skinning.

2.2. Sampling procedure and preparation of the swab and muscle samples

Meat surface samples of the body cavity and from the freshly skinned surface of the carcasses (leg and back) were taken with a moistened swab (3.8 × 7.6 cm; 3M Sponge-Stick, Mercateo Deutschland AG, Munich, Germany), followed by a dry swab (16 × 152 mm, Greiner Bio-One cotton swab, Altmann Analytik GmbH & Co. KG, Munich, Germany) for each area according to ISO 17604:2015. The sampling area was 50 cm^2 for belly flaps, and freshly skinned surface of the carcasses and 20 cm^2 for fillets. The moistened and dry swabs of each roe deer carcass area were combined in a sterile bag to form a single sample. Sampling of back and leg muscles was conducted after flaming and sterile removal of the muscle surface taking a deep muscle sample of approximately 50 g in accordance with ISO 6887-2:2017. The swab samples or 10 g of muscle samples were diluted with 90 ml diluent (Maximum Recovery Diluent for microbiology, Merck, Darmstadt, Germany) according to ISO 6887-1:2017 and homogenized using a bag mixer (BagMixer® 400, step 3, 120 s, Interscience, Saint Nom, France).

2.3. Microbiological analyses

The total aerobic colony count was determined according to DIN ISO 4833-2:2014 on Plate Count Agar (Carl Roth, Karlsruhe, Germany), *Pseudomonas* spp. were quantified following specifications of the manufacturer on Pseudomonas/Aeromonas selective agar (Sigma-Aldrich, Darmstadt, Germany), *Lactobacillus* spp. were quantified in accordance with DIN 10109:2017 on de Man Rogasa and Sharpe agar (Carl Roth, Karlsruhe, Germany). Total aerobic colony count, *Pseudomonas* spp. and *Lactobacillus* spp. were analyzed by the spread plate method and after aerobic incubation for 72 h at +30 °C. Prior to the calculation of the number of *Pseudomonas* spp. presumptive colonies were confirmed by oxidase testing (ROTITEST®Oxidase strips, Carl Roth, Karlsruhe, Germany). *Enterobacteriaceae* were analyzed in accordance with DIN 10164:2019 with the spread plate method on Violet Red Bile Dextrose agar (Merck, Darmstadt, Germany) after anaerobic incubation for 24 h at +37 °C. Determination of *Escherichia coli* was done in accordance with DIN ISO 16649-2:2010 by using the pour plate method in Tryptone Bile X-glucuronide Agar (Carl Roth, Karlsruhe, Germany) after incubation for 24 h at +44 °C. Finally, all bacterial counts were calculated per surface of the swab samples in $\log_{10}$ CFU/cm^2 and for the muscle samples in $\log_{10}$ CFU/g.

2.4. Statistical analyses of data

The information on the animals, possible influencing factors of hunting and the environmental factors were summarized descriptively using SPSS Software version 26 (IBM, Ehningen, Germany). The relevant time spans for handling of the carcass were related to the time of killing using Microsoft Office Excel (Microsoft® Office Professional Plus 2016; Microsoft Corporation, Redmond, USA) and box plots were prepared using SigmaPlot 14.0 (Inpixon GmbH, Düsseldorf, Germany). These relative times were compared with the variability of the handling conditions during the hunting supply chain for the rinsed and unrinsed carcasses by using a *t*-test ($p < 0.05$) with the SPSS Software. Charts were created with Microsoft Office PowerPoint, SigmaPlot 14.0 or GraphPad Prism 8.2.0 (GraphPad Software, San Diego, USA). Statistical analysis of the ML data was performed using SAS 9.4, 2016 (SAS Institute GmbH, North Carolina, USA). Results are presented as Least Squares Means (LS mean) ± standard error (SE) or as dot plots. Logarithmic transformation was used to ensure a normal distribution. In the LS mean calculation, the values below the limit of detection (LOD) were replaced by zero. ML data were analyzed using a mixed model with rinsing group (rinsing), visible soiling with intestinal content (soiling), and sampling point (time II vs. IV (all) or I – IV (only rinsed)) as fixed effects and individual roe deer as a random effect.

3. Results

3.1. Animals and possible influencing hunting and environmental factors

In the hunting season 2020/21, roe deer were shot on 14 hunting days during group, stalking, or drive hunts in Brandenburg, Germany. A

Table 1
Information on rinsed (n = 12) and unrinsed (n = 11) roe deer carcasses and possible influencing factors from hunting and the environment.

Parameter	Category	Rinsed	Unrinsed
Sex	Male	3 (25%)	4 (36%)
	Female	9 (75%)	6 (55%)
	No data	–	1 (9%)
Age (estimated)	Under 1 year	4 (33%)	4 (37%)
	1 - 2 years	3 (25%)	3 (27%)
	Above 2 years	5 (42%)	3 (27%)
	No data	–	1 (9%)
Type of hunting	Drive hunt	12 (100%)	7 (64%)
	Sitting game hunt in a group	–	1 (9%)
	Stalking	–	3 (27%)
Shot accuracy	Damage to the gastrointestinal tract	4 (33%)	1 (9%)
	No damage to the gastrointestinal tract	8 (67%)	10 (91%)
Position of game	Hanging	1 (8%)	1 (9%)
during evisceration	Lying on the ground	11 (92%)	10 (91%)
Visible soiling with intestinal content[a]	Yes	4 (33%)	4 (36%)
	No	8 (67%)	7 (64%)
Visible soiling with plant material	Yes	1 (8%)	2 (18%)
	No	11 (92%)	9 (82%)
Visible soiling with blood	Yes	2 (17%)	5 (46%)
	No	10 (83%)	6 (54%)
Visible soiling with fur	Yes	3 (25%)	1 (9%)
	No	9 (75%)	10 (91%)

[a] The visible soiling of the carcasses with intestinal content was influenced by both the shot accuracy and the handling process.

total of 23 roe deer with an eviscerated bodyweight mean of 13.2 ± 0.6 kg were examined. Based on local routine, roe deer carcasses were eviscerated by the hunter either hanging or lying on the ground before the carcasses were handed over to the sampling person. The *postmortem* body temperature mean value during the sampling in the field (sampling II, Fig. 1) was 25.7 ± 0.8 °C. Additional information was collected on roe deer carcasses (Table 1).

