Aus dem Institut für Lebensmittelsicherheit und -hygiene
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

und dem
Bundesinstitut für Risikobewertung

Influencing factors and reduction measures affecting the microbial load of hunted roe deer carcasses and meat with special emphasis on rinsing

Inaugural-Dissertation
zur Erlangung des Grades eines
Doctor of Philosophy (PhD)
in Biomedical Sciences
an der
Freien Universität Berlin

vorgelegt von
Birsen Korkmaz
Lebensmitteltechnologin aus Berlin

Berlin 2023
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List of Abbreviations

Abbreviation	Explanation
AVV LmH	Allgemeine Verwaltungsvorschrift über die Durchführung der amtlichen Überwachung der Einhaltung von Hygienevorschriften für Lebensmittel und zum Verfahren zur Prüfung von Leitlinien für eine gute Verfahrenspraxis (AVV Lebensmittelhygiene)
BfJ	Bundesamt für Justiz
BfR	Bundesinstitut für Risikobewertung
BJagdG	Bundesjagdgesetz
BMI	Bundesministerium des Innern und für Heimat
BW	Body weight
CFU	Colony forming unit
CI	Confidence interval
DFD	Dark, firm and dry meat
DIN e.V.	Deutsches Institut für Normung eingetragener Verein
DJV	Deutscher Jagdverband
E 100	Impact energy of ammunition at 100 m distance
EC	European Community
EU	European Union
FMEA	Failure Mode and Effects Analysis
GHE	Game handling establishment
GHEs	Game handling establishments
GHP	Good Hygiene Practice
GIC	Gastrointestinal content
GICs	Gastrointestinal contents
GIT	Gastrointestinal tract
HACCP	Hazard Analysis Critical Control Point
IF	Influencing factor
IFs	Influencing factors

Abbreviation	Explanation
IML	Initial microbial load
IMLs	Initial microbial loads
LMHV	Verordnung über Anforderungen an die Hygiene beim Herstellen, Behandeln und Inverkehrbringen von Lebensmitteln (Lebensmittelhygiene-Verordnung - LMHV)
LOD	Limit of detection
LS mean	Least Squares mean
ML	Microbial load
MLs	Microbial loads
n	Sample size
pH	Potential hydrogen
PSE	Pale, soft and exudative meat
SD	Standard deviation
SE	Standard error
spp.	species pluralis
STEC	Shiga toxin-producing *Escherichia coli*
TAC	Total aerobic colony count
Tier-LMHV	Verordnung über Anforderungen an die Hygiene beim Herstellen, Behandeln und Inverkehrbringen von bestimmten Lebensmitteln tierischen Ursprungs (Tierische Lebensmittel-Hygieneverordnung)
TVC	Total viable count

List of Definitions

Term	Definition/Explanation
Adverse effect*	Any nauseating or other undermining factor of the hygienic condition of foodstuffs, e.g., by microorganisms, contaminants, weather conditions, odors, unsuitable temperatures, gases, vapors, smoke, aerosols, animal pests, human and animal excreta, as well as by waste, sewage, cleaning agents, pesticides, veterinary medicines, biocidal products, or improper treatment and treatment processes (BfJ 2018).
Contaminant	Means any substance not intentionally added to food that is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination. Extraneous matter, such as, for example, insect fragments, animal hair, etc., is not covered by this definition (European Commission 1993).
Contamination	The presence or introduction of a hazard (European Commission 2004a).
Food or Foodstuff	Any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans (European Commission 2002).
Food business	Means any undertaking, whether for profit or not and whether public or private, carrying out any of the activities related to any stage of production, processing and distribution of food (European Commission 2002).
Food business operator	The natural or legal persons responsible for ensuring that the requirements of food law are met within the food business under their control (European Commission 2002).
Food hygiene	The measures and conditions necessary to control hazards and to ensure fitness for human consumption of a foodstuff taking into account its intended use (European Commission 2004a).

* Own translation from German language

Term	Definition/Explanation
Game handling establishment	Any establishment in which game and game meat obtained after hunting are prepared for placing on the market (European Commission 2004b).
Hazard	Means a biological, chemical or physical agent in, or condition of, food or feed with the potential to cause an adverse health effect (European Commission 2002).
Kill*	Killing of big and small game according to hunting regulations (BfJ 2018).
Perishable food*	A foodstuff that, from a microbiological point of view, is especially perishable within a short period of time and whose perishability and marketability can only be maintained if certain temperatures or other conditions can be maintained (BfJ 2018).
Primary products	Products of primary production including products of the soil, of stock farming, of hunting and fishing (European Commission 2004a).
Primary production	The production, rearing or growing of primary products including harvesting, milking and farmed animal production prior to slaughter. It also includes hunting and fishing and the harvesting of wild products (European Commission 2002).
Risk	A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard (European Commission 2002).
Small quantities*	Amount of wild game carcasses that are harvested on a single hunting day (BfJ 2020b).

* Own translation from German language

Term	Definition/Explanation
Trained person	In order for hunters to become trained person, training must be provided to the satisfaction of the appropriate authority. It should cover at least the following subjects: (a) the normal anatomy, physiology and behavior of wild game; (b) abnormal behavior and pathological changes in wild game due to diseases, environmental contamination or other factors which may affect human health after consumption; (c) the hygiene rules and proper techniques for the handling, transportation, evisceration etc. of wild game animals after killing; and (d) legislation and administrative provisions on the animal and public health and hygiene conditions governing the placing on the market of wild game (European Commission 2004b).
Trained person*	Small quantities of killed game or meat from killed game may only be distributed by persons who are adequately trained in the areas of body structure (anatomy), vital functions (physiology), normal and abnormal behavior and pathological changes of game, as well as in the hygienic requirements for handling game (BfJ 2020b).
Wild game	Wild ungulates, lagomorphs, other land mammals and wild birds that are hunted for human consumption and are considered to be wild game and wild birds under the applicable law in the Member State concerned, including mammals living in enclosed territory under conditions of freedom similar to those of wild game. (European Commission 2004b).
Zoonosis	An infectious disease that can be transmitted from a nonhuman animal to a human by bacteria, animals, parasites, or unconventional pathogens (WHO 2020). However, transmission can also occur from human to animal (Groschup 2022).

* Own translation from German language

1 General introduction

Food hygiene encompasses the measures and conditions necessary to control hazards and ensure that food is fit for human consumption (European Commission 2004a). To obtain safe game meat, there are established traditions and recommendations for handling game carcasses as a part of good game meat hygiene, which are reported in guidelines for hunters (Bert 2008, Bildungs- und Wissenszentrum Aulendorf 2008, Amt für Landschaft und Natur 2019, Pegel and Schreiber unknown) and books on good game meat hygiene (Winkelmayer et al. 2007, Martini 2008, Winkelmayer et al. 2008, Deutz 2012a). These recommendations are usually based on the experience of hunters, which provides good indications regarding which influencing factors (IFs) and measures can improve or degrade game meat quality (Marescotti et al. 2016). Scientific studies have also been conducted to provide new data on the IFs and measures to better understand the effectiveness of food hygiene measures against bacterial contamination of game carcasses (Slowak 1986, Paulsen et al. 2008, Branciari et al. 2020). However, several recommendations for handling game carcasses are difficult to generalize because they can be partially contradictory. In contrast, there are already summary review articles covering various studies that address cleaning measures and their effects on reducing the microbial load (ML) of carcasses under slaughterhouse conditions (Dickson and Anderson 1992, Gill 2004). However, producing meat from slaughtered animals in slaughterhouses is conducted under controlled conditions where food safety and management concepts such as Good Hygiene Practice (GHP) and Hazard Analysis Critical Control Point (HACCP) are established. Under field conditions, the harvesting process is highly dependent on environmental, hunting, and handling conditions and can impact bacterial growth through several IFs (Branciari et al. 2020, Ranucci et al. 2021).

During the early steps of the hunting chain, primary products such as roe deer (*Capreolus capreolus*) carcasses can be contaminated by various bacterial agents (Deutz and Fötschl 2014, Mirceta et al. 2015), including pathogens (Riemer and Reuter 1979, Branciari et al. 2020). This can pose a risk to public health when game meat is consumed. The effects of bacterial activity, e.g. in the form of faulty meat maturation or spoilage, have been observed in the sensory characteristics of food when bacterial concentrations increase during manufacturing and cold storage (Iulietto et al. 2015, Odeyemi et al. 2020). Bacterial concentrations can rise relatively quickly in perishable foods such as meat, as such foods provide an ideal growing habitat for bacteria (Sofos 2014). Therefore, microbial limits for process hygiene have been set by the European Union in Commission Regulation (EC) No. 2073/2005 for meat and meat products (European Commission 2020). However, a challenge for microbiological assessments of game carcasses, game meat and game meat products is

that there is no limiting value as there is with meat obtained from slaughtered animals. Bacteria have specific requirements and growth condition needs, such as those related to temperature, for survival, growth, and multiplication (BfR 2006, Iulietto et al. 2015). When these requirements and needs are not met, bacteria can be reduced in number or eliminated (Gill and Newton 1978a, Sperber 1983, Odeyemi et al. 2020). Therefore, hunters must follow strict food hygiene requirements during primary production in the field and overcome various food hygiene challenges to produce safe game meat with the lowest possible ML (Slowak 1986, Apelt 2007, Van Schalkwyk and Hoffman 2011). For example, handling practices that are highly likely to transmit bacteria to game meat must be avoided.

Currently, the game meat market is becoming increasingly popular because game animals can be hunted regionally, which is in line with the principle of sustainability (Bruckner 2007). The ethical aspect of game meat on the market is ranked by consumers as one of the most important characteristics when deciding to consume game meat (Niewiadomska et al. 2020). Furthermore, game meat has a valuable nutritional composition (Daszkiewicz et al. 2012, Dannenberger et al. 2013, Klupsaite et al. 2020). The number of roe deer hunted in Germany in 2020/21 increased by an additional 4.8 % over the previous year to 1,285.562 (DJV 2022). Since the number of hunted roe deer carcasses continues to increase during the annual hunting seasons in Germany, the original studies presented in this thesis are limited to roe deer carcasses and their meat.

The aim of this thesis is to identify the factors and measures that may influence the initial microbial load (IML) and subsequent ML of roe deer carcasses during the early steps of the hunting chain. This is to verify whether it is possible to provide recommendations to hunters during the early steps of the hunting chain in the field to ensure a higher microbiological quality of roe deer carcasses. In particular, the effect of rinsing on the IML of visibly clean roe deer carcasses or on carcasses soiled with gastrointestinal content (GIC) was investigated. Rinsing has been recommended, but to date, the effectiveness of this measure — including the effects of rinsing on the subsequent ML of carcasses after cold storage — in reducing the IMLs of game carcasses has not been verified. Therefore, selected bacterial groups that are relevant as environmental, fecal and spoilage indicators in game carcasses and meat were studied. Furthermore, a review and Failure Mode and Effects Analysis (FMEA) were performed to determine the effects of potential IFs and failures on carcass IML in the handling of carcasses during the game carcass harvesting process.

2 Background

2.1 Placing game meat on the German market in accordance with European and national legal bases

This chapter provides an overview of the relevant definitions, legal bases and distribution channels for placing game meat from wild game, specifically roe deer, on the German market. Wild game as defined in Regulation (EC) No. 853/2004, Annex I, point 1.5 are: “wild ungulates and lagomorphs, as well as other land mammals that are hunted for human consumption and are considered to be wild game under the applicable law in the Member State concerned. Furthermore, mammals living in enclosed territory under conditions of freedom similar to those of wild game; and wild birds that are hunted for human consumption” (European Commission 2004b). Different legal bases apply depending on whether the hunting products are primary products or foodstuffs. Primary products are “products of primary production including products of the soil, of stock farming, of hunting and fishing” (European Commission 2004a). According to Regulation (EC) No. 178/2002, food or foodstuff is “any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans”. Furthermore, different legal regulations apply when distributing hunting products in Germany depending on the state of preparation (Figure 1) and product quantity. Small quantities of primary products are considered to be the amounts of game carcasses that are harvested on a single hunting day (BfJ 2018).

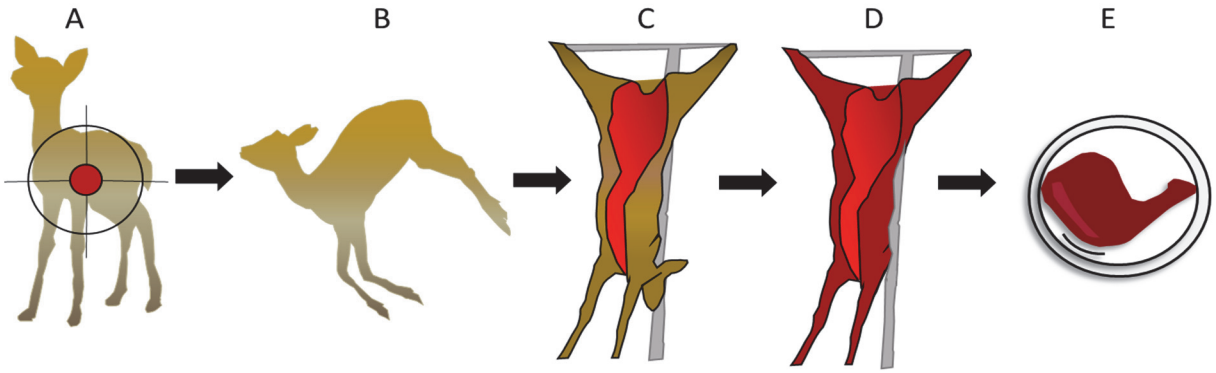


Figure 1: Schematic overview of the hunting chain beginning with observation (A) and killing (B) of wild game (roe deer), followed by evisceration (C), skinning (D) and cutting (E) of a roe deer carcass.

The legal requirements for the marketing of game meat in Europe are laid down in Regulation (EC) No. 178/2002, Regulation (EC) No. 852/2004 and Regulation (EC) No. 853/2004. These regulations aim to improve consumer health protections. Regulation (EC)

No. 178/2002 stipulates that only safe food may be placed on the market and that the responsibility lies with the food business operator (natural or legal persons). In addition to identifying the responsible entities, other general requirements of food law, such as food safety requirements and traceability of foods, are also defined here (European Commission 2002). Traceability requirements cover all aspects of the food production chain up to delivery to the consumer, including primary production. However, Regulation (EC) No. 178/2002 excludes primary production for private domestic use, as well as foods that are processed, handled or stored in the home and are intended for private domestic consumption. Regulation (EC) No. 852/2004 regulates general and specific hygiene requirements for the production of safe food. An example of these would be, maintaining the cold chain for certain foods. Good manufacturing practices and HACCP principles in food businesses are also regulated (European Commission 2004a). More specific requirements for foods of animal origin are described in Regulation (EC) No. 853/2004, which lays down specific hygiene rules. For example, food business operators may use drinking water to remove surface contamination from products of animal origin (European Commission 2004b). Regulation (EC) No. 852/2004 excludes “primary production for private domestic use; the domestic preparation, handling or storage of food for private domestic consumption; the direct supply, by the producer, of small quantities of primary products to the final consumer or to local retail establishments directly supplying the final consumer” (European Commission 2004a). Regulation (EC) No. 853/2004 excludes “primary production for private domestic use; the domestic preparation, handling or storage of food for private domestic consumption; the direct supply, by the producer, of small quantities of primary products to the final consumer or to local retail establishments directly supplying the final consumer; hunters who supply small quantities of wild game or wild game meat directly to the final consumer or to local retail establishments directly supplying the final consumer” (European Commission 2004b). Therefore, primary products that are supplied directly by the producer in small quantities to the final consumer or to local retail establishments that supply the products directly to the final consumer are covered by national regulations.

Placing game carcasses and game meat on the German market must be conducted in accordance with the national regulations “Lebensmittelhygiene-Verordnung” (LMHV) and “Tierische Lebensmittel-Hygieneverordnung” (Tier-LMHV). The LMHV clarifies terms such as adverse effects and perishable food (BfJ 2018). An adverse effect occurs when the proper hygienic condition of a food is not maintained due to nauseating or other undermining factors (BfJ 2018). Such factors that undermine the hygienic conditions may be caused by microorganisms, contaminants, weather conditions, unsuitable temperatures, animal pests, human and animal excreta, as well as pesticides or improper treatment and preparation methods (BfJ 2018). The term “perishable foods” refers to foods that, from a microbiological

point of view, are especially perishable within a short period of time. In addition, the marketability of these foods can only be ensured if certain temperatures or other conditions can be maintained. The national requirement to use water of drinking water quality for the hygienic production of food is also given here (BfJ 2018). Tier-LMHV establishes the obligation to chill game carcasses (large game: max. +7 °C) and the requirement of official meat inspection in the event of the presence of any abnormal characteristic (BfJ 2020b). According to this regulation (Annex 4 No. 1.3), abnormal characteristics include abnormal behavior of game animal, tumors, abscesses, foreign content in the body cavity (stomach and intestinal contents), discoloration of the internal organs or significant emaciation of the game carcass (BfJ 2020b).

In Germany, shooting of game animals must be conducted in accordance with the “Bundesjagdgesetz” (BJagdG). In the BJagdG, the right to hunt is defined as the authorization to keep, hunt and obtain wild animals subject to the right to hunt in certain areas according to Section 1 paragraph 1 (BfJ 2020a). Therefore, it is forbidden to shoot at roe deer with rifle cartridges whose impact energy at 100 m (E 100) is less than 1000 J. For ungulates in general, it is forbidden to shoot with rifle cartridges of caliber less than 6.5 mm. For all other ungulates, except roe deer, a caliber of 6.5 mm and more with an E 100 of at least 2000 J is to be used. Shooting at game is also prohibited during the closed season, which is regulated differently in the German federal states (BfJ 2020a).

Considering the legal bases summarized above, there are five main distribution channels through which game meat can reach consumers, either directly or through the German market (Figure 2):

1. Private domestic consumption by the hunter and his or her family,
2. Handover of eviscerated large game carcasses with fur to local retail establishments or for direct handover to final consumers,
3. Handover of eviscerated and skinned large game carcasses to final consumers or to local retail establishments for direct handover to final consumers.
4. Handover of meat cuts or other meat products obtained from large game carcasses to final consumers or local retail establishments for direct handover to final consumers,
5. Handover of eviscerated large game carcasses with fur to approved game handling establishments (GHEs) by trained persons according to Regulation (EC) No. 853/2004

The order of the distribution channels has been arranged from the national to international legal bases and from fewer to more mandatory requirements according to the legal bases.

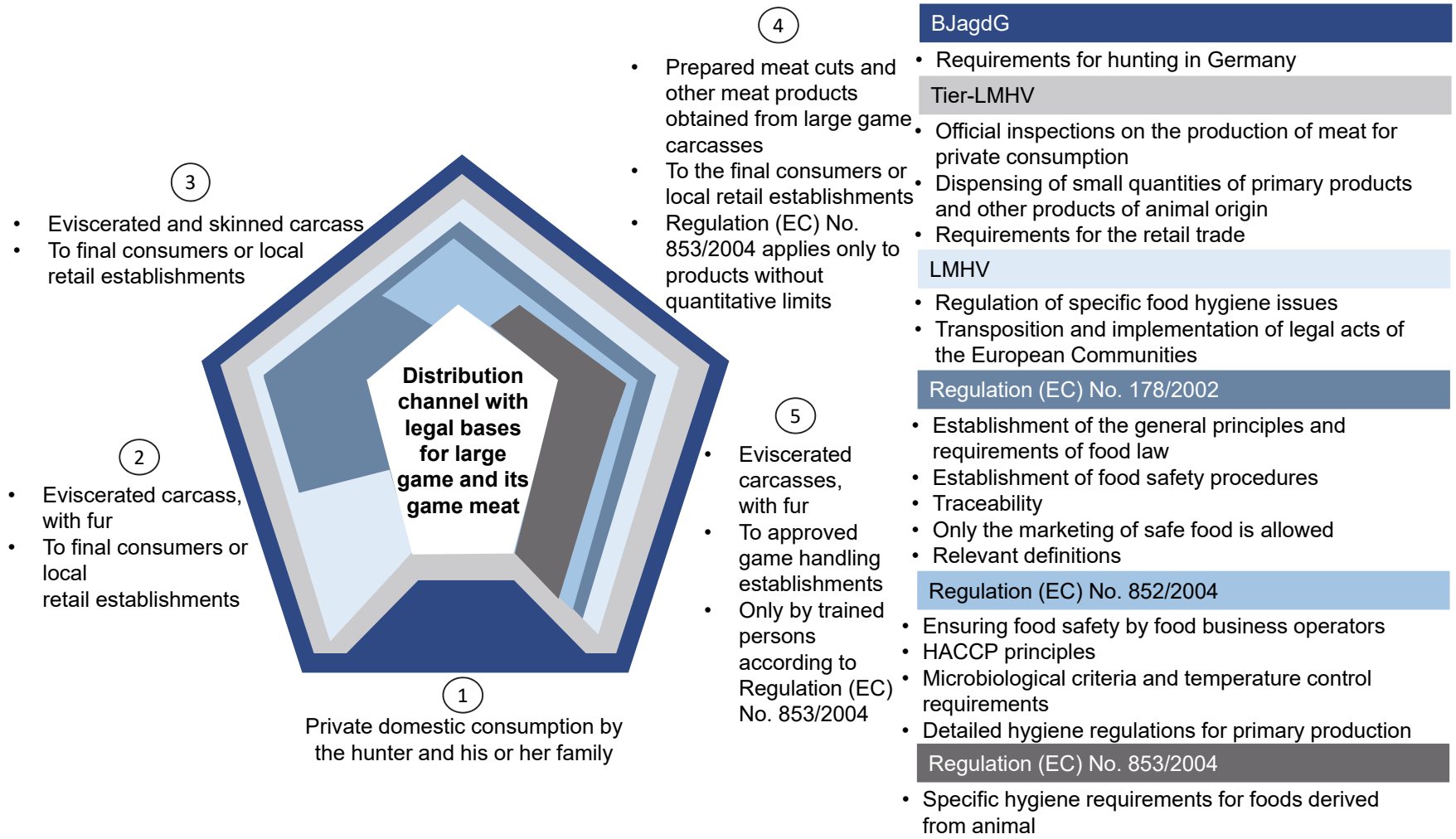


Figure 2: Distribution channels for large game from the hunter's perspective with the legal bases to comply with in Germany through which game carcasses and game meats can reach consumers depending on the stage of preparation.

2.2 Hunted roe deer (*Capreolus capreolus*) carcass and obtained game meat

2.2.1 Microbial aspects

2.2.1.1 Meat muscle surfaces and tissues

The meat muscle surfaces of roe deer carcasses might already be contaminated in the field due to environmental (Branciari et al. 2020), hunting (Deutz and Fötschl 2014), and handling conditions (Mirceta et al. 2017, Gomes-Neves et al. 2021). For example, a shot to the gastrointestinal tract (GIT) can soil the body cavity with gastrointestinal contents (GIC) (Avagnina et al. 2012). In addition, bacterial cross-contamination between animal skin and the meat surface during handling is possible, as found in the study by Paulsen et al. (2022) for game carcasses in an approved game handling establishment (GHE). These bacteria can multiply with elapsed time on meat surfaces, including cold-tolerant bacteria during cold storage (Labuza and Fu 2005). Therefore, the IML of primary products, such as roe deer carcasses, are of utmost relevance to the subsequent ML of the meat obtained from them (Ayres 1960, Doulgeraki et al. 2012, Odeyemi et al. 2020). Meat is a perishable foodstuff (BfJ 2018) that has a limited shelf life.

Microbial condition and shelf life of game carcasses and meat are typically assessed by microbial examinations and can be investigated using different sampling methods (Merwe et al. 2013). The microbial condition of game carcasses is strongly dependent on the processing conditions (Bell 1997, Blagojevic et al. 2011) and can be examined by sampling the meat surfaces using the wet-dry sponge method (DIN e.V. 2015). In contrast, the sampling method for muscle tissues involves destructive sampling from which the microbial condition of meat can be determined (DIN e.V. 2017). In general, in the deep tissues of healthy animal muscles, bacteria were found to be absent (Gill et al. 1978b, Anderson et al. 1991, Dickson and Anderson 1992, Gill 2007). However, bacteria could be present in the deep muscle tissue of animals that are shot but not immediately killed by injury to vital organs (Gill 2007).

2.2.1.2 Bacterial groups and investigated microbial loads

The microbiota of roe deer carcasses or meat includes ubiquitous (Branciari et al. 2020), fecal (Paulsen and Winkelmayer 2004) and meat-specific spoilage bacteria (Riemer and Reuter 1979); examples are presented in Table 1 (BfR 2006). The IML and subsequent ML of game carcasses or meat could be dependent on the ambient temperature of the hunting day (Paulsen and Winkelmayer 2004, Branciari et al. 2020) due to the specific growth temperatures of the bacterial groups present (Ayres 1955, Labuza and Fu 2005, Lawrie and Ledward 2014, Sofos 2014). Therefore, the IML and subsequent ML levels determined in roe deer carcasses or meat are highly variable (Tables 2 – 3).

Table 1: Growth temperatures and generation times of relevant bacteria for deer and roe deer meat reviewed by the German Federal Institute for Risk Assessment (BfR) (BfR 2006) using data from various authors (references were not given and the classification of bacteria into ubiquitous and fecal (Halkman and Halkman 2014) and meat-specific spoilage bacteria (Nychas and Panagou 2011) has been conducted on the basis of information from other literature).

Bacterial group	**Growth temperatures, °C**			**Generation time (min) at optimum temperature**
	Minimum	Optimum	Maximum	
*Yersinia enterocolitica***	0	27	45	
Listeria spp.*	1	34	45	
Pseudomonas spp.*,***	(-3) 4	not specified	41	
Salmonella spp.**	6	37	47	40
Campylobacter spp.**	30	42 – 45	47	20
*Escherichia coli***	4	37	46	
*Bacillus cereus**	5	32	50	35
*Staphylococcus aureus**	6	37	46	20 (35 °C)
Moraxella spp.	4	not specified	45	
Lactobacillus spp.***	1	not specified	53	
*Brochothrix termosphacta****	1	20	30	
Klebsiella spp*.	3	10 – 44	44	

* ubiquitous bacteria, ** fecal bacteria, and *** meat-specific spoilage bacteria

Table 2: Reviewed bacterial groups and loads examined in roe deer carcasses and meat

Reference	Microorganism examined	Sampling time point	Sample size	Sample	Method	Statistical parameter	Bacterial load
Atanassova et al. 2008	Aerobic mesophilic plate count	After the end of the hunt at the collection point	95	Meat samples from the area of belly/flank, breast, shoulder and throat (punch samples of 4x5 cm², thickness of 5 mm)	ISO 4833:2003	Geometrical mean (range)	2.6 (1.0 – 5.7) log_{10} CFU/cm²
Avagnina et al. 2012	Aerobic viable count	Upon the arrival of the carcasses at the collection point (1 – 6 h after shooting)	61	4 samples by swabbing a 25 cm² area on the surface of the muscles within the anatomical region of the medial hind limb	ISO 4833:2004	Median	3.46 log CFU/cm²
Bandick et al. 1995	Aerobic colony count	Freshly killed carcasses sampled in the field	50	50x50x5 mm of adductor muscles	Standard pour plate method	Geometrical mean	3.9 lg CFU/g
Branciari et al. 2020	Aerobic colony count	Roe deer carcasses were skinned and sampled after 2, 4 and 6 days of cold storage at 5 ± 1 °C within skin, respectively, at the local game handling establishment	64	Four tissue samples of 5 cm² each were obtained from four different parts: hind leg (rump), flank, brisket and foreleg (total surface area of 20 cm²).	ISO 4833-1:2013	Geometrical mean ± standard error	3.39 ± 1.06 log CFU/cm²
Irschik et al. 2012	Total aerobic colony count	Roe deer carcasses dressed and sampled 24 – 48 h after killing and chilling (+7 °C)	10	*M. longissimus lumborum*, *M. semitendinosus,* Goulash, and Ragout	TEMPO®, Bio Merieux, Marcy l´Etoile, F)	Geometrical mean ± standard deviation	5.6 ± 1.3 log_{10} CFU/g
Irschik et al. 2012	Total aerobic colony count	Meat cuts after 7 days chilling at 0 – 2 °C in vacuum packages	10	*M. longissimus lumborum*, *M. semitendinosus,* Goulash, and Ragout	TEMPO®, Bio Merieux, Marcy l´Etoile, F)	Geometrical mean ± standard deviation	5.9 ± 1.5 log_{10} CFU/g
Atanassova et al. 2008	*Enterobacteriaceae*	After the end of the hunt at the collection point	95	Meat samples from the area of belly/flank, breast, shoulder and throat (punch samples of 4x5 cm², thickness of 5 mm)	ISO 21528-2:2004	Geometrical mean (range)	2.1 (1.7 – 2.6) log_{10} CFU/cm²
Avagnina et al. 2012	*Enterobacteriaceae*	Upon the arrival of the carcasses at the collection point (1 – 6 h after shooting)	61	4 samples by swabbing a 25 cm² area on the surface of the muscles within the anatomical region of the medial hind limb	ANOR NF V08-054 (1999)	Median	2.47 log CFU/cm²
Branciari et al. 2020	*Enterobacteriaceae*	Roe deer carcasses were skinned and sampled after 2, 4 and 6 days of cold storage at 5 ± 1 °C within skin, respectively, at the local game handling establishment	64	Four tissue samples of 5 cm² each were obtained from four different parts: hind leg (rump), flank, brisket and foreleg (total surface area of 20 cm²).	ISO 21528-2:2017	Geometrical mean ± standard error	2.27 ± 1.11 log CFU/cm²
Irschik et al. 2012	*Enterobacteriaceae*	Roe deer carcasses dressed and sampled 24 – 48 h after killing and chilling (+7 °C)	10	*M. longissimus lumborum*, *M. semitendinosus,* Goulash, and Ragout	TEMPO®, Bio Merieux, Marcy l´Etoile, F)	Geometrical mean ± standard deviation	2.7 ± 0.7 log_{10} CFU/g
Irschik et al. 2012	*Enterobacteriaceae*	Meat cuts after 7 days chilling at 0 – 2 °C in vacuum packages	10	*M. longissimus lumborum*, *M. semitendinosus,* Goulash, and Ragout	TEMPO®, Bio Merieux, Marcy l´Etoile, F)	Geometrical mean ± standard deviation	3.6 ± 0.9 log_{10} CFU/g

Table 2: Continued

Reference	Microorganism examined	Sampling time point	Sample size	Sample	Method	Statistical parameter	Bacterial load
Irschik et al. 2012	*Escherichia coli*	Roe deer carcasses dressed and sampled 24 – 48 h after killing and chilling (+7 °C)	10	*M. longissimus lumborum*, *M. semitendinosus,* Goulash, and Ragout	TEMPO®, Bio Merieux, Marcy l´Etoile, F)	Geometrical mean ± standard deviation	1.8 ± 0.8 $\log_{10}$ CFU/g
Irschik et al. 2012	*Escherichia coli*	Meat cuts after 7 days chilling at 0 – 2 °C in vacuum packages	10	*M. longissimus lumborum*, *M. semitendinosus,* Goulash, and Ragout	TEMPO®, Bio Merieux, Marcy l´Etoile, F)	Geometrical mean ± standard deviation	2.2 ± 1.1 $\log_{10}$ CFU/g
Atanassova et al. 2008	Coagulase positive staphylococci	After the end of the hunt at the collection point	95	Meat samples from the area of belly/flank, breast, shoulder and throat (punch samples of 4x5 cm², thickness of 5 mm)	ISO 6888-1:2003	Range	2.0 – 2.8 $\log_{10}$ CFU/cm²
Atanassova et al. 2008	*Salmonella*	After the end of the hunt at the collection point	95	Meat samples from the area of belly/flank, breast, shoulder and throat (app. 100 cm² in size)	ISO 6579:2003	Prevalence	Not detected
Avagnina et al. 2012	*Salmonella*	Upon the arrival of the carcasses at the collection point (1 – 6 h after shooting)	61	4 samples by swabbing a 25 cm² area on the surface of the muscles within the anatomical region of the medial hind limb	ISO 6579 (1993)	Number of animals	Not detected in animals
Branciari et al. 2020	*Salmonella*	Roe deer carcasses were skinned after 2, 4 and 6 days of cold storage at 5 ± 1 °C within skin in the local game handling establishment	64	4 tissue samples of 5 cm² each were obtained from 4 different parts: hind leg (rump), flank, brisket and foreleg (total surface area of 20 cm²).	ISO 6579-1:2017	Number of animals	Not detected
Atanassova et al. 2008	*Campylobacter*	After the end of the hunt at the collection point	95	Meat samples from the area of belly/flank, breast, shoulder and throat (app. 100 cm² in size)	ISO 10272:2002	Prevalence	Not detected
Atanassova et al. 2008	*Listeria* spp.	After the end of the hunt at the collection point	95	Meat samples from the area of belly/flank, breast, shoulder and throat (app. 100 cm² in size)	ISO 11290-1:2005	Prevalence	4 animals
Avagnina et al. 2012	*Listeria monocytogenes*	Upon the arrival of the carcasses at the collection point (1 – 6 h after shooting)	61	4 samples by swabbing a 25 cm² area on the surface of the muscles within the anatomical region of the medial hind limb	ISO 11290-1:1996	Number of animals	Not detected
Branciari et al. 2020	*Listeria monocytogenes*	Roe deer carcasses were skinned and sampled after 2, 4 and 6 days of cold storage at 5 ± 1 °C within skin, respectively, at the local game-handling establishment	64	4 tissue samples of 5 cm² each were obtained from 4 different parts: hind leg (rump), flank, brisket and foreleg (total surface area of 20 cm²).	ISO 11290-1:2017	Number of animals	Not detected
Avagnina et al. 2012	*Yersinia* spp.	Upon the arrival of the carcasses at the collection point (1 – 6 h after shooting)	61	4 samples by swabbing a 25 cm² area on the surface of the muscles within the anatomical region of the medial hind limb	Published protocol by Niskanen et al. 2003	Number of animals	3 animals

2.2.2 Meat quality characteristics, meat maturation and spoilage

The quality of roe deer meat is described in this thesis on the basis of its microbial (Chapter 2.2.1), chemical, physical, and sensory properties (Figure 3). Microbial, chemical and physical properties interact with each other and can influence the sensory properties of game meat (Lawrie and Ledward 2014, Sofos 2014).

The chemical composition of game meat is characterized by the macronutrient, fatty acid, vitamin and trace element contents. Macronutrients are, for example, the protein, fat or water contents (Daszkiewicz et al. 2012, Dannenberger et al. 2013, Klupsaite et al. 2020). Furthermore, roe deer meat contains fatty acids, such as long-chain n-3 polyunsaturated fatty acids, vitamin E and trace metals (e.g., iron and selenium) as valuable nutrients (Dannenberger et al. 2013). Daszkiewicz et al. (2012) presented their findings on the chemical composition of game meat grouped by sex due to significant differences in the total protein and fat contents (Figure 3). Evidence that, for example, the intramuscular fat contents of male and female roe deer muscles were significantly dependent on the geographical region and age of the animals was provided a year later in another study (Dannenberger et al. 2013).

The physical property of the pH value is a good parameter for estimating and assessing the quality of meat. During post-mortem metabolism (glycolysis), the pH of meat decreases due to the conversion of glycogen from muscle to lactic acid (Lawrie and Ledward 2014). The final pH value of meat after meat maturation is influenced by the rate of decrease in pH with time, which depends on the premortem stress, available glycogen and other factors (Bareuther 1984, Viganò et al. 2019). Bareuther (1984) reported a pH of 6.6 for roe deer meat when measured 0.6 h post-mortem. After 3.4 h, the pH dropped to 6.0, after 7 h to 5.7, and after 24 h to 5.5 (Bareuther 1984). This is in line with the findings of the study by Avagnina et al. (2012), where a mean pH of 5.97 (95% CI 5.92 – 6.02) was measured between 30 min and 6 h after killing the game (roe deer, red deer). After meat maturation, for example after 7 days, the pH ranged between 5.55 and 5.83 (Irschik et al. 2012). In the study by Daszkiewicz et al. (2012), the pH was 5.48 ± 0.05 for meat from female roe deer carcasses and 5.47 ± 0.05 for meat from male roe deer carcasses at 54 h post-mortem. Another factor that can influence the pH of ruminant meat is the diet or feeding regime (Priolo et al. 2002).

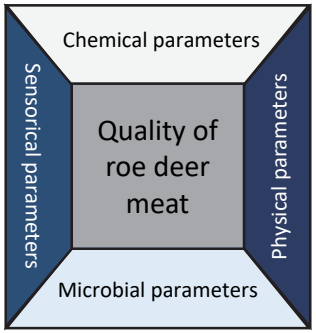
As with all foods, the sensory aspect of roe deer meat plays an important role in meat quality. In the review by Neethling et al. (2016), the sensory quality of game meat is often described in the literature as “an aroma and flavor associated with a wild animal species”. However, the sensory quality of individual roe deer carcasses was found to vary depending on the animal ages and muscle types (Daszkiewicz et al. 2012).