The handling processes and sampling points were defined (Fig. 1), but the resulting time spans relative to the time of killing during the hunting supply chain are mostly externally influenced and could therefore not be standardized. Despite randomized grouping, some relative time spans differed significantly between rinsed and unrinsed carcasses for evisceration, handover, sampling in the research facility, and start of the cold storage at +4 °C (Fig. 2).

3.2. Comparison of microbial loads in rinsed and unrinsed roe deer carcasses

3.2.1. Microbial load of the body cavity

In a few cases, the after-rinse sample swabs of 12 roe deer carcasses appeared soaked with blood when compared with the initial swab samples. Among 11 roe deer carcasses sampled without rinsing, four were visibly soiled with intestinal contents. Of the total of four soiled, unrinsed carcasses, the shot channels and visible soiled parts of two carcasses were trimmed by the hunters after evisceration in deviation from the study specifications.

Microbial load (ML) was used as a comprehensive term for the total aerobic colony count, the counts of *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae*, and *E. coli*. On rinsed and unrinsed carcasses, the initial ML and ML after meat maturation of belly flap samples from soiled carcasses had a lower ML LS mean than the unsoiled carcasses (Supplementary Tables S1 and S2). There was a trend for higher levels of *Lactobacillus* spp. in rinsed belly flap than in unrinsed (sampling point II vs. IV, Fig. 3 C). The total aerobic colony count and counts of *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae*, and *E. coli* on belly flap surfaces from soiled carcasses were 4.70 ± 0.39 $\log_{10}$ CFU/cm^2, 2.51 ± 0.72 $\log_{10}$ CFU/cm^2, 2.56 ± 0.31 $\log_{10}$ CFU/cm^2, 2.32 ± 0.43 $\log_{10}$ CFU/cm^2 and 1.82 ± 0.42 $\log_{10}$ CFU/cm^2, respectively. The count levels on unsoiled carcasses were 5.40 ± 0.36 $\log_{10}$ CFU/cm^2, 3.76 ± 0.71 $\log_{10}$ CFU/cm^2, 3.34 ± 0.23 $\log_{10}$ CFU/cm^2, 3.44 ± 0.39 $\log_{10}$ CFU/cm^2, and 2.53 ± 0.38 $\log_{10}$ CFU/cm^2, respectively (Fig. 3 A - E). The level of *Enterobacteriaceae* on fillets (Fig. 4) was lower in soiled carcasses (2.50 ± 0.44 $\log_{10}$ CFU/cm^2) than in unsoiled carcasses (3.45 ± 0.41 $\log_{10}$ CFU/cm^2).

Since visible soiling of the carcass with intestinal contents was found to be one of the most relevant factors influencing the initial ML, it was included as a fixed parameter in the statistical model. On rinsed carcasses with soiling, the number of *Pseudomonas* spp. on the belly flaps tended to be lowest, whereas unrinsed and unsoiled belly flaps showed the highest numbers (Fig. 3 B). The same interaction was observed for *Pseudomonas* spp. on fillet (Fig. 4 B). The counts of *Pseudomonas* spp. on belly flaps tended to be lower after rinsing and ranged from 3.04 ± 0.3 $\log_{10}$ CFU/cm^2 to 2.48 ± 0.3 $\log_{10}$ CFU/cm^2; the counts were higher after cold storage for all carcasses. In rinsed carcasses, the levels of *Enterobacteriaceae* tended to decrease over time during cold storage on the belly flap (Fig. 3). An assignment of initial time point of unrinsed (time II) to initial time point of rinsed (time I) was also analyzed with a mixed model as described and resulted in comparable findings but with less information about rinsing.

3.2.2. Microbial load of the skinned carcass surface and muscle samples

The ML for most muscle samples was below the limit of detection (LOD, Supplementary Tables S3 and S4). Very high variations of initial bacterial counts were determined on leg and back meat surfaces after skinning on the day of hunting and after cold storage. The total aerobic colony count ranged from below the LOD to a maximum of 6.1 $\log_{10}$ CFU/cm^2 on the leg meat surface of skinned carcasses (Fig. S1) and a maximum of 5.6 $\log_{10}$ CFU/cm^2 on the skinned back meat surface (Fig. S2). Time of cold storage influenced the total aerobic colony count on backs of rinsed and unrinsed skinned carcasses. After meat maturation, the total aerobic colony count tended to be lower in rinsed carcasses with soiling (n = 3).