Chemical composition and energy value of roe deer meat aged 3 – 4 years (Daszkiewicz et al. 2012):

	Sex (mean ± SE)	
Parameter	Female (n = 25)	Male (n = 16)
Dry matter (%)	26.20 ± 0.56	24.68 ± 0.46
Total protein (%)	22.79 ± 0.67	21.84 ± 0.32
Fat (%)	1.46 ± 0.55	0.83 ± 0.39
Water/protein ratio	3.24 ± 0.10	3.45 ± 0.06
Ash (%)	1.12 ± 0.05	1.13 ± 0.17
Energy value (kJ)	437.50 ± 24.49	397.63 ± 14.73

Sensorical properties of roe deer meat (Daszkiewicz et al. 2012):

	Sex (mean ± SE)	
Parameter	Female (n = 25)	Male (n = 16)
Aroma-intensity	4.18 ± 0.63	4.12 ± 0.85
Aroma-desirability	4.92 ± 0.28	5.00 ± 0.00
Taste-intensity	4.32 ± 0.50	4.09 ± 0.52
Taste-desirability	4.90 ± 0.29	5.00 ± 0.00
Juiciness	4.04 ± 0.32	3.59 ± 0.37
Tenderness	4.66 ± 0.40	4.44 ± 0.48



pH of meat from roe deer killed by chest hit, after cutting and after 7 days of storage in vacuum packaging at 0 – 2 °C (Irschik et al. 2012):

	pH (mean)	
Samples (n = 5)	Sampling day 0	Sampling day 7
M. Longissimus lumborum	5.55	5.55
M. supraspinatus	5.74	5.83
M. semitendinosus	5.61	5.60

Bacterial load of freshly shot roe deer (Atanassova et al. 2008):

	Bacterial load (geometrical mean $\log_{10}$ CFU/cm², range)	
Samples (n = 95)	Measophilic aerobic plate count	*Enterobacteriaceae*
Meat samples from the area of the belly/flank, breast, shoulder and throat	2.6 (1.0 – 5.7)	2.1 (1.7 – 2.6)

Figure 3: Quality of roe deer meat (microbial, physical, chemical, and sensory properties) with relevant parameters from the literature (Atanassova et al. 2008, Daszkiewicz et al. 2012, Irschik et al. 2012).

After animal death, various biochemical, chemical and physical processes begin in the skeletal muscles (Sielaff 1996, Lawrie and Ledward 2014). These processes continue during cold storage until the meat is consumed and are termed meat maturation or aging (Lawrie and Ledward 2014). During this period, the juiciness, tenderness, color, taste and aroma of meat change, leading to the conversion of muscle into meat (Casoli et al. 2005, Lawrie and Ledward 2014, Brad Kim et al. 2018). Targeted meat maturation can be controlled using defined conditions, e.g., controlled temperature and duration of cold storage (Sielaff 1996, Lawrie and Ledward 2014). In addition, metabolic catabolites and toxins produced by certain bacterial groups, such as lactic acid and bacteriocins from lactic acid bacteria, are involved in targeted meat maturation and may be beneficial for meat quality (Sofos 2014). However, meat maturation can also be faulty or can seamlessly transition to meat spoilage (Sielaff 1996, Lawrie and Ledward 2014). An example of faulty meat maturation is when the pH value decreases or increases uncharacteristically (Lawrie and Ledward 2014). Meat properties, specifically meat colors, textures and water-holding capacities, are negatively affected by this (Lawrie and Ledward 2014). If the pH is too high, dark, firm and dry (DFD) meat may result (Lawrie and Ledward 2014, Neethling et al. 2016); if it is too low, pale, soft and exudative (PSE) meat may result (Bareuther 1984, Lawrie and Ledward 2014). These deviations are meat quality defects (Lawrie and Ledward 2014).

Spoilage is the loss of acceptable meat quality (Sofos 2014) that renders the product unsuitable for consumption (Nychas and Panagou 2011). Manifestations of spoilage in meat are those such as putrefaction, acidification, graying/greening or mold growth (Baumgart et al. 2015). Therefore, the type of spoilage depends on the predominant bacterial or mold species and their concentrations and metabolic catabolites (Lawrie and Ledward 2014). *Enterobacteriaceae*, *Pseudomonas* spp. or *Brochothrix thermosphacta* may be responsible for putrefaction; lactic acid bacteria, *Carnobacterium* spp. or *B. thermosphacta* may be responsible for acidification; *Shewanella putrefaciens*, *Lactobacilli, Enterococcus faecalis*, or *Enterococcus faecium* may be responsible for graying/greening; and *Thomnidium, Mucor, Rhizipos, Cladoporidium*, or *Sporotriculum* may be responsible for mold growth (Sofos 2014, Baumgart et al. 2015). In general, meat begins to spoil at a microbial count of $10^7/cm^2$, which is also sensorily noticeable (Baumgart et al. 2015). Bacterial growth and the resulting spoilage types are also influenced by the chemical and physical properties of the meat and by the conditions and duration of cold storage (Sofos 2014). For example, bacterial growth on meat is promoted by meat-specific water activity and pH or at higher storage temperatures (Sperber 1983, Sofos 2014). In addition, the noticeable sensory spoilage of roe deer meat that can occur immediately after killing the game without bacterial action, is the so-called “stickige Reifung” (“stuffy maturation”) (Bauer et al. 2014). The color of the meat

surface turns to copper-red, the consistency becomes doughy, and the odor has a typical acidic and butyric acid smell (Bauer et al. 2014).

2.3 Process of obtaining roe deer carcasses and game meat

2.3.1 Hunting and influencing factors on the microbial load of game carcasses

Hunting is a process that is challenging to define and standardize in various countries but that requires plausible reasons for killing wild game in every case. The major reasons are the use of meat and fur, the control of animal disease and epidemics, the regulation of the population density of wild game, and the protection of forestry (BfJ 2020a). Hunts are conducted in different seasons or scenarios. In Germany, for example, in Brandenburg, the annual drive hunt season typically begins in October and ends in January (Maaz et al. 2022). However, depending on the released hunting period, game animals can also be hunted in the remaining months of the year according to species, sex and/or age. These periods that are open or closed to hunting are set by the German federal states (BfJ 2020a). Depending on the type of hunting, different hunting practices are used. In Germany and Austria, hunting can be organized as still (Paulsen 2011) or drive hunts (Maaz et al. 2022). In contrast, in Italy, hunting can be conducted as spot and stalk hunts (Avagnina et al. 2012). Furthermore, hunting can be performed with different numbers of hunters, and with/without the use of dogs (Alberto et al. 2011, Serrano et al. 2020). However, every hunt includes these general major stages: observation of the game, shooting/killing/salvaging, evisceration, removal of soiling matter from game carcass, and transport of the game carcasses to the GHE or storage location. Each main step introduces additional factors that can affect the IML and subsequent ML of roe deer carcasses or their meat (Figure 4). However, the order of these major stages may change nationwide or worldwide or may be supplemented by additional intermediate stages such as bleeding (Branciari et al. 2020).

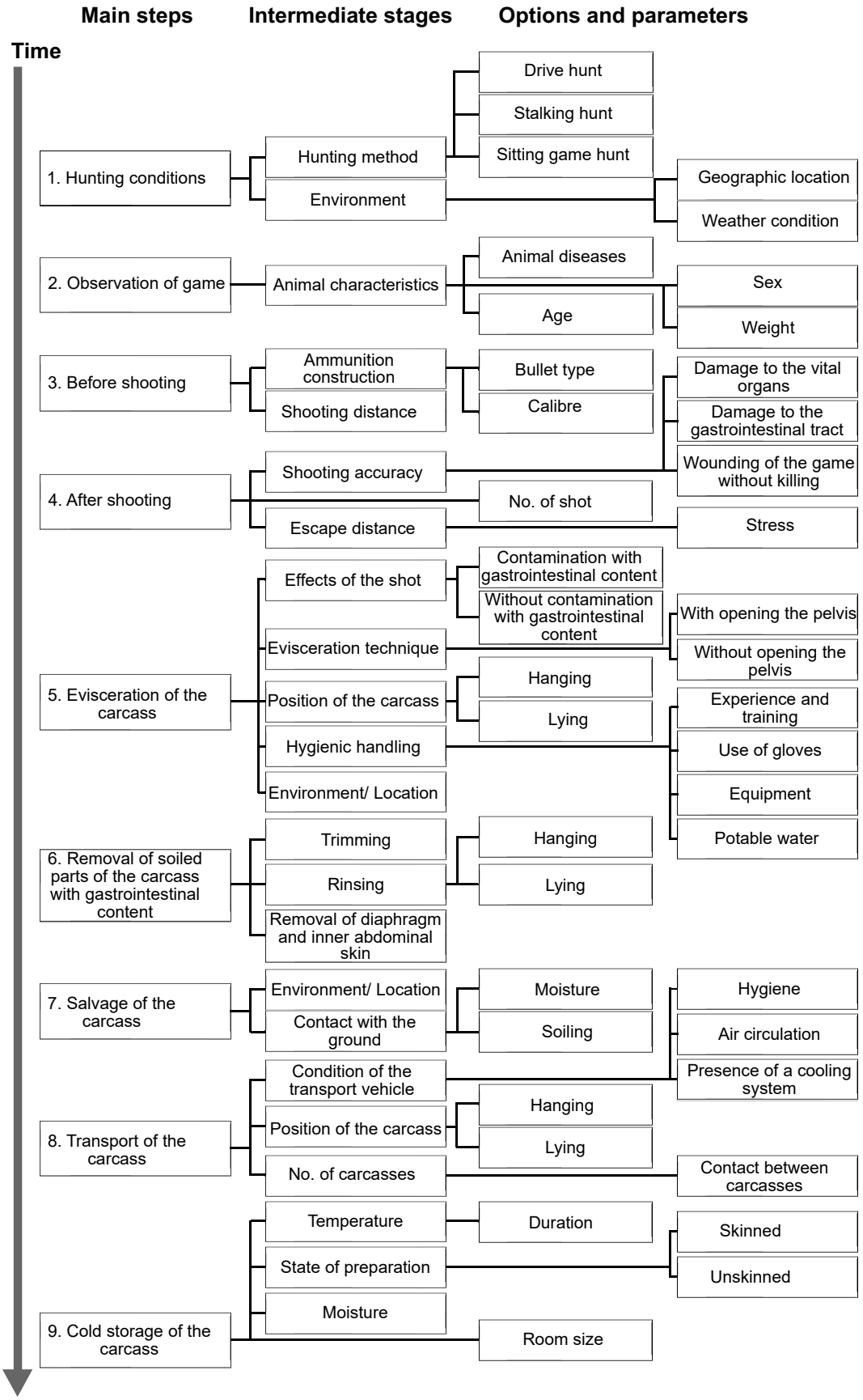


Figure 4: Main hunting steps to obtain game carcasses with intermediate stages, options and parameters described as factors that could influence the microbial load of game carcasses. References are listed in Table 3.

Table 3: Reference list for the main hunting steps to obtain game carcasses with intermediate stages, options and parameters described as factors that could influence the microbial load of game carcasses (presented in Figure 4).

Major step of hunt	Intermediate stage/option/parameter	Reference/s
Hunting condition	Hunting method	Deutz and Fötschl 2014
Hunting condition	Environment/geographic location	Deutz and Fötschl 2014, Mateus-Vargas et al. 2022
Hunting condition	Environment/weather condition	Paulsen and Winkelmayer 2004, Stella et al. 2018, Sauvala et al. 2019, Ranucci et al. 2021
Observation	Animal characteristics, animal disease	Hedman et al. 2020
Observation	Animal characteristics, sex	Sauvala et al. 2019, Ranucci et al. 2021, Peruzy et al. 2022
Observation	Animal characteristics, age	Stella et al. 2018
Observation	Animal characteristics, weight	Branciari et al. 2020, Orsoni et al. 2020, Peruzy et al. 2022
Before shooting	Ammunition construction	Branciari et al. 2020
Before shooting	Shooting distance	Deutz and Fötschl 2014
After shooting	Shooting accuracy	Branciari et al. 2020, Cenci-Goga et al. 2021, Peruzy et al. 2022
After shooting	No. of shot	Cenci-Goga et al. 2021, Peruzy et al. 2022
After shooting	Escape distance	Bandick and Ring 1995
After shooting	Escape distance, stress	Deutz and Fötschl 2014, Tomljanović et al. 2022
Evisceration of the carcass	Effects of the shot	Bandick and Ring 1995, Mirceta et al. 2015, Sauvala et al. 2019
Evisceration of the carcass	Evisceration technique	Cenci-Goga et al. 2021
Evisceration of the carcass	Position of the carcass	Mirceta et al. 2017
Evisceration of the carcass	Hygienic handling	Mirceta et al. 2017
Evisceration of the carcass	Environment/Location	Deutz and Fötschl 2014
Removal of soiled parts of the carcass with gastrointestinal content	Trimming	Van Schalkwyk 2010, Deutz 2012b
Removal of soiled parts of the carcass with gastrointestinal content	Rinsing	Bildungs- Und Wissenszentrum Aulendorf 2008, Rheinisch-Westfälischer Jäger 2017, Amt Für Landschaft Und Natur 2019
Removal of soiled parts of the carcass with gastrointestinal content	Removal of diaphragm and inner abdominal skin	Kujawski and Heintges 1984, Scherling 1989
Salvage of the carcass	Environment	Cenci-Goga et al. 2021
Salvage of the carcass	Contact with ground	Cenci-Goga et al. 2021
Transport	Condition of the transport vehicle	Van Schalkwyk and Hoffman 2011, Deutz and Fötschl 2014, Cenci-Goga et al. 2021
Transport	Position of game	Van Schalkwyk et al. 2011
Transport	No. of carcasses	Deutz and Fötschl 2014
Cold storage of the carcass	Temperature, moisture, room size	Deutz and Fötschl 2014
Cold storage of the carcass	State of preparation	Cenci-Goga et al. 2021
Cold storage of the carcass	Moisture	Deutz and Fötschl 2014
Cold storage of the carcass	Room size	Deutz and Fötschl 2014

2.3.2 Management of food safety and quality

Foods such as meat undergo changes over time that affect their quality, such as meat maturation or spoilage (Gill et al. 1976a, Gill 1983, Farouk et al. 2007, Irschik et al. 2012). To prevent undesirable changes from occurring too quickly, which can decrease meat quality and lead to premature spoilage (Gill 1983), GHP must begin to be followed when harvesting carcasses in the field (Van Schalkwyk et al. 2011, Deutz and Fötschl 2014). GHP means the application of hygiene rules by a food business operator in accordance with the legal requirements, standards and guidelines (Zschaler and Heeschen 2015). The aim is to ensure food safety and quality. When harvesting game carcasses, it is hard to follow GHP due to the challenges present under field conditions and missing standards (Van Schalkwyk et al. 2011, Deutz and Fötschl 2014, Mirceta et al. 2017), such as a lack of warm running water (Paulsen 2011). Therefore, additional hygiene measures must be taken during game meat production as part of good game meat hygiene. For example, different measures apply to the processing of carcasses that have already been soiled with GIC due to the shot than those that have been properly shot. One measure could be trimming of the soiled carcass parts followed by washing with water at ambient temperature (Paulsen et al. 2012) or shortening the cold storage time of the carcass (Borilova et al. 2016). These measures depend on the expertise and experience of the certified hunter as well as on the given circumstances (Deutz and Fötschl 2014, Mirceta et al. 2017).

In addition to good game meat hygiene in the field, other preventive measures to avoid contamination, such as the HACCP concept must be considered when processing carcasses, e.g., in GHEs (European Commission 2004a, European Commission 2004b). This is because the HACCP concept identifies, evaluates and controls hazards to consumer health (e.g., pathogenic bacteria in meat) in advance (Pierson and Corlett 1993, Mortimore et al. 2002, Zschaler and Heeschen 2015). Another quality management tool used to test the production of safe foods is the FMEA (DIN e.V. 2018). Failure Mode and Effects Analysis is used to avoid potential failures, such as harvesting contaminated game carcasses, by systematically analyzing, reducing, and ideally eliminating all possible failures that could lead to them in advance (Andrée et al. 2010). The evaluation of hazards and possible sources of failures for safe food can be performed using quantitative and/or qualitative parameters. Quantitative parameters include the levels of certain bacterial species in carcasses, which serve as indicator microorganisms (Halkman and Halkman 2014) and present reference values to evaluate the hygienic processing conditions of carcasses (Baumgart et al. 2015). The TVC, levels of *Enterobacteriaceae* and the presence of pathogenic bacteria (e.g., coagulase positive staphylococci, *Campylobacter*, *L. monocytogenes*, *Salmonella* spp. and *Y. enterocolitica*) are used to study the microbial condition (Paulsen and Winkelmayer 2004, Atanassova et al. 2008, Avagnina et al. 2012) and the presence of *E. coli* (Irschik et al. 2012,

Asakura et al. 2017) is determined to be an indicator of fecal contamination in game carcasses. Pathogenic bacteria such as Shiga toxin-producing *E. coli* (STEC) are an important group of food-borne zoonotic pathogens (Díaz-Sánchez et al. 2012). Furthermore, *Pseudomonas* spp. and *Lactobacillus* spp. are relevant meat-specific spoilage bacteria that may adversely affect the sensory characteristics of meat (Ayres 1955, Sofos 2014). Sensory characteristics such as appearance, color and odor can therefore serve as parameters for evaluating meat quality (BfR 2006). This is because they are attributable, for example, to the ML of the meat (BfR 2006, Baumgart et al. 2015). Other qualitative parameters that can be determined in the field and that may affect meat quality are the shooting accuracy and the presence of soiling on the carcass (BfR 2006, Avagnina et al. 2012, Branciari et al. 2020, Ranucci et al. 2021). These parameters could be used for evaluating meat quality.

3 Content and research aims of this thesis

As presented in previous chapters, the production of game meat from game carcasses is a relatively complex process in terms of food hygiene and quality, especially microbial condition aspects. This process begins with hunting in the field, continues at the GHE, and ends when the game meat reaches the consumer via the distribution channels described in Chapter 2.1. The focus of this thesis is only on a part of this food chain, beginning with primary production in the field and ending with meat maturation in a research facility as a model for a GHE. This is because the microbial condition of game meat depends on the circumstances that occurred during hunting (Ranucci et al. 2021) and can be negatively affected by improper handling practices in the field (Mirceta et al. 2017, Orsoni et al. 2020). However, the effects of handling practices, such as removing visible soiling from game carcasses, particularly by rinsing the body cavities, on the IML and subsequent ML of game carcasses and meat have not been fully elucidated.

This thesis is based on the reports of a preliminary test (Chapter 3.1) and three studies, the results of which were published in two research articles (Chapters 3.2 and 3.4) and a short communication (Chapter 3.3). The preliminary test was used to develop the rinsing process for Studies I – II (Chapters 3.2 – 3.3) and was not published as an independent study. The research papers and short communication on the IML and subsequent ML of roe deer carcasses were published in peer-reviewed journals at the time of submission of this thesis. This chapter provides a summary of the objectives of the investigations.

To standardize the rinsing process of the game carcasses used in Studies I – II, the objective of the preliminary test was to investigate the performance of two rinsing devices under defined conditions at a research facility and to determine the appropriate rinsing conditions. The aim of Studies I and II was to investigate whether rinsing the body cavities of roe deer carcasses with drinking water affects the IML and subsequent ML of the carcasses and/or edible meat. For this purpose, the microbial condition of visibly soiled and not visibly soiled carcasses were compared, and carcasses whose body cavities were intentionally soiled with a gastrointestinal mixture were also examined. The aim of intentional contamination was to achieve a higher IML to demonstrate the assumed ML-reducing effect of rinsing. Regarding the methodology, in both studies, the IMLs and subsequent MLs of rinsed and unrinsed carcasses were determined and compared on the day of killing and after three days of meat maturation at + 4 °C. During the primary production of roe deer carcasses in the field, other handling practices besides rinsing are handled differently, e.g., wearing or not wearing gloves during evisceration. Whether these handling practices and other options and parameters of the intermediate steps of the hunt have an influence on the IML of roe deer carcasses was investigated in Study III.

3.1 Preliminary test

Abstract

Several varying practices and measures used during the handling of hunted roe deer (*Capreolus capreolus*) carcasses, such as rinsing the body cavities, can affect their microbial condition. To investigate the effects of rinsing on the initial microbial load (IML) and subsequent microbial load (ML) of roe deer body cavities in the studies, the development of a standardized rinsing procedure was essential. Therefore, the aim of this preliminary test was to determine, for example, the appropriate water application to the body cavities during rinsing (n = 2) to establish a standardized rinsing procedure. This would include, for example, the position of the carcasses during rinsing. Thus, it was specified that the body cavities of roe deer carcasses used in the studies were to be rinsed using a low-pressure device while hanging from their hind legs on a game gallows. During rinsing, the body cavities were to be kept open with a rib spreader.

1. Introduction

Production of hygienic game meat is challenging due to the various practices and parameters related to hunting conditions that can contaminate game carcasses (Orsoni et al. 2020, Ranucci et al. 2021). This can result in a higher microbiological load in the game meat obtained (Orsoni et al. 2020). Rinsing the game body cavities, especially those soiled with gastrointestinal contents (GIC), is recommended to remove soiling from hunted game carcasses (Deutz and Fötschl 2014). There is no standardized rinsing process, such as using a tested rinsing device with a defined water pressure or temperature control system, for game carcasses in the field. In practice, rinsing water is applied using, for example, drinking water bottles or electric outdoor cleaning devices (Anonymous 2015).

The aim of this preliminary test was to compare two devices for water application when rinsing freshly slaughtered pig carcasses. Therefore, the distribution of rinsing water in the body cavity, the amount of rinsing water used, the flow rate of the equipment and the amount of water remaining in the body cavity were considered. Pigs were chosen as a model animal because of their availability.

2. Materials and Methods

To compare the rinsing performance of the two rinsing devices, two pig carcasses were rinsed under standardized conditions. Freshly slaughtered and eviscerated pig carcasses were hung by their hind legs, and rinsing extended from the pelvis to the ribcage of the pig carcasses. The body cavities of the carcasses with body weights of approximately 25 to 30 kg were held open with a rib spreader during rinsing. To visualize the distribution and coverage of the rinse, 0.5 % methylene blue was added to deionized water. Water was

applied to the first pig body cavity using a manual spray bottle (countryside, 2 L, 0 bar, Germany) and to the second pig body cavity using an electric low pressure device (Fontus, Bosch, 15 L, 1st stage: low pressure, data on the exact pressure were not available, Germany). The pig body cavities were rinsed for 10 seconds following the method of an instructional video on hygienic evisceration of game carcasses provided by the German Hunting Association (DJV 2017a). Based on the duration of the rinsing process and the previously determined flow rate, the amount of rinsing water used to rinse the pig carcasses was calculated. The flow rate was determined to be 238 mL/min for the manual spray bottle and 1460 mL/min for the electric low pressure device. The amount of water drained from the pig body cavities was measured 17 min after rinsing. The drained rinsing water was collected in a plastic tray, then transferred into a graduated cylinder and measured in mL.

3. Results

The results of this preliminary test on pig carcasses showed that less water was applied to the body cavity in the defined time of 10 seconds when using the manual spray bottle than with the rinsing spray produced by the electric low pressure device (Table 1). In addition, more extensive and uniform rinsing of the pig body cavity was achieved by the rinsing jet of the low-pressure device.

Table 1: Comparison of rinsing performances based on the rinsing parameters of the manual spray bottle and electric low pressure device

Parameter	Manual spray bottle	Electric low pressure device
Flow rate, mL/min	238	1460
Rinsing water used, mL	40	244
Drained rinsing water after rinsing, mL	30	200
Amount of water remaining in the body cavity in relation to the amount of water used, %.	25	18
Distribution of rinsing water in the body cavity	Uneven coverage	Even coverage

4. Discussion

In this preliminary test using pig carcasses as model animal carcasses, the rinsing performances of two different rinsing devices were compared. In practice, there is no standardized rinsing procedure with a specified rinsing device for game carcasses (Anonymous 2015). The electric low pressure device had a larger capacity, higher flow rate, and a water spray distribution that caused a more even distribution of water in the pig body cavity. Thus this device was identified as ideal for rinsing roe deer body cavities in future studies. It was assumed that the larger capacity of the rinsing device would be necessary if several roe deer carcasses needed to be rinsed on the same hunting day. Furthermore, it was assumed that heavily soiled carcasses could also be cleaned more easily by visual means due to the low pressure and higher flow rate of the electric low pressure device compared to those of the manual spray bottle.

In this preliminary test of rinsing with the electric low pressure device, after a 15 min hanging time of the pig carcasses, no visual changes in the color intensity could be observed in the pig body cavity and the remaining residual water almost ceased dripping from the meat surface. After 17 min of hanging time, 85 % of the rinse water used had drained from the pig carcass. To ensure that as little residual rinsing water as possible would remain in a roe deer body cavity within a manageable time under hunting conditions, an additional 3 min extension was planned for future studies. Thus, it was specified that roe deer carcasses must hang for 20 min after rinsing with the electric low pressure device to reduce the amount of residual water as much as possible. Hanging the carcasses by their hind legs also ensured that the legs, which are valuable as food, remained dry.

5. Conclusion

Based on these research results, the rinsing procedure performed in the future studies is to be performed with an electric low pressure device.

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3.2 Study I - 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.

Keywords

Game meat hygiene, hunting, meat maturation, microbial quality, soiling, washing

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 852, 2004; Regulation (EC) No 853, 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, 2019; Rheinisch-Westfälischer Jäger, 2017). Others recommend a general rinsing of all game carcasses (Bildungs- und Wissenszentrum Aulendorf, 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, 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 (Figure 1).

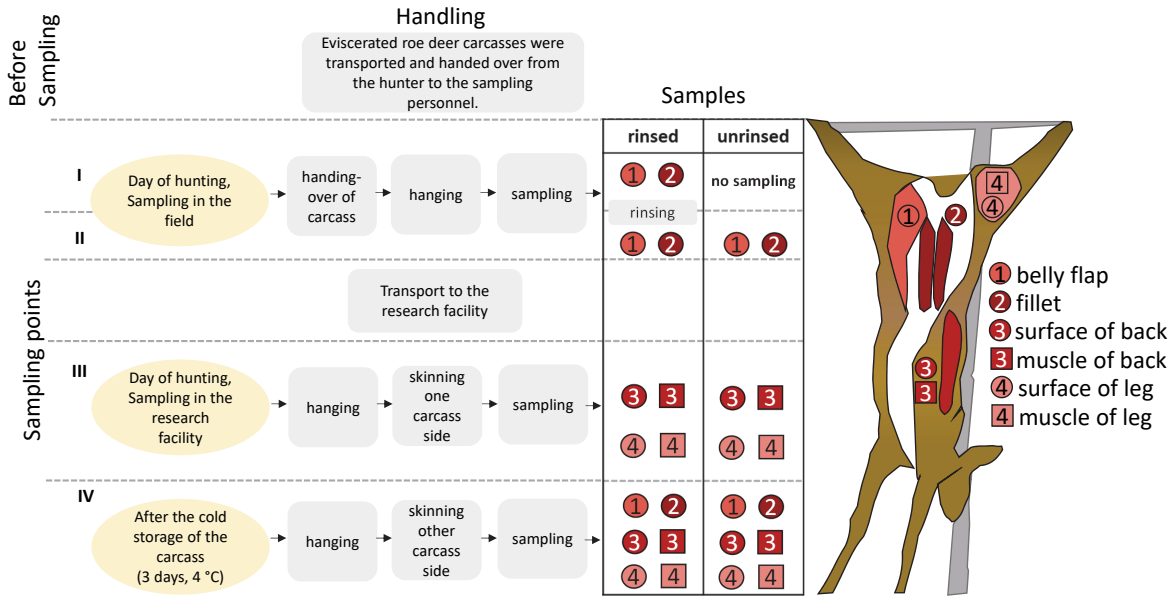

Figure 1. Study design and sampling of rinsed and unrinsed roe deer carcasses. Gray fields indicate the handling process. The yellow ovals mark the sampling points. The sampled areas of the roe deer carcasses were numbered (1 - 4, meat surface samples were arranged in circular fields and muscle samples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

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 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 x 7.6 cm; 3M Sponge-Stick, Mercateo Deutschland AG, Munich, Germany), followed by a dry swab (16 x 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 cm² for belly flaps, and freshly skinned surface of the carcasses and 20 cm² 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/cm² 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 value 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 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 after the sampling in the field (sampling II, Figure 1) was 25.7 ± 0.8 °C. Additional information was collected on roe deer carcasses (Table 1).

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 during evisceration	Hanging	1 (8 %)	1 (9 %)
	Lying on the ground	11 (92 %)	10 (91 %)
Visible soiling with intestinal content[1]	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 %)

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

The handling processes and sampling points were defined (Figure 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 (Figure 2).

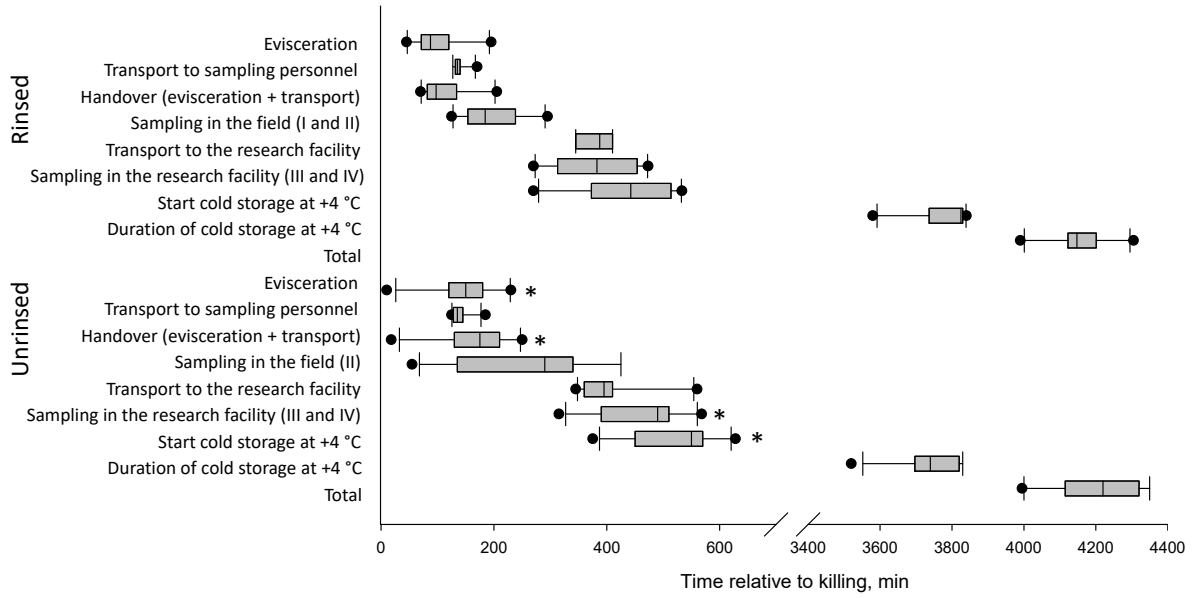

Figure 2. Comparison of the relative time spans during the hunting supply chain of randomly assigned rinsed (n = 12) and unrinsed (n = 11) roe deer carcasses using t-test for independent variables. All time data were set in relation to the killing time (time = 0). A star (*) indicates statistically different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

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, Figure 3C). 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/cm², 2.51 ± 0.72 $\log_{10}$ CFU/cm², 2.56 ± 0.31 $\log_{10}$ CFU/cm², 2.32 ± 0.43 $\log_{10}$ CFU/cm² and 1.82 ± 0.42 $\log_{10}$ CFU/cm², respectively. The count levels on unsoiled carcasses were 5.40 ± 0.36 $\log_{10}$ CFU/cm², 3.76 ± 0.71 $\log_{10}$ CFU/cm², 3.34 ± 0.23 $\log_{10}$ CFU/cm², 3.44 ± 0.39 $\log_{10}$ CFU/cm², and 2.53 ± 0.38 $\log_{10}$ CFU/cm², respectively (Figure 3 A - E). The level of *Enterobacteriaceae* in fillets (Figure 4) was lower in soiled carcasses (2.50 ± 0.44 $\log_{10}$ CFU/cm²) than in unsoiled carcasses (3.45 ± 0.41 $\log_{10}$ CFU/cm²).

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 (Figure 3B). The same interaction was observed for *Pseudomonas* spp. in fillet (Figure 4B). The counts of *Pseudomonas* spp. on belly flaps tended to be lower after

rinsing and ranged from 3.04 ± 0.3 log_{10} CFU/cm² to 2.48 ± 0.3 log_{10} CFU/cm²; 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 (Figure 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.

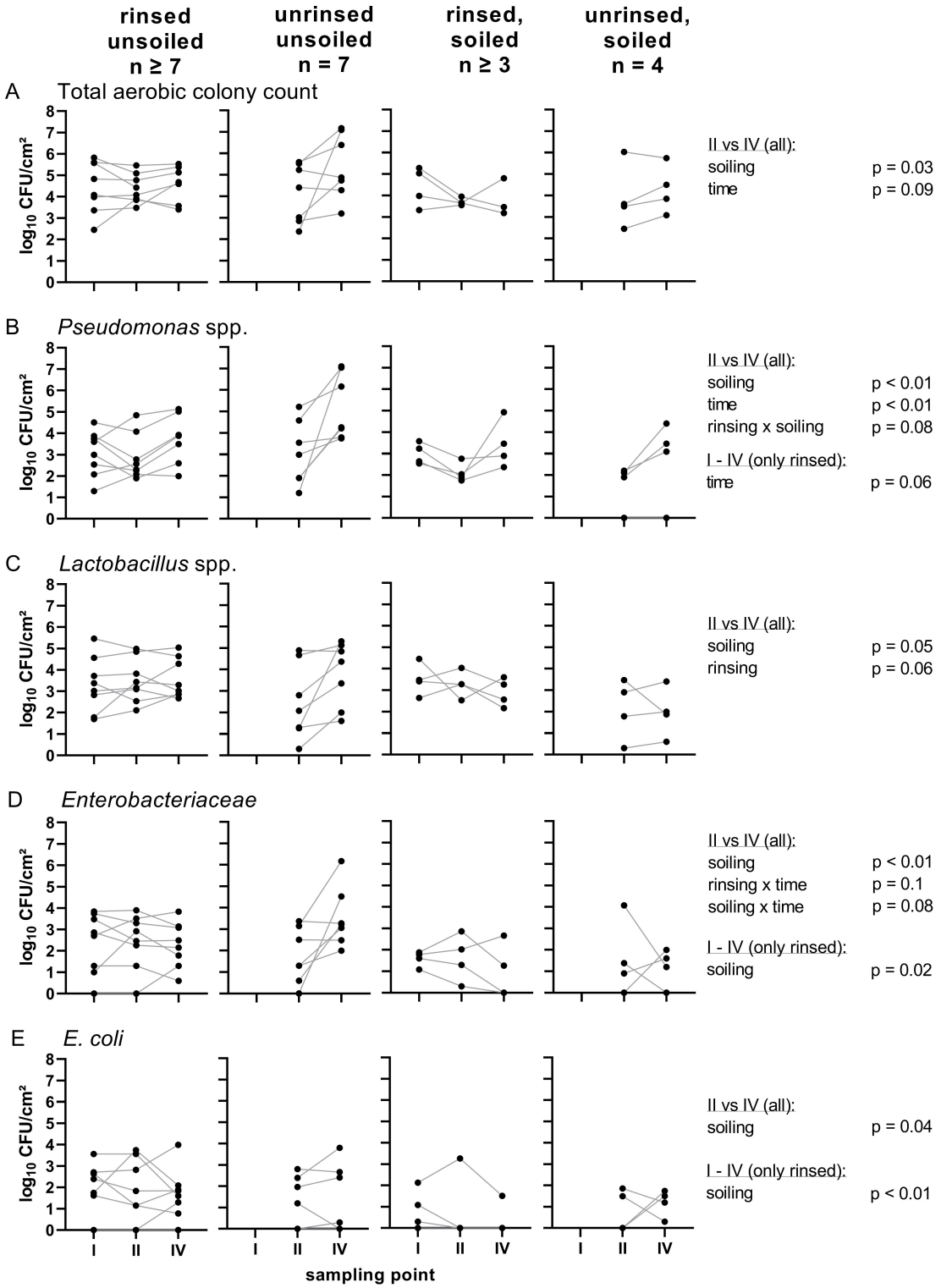

Figure 3. Belly flaps. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp., C: *Lactobacillus* spp., D: *Enterobacteriaceae*, E: *E. coli*) on the meat surface of belly flaps of rinsed and unrinsed carcasses at the sampling points: before rinsing (I), after rinsing or not

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.