4. Discussion

4.1. Microbial load of meat surface and meat samples of rinsed and unrinsed carcasses

Since the initial ML of the meat surface samples had a widely scattered LS mean within a small sample size, examining the effects of

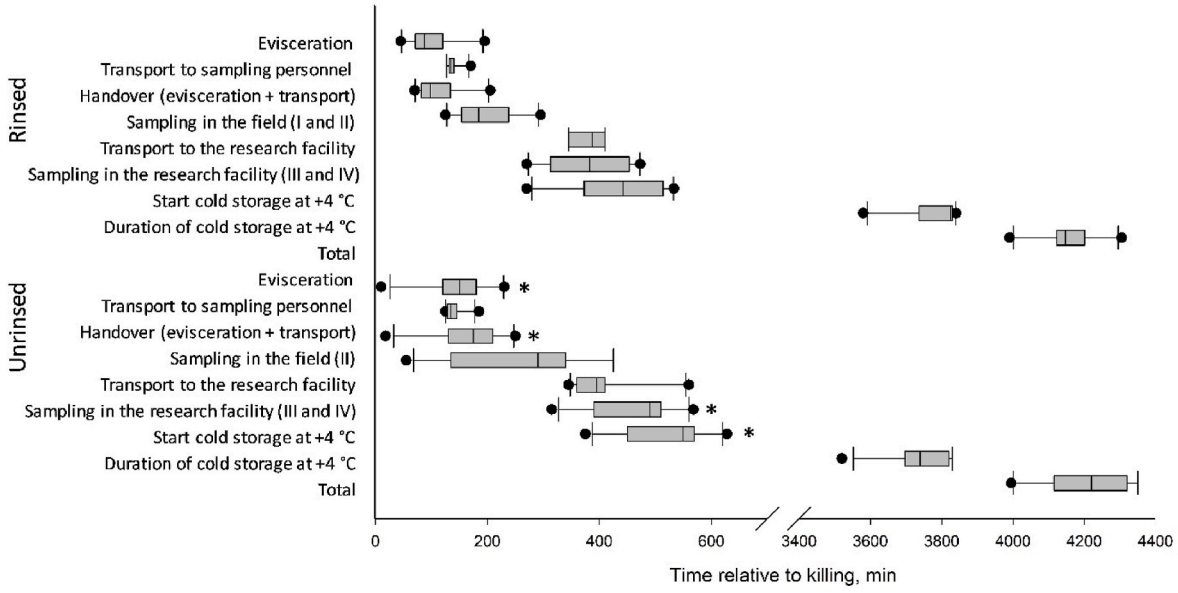

Fig. 2. Comparison of the relative time spans during the hunting supply chain of randomly assigned rinsed (n = 12) and unrinsed (n = 11) roe deer carcasses using *t*-test for independent variables. All time data were set in relation to the killing time (time = 0). A star (*) indicates statistically different mean values ($p < 0.05$).