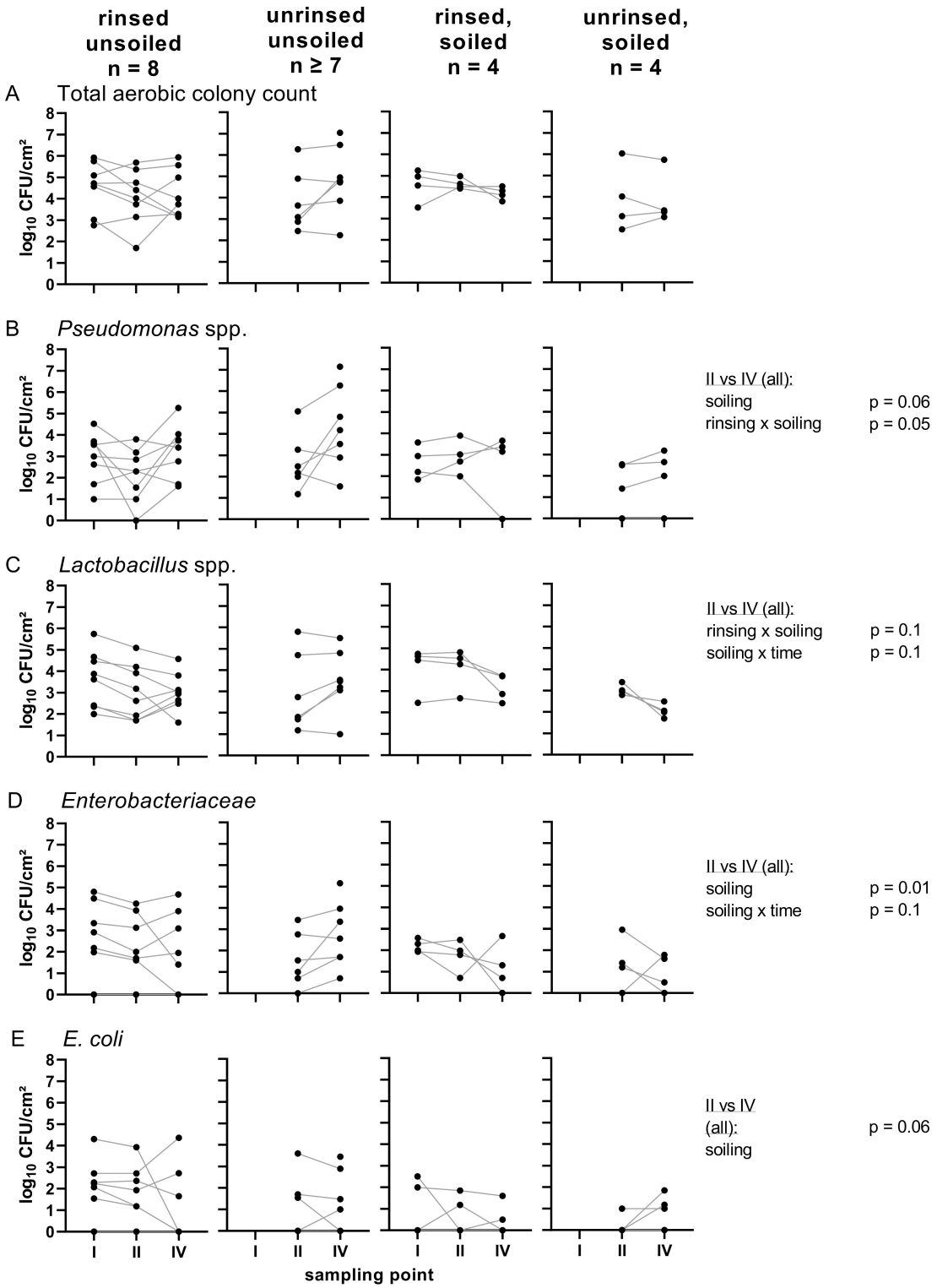

Figure 4. Fillets. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp., C: *Lactobacillus* spp., D: *Enterobacteriaceae*, E: *E. coli*) on the meat surface of fillets of rinsed and unrinsed carcasses at the sampling points: before rinsing (I), after rinsing or not rinsing

(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.

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/cm² on the leg meat surface of skinned carcasses (Figure S1) and a maximum of 5.6 $\log_{10}$ CFU/cm² on the skinned back meat surface (Figure 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 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 hours 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 to 7 $\log_{10}$ CFU/cm² (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 et al. (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/cm² and 4.1 $\log_{10}$ CFU/cm²), in contrast to the samples that were collected without rinsing (5.2 $\log_{10}$ CFU/cm² and 3.6 $\log_{10}$ CFU/cm²). 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/cm² and 2.3 $\log_{10}$ CFU/cm²) than carcasses rinsed lying on the field (6.0 $\log_{10}$ CFU/cm² and 4.4 $\log_{10}$ CFU/cm²) (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 during 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.

Supplementary Material

Table S1. Least Squares Mean (LS mean) and standard error (SE) of the microbial load (total aerobic colony count, *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae*, *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

Table S2. Least Squares Mean (LS mean) and standard error (SE) of the microbial load (total aerobic colony count, *Pseudomonas* spp*., Lactobacillus* spp., *Enterobacteriaceae*, *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.

Table S3. Frequency of bacterial counts above the limit of detection (LOD) in leg muscles of rinsed and unrinsed roe deer carcasses. The groups were classified in carcasses with and without visible soiling with intestinal content. Samples were taken from leg muscles before (III) and after (IV) cold storage for 3 days at +4 °C. Values are presented as positive when the bacterial count of the samples was above the LOD of 10 CFU/g.

Table S4. Frequency of bacterial counts above the limit of detection (LOD) in back muscles of rinsed and unrinsed roe deer carcasses. The groups were classified in carcasses with and without visible soiling with intestinal content. Samples were taken from leg muscles before (III) and after (IV) cold storage for 3 days at +4 °C. Values are presented as positive when the bacterial count of the samples was above the LOD of 10 CFU/g.

Figure S1. Skinned meat surface of leg muscle. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp., C: *Lactobacillus* spp., D: *Enterobacteriaceae*, E: *E. coli*) on the skinned meat surface of leg muscle of rinsed and unrinsed carcasses at the sampling points: before (III) and after (IV) cold storage for 3 days at +4 °C. 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 III and IV. The values are presented for individual carcasses; values below the LOD are given as 0.

Figure S2. Skinned meat surface of back muscle. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp., C: *Lactobacillus* spp., D: *Enterobacteriaceae*, E: *E. coli*) on the skinned meat surface of back muscle of rinsed and unrinsed carcasses at the sampling points: before (III) and after (IV) cold storage for 3 days at +4 °C. 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 III and IV. The values are presented for individual carcasses; values below the LOD are given as 0.

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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.

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Table S1. Least Squares Mean (LS mean) and standard error (SE) of the microbial load (total aerobic colony count, *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae, 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

Parameter	**Rinsed**		**Unrinsed**	
	unsoiled	**soiled**	**unsoiled**	**soiled**
	n ≥ 7	**n ≥ 3**	**n = 7**	**n = 4**
Total aerobic count in CFU/cm²				
Sampling I	4.5 ± 0.4	4.4 ±0.5	-	-
Sampling II	4.4 ± 0.2	3.7 ± 0.1	4.1 ± 0.5	3.9 ± 0.8
Sampling IV	4.6 ± 0.3	3.8 ± 0.5	5.4 ± 0.6	4.3 ± 0.6
Pseudomonas spp. in CFU/cm²				
Sampling I	3.1 ± 0.4	3.0 ± 0.3	-	-
Sampling II	2.9 ± 0.4	2.1 ± 0.2	3.0 ± 0.6	1.6 ± 0.5
Sampling IV	3.7 ± 0.4	3.4 ± 0.6	5.2 ± 0.6	2.7 ± 1.0
Lactobacillus spp. in CFU/cm²				
Sampling I	3.3 ± 0.5	3.5 ± 0.4	-	-
Sampling II	3.5 ± 0.4	3.3 ± 0.3	2.5 ± 0.7	2.1 ± 0.7
Sampling IV	3.6 ± 0.3	2.9 ±0.3	3.8 ± 0.6	2.0 ± 0.6
Enterobacteriaceae in CFU/cm²				
Sampling I	2.4 ± 0.5	1.6 ±0.2	-	-
Sampling II	2.5 ±0.5	1.6 ± 0.5	1.8 ± 0.5	1.6 ± 0.9
Sampling IV	2.3 ± 0.4	1.0 ± 0.6	3.5 ± 0.5	1.2 ± 0.4
E. coli in CFU/cm²				
Sampling I	1.8 ± 0.5	0.9 ± 0.5	-	-
Sampling II	1.8 ± 0.5	0.8 ± 0.8	1.2 ± 0.5	0.8 ± 0.5
Sampling IV	1.7 ± 0.4	0.4 ± 0.4	1.6 ± 0.6	1.2 ± 0.3

Table S2: Least Squares Mean (LS mean) and standard error (SE) of the microbial load (total aerobic colony count, *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae, 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

Parameter	**Rinsed**		**Unrinsed**	
	unsoiled	**soiled**	**unsoiled**	**soiled**
	n = 8	**n ≥ 6**	**n = 4**	**n = 4**
Total aerobic count in CFU/cm²				
Sampling I	4.6 ± 0.4	4.6 ± 0.4	-	-
Sampling II	4.1 ± 0.5	4.6 ± 0.1	3.9 ± 0.6	3.9 ± 0.8
Sampling IV	4.2 ± 0.4	4.2 ± 0.2	4.9 ± 0.6	3.9 ± 0.6
Pseudomonas spp. in CFU/cm²				
Sampling I	3.0 ± 0.4	2.6 ± 0.4	-	-
Sampling II	2.1 ± 0.4	2.9 ± 0.4	2.7 ± 0.6	1.6 ± 0.6
Sampling IV	3.3 ± 0.4	2.6 ± 0.9	4.3 ± 0.7	2.0 ± 0.7
Lactobacillus spp. in CFU/cm²				
Sampling I	3.7 ± 0.5	4.1 ± 0.5	-	-
Sampling II	3.0 ± 0.5	4.1 ± 0.5	3.0 ± 0.8	3.0 ± 0.1
Sampling IV	3.0 ± 0.3	3.2 ± 0.3	3.5 ± 0.5	2.1 ± 0.2
Enterobacteriaceae in CFU/cm²				
Sampling I	2.4 ± 0.6	2.2 ± 0.2	-	-
Sampling II	2.1 ± 0.6	1.7 ± 0.4	1.6 ± 0.5	1.4 ± 0.6
Sampling IV	1.9 ± 0.7	1.2 ± 0.6	2.7 ± 0.6	1.0 ± 0.4
E. coli in CFU/cm²				
Sampling I	1.9 ± 0.5	1.1 ± 0.7	-	-
Sampling II	1.7 ± 0.5	0.8 ± 0.5	1.1 ± 0.6	0.3 ± 0.3
Sampling IV	1.1 ± 0.6	0.5 ± 0.4	1.3 ± 0.5	1.0 ± 0.4

Table S3: Frequency of bacterial counts above the limit of detection (LOD) in leg muscles of rinsed and unrinsed roe deer carcasses. The groups were classified in carcasses with and without visible soiling with intestinal content. Samples were taken from leg muscles before (III) and after (IV) cold storage for 3 days at +4 °C. Values are presented as positive when the bacterial count of the samples was above the LOD of 10 CFU/g.

Parameter	Rinsed				Unrinsed			
	unsoiled		soiled		unsoiled		soiled	
	total	positive	total	positive	total	positive	total	positive
Total aerobic count, n > 10 CFU/g								
Sampling III	8	5	4	4	7	2	4	1
Sampling IV	8	7	3	2	7	3	4	1
Pseudomonas spp., n > 10 CFU/g								
Sampling III	8	1	4	0	7	0	4	0
Sampling IV	8	0	4	0	7	0	4	1
Lactobacillus spp., n > 10 CFU/g								
Sampling III	8	1	4	0	7	0	4	0
Sampling IV	8	2	4	1	7	1	4	0
Enterobacteriaceae, n > 10 CFU/g								
Sampling III	8	0	4	0	7	0	4	0
Sampling IV	8	0	4	0	7	0	4	1
E. coli, n > 10 CFU/g								
Sampling III	8	0	4	0	7	0	4	0
Sampling IV	8	0	4	0	7	0	4	1

Table S4. Frequency of bacterial counts above the limit of detection (LOD) in back muscles of rinsed and unrinsed roe deer carcasses. The groups were classified in carcasses with and without visible soiling with intestinal content. Samples were taken from leg muscles before (III) and after (IV) cold storage for 3 days at +4 °C. Values are presented as positive when the bacterial count of the samples was above the LOD of 10 CFU/g.

Parameter	Rinsed				Unrinsed			
	unsoiled		soiled		unsoiled		soiled	
	total	positive	total	positive	total	positive	total	positive
Total aerobic count, n > 10 CFU/g								
Sampling III	8	5	4	4	7	6	4	1
Sampling IV	8	6	4	4	7	6	4	0
Pseudomonas spp., n > 10 CFU/g								
Sampling III	8	0	4	0	7	1	4	0
Sampling IV	8	1	4	0	7	1	4	0
Lactobacillus spp., n > 10 CFU/g								
Sampling III	8	0	4	2	7	2	4	0
Sampling IV	8	1	4	0	7	4	4	0
Enterobacteriaceae, n > 10 CFU/g								
Sampling III	8	0	4	0	7	0	4	1
Sampling IV	8	1	4	0	7	1	4	0
E. coli, n > 10 CFU/g								
Sampling III	8	0	4	0	7	0	4	0
Sampling IV	8	0	4	0	7	0	4	1

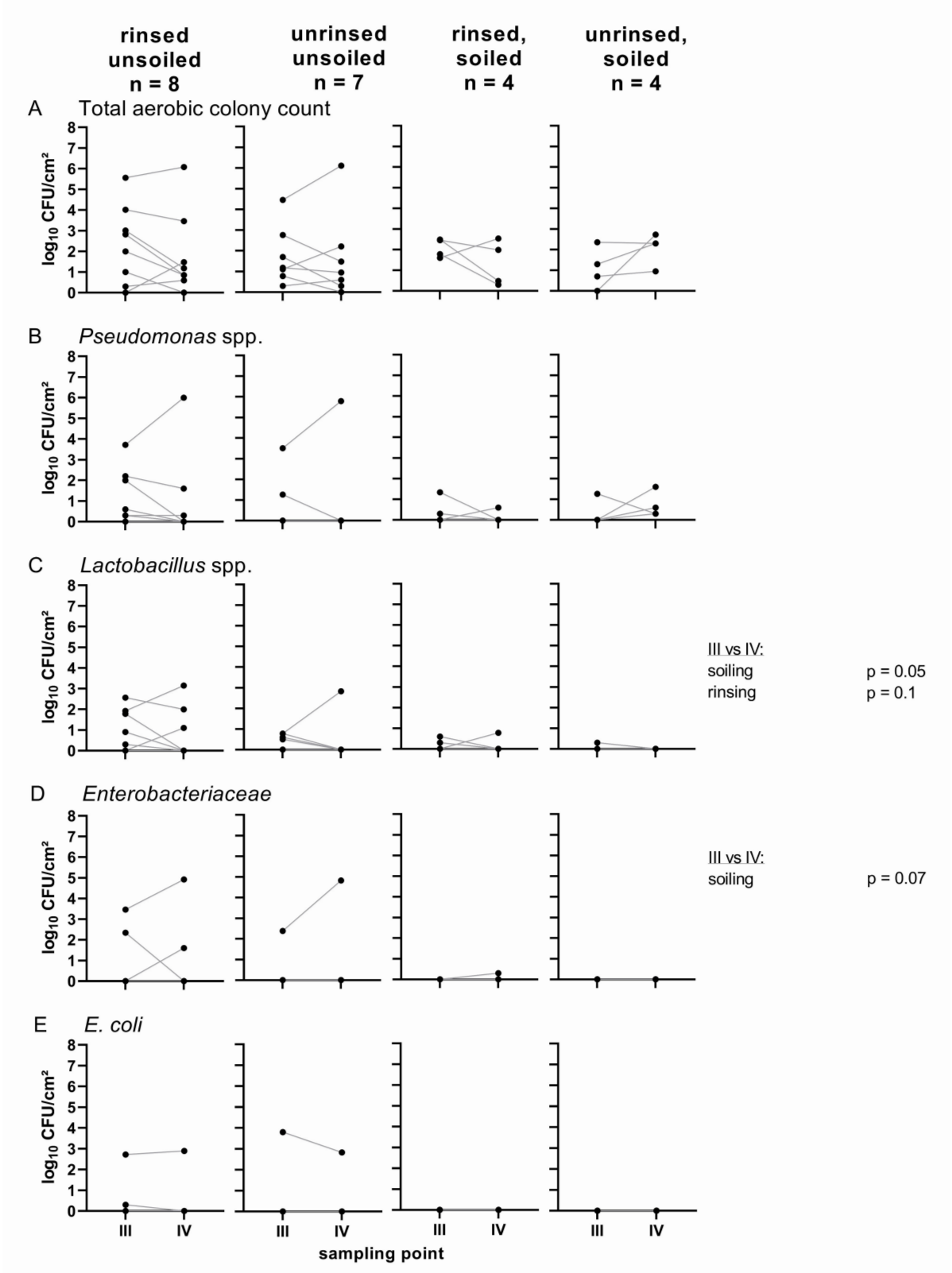

Figure S1. Skinned meat surface of leg muscle. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp., C: *Lactobacillus* spp., D: *Enterobacteriaceae*, E: *E. coli*) on the skinned meat surface of leg muscle of rinsed and unrinsed carcasses at the sampling points: before (III) and after (IV) cold storage for 3 days at +4 °C. 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 III and IV. The values are presented for individual carcasses; values below the LOD are given as 0.