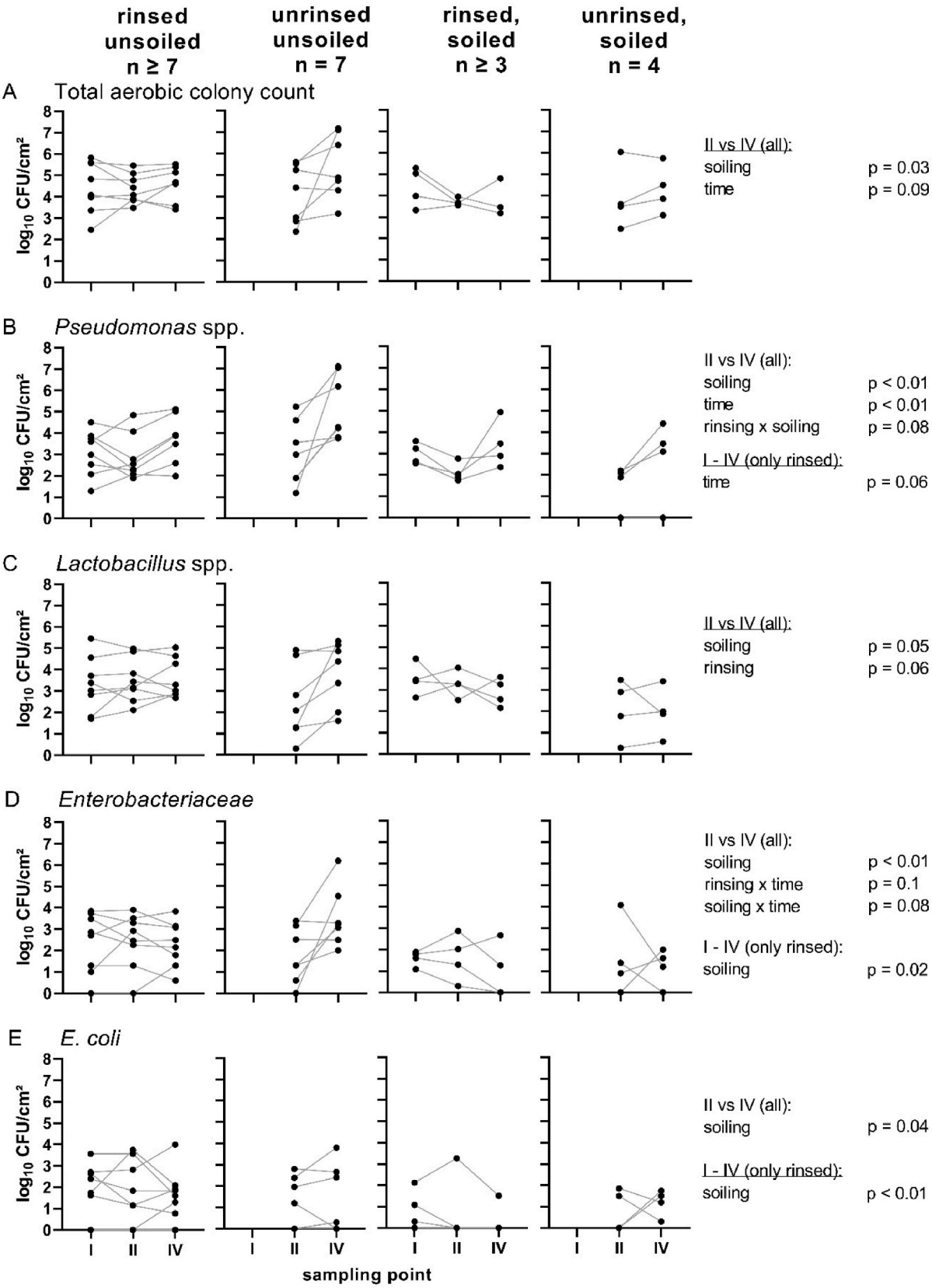

Fig. 3. Belly flaps. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp. C: *Lactobacillus* spp. D: Enterobacteriaceae, E: *E. coli*) on the meat surface of belly flaps of rinsed and unrinsed carcasses at the sampling points: before rinsing (I), after rinsing or not rinsing (II), and after cold storage for 3 days at +4 °C (IV) respectively. The rinsed and unrinsed groups were classified as carcasses with and without "soiling" by intestinal contents and by the "time" of sampling points. Statistics comparing sampling points II and IV for group "all" or sampling points I, II and IV for rinsed roe deer. The values are presented for individual carcasses; values below the limit of detection are given as 0.

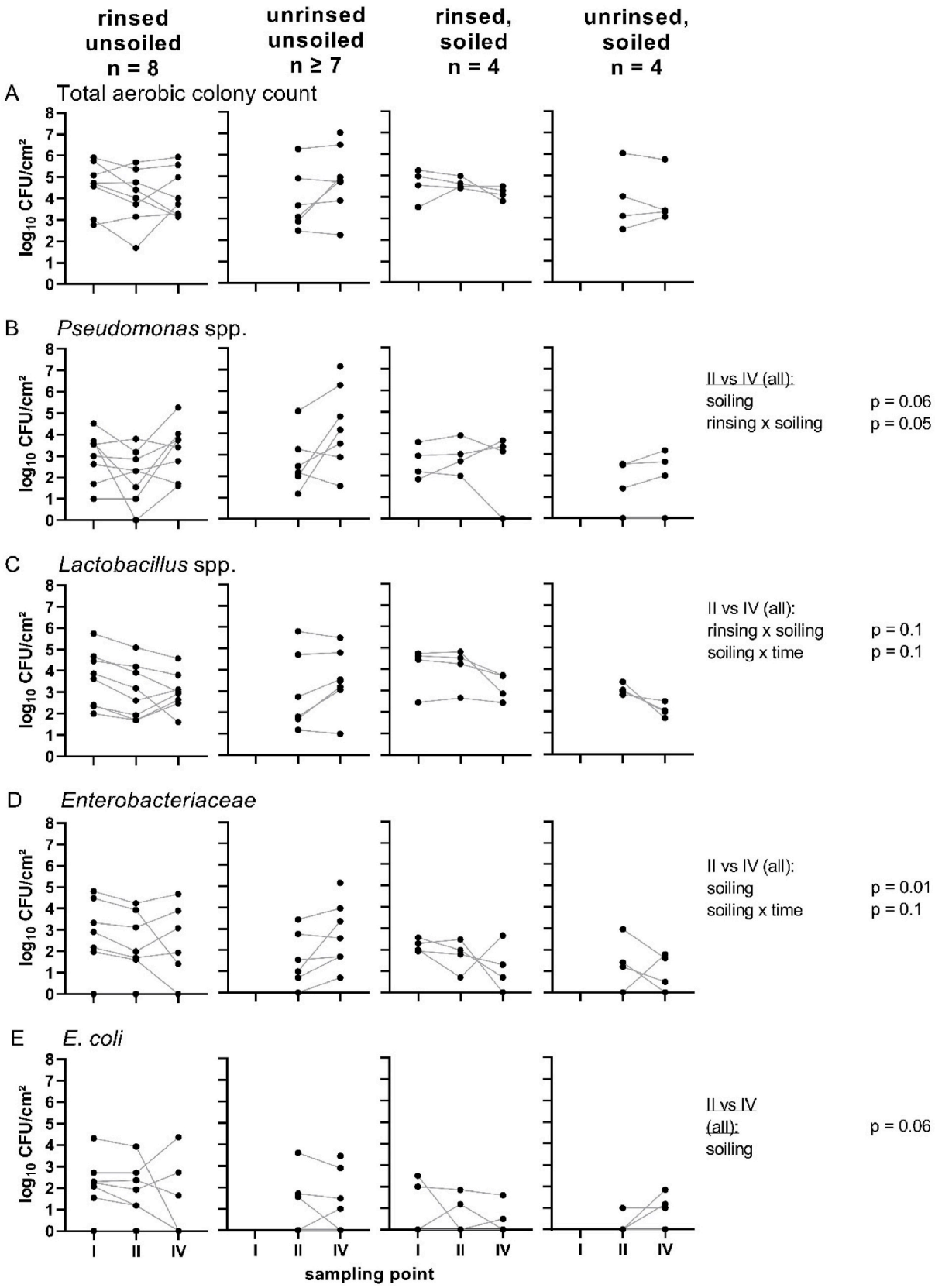

Fig. 4. Fillets. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp. C: *Lactobacillus* spp. D: Enterobacteriaceae, E: *E. coli*) on the meat surface of fillets of rinsed and unrinsed carcasses at the sampling points: before rinsing (I), after rinsing or not rinsing (II), and after cold storage for 3 days at +4 °C (IV) respectively. The rinsed and unrinsed groups were classified as carcasses with and without "soiling" by intestinal contents and by the "time" of sampling points. Statistics comparing sampling points II and IV for group "all" or sampling points I, II and IV for rinsed roe deer. The values are presented for individual carcasses; values below the limit of detection are given as 0.