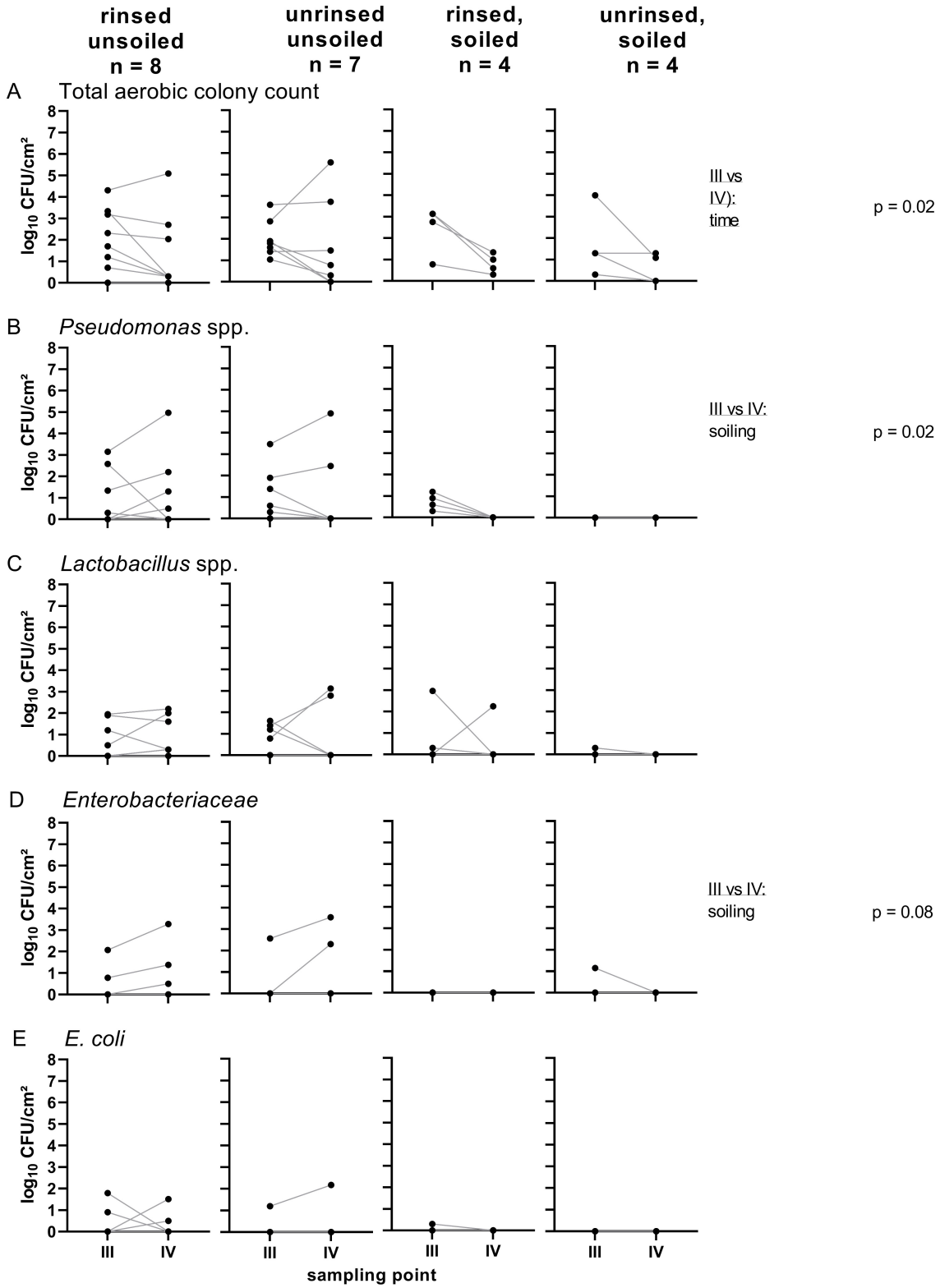

Figure S2. Skinned meat surface of back muscle. Microbial load (A: total aerobic colony count, B: *Pseudomonas* spp., C: *Lactobacillus* spp., D: *Enterobacteriaceae*, E: *E. coli*) on the skinned meat surface of back muscle of rinsed and unrinsed carcasses at the sampling points: before (III) and after (IV) cold storage for 3 days at +4 °C. 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 III and IV. The values are presented for individual carcasses; values below the LOD are given as 0.

3.3 Study II - Microbiological investigation on the effect of rinsing of intentionally soiled roe deer carcasses

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Niels Bandick: conceptualization, methodology resources, writing – review & editing.

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Abstract

Reduction of the microbial load of soiled carcasses is essential in the production of game meat. Rinsing with water is a common practice in handling game carcasses to remove any visible contamination. In this study, microbiological investigations were performed on carcasses of roe deer (*Capreolus capreolus*), inoculated with a mixture of gastrointestinal content and then either rinsed (n = 3) or unrinsed (n = 3). Water rinsing may have short-term effects on bacterial contamination related to soiling. However, introducing water into the body cavity may promote bacterial growth during cold storage.

Keywords

Game meat, Microbial contamination, Gastrointestinal content, Water washing, Soiled carcasses

1 Introduction

In contrast to livestock meat production, several uncontrollable factors influence the microbiological quality of game meat products at the primary production stage. Disregarding the level of influence of natural conditions, factors such as damage to the abdominal area or an inadequate evisceration technique affect the microbial load (ML) of the muscle surfaces (Branciari et al. 2020; Mirceta et al. 2017). In the field, the presence of soiling is one of the most noticeable indications of the unsuccessful shot or inadequate evisceration practice (Avagnina et al. 2012; Paulsen and Winkelmayer 2004). Nevertheless, carcasses that are visually clean may also contain relevant MLs (Korkmaz et al. 2022a; Paulsen and Schopf 2016; Paulsen et al. 2022). Rinsing with water is one of the most recommended corrective measures to reduce the visible soiling of carcasses as well as the resulting ML (Deutz 2014). As previously discussed for roe deer shot by certified hunters in Germany, rinsing with drinking water at ambient temperature was not always effective, and the corresponding reduction of the ML was not always reproducible (Korkmaz et al. 2022a). The latter is partly due to the fact that the initial ML of surfaces of roe deer's abdominal cavity (belly flap or fillet) was not always visibly associated with soiling. Thus, this incongruence impeded a clear statement on the effectiveness of rinsing on the ML reduction of soiled roe deer carcasses (Korkmaz et al. 2022a). In this study, we aimed to assess the effect of water rinsing on the ML of carcasses directly at harvesting in the field, with a limited set of samples. By experimentally soiling with gastrointestinal content (GIC), we intended to reproduce similar MLs for the rinsed and the unrinsed carcasses. And thus, eliminating this confounder and achieving comparability of ML values prior to treatment at a single hunting day with its particular weather conditions.

2 Results and discussion

Using deliberate contamination of the roe deer body cavities with a freshly prepared mixture of GIC, we intended to standardize the initial microbial conditions of body cavities to better elucidate the impact of rinsing on the ML of game carcasses. Experiments were conducted directly in the hunting ground using freshly shot carcasses under field conditions. Table 1 shows an overview of the basic experimental conditions of each trial. In the context of the practical focus of the experiments, some ambient as well as individual factors could not be controlled, and consequently differed between trials (Table 1). Since ambient temperature, have been described to affect the microbiological quality of game carcasses during the harvesting (Korkmaz et al. 2022b; Paulsen and Winkelmayer 2004; Branciari et al. 2020), the comparing of rinsed and unrinsed carcasses were evaluated separately within each trial. Further influencing factors such as body weight, elapsed time between shot and evisceration of the animals, and the time between the evisceration and the further processing (Korkmaz et al. 2022b; Paulsen and Winkelmayer 2004; Paulsen et al. 2022) are additional challenges

for the interpretation of the outcome of the experiments. Despite the low number of carcasses and the different conditions between the hunts for the microbial contamination and development, the subsequent inclusion of further animals for experimental soiling was not undertaken for ethical reasons. The number of animals was also chosen based on results of a previous study, where a higher sample number did not allow further insights on the microbiological effects of rinsing soiled body cavities (Korkmaz et al. 2022a). Nevertheless, we stress that the practical context of this study as well as the use of 2 different muscle surfaces bring valuable results. We obtained baseline data in the field for a basic measure of hygiene that is important for hunters and stakeholders alike. It could be a critical point in the primary production chain of game meat.

In accordance with previous reports (Korkmaz et al. 2022a; Paulsen and Schopf 2016), the ML of visually clean body cavities considerably differed prior to soiling both between and within the trials. The bacteriological load on the surface samples for every trial are presented in Fig. 1. According to an investigation of Paulsen et al. (2022) in 352 hunted roe deer, bacterial counts of clean body cavities can differ considerably between animals even without perforation of structures of the gastrointestinal tract. As expected, sampling before soiling (BS) vs. sampling after soiling (AS) resulted in a general increase of the ML in belly flaps and fillets of soiled carcasses (Fig. 1). The increase in bacterial counts occurred independently from the initial ML and with single exceptions for the *Enterobacteriaceae* and *E. coli*. However, the level of increased ML differed between trials, which may be related to the different bacterial composition of GIC mixtures. In trial 1 and 2, the bacterial load in the mixture ranged from 4.3 to 7.1 $\log_{10}$ CFU/g for the total aerobic colony count (TAC), from 3.7 to 4.4 $\log_{10}$ CFU/g for *Pseudomonas* spp., from 2.5 to 4.2 $\log_{10}$ CFU/g for *Lactobacillus* spp., and from 2.5 to 4.7 $\log_{10}$ CFU/g for *Enterobacteriaceae*. *E. coli* was either below the limit of detection or reached counts of 4.7 $\log_{10}$ CFU/g. Due to technical issues, data of trial 3 was not considered. Although, the proportions of the GIC for preparation of the mixture was comparable between trials, divergence on bacterial content of the mixtures may have occurred due to differences in the microbial content in the segments of the gastrointestinal tract, and may explain the apparent incongruences BS to AS in unrinsed carcasses, especially for both fecal indicators (Fig. 1). The microbial communities may differ between the sections of the gastrointestinal tract as well as between the studied roe deer individuals (Li et al. 2014), which may be influenced by the diet composition in different habitats (König et al. 2020; Liu et al. 2019).

Rinsing soiled belly flap surfaces consistently reduced the TAC as well as the *Pseudomonas* spp. count to a level similar to or lower than the initial ML, as determined by sampling 20 min after rinsing (Fig. 1). However, the effects of rinsing were incongruent between trials for *Lactobacillus* spp., *Enterobacteriaceae*, and *E. coli* on the same surfaces. In contrast to belly flaps and with one exception for *E. coli* (trial 1), the ML on rinsed fillet surfaces remained above the initial bacterial counts, with a maximal difference of 1.40 $\log_{10}$ CFU/cm^2 observed for *Lactobacillus* spp. in trial 1 (Fig. 1). Differences on the effect of rinsing are possibly due to the more irregular surface of fillets compared to belly flaps after field evisceration. The irregular surface may have promoted bacterial attachment and consequently reduced the short-term effects of rinsing (Delaquis and Mccurdy 1990; Dickson 1988).

Regarding the MLs after cold storage, bacterial development during 3 days at+4 °C did not only differ between rinsed and unrinsed body cavities, but also between meat cuts of single animals. While counts for TAC (trial 1 − 3), *Pseudomonas* spp. (trial 2 and 3), *Lactobacillus* spp. (trial 1 and 3) and *Enterobacteriaceae* (trial 1 and 3) considerably increased on the rinsed belly flap surfaces, slight reductions of the TAC (trial 1 and 2), the counts of *Lactobacillus* spp. (trial 1 and 2) as well as the counts of *E. coli* (trial 1 and 2) were observed for rinsed fillets after the storage (Fig. 1). Interestingly, the ML decreased in 2 of 3 unrinsed

body cavities, disregarding the meat cuts after the 3-day cold storage, except for the counts of *Pseudomonas* spp. on fillets. Thus, these results support the hypothesis that residual water may promote bacterial growth on meat surfaces (Sofos 2014).

Table 1 Description of natural experimental conditions for inoculated and rinsed (n = 3) or unrinsed (n = 3) roe deer carcasses at each trial

Trial no.	Carcass no.	Sex	Body-weight (kg[1])	Time (min[2])	Water volume (ml[3])	Temperature (°C)					pH			
						Ambient	Pelvis[4]		Back[5]		Pelvis[4]		Back[5]	
							At hunting day	After cold storage	At hunting day	After cold storage	At hunting day	After cold storage	At hunting day	After cold storage
1	1[6]	Male	11	145	1680	11	33	7	28	6	6.7	6.4	5.8	6.2
	2	Female	18	180	-		27	4	28	4	5.5	5.8	5.5	6.0
2	3	Female	9	75	1840	0	20	3	19	2	5.7	5.7	5.5	5.6
	4	Male	13	150	-		14	4	10	2	5.5	5.7	5.5	5.9
3	5	Male	14	240	7200	6	34	5	29	5	5.6	5.8	5.7	5.7
	6	Female	12	45	-		19	5	19	5	5.8	6.1	5.7	6.0

[1]after evisceration of the carcass
[2]elapsed between killing and evisceration
[3]used for rinsing the carcass
[4]temperature or pH measured in the muscle close to the pelvis
[5]temperature or pH measured in the muscle between the 13th and 14th spinous process of the thoracic spine
[6]eviscerated without opening the pelvis, all other carcasses were eviscerated with opening pelvis

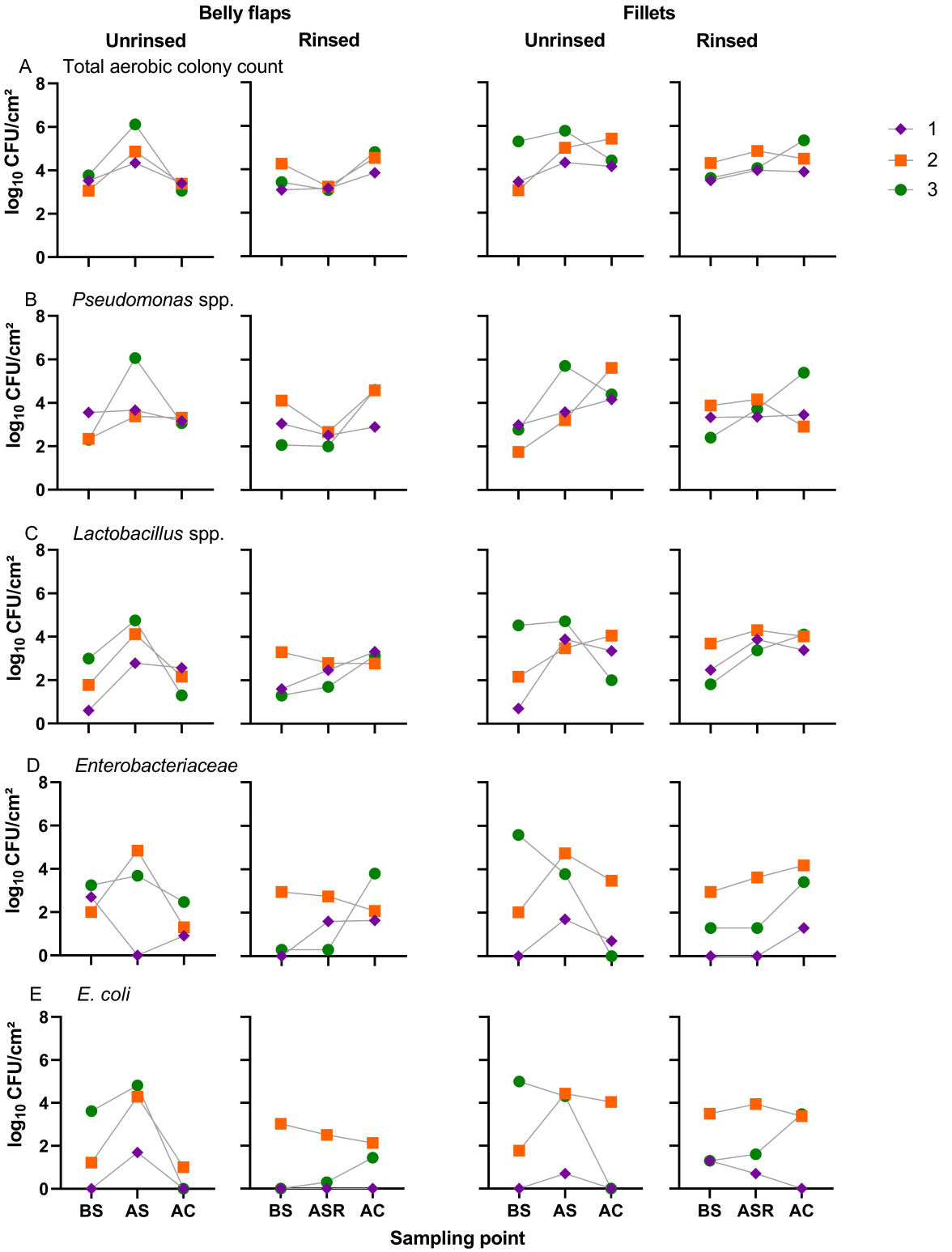

Fig. 1 Bacterial counts determined on rinsed and unrinsed meat surface of roe deer body cavities that were intentionally soiled with GIC mixture in 3 diferent experimental trials (1 – 3). Total aerobic colony count (A), *Pseudomonas* spp. (B), *Lactobacillus* spp. (C), *Enterobacteriaceae* (D), *E. coli* (E). Sampling was performed before soiling (BS), after soiling (AS) or after soiling and rinsing (ASR), and after cold storage for 3 days at +4 °C (AC). The values are presented for individual carcasses; values below the limit of detection are given as 0.

3 Conclusions

In conclusion, the microbiological investigation after this experimental approach showed that rinsing of soiled roe deer body cavities may acutely reduce the bacterial load directly caused by fresh soiling under field conditions. However, rinsing with water may further facilitate the growth of remaining bacteria during cold storage. Further experimental studies are required to better understand the effects of rinsing on the shelf life of game meat under different storage temperatures. Based on this and previous observations (Korkmaz et al. 2022a) as well as considering ethical issues, soiling complete body cavities should be avoided in future studies, since the practice compromises the hygienic quality of the whole carcass and the obtained information is limited. Instead, similar to previous studies that examined the effect of washing meat from slaughtered animals (Castillo et al. 1998), future studies should rather use meat cuts of game carcasses including muscles with different surface characteristics and perform them under controlled laboratory conditions. Because there, potential influencing factors such as bacterial contamination load, rinsing regime or temperature can be modulated. This may also permit i.e. the examination of hot water rinsing, which was reported to reduce bacterial counts on livestock carcasses (Bosilevac et al. 2006). Overall, regardless of whether a carcass is visually clean or whether rinsing successfully removed visual soiling, all game products should be cooked to a core temperature of 70 °C for at least 2 min prior consumption.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00003-023-01417-0

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Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Publisher's Note

Supplementary Material

Materials and methods

During the hunting season between October 2021 and January 2022, 6 roe deer carcasses were obtained on 3 different days (2 carcasses per trial) from drive hunts. Animals were hunted by certified hunters (Maaz et al. 2022). After field evisceration by hunters, carcasses without visible soiling of the body cavity were handed over and hung up on game hoists at the gathering place of the hunting ground. Prior to soiling, temperature and pH of each carcass was measured in muscle tissue close to the pelvis and between the 13th and 14th spinous process of the thoracic spine. Afterwards, as previously described by Korkmaz et al. (2022), meat surfaces of the belly flaps (*Musculus (M.) obliquus internus abdominis*) and the fillets (*M. psoas major*) were sampled with swabs (ISO 17604:2015) to determine the original microbiological condition of selected carcasses before intentional soiling (sampling point before soiling; BS).