rinsing was challenging for all bacterial species studied. It was observed that a) the initial level of *Pseudomonas* spp. on belly flaps tended to be lower after rinsing and b) increased during cold storage. A comparable observation was made by Orsoni et al. (2020), who found that the total aerobic colony count increased faster in unrinsed carcasses than rinsed carcasses 160 h after evisceration and cold storage in a game handling establishment (no storage temperature information was provided), although the initial bacterial count was lower on average in unrinsed and unsoiled carcasses than in rinsed carcasses than in the present study. The higher bacterial load in the body cavities of unrinsed carcasses could be related to blood that has dried on the surface of the body cavity during cold storage, which can lead to higher bacterial growth and consequently result in a reduced meat quality or shelf life (Casoli et al., 2005; Sofos, 2014). Blood provides an excellent environment for bacterial growth. Bacteria of concern for meat quality include those bacterial species that can survive and multiply during the meat maturation process e.g. pseudomonads, lactic acid bacteria and cold-tolerant *Enterobacteriaceae* (Sofos, 2014). Noticeable spoilage of meat usually starts at mesophilic bacterial counts of 6 – 7 $\log_{10}$ CFU/cm^2 (BfR, 2006; Paulsen, 2019) as observed in the present study at such bacterial counts in individual body cavity samples that were unrinsed.

Unexpectedly, lower initial microbial counts were found on all surfaces of roe deer body cavities that were soiled with intestinal contents compared to unsoiled carcasses. This finding was similar to a study by Paulsen & Schopf, 2016, where the microbiological condition of roe deer carcasses was examined in relation to the presence of visible soiling (aerobic mesophilic count, *Enterobacteriaceae*) (Paulsen & Schopf, 2016). These carcasses were divided into four groups (no contamination; single green particles; clearly visible fecal soiling of about 2 cm in diameter, max. 1/8 of the thoracic and abdominal cavity soiled; higher degree of soiling or putrefaction) and it was found that the carcasses appearing visually clean showed high surface microbial counts in some cases (Paulsen & Schopf, 2016). No significant relationship was found between surface microbial counts and visual assessments of carcasses in that study (Paulsen & Schopf, 2016). The soiling with intestinal contents was considered more in detail, because this contamination was more evenly distributed on the meat surface. This could be reflected by changes in initial ML. Soiling with plant material, blood and fur appeared more in spots and could only be randomly caught by the systematic sampling method. Visible soiling of the body cavity with intestinal contents is apparently not necessarily associated with higher bacterial concentration. Therefore, other parameters than just the visual classification of soiling are needed to assess the initial bacterial load of freshly killed game.

Factors causing the higher initial bacterial counts on rinsed compared to unrinsed carcasses in the present study and in the study by Orsoni et al. (2020) could be an actually higher initial ML of the game carcasses, or an improved transfer of bacteria to the swab from wet and dirty hides of the rinsed carcasses than from dry and dirty hides, which has been described for slaughtered animal carcasses (Blagojevic et al., 2012). Furthermore, the rinsing water may lead to bacterial cross-contamination to other areas of the body cavity. For the present study, this could indicate a higher bacterial recovery from rinsed carcass surfaces on the hunting day than from unrinsed carcass surfaces (sampling point II) or from carcass surfaces dried after cold storage (sampling point IV), leading to higher levels of ML for the freshly rinsed meat surfaces.

As the roe deer carcasses generally showed a low initial bacterial load in the meat samples, no effects of rinsing on meat quality can be assumed. However, in this study, this does indicate the very high microbial quality of game meat. Additionally, the study ended with three days of cold storage at the relatively low temperature of +4 °C. Longer storage or higher temperatures during storage may impair the outcome. This study was performed during winter, which can be considered as a low risk scenario for bacterial growth. Game meat must be stored below +7 °C (Regulation (EC) No 853, 2004; Tier-LMHV, 2018), but even then several bacterial species can grow and have an influence on meat quality and therefore lower temperatures are preferable (Maahs, 2010).

4.2. *Influencing factors and conditions of the carcass on the microbial load of body cavities of rinsed roe deer carcasses*

Different rinsing parameters (e.g. water temperature, pressure and flow rate of the water) or carcass conditions (e.g. *postmortem* body temperature, occurrence and extent of soiling, position of the carcass during rinsing) can affect the effectiveness of ML reduction, as has been described in articles on slaughtered animal carcasses (Gill, 2004; Kotula et al., 1974). An example of different effects of rinsing of wild boar carcasses in relation to rinsing parameters and carcass conditions was reported by Mirceta et al. (2017). In that study, a portion of the samples was collected from wild boar carcasses in the field that were rinsed after evisceration with a high-pressure outdoor cleaner while lying on the ground. Another group of wild boar carcasses was sampled after transport to a game handling establishment where the carcasses were eviscerated while hanging and then rinsed. Mirceta et al. (2017) compared the bacterial counts of field-collected samples and found significantly higher total bacterial counts and *Enterobacteriaceae* counts on the wild boar carcasses when they were rinsed on the ground after evisceration (5.8 $\log_{10}$ CFU/cm^2 and 4.1 $\log_{10}$ CFU/cm^2), in contrast to the samples that were collected without rinsing (5.2 $\log_{10}$ CFU/cm^2 and 3.6 $\log_{10}$ CFU/cm^2). The bacterial counts of wild boar carcasses rinsed hanging in the game handling establishment was described as having, on average, lower total bacterial counts and *Enterobacteriaceae* counts (4.3 $\log_{10}$ CFU/cm^2 and 2.3 $\log_{10}$ CFU/cm^2) than carcasses rinsed lying on the field (6.0 $\log_{10}$ CFU/cm^2 and 4.4 $\log_{10}$ CFU/cm^2) (Mirceta et al., 2017). Those bacterial counts of hanged, rinsed carcasses were similar to the results in this study. The position of the game carcass during rinsing and the resulting amount of rinsing water remaining in the body cavity can affect the ML. Mirceta et al. (2017) hypothesized that the higher bacterial counts of carcasses rinsed lying on the ground in the field were due to increased aerosol formation through rinsing with a high-pressure outdoor cleaner. The rinsing in this study was done with a low pressure outdoor cleaner and could be a reason for the difference. Mirceta et al. (2017) did not describe the water quality. In the present study, the low-pressure outdoor cleaner was cleaned before each hunt and water samples were analyzed to ensure drinking water quality and to avoid biofilm formation. It is to be assumed that the quality and condition of the rinsing water will have an influence on the rinsing effect and it therefore needs to be monitored.

The time between killing and evisceration of carcasses is also thought to influence ML and meat quality, but several articles could not show a significant correlation between ML and the time between killing and evisceration time points in roe deer (Avagnina et al., 2012), red deer (Soriano et al., 2016) or wild boar (Orsoni et al., 2020; Peruzy et al., 2022). In contrast, Branciari et al., 2020 reported a significant effect of the time elapsed between killing and evisceration of roe deer carcasses on the total aerobic colony count (Branciari et al., 2020). It was assumed that the ML would rise with time. In this study, the unrinsed carcasses were eviscerated after killing later than the rinsed carcasses and as a result of that also the handover or the start of the cold storage of the unrinsed carcasses occurred later. These differences resulted from hunting practice and not from the rinsing process. Although unrinsed carcasses were eviscerated later, the detected initial ML was lower in the unrinsed carcasses than in rinsed carcasses. Beside the rinsing process, there are several unknown factors that can affect the initial ML. In addition to the influence of environmental or handling factors on bacterial load (Branciari et al., 2020), the impact of *premortal* stress on pH, water holding capacity, water content, and color of roe deer carcasses has been shown to be an influencing factor (Tomljanović et al., 2022).

5. Conclusions

In this study, the impact of the rinsing of the body cavity of eviscerated roe deer carcasses on game meat hygiene and quality was examined based on the ML. It is challenging to make a clear and general recommendation for rinsing game body cavities with defined rinsing parameters. The initial ML of unrinsed carcasses was lower than of rinsed carcasses. However, bacterial counts tended to be higher in unrinsed carcasses than in rinsed carcasses after cold storage.

Adequate estimation of the initial ML would be required to predict the effect of rinsing on bacterial contamination on game carcasses. Factors affecting the initial ML during the hunting supply chain should be identified using information on environmental, hunting and handling practices. Bacterial counts may increase with higher outside temperatures, delayed cooling or ineffective air flow to cool carcasses due to delayed salvage, evisceration, or transport of carcasses. Factors that increase the bacterial counts of game carcasses could mask the reducing effect of the rinsing process. For example, when the carcass is trimmed, contamination can be spread to other areas of the carcass meat surface. Therefore, carcass rinsing should be considered and examined in the context of the aforementioned factors.

To ensure the safety and hygiene of game meat, the hunter must be aware of several hurdles in the hunting supply chain. Removing contamination from game carcasses by rinsing is part of the "from farm to fork" principle for game meat hygiene. Further parameters need to be determined before, during, and after the rinsing process to achieve the best possible efficacy in reducing bacterial counts in future studies.

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CRediT authorship contribution statement

B. Korkmaz: Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **F. Reich:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision. **T. Alter:** Conceptualization, Methodology, Writing – review & editing, Supervision. **J. Steinhoff-Wagner:** Methodology, Software, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **D. Maaz:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **C. Gremse:** Conceptualization, Investigation, Writing – review & editing. **A. Haase:** Investigation, Writing – original draft, Writing – review & editing, Visualization. **A. Mader:** Methodology, Validation, Investigation, Resources, Writing – review & editing, Project administration. **H.A. Schafft:** Conceptualization, Writing – review & editing. **N. Bandick:** Conceptualization, Resources, Writing – review & editing. **K. Nöckler:** Conceptualization, Resources, Writing – review & editing. **M. Lahrssen-Wiederholt:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability statement

The datasets generated for this study are available upon reasonable request from the corresponding author.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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References

Amt für Landschaft und Natur, F.-u. J. H. (2019). *Leitfaden für Jägerinnen und Jäger, Selbstkontrolle zur Wildbrethygiene*. Retrieved 28.09.2021 from https://aln.zh.ch/internet/baudirektion/aln/de/fjv/Jagd/wildbrethygiene/_jcr_content/contentPar/downloadlist/downloaditems/169_1553864685118.spooler.download.1553864843168.pdf/20190326_Leitfaden_Selbstkontrolle_zur_Wildbrethygiene_Version_1_0_FJV.pdf.

Amt für Landwirtschaft, V.-u. L., & Havelland, L. (2007). *Hygienevorschriften beim Umgang mit Wildbret*. Retrieved 25.08.2020 from https://www.havelland.de/fileadmin/dateien/amt83/Formulare/Hygienevorschriften_beim_Umgang_mit_Wildbret_20071212155927.pdf.

Anonymous. (2015). *Jäger, revierpraxis, wasser marsch, praxistest* (Vol. 9). Wild und Hund.

Avagnina, A., Nucera, D., Grassi, M. A., Ferroglio, E., Dalmasso, A., & Civera, T. (2012). The microbiological conditions of carcasses from large game animals in Italy. *Meat Science, 91*(3), 266–271. https://doi.org/10.1016/j.meatsci.2012.01.025

Awa, A. M.-u. W. (2021a). *Anzahl der Personen in Deutschland, die beim Einkauf regionale Produkte aus der Heimat bevorzugen, von 2017 bis 2021 (in Millionen)*. Statista. Retrieved 02.01.2022 from https://de.statista.com/statistik/daten/studie/264557/umfrage/kaeufertypen-bevorzugung-von-produkten-aus-der-region/.