Then, the carcasses were inoculated with a mixture of gastrointestinal content (GIC), which was prepared from one of the animals at the day of the hunt. Portions of the content were collected from different sections of the gastrointestinal tract (reticulum 15 %, rumen 40 %, omasum 5 %, abomasum 2 %, small intestine 10 %, large intestine 8 %, caecum 20 %). After thorough mixing, approximately 250 ml of the mixture were evenly distributed in the body cavity of each carcass using latex hand gloves until the surface was completely covered. Aliquots of GIC mixture were cold transported and microbiologically examined within the next 24 hrs. For microbiological examination, 10 g of GIC mixture were diluted with 90 ml diluent (Maximum Recovery Diluent for microbiology, Merck, Darmstadt, Germany) according to ISO 6887-1:2017 and were investigated after homogenization using a bag mixer (BagMixer® 400, step 3, 120 s, Interscience, Saint Nom, France).

15 min after application of the GIC mixture, the body cavity of one of the two carcasses was sampled for microbiological examination without further treatment (sampling point after soiling; AS). Similarly, after the same period, the body cavity of the second carcass was

rinsed by a certified hunter following recommendations using water of drinking quality at environmental temperature with a low-pressure outdoor cleaner (Fontus, Bosch, Gerlingen, Germany, pressure setting 3, 2400 ml/min). Rinsing was performed until all visible soiling was removed or no further cleaning was achievable. The rinsed carcass was left hanging for additional 20 min to allow the applied water to drain and was then swab sampled (sampling point after soiling and rinsing; ASR). The carcasses were then transported to the research facility, hung up for 3 days at +4 °C, and sampled one last time (sampling point after cold storage; AC). At sampling point AC, sampling was carried out on a different half of the body for each sampling to avoid repeated sampling of the same meat surface. The Graphic was created with GraphPad Prism 9.0 (GraphPad Software, San Diego, USA).

Analyses included the total aerobic colony count (TAC) as well as the counts of *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae*, and *Escherichia (E.) coli*, per surface area (cm²) or GIC sample (g). Bacteriological results were log transformed for data evaluation. Additionally, background data on natural conditions were measured or recorded at each experimental trial.

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3.4 Study III - Cause and Effect Analysis between Influencing Factors Related to Environmental Conditions, Hunting and Handling Practices and the Initial Microbial Load of Game Carcasses

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Abstract

Environmental, hunting and handling factors affect the microbial load of hunted game and the resulting meat products. The aim of this study was to systematically investigate the influence of several factors on the initial microbial load (IML) of game carcasses during the early hunting chain. Eviscerated roe deer body cavities (n = 24) were investigated in terms of total viable count and the levels of *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae* and *Escherichia coli* (*E. coli*). Furthermore, a risk analysis based on the obtained original IML data, literature search and a Failure Mode and Effects Analysis (FMEA) was performed. The IML could be explained in a regression model by factors including the higher body weight (BW), damaged gastrointestinal tract by the shot, ambient temperature or rain. The levels of *Lactobacillus* spp. (p = 0.0472), *Enterobacteriaceae* (p = 0.0070) and *E. coli* (p = 0.0015) were lower on the belly flap surface when gloves were used during evisceration. The literature search revealed that studies examining influencing factors (IF) on the IML of game carcasses found contradictory effects of the comparable IF on IML. Potential handling failures may lead to a higher IML of game carcasses during the early hunting chain ranked by FMEA. Several handling practices for game carcasses are recommended, such as ensuring efficient cooling of heavier BW carcasses to limit bacterial growth or eviscerating heavier carcasses before lighter ones.

Keywords

microbial growth; *Enterobacteriaceae*; *Escherichia coli*; body weight; ambient temperature; shooting accuracy; evisceration method; meat hygiene; FMEA

1. Introduction

Game meat is becoming increasingly popular due to its beneficial nutritional [1,2,3], ethical and sustainability aspects [4]. Since game animals inhabit various territories with different environmental conditions, the initial microbial load (IML) of game meat is influenced by the circumstances before and after the animal is hunted [5,6,7,8,9,10]. For example, hunting can be performed using different hunting methods, which may result in varying IML [11,12]. The

stages of a hunt include observation, killing, salvage (recovery from the place of killing), evisceration and transport of the game in the field to a collection point or direct to the game-handling establishment or another storage location. Other steps may be implemented, such as bleeding of the carcass before evisceration [5,6,11]. Besides the hunting method and several published factors such as the ambient temperature on the hunting day [13,14,15], other factors may have a high impact on the IML of game carcasses. One example is the killing process itself. Several studies have reported that the shooting accuracy affects IML [16,17], while other studies have found no influence of this factor [5,18]. In Germany, hunters must pass an examination that tests knowledge and skills such as shooting, game hygiene and other topics before they are allowed to hunt. Subsequently, however, they are not normally required to demonstrate regular practice or further training. When killing game animals, hunters aim to shoot the game animal in the heart. Other factors related to the killing process that have been discussed but not confirmed as influencing bacterial load include ammunition construction [5], the shooting or escape distance [16] of the game. It is important to examine the conditions of the early steps of the hunting chain in their entirety and their effect on IML to improve game meat quality through handling recommendations or prevention strategies when handling game carcasses.

According to Regulation (EC) No. 178/2002, only safe products may be placed on the market in the European Union [19]. Obtaining and producing safe food with limited equipment and in non-standardized conditions, such as natural environments, is a challenge for game meat hygiene. In this regard, quality assurance and management concepts such as Hazard Analysis Critical Control Point (HACCP) in accordance with European Regulation (EC) 852/2004 [20] could help food business operators in producing safe food. However, this concept is hard to apply to the hunting chain. This is because the HACCP analysis begins with the identification of potential hazards to consumer health along a standardized production process, but no standardized process exists for obtaining game carcasses as primary products in the field. Each hunt is unique due to animal-related parameters, environmental conditions and the killing process; also, the hunting and handling practices are variable. For example, during drive hunts in Germany, the time that elapses between killing and eviscerating the game could be very different [5]. In this matter, a Failure Mode and Effects Analysis (FMEA) can be used to generate a preliminary impression of the potential failures in handling game carcasses during the hunting chain and to estimate their impact on the IML of game carcasses. FMEA is a powerful method for identifying critical points in a process and preventing failures [21] that may result in a high IML of game carcasses and meat.

A high IML of carcasses is a potential risk for low-quality game meat [22]. Nevertheless, there are still no microbial limits for game meat as exist for meat obtained from livestock [23]. Data on bacterial loads in game carcasses have been published, e.g., for environmental bacteria, fecal bacteria [5,6] and/or pathogens [15] under a variety of environmental and hunting conditions and using different sampling methods and matrices, depending on the objective of each study. This complicates the comparability of the microbial data and the specification of a generally valid microbial limit or warning value for the different animal species. However, Paulsen et al. [24] propose a total bacterial count of 10^6 CFU/cm² as a provisional warning limit for roe deer (*Capreolus capreolus*) carcasses based on a veterinary post-mortem inspection of “conspicuous” roe deer carcasses.

In the present study, animal-related parameters, environmental conditions, factors of the killing process as well as hunting and handling practices were investigated to identify which parameters most strongly affect the IML of hunted and eviscerated roe deer carcasses from Brandenburg, Germany. The magnitude of each identified influencing factor (IF) on IML was assessed in this study in the context of a statistical risk analysis, literature search and an

FMEA. Based on the IFs that can lead to higher IMLs, potential handling failures were identified. Conversely, recommendations for the handling of game carcasses were provided on the basis of IFs that may lead to a reduction in IML.

2. Materials and Methods

2.1. Collection of Data on Animal-Related, Environmental, Ammunition and Shooting, as Well as Hunting and Handling Parameters

This study was conducted complying with ethical standards, the data privacy agreement of the German Federal Institute for Risk Assessment, and with federal and institutional animal use guidelines. Roe deer (*n* = 24) were shot within the framework of wildlife management [25] and for human consumption in the hunting season 2020–2021 (*n* = 19) and 2021–2022 (*n* = 5) by several hunters on 12 hunting estates in Brandenburg, Germany. Roe deer carcasses were obtained during the annual drive hunt-season (autumn and winter season in the Northern hemisphere) at comparably low ambient temperatures organized by the German Federal Forestry Service at hunting districts administered by the German Federal Institute for Real Estate (BImA) or at hunting districts of the state forest of Brandenburg. Data on the hunted roe deer were recorded for the early steps of hunting chain and contained information on sex, body weight (BW) after evisceration, weather conditions (especially ambient temperature and rain on the day of hunt), ammunition used, duration between killing and evisceration, technique of evisceration, use of gloves during evisceration and presence of visible soiling on the roe deer body cavity with gastrointestinal contents. Parts of this study with a total of 23 roe deer carcasses were previously published as a set of 19 roe deer from the season 2020–2021 by Korkmaz et al. [26]. The data for four carcasses from that study were statistically incomplete, so five additional roe deer carcasses were sampled in the hunting season 2021–2022 including all required data to reach a comparable sample size.

2.2. Sampling and Microbial Investigation of Swab Samples from Roe Deer

Swab samples according to ISO 17604:2015 were taken from the meat surface of the belly flaps (*M. obliquus internus abdominis*) and the fillets (*M. psoas major*) 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). Sampling of the belly flap and fillet surface was executed in the center of the indicated region with an area of 50 cm² or 20 cm², respectively. Swab samples were rinsed with 90 mL diluent (Maximum Recovery Diluent for microbiology; Merck, Darmstadt, Germany) according to ISO 6887-1:2017 in a bag mixer (BagMixer® 400, step 3, 120 s; Interscience, Saint Nom, France).

The total viable count (DIN ISO 4833-2:2014, Plate Count Agar; Carl Roth, Karlsruhe, Germany), the levels of *Pseudomonas* spp. (specifications of the manufacturer, Pseudomonas/Aeromonas selective agar; Sigma-Aldrich, Darmstadt, Germany), *Lactobacillus* spp. (DIN 10109:2017, de Man Rogasa and Sharpe agar; Carl Roth, Karlsruhe, Germany) and *Enterobacteriaceae* (DIN 10164:2019, Violet Red Bile Dextrose agar; Merck, Darmstadt, Germany) were analyzed by the spread plate method. After aerobic incubation at 30 °C for 72 h or anaerobic incubation at 37 °C for 24 h for *Enterobacteriaceae*, counts of the respective bacterial groups were calculated. Presumptive colonies of *Pseudomonas* spp. were confirmed by positive oxidase testing (ROTITEST® Oxidase strips; Carl Roth, Karlsruhe, Germany). The level of *Escherichia coli* (*E. coli*, DIN ISO 16649-2:2010, Tryptone Bile X-glucuronide Agar; Carl Roth, Karlsruhe, Germany), was determined by the pour plate method after aerobic incubation at 44 °C for 24 h. The counts of bacteria examined were given in $\log_{10}$ CFU/cm².

2.3. Statistical Risk Analysis

Linear regressions with backward variable elimination were performed to identify potential factors affecting IML as target variables. The target variables for every regression included the total viable counts, the counts of *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae* and *E. coli* on the meat surface of the belly flap and fillet, respectively. The normality of the target variable distributions was examined with the Shapiro–Wilk Test after logarithmic transformation. Potential factors affecting IML included BW after evisceration, sex of roe deer carcasses, ambient temperature and occurrence of rain on the hunting day, ammunition construction used with assigned impact energy at 100 m distance, shooting accuracy, shooting distance between hunter and roe deer, escape distance of roe deer, duration between killing and evisceration, evisceration technique and position of carcass during this process, usage of gloves, as well as presence of visible soiling of the roe deer body cavity with gastrointestinal content as independent factors. All regressions were calculated in R Statistics (R-Version 4.1.2., R Core Team 2022) using the function “lm” (package stats). Backward variable elimination was performed using the “step” function (package stats). Variables were excluded stepwise until the Akaike Information Criterion (AIC) could not be improved further. All of the resulting “best models” for every regression (every combination of bacterial group and sampled muscle) revealed p-values ≤ 0.05 in the F-statistic. In order to quantify the magnitude of the effects of the resulting IF in the “best models” on the IML, Rate Ratios (RR) were determined by calculating the exponential function of the model estimates. A RR corresponds to a factor by which, according to the model, the IML ($\log_{10}$ CFU/cm²) increases (if RR > 1) or decreases (if RR < 1) if a specific level of an IF (e.g., animal sex: female) occurs in comparison to a reference level (e.g., male), or if an increase in a metric IF occurs (e.g., +1 kg body weight). To make the effect statements more tangible, RRs to ambient temperature, shooting and escape distance, duration between killing and evisceration were calculated for increments of ten. The data on, e.g., animal-related parameters or the IML examined of the carcasses were summarized descriptively using SPSS Software version 26 (IBM, Ehningen, Germany). Heat maps and stacked bar graphs were created in GraphPad Prism 9.3.1 (GraphPad Software, San Diego, CA, USA).

2.4. Literature Search of Factors Affecting the Initial Microbial Load of Game Carcasses Based on Previously Published Data in Original Research Articles

The literature was screened on 23 May 2022 for previously reported IFs on IMLs of game carcasses. The search was conducted using Google Scholar with the English search terms “weight bacteria game meat” or “carcass microbial contamination game” without any restriction. The articles to be screened were selected according to the relevance of their titles. Additional articles cited by the initially screened articles were considered and checked for relevance. Results on the identified IFs and on the bacterial load of game carcasses were classified by animal species, sample size, significance and bacterial group studied.

2.5. Failure Mode and Effects Analysis Based on Authors’ Expertise or on a Defined Stepwise Search

A flowchart of obtaining game carcasses along the early steps of the hunting chain was created and used for the two FMEA approaches: one based on the authors’ expertise and one based on a defined stepwise search (Figure 1). In this study, the assessed part of the hunting chain started with game observation and ended with the collection of samples from the killed and eviscerated carcasses in the field. Potential failures during handling of game carcass were identified based on the results of this study and the literature search.

To assess the impact of IFs on IML of game carcasses during each step of the hunting chain, a Risk Priority Number (RPN) was calculated. The RPNs for each possible failure was calculated by multiplication of the estimated values from 1 to 5 for the probability of occurrence (O), the significance (S) and the probability of detection (D). The calculated RPN can range from 1 to 125, with the failure or risk becoming less acceptable as the RPN increases. In this study, the risk of adverse impact of handling failures on IML was classified as low risk with an RPN of <19, medium risk with 29 > RPN > 20 and high risk with an RPN ≥30 based on the FMEA performed.

There is no single standard for the rating scale of an FMEA. However, the scale of 1 to 5 is preferred because it allows for the easy interpretation of a possible failure during a process [21]. Furthermore, the weighting of the rating scale is process-dependent and related to a meaningful class formation. In this study, O, S and D were each divided into five classes of the rating scales (Table 1). Two FMEAs were performed based on either the authors' expertise or a defined stepwise search, described in Section 2.5.2. The RPNs of both FMEAs were graphically compared for the same defined handling failures in stacking bars. Variability of the given RPNs by experts were presented as boxplots. The illustrations were created in GraphPad Prism 9.3.1 (GraphPad Software, San Diego, CA, USA).

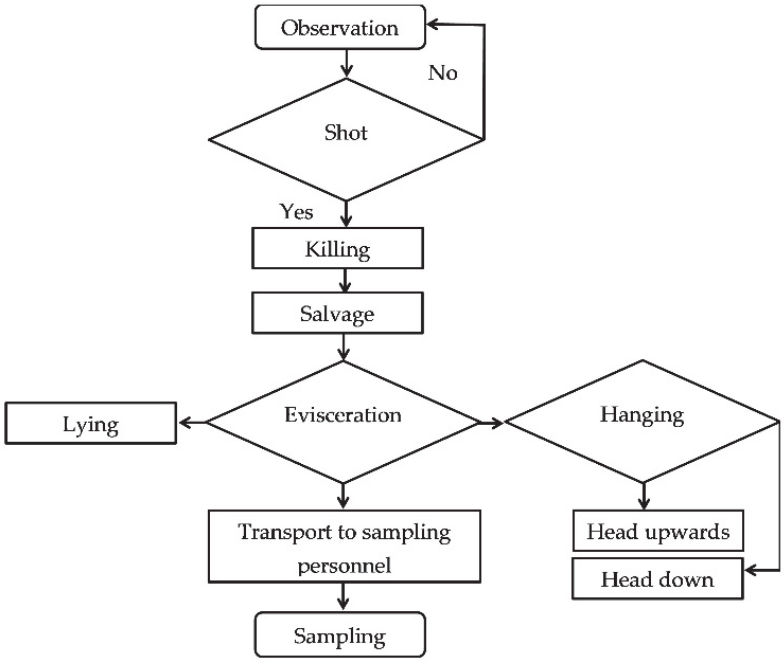

Figure 1. Flowchart for the investigated early steps of the hunting chain from observation of the living game animal to the sampling of the carcasses.

Table 1. Rating scales used in Failure Mode and Effects Analysis (FMEA) to classify the probability of occurrence, significance and probability of detection to assess the impact of handling failures during the hunting chain on the initial microbial load (IML) of game carcasses.

Classes of the Rating Scale	Rating Scale
Probability of occurrence	
1	Very unlikely to occur in the hunting practice
2	Unlikely to occur in the hunting practice
3	Possible to occur in the hunting practice
4	Likely to occur in the hunting practice
5	Very likely to occur in the hunting practice
Significance	
1	Very unlikely to have an impact on IML (very low probability of contamination and distribution of bacteria on/in the carcass) *
2	Unlikely to have an impact on IML (low probability of contamination and distribution of bacteria on/in the carcass) *
3	An impact on the IML is possible (contamination and distribution of bacteria on/in the carcass is probable) *
4	Likely to have an impact on IML (high probability of contamination and distribution of/in bacteria on the carcass) *
5	Very likely to have an impact on IML (very high probability of contamination and distribution of/in bacteria on the carcass) *
Probability of detection	
1	Detection of failure is very likely
2	Detection of failure is likely
3	Detection of failure is possible
4	Detection of failure is unlikely
5	Detection of failure is very unlikely

* For classification of significance on the IML, bacteria were assumed to have been transferred or distributed by contact or through animal metabolism.

2.5.1. Failure Mode and Effects Analysis Based on Authors' Expertise

The consultation of the authors for the FMEA was performed by using a survey. The possible handling failures were formulated openly, so that the experts had to prioritize based on their own experiences. This was executed to ensure that the evaluation is based on the aspect that seems the most critical for the respective author and covers as many sources of failures as possible while obtaining game carcasses in a common context. Therefore, the authors ranked RPNs using multiple scenarios and viewpoints and considering various potential IFs.

2.5.2. Failure Mode and Effects Analysis Based on Defined Stepwise Search

Since the FMEA based on the authors' expertise included personal bias, it was complemented by an FMEA based on a defined stepwise search of scientific evidence. Therefore, values of O, S and D were first classified based on the effects of IFs on IML determined by linear regression and RRs in the context of a risk analysis in this study. When the classification of factors affecting IML could not be explained by the results of original IML data, other original research articles were reviewed for evidence as a second step. This was the case when data for the relevant IF were not obtained in our own study. As a third step, when there was a lack of published evidence (either in this or in another study), the classification was based on experience reported by hunters in grey literature.