Awa, A. M.-u. W. (2021b). *Interesse der Bevölkerung in Deutschland an gesunder Ernährung und gesunder Lebensweise von 2016 bis 2020 (Personen in Millionen). IfD Allensbach*. Retrieved 24.06.21 from https://de.statista.com/statistik/daten/studie/170913/umfrage/interesse-an-gesunder-ernaehrung-und-lebensweise/.

Bandick, N., & Ring, C. (1995). Zur Bedeutung exogener Einflussfaktoren auf den Hygienestatus von Reh-und Schwarzwildbret. In *Conference Paper presented in DVG, Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene* (Garmisch-Partenkirchen, Germany).

BfR. (2006). *Leitfaden für die sensorische Untersuchung und Beurteilung von Wild; Stellungnahme Nr. 047/2006 des BfR vom 28.06.2006*. Retrieved 25.08.2020 from https://www.bfr.bund.de/cm/343/leitfaden_fuer_die_sensorische_untersuchung_und_beurteilung_von_wild.pdf.

Bildungs- und Wissenszentrum Aulendorf, V., Grunlandwirtschaft, Wild, Fischerei. (2008). Wildbrethygiene, Begleitheft zur landeseinheitlichen Schulung „Kundige Person" nach EU-Recht (B. S. VeBu Druck + Medien GmbH, Ed.) https://docplayer.org/263197-Wildbrethygiene-begleitheft-zur-landeseinheitlichen-schulung-kundige-person-nach-eu-recht.html.

Blagojevic, B., Antic, D., Ducic, M., & Buncic, S. (2012). Visual cleanliness scores of cattle at slaughter and microbial loads on the hides and the carcases. *The Veterinary Record, 170*(22). https://doi.org/10.1136/vr.100477, 563-563.

Branciari, R., Onofri, A., Cambiotti, F., & Ranucci, D. (2020). Effects of animal, climatic, hunting and handling conditions on the hygienic characteristics of hunted roe doer (caprelous capreolus L.). *Foods, 9*(8), 1076. https://doi.org/10.3390/foods9081076

Casoli, C., Duranti, E., Cambiotti, F., & Avellini, P. (2005). Wild ungulate slaughtering and meat inspection. *Veterinary Research Communications, 29*(2), 89–95. https://doi.org/10.1007/s11259-005-0028-y

Deutz, A. (2012a). Die 10 gebote der Wildbrethygiene. In *Wildbrethygiene heute, beurteilung, versorgung, rechtslage*. BLV Buchverlag GmbH & Co.KG.

Deutz, A. (2012b). Optische beurteilung von Schalenwild in der Decke. In *Wildbrethygiene heute, beurteilung, versorgung, rechtslage*. BLV Buchverlag GmbH & Co.KG.

DJV, D. J.e. V. (2017). *Pressemitteilung des Deutschen Jagdverbands: Wildfleisch liegt im Trend*. Retrieved 25.08.2020 from https://www.jagdverband.de/wildfleisch-liegt-im-trend.

DJV, D. J.e. V. (2021). *Anzahl der Jagdscheininhaber in Deutschland in den jagdjahren 1968/69 bis 2019/20*. Retrieved 2021 from https://de.statista.com/statistik/daten/studie/161126/umfrage/anzahl-der-jagdscheininhaber-in-deutschland-seit-1968/ https://de.statista.com/statistik/daten/studie/161126/umfrage/anzahl-der-jagdscheininhaber-in-deutschland-seit-1968/.

Frank, E., Bonke, R., Drees, N., Heurich, M., Märtlbauer, E., & Gareis, M. (2019). Shiga toxin-producing Escherichia coli (STEC) shedding in a wild roe deer population. *Veterinary Microbiology, 239*, Article 108479. https://doi.org/10.1016/j.vetmic.2019.108479

Gill, C. (2004). Visible contamination on animals and carcasses and the microbiological condition of meat. *Journal of Food Protection, 67*(2), 413–419. https://doi.org/10.4315/0362-028X-67.2.413

Hadlok, R. M., & Bert, F. (1988). Gesetzliche Grundlagen und praktische Durchführung der Schlachttier- und Fleischuntersuchung bei Haarwild (Fortsetzung). *Tierarztliche Praxis, 16*, 343–350.

Hoffman, L. C., & Wiklund, E. (2006). Game and venison – meat for the modern consumer. *Meat Science, 74*(1), 197–208. https://doi.org/10.1016/j.meatsci.2006.04.005

IFAK Institut, T. (2021). *Bevölkerung in Deutschland nach Einstellung zur Aussage "Ich lege Wert auf gesunde Ernährung, auch wenn es mehr kostet" von 2016 bis 2020 (in Millionen)*. VuMA 2021. Retrieved 24.06.2021 from https://de.statista.com/statistik/daten/studie/172237/umfrage/gesunde-ernaehrung-wichtigkeit-trotz-hoeherer-kosten/.

Kappelhoff, W. (1999). Wildbrethygiene in der jagdlichen Praxis. *Amtstierärztlicher Dienst und Lebensmittelkontrolle, 6. Amtstierärztlicher Dienst und Lebensmittelkontrolle, 6*, 272–276.

Kotula, A., Lusby, W., Crouse, J., & de Vries, B. (1974). Beef carcass washing to reduce bacterial contamination. *Journal of Animal Science, 39*(4), 674–679.

Kujawski, O. E. J. G., & Heintges, F. (1984). *Wildbrethygiene-fleischbeschau, versorgen, verwerten, trophäenbehandlung*. BLV Verlagsgesellschaft.