3. Results

3.1. Animal-Related Parameters, Environmental Factors, Ammunition and Shooting, as Well as Hunting and Handling Parameters

Freshly eviscerated roe deer carcasses (*n* = 24) were examined from 2020 to 2022 by taking swab samples of the belly flap (*n* = 24) and fillet (*n* = 23) surfaces on hunting day. Roe deer carcasses were obtained on six rainy hunting days (*n* = 13) and eight dry hunting days (*n* = 11). Of the roe deer carcasses, 21% showed damage to the gastrointestinal tract. However, visible soiling of the body cavity by gastrointestinal contents after the handover appeared in 46% of the carcasses (Figure 2).

The BW of the roe deer carcasses after evisceration varied from 8.4 to 18.2 kg (median 13.5 kg, 95% confidence interval (CI) 11.0–15.1 kg). The ambient temperature measured during the sampling of the carcasses ranged from 0 to 13 °C (median 5 °C, 95% CI 2.0–10.3 °C). Based on manufacturer's specifications, the impact energy of ammunition at 100 m distance ranged from 2358 to 3484 J (median 2765 J, 95% CI 2759–3247 J). The time from killing the roe deer until evisceration ranged from 5 to 240 min (median 148 min, 95% CI 78–180 min). The shooting distance between the hunter and the roe deer was estimated to be up to 60 m (median 40 m, 95% CI 20–50 m). Half of the sampled roe deer were killed by the shot directly in place, the other half after an escape distance between 2 and 50 m.

3.2. Initial Microbial Load of Meat Surfaces of the Body Cavity

The total viable count mean and standard deviation (SD) were similar on both meat surfaces: 3.8 ± 1.0 $\log_{10}$ CFU/cm² on the belly flap surface and 4.0 ± 1.1 $\log_{10}$ CFU/cm² on the fillet surface. The counts of *Pseudomonas* spp., *Enterobacteriaceae* and *E. coli* were also similar in both sample matrices. The levels of *Lactobacillus* spp. were higher in the fillet than in the belly flap (Table 2).

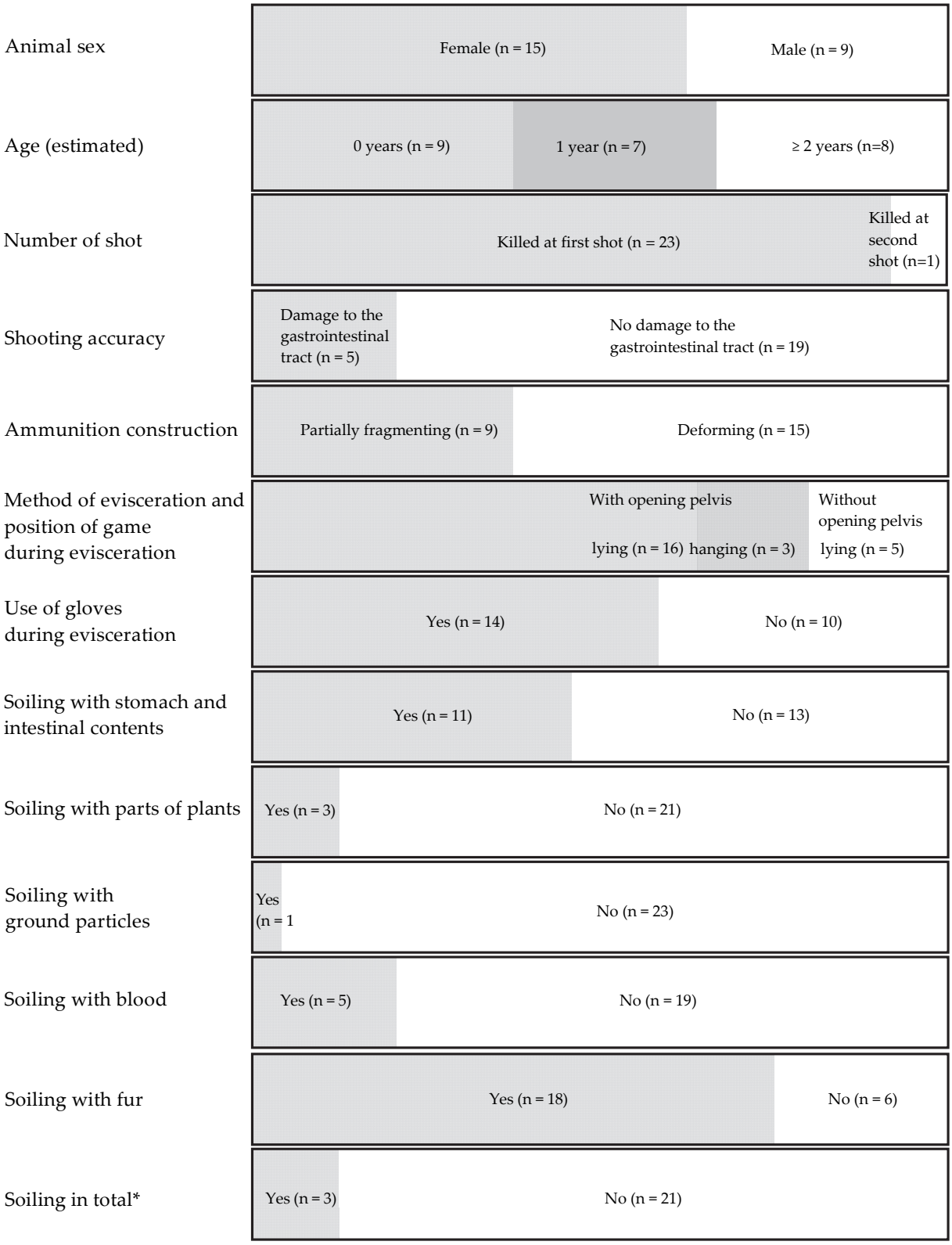

Figure 2. Animal-related parameters and parameters representing shooting-related factors, handling of roe deer carcasses as well as visual evaluation of the body cavity. * Soiling in total was classified as “no” if no visible contamination was present in the body cavity and “yes” if one or more types of contamination were present in the body cavity.

Table 2. Mean, standard deviation (SD) and 95% confidence intervals (95% CI) of the initial microbial load on the meat surface of belly flap (n = 24) and fillet (n = 23) of roe deer carcasses

Initial Microbial Load, $\log_{10}$ CFU/cm²	*n*	Mean	SD	95% CI
Belly flap				
Total viable count	24	3.8	1.0	3.4–4.3
Pseudomonas spp.	24	2.6	0.8	2.3–3.0
Lactobacillus spp.	24	2.4	1.3	1.8–3.0
Enterobacteriaceae	24	1.6	1.2	1.1–2.1
E. coli	24	1.0	1.2	0.5–1.5
Fillet				
Total viable count	23	4.0	1.1	3.5–4.5
Pseudomonas spp.	23	2.6	0.9	2.2–3.0
Lactobacillus spp.	23	3.1	1.4	2.5–3.7
Enterobacteriaceae	23	1.8	1.6	1.1–2.5
E. coli	23	1.3	1.4	0.7–2.0

3.3. Factors Influencing the Initial Microbial Load of Freshly Eviscerated Roe Deer Carcasses

IFs were defined in this study as parameters that could have an impact on IML, are measurable or categorizable or could be managed in hunting practice. Ambient temperature (0–13 °C) had an effect on the bacterial load of carcasses. Keeping other variables constant, an increase in ambient temperature may result in a 3.2-fold higher total viable count and a 4.1-fold higher *Pseudomonas* spp. count in the belly flap (95% CI 1.3–7.7 $\log_{10}$ CFU/cm²; 95% CI 1.9–8.9 $\log_{10}$ CFU/cm²) and a 3.4-fold higher *Pseudomonas* spp. count in the fillet (95% CI 1.5–7.7 $\log_{10}$ CFU/cm², Figure 3). Damage to the gastrointestinal tract resulted in a higher bacterial load by 5.1 for total viable count, by 2.3 for *Pseudomonas* spp. and by 8.4 for *Lactobacillus* spp. in the belly flap (95% CI 2.1–12.4 $\log_{10}$ CFU/cm²; 95% CI 1.1–5.0 $\log_{10}$ CFU/cm²; 95% CI 2.9–24.2 $\log_{10}$ CFU/cm²) compared to carcasses shot without gastrointestinal damage. Likewise, the total viable count may be 3.4-fold and the *Lactobacillus* spp. counts 5.7-fold higher in the fillet (95% CI 1.1–10.1; 95% CI 1.8–18.1 $\log_{10}$ CFU/cm²).

Furthermore, carcasses eviscerated with opening in a hanging position had higher values of *E. coli* in the belly flap (RR = 12.1, 95% CI 4.6–31.6 $\log_{10}$ CFU/cm²) and higher values of *Enterobacteriaceae* and *E. coli* in the fillet (RR = 11.4, 95% CI 1.4–90.1 $\log_{10}$ CFU/cm²; RR = 10.4, 95% CI 2.4–44.4 $\log_{10}$ CFU/cm²) than carcasses eviscerated lying on the ground (n = 16). Carcasses eviscerated without opening the pelvis (n = 5) had higher levels of *Pseudomonas* spp. in the fillet (RR = 3.2, 95% CI 1.5–6.6 $\log_{10}$ CFU/cm²) than carcasses with the pelvis opened (n = 16).

When hunters eviscerated carcasses using gloves, levels of *Lactobacillus* spp., *Enterobacteriaceae* and *E. coli* in the belly flap (RR = 0.4, 95% CI 0.2–0.99 $\log_{10}$ CFU/cm²; RR = 0.2, 95% CI 0.1–0.5 $\log_{10}$ CFU/cm²; RR = 0.2, 95% CI 0.1–0.6 $\log_{10}$ CFU/cm²) and the values of *E. coli* in the fillet (RR = 0.3, 95% CI 0.1–0.9 $\log_{10}$ CFU/cm²) were lower than in carcasses eviscerated without using gloves.

Compared to partially fragmenting bullets, use of deforming bullets caused higher initial levels for *Enterobacteriaceae* and *E. coli* in the belly flap (RR = 2.5, 95% CI 1.0–6.1 $\log_{10}$ CFU/cm²; RR = 2.6, 95% CI 1.4–4.7 $\log_{10}$ CFU/cm²) and higher levels of *E. coli* in the fillet (RR = 3.1, 95% CI 1.3–7.6 $\log_{10}$ CFU/cm²).

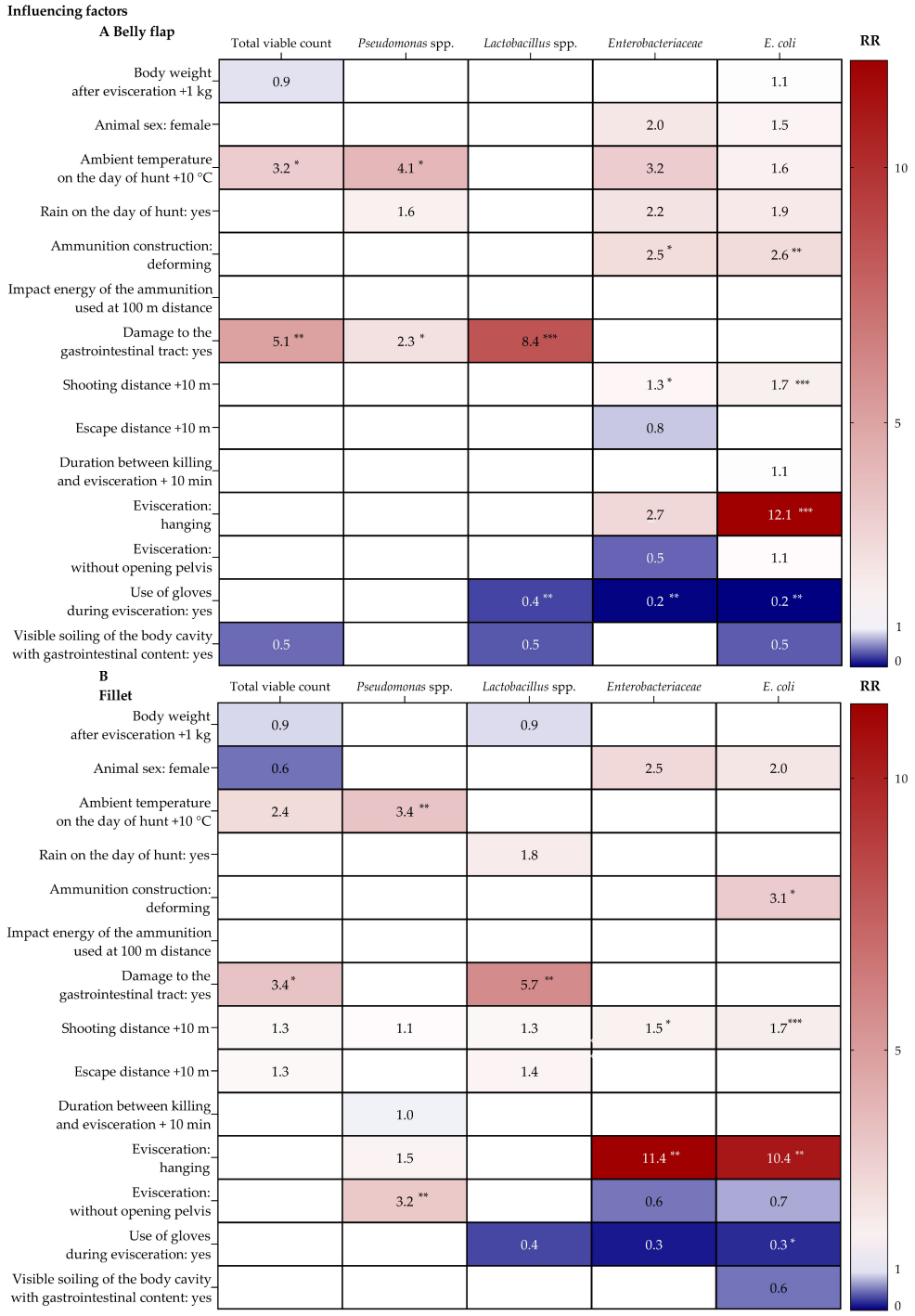

Figure 3. Heat maps for influencing factors (IFs) affecting initial microbial load (IML; total viable colony count, *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae*, *E. coli*) of roe deer belly flap ((**A**), n = 24) and fillet ((**B**), n = 23) with resulting Rate Ratios (RRs) shown in each cell. The heat maps have been created using the RRs of variables identified as IFs by linear regression with backward selection. An RR of 1 were presented as empty cells and means no effect. Significance levels of RRs were highlighted by stars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). To make the effect statements more tangible, RRs were calculated for ambient temperature, shooting distance, escape distance and duration between killing and evisceration in increments of ten.

When the shooting distance between the hunter and the roe deer increased, the values of *Enterobacteriaceae* and *E. coli* in the belly flap (RR = 1.4, 95% CI 1.0–1.8 $\log_{10}$ CFU/cm²;

RR = 1.7, 95% CI 1.4–2.0 log_{10} CFU/cm²) and in the fillet (RR = 1.5, 95% CI 1.1–2.2 log_{10} CFU/cm²; RR = 1.7, 95% CI 1.3–2.2 log_{10} CFU/cm²) were elevated (Figure 3; Tables S1 and S2 (Supplementary Materials)).

3.4. Factors Influencing the Initial Microbial Load of Game Carcasses Based on a Literature Search

During the literature search, 34 articles with relevant titles on microbial investigation of game carcasses were reviewed twice using the selected terms in Google Scholar (Figure 4). Of these, 13 articles were considered in more detail as they contained results on IF on the bacterial load of game carcasses (Table 3–5). Articles with a focus on the IML and with using a convincing statistical method were included in the stepwise FMEA, whereas e.g., descriptive papers were only used as an alternative groundwork for the discussion of observations.

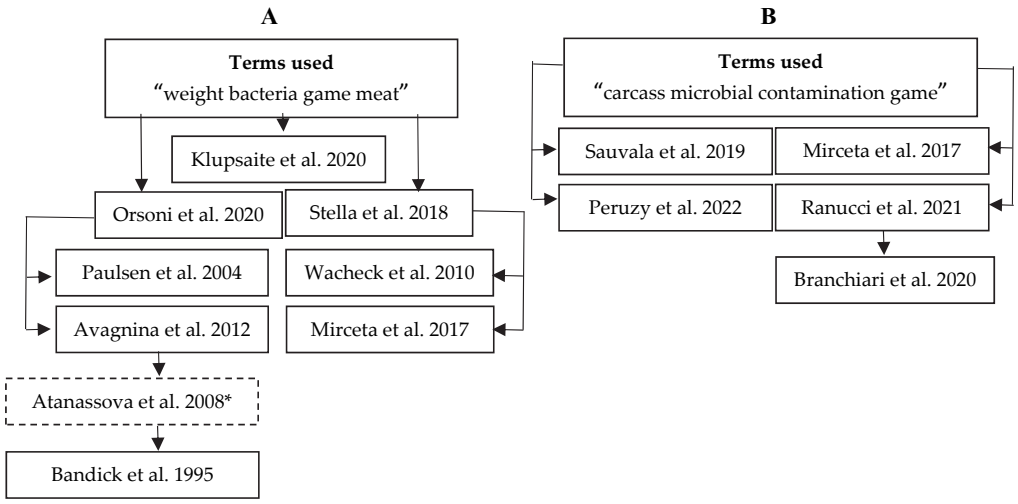

Figure 4. Original research articles found through the literature search in Google Scholar with the terms "weight bacteria game meat" (**A**) [3,7,9,14,15,16,18,27,28] and "carcass microbial contamination game" (**B**) [5,6,8,9,13]. Arrows represent direct hits of the search term or the primary reference that cited the related study. ***** The discontinuous frame indicates a reference that was excluded by the described criteria, but served as a lead for another reference.

3.5. Failure Mode and Effects Analysis

The FMEA based on the authors' expertise and the defined stepwise search identified the shooting/killing, salvage, evisceration and transport steps as having the greatest potential for failure. Handling failures that can affect carcass IML are e.g., lack of awareness of hygienic handling of game carcasses (contamination of carcass by, e.g., unwashed hands in the absence of running water or improper handling with gloves or by using improperly cleaned or unsuitable equipment, e.g., unclean or blunt knives); pulling/dragging the game on the ground during salvage; contamination of the carcass (not only musculature, but also the fur) by various factors, e.g., rain, grass, leaves, surface water, etc., on the ground when the tarpaulin is not in use or when the stomach and intestinal tract of the game is damaged during evisceration and the contents contaminate the carcass; cross-contamination of carcasses by e.g., other animals (stacking or too close placement of several killed animals on a transport vehicle) or due to insufficient hygienic