Lenze, W. (1977). In *Fleischhygienische Untersuchungen an Rehwild, Einfluss von Gesundheitszustand, Herkunft, Erlegungs- und Versorgungsmodalitäten auf Keimgehalt und pH-Wert*. München.

Maahs, C. (2010). In T. H. H. Institut für Lebensmittelqualität und- sicherheit (Ed.), *Untersuchung zur mikrobiologischen Qualität von Rehwild unter verschiedenen Kühlbedingungen*. Hannover.

Mirceta, J., Petrovic, J., Malesevic, M., Blagojevic, B., & Antic, D. (2017). Assessment of microbial carcass contamination of hunted wild boars. *European Journal of Wildlife Research, 63*(2), 37. https://doi.org/10.1007/s10344-017-1096-3

Orsoni, F., Romeo, C., Ferrari, N., Bardasi, L., Merialdi, G., & Barbani, R. (2020). Factors affecting the microbiological load of Italian hunted wild boar meat (Sus scrofa). *Meat Science, 160*, Article 107967. https://doi.org/10.1016/j.meatsci.2019.107967

Paulsen, P. (2019). *Wildfleischhygiene: Ein duett von Lebensmittelwissenschaft und jagdpraxis. WALD.WILD.verBUND.FORSCHUNG am 25.11.2019*. Berlin: Bundesinstitut für Risikobewertung, Standort Marienfelde.

Paulsen, P., & Schopf, E. (2016). Wildbrethygiene bei Rehen - lage der Schusswunden bei Rehen, Bewertung von Verschmutzungen und mikrobielle Belastung der Brust- und Bauchhöhle. *Rundschau für Fleischhygiene und Lebensmittelüberwachung, 68*, 431–433.

Pegel, M., & Schreiber, W. (unknown). Leitfaden zur hygienischen Gewinnung von Wildbret im Rahmen der Primärproduktion Vom Ansprechen des Wildes bis zur Lagerung des Wildkörpers in der Decke/Schwarte bzw. im Federkleid. Retrieved 26.08.2020 from https://www.landesjagdverband.de/fileadmin/Medien/LJV/Dokumente/3_Jagdpraxis/Wildbrethygiene_und_Wildkrankheiten/leitfadengewinnung_von_wildbret.pdf.

Peruzy, M. F., Murru, N., Smaldone, G., Proroga, Y. T. R., Cristiano, D., Fioretti, A., & Anastasio, A. (2022). Hygiene evaluation and microbiological hazards of hunted wild boar carcasses. *Food Control, 135*, Article 108782. https://doi.org/10.1016/j.foodcont.2021.108782

Regulation (EC) No 178. (2002). *Regulation (EC) No 178/2002 of the European Parliament and of the council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety*.

Regulation (EC) No 852. (2004). *Regulation (EC) No 852/2004 of the European parliament and of the council of 29 april 2004 on the hygiene of foodstuffs*. https://eur-lex.europa.eu/legal-content/DE/TXT/PDF/?uri=CELEX:32004R0852&qid=1598435714806&from=DE.

Regulation (EC) No 853. (2004). *Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin*. https://eur-lex.europa.eu/legal-content/DE/TXT/PDF/?uri=CELEX:32004R0853&qid=1537133433900&from=EN.

Rheinisch-Westfälischer Jäger, L. (2017). *Wildbrethygiene– ein Praxis-Leitfaden für NRW. Landwirtschaftsverlag GmbH*. Retrieved 25.08.2020 from https://www.ljv-nrw.de/media/1562836190_wildbrethygiene_2017.pdf.

Scherling, L. (1989). In *Zum Hygienestatus von Schwarzwildbret aus dem Staatsrevier Forstenried*. München.

Sofos, J. N. (2014). Chapter 6 - meat and meat products. In Y. Motarjemi, & H. Lelieveld (Eds.), *Food safety management* (pp. 119–162). Academic Press. https://doi.org/10.1016/B978-0-12-381504-0.00006-8.

Soriano, A., Montoro, V., Vicente, J., Sánchez-Migallón, B. F., Benítez, S., Utrilla, M. C., & García Ruiz, A. (2016). Influence of evisceration time and carcass ageing conditions on wild venison quality. Preliminary study. *Meat Science, 114*, 130–136. https://doi.org/10.1016/j.meatsci.2015.12.021

Tier-LMHV. (2018). *Verordnung über Anforderungen an die Hygiene beim Herstellen, Behandeln und Inverkehrbringen von bestimmten Lebensmitteln tierischen Ursprungs (Tierische Lebensmittel-Hygieneverordnung - Tier-LMHV)*. Bundesamt für Justiz https://www.gesetze-im-internet.de/tier-lmhv/BJNR182800007.html: Bundesministerium der Justiz und für Verbraucherschutz.

Tomljanović, K., Grubešić, M., Medić, H., Potočnik, H., Topolovčan, T., Kelava Ugarković, N., & Marušić Radovčić, N. (2022). The impact of premortality stress on some quality parameters of roe deer, wild boar, and red deer meat. *Foods, 11*(9), 1275. https://doi.org/10.3390/foods11091275

Van Schalkwyk, H. (2010). *Guidelines for the harvesting of game for meat export 2010. Ministry of environment and tourism ministry of agriculture, water and Forestry*. Retrieved 24.06.2021 from https://core.ac.uk/download/pdf/37410361.pdf.

Wongprawmas, R., Mora, C., Pellegrini, N., Guiné, R. P. F., Carini, E., Sogari, G., & Vittadini, E. (2021). Food choice determinants and perceptions of a healthy diet among Italian consumers. *Foods, 10*(2), 318. https://www.mdpi.com/2304-8158/10/2/318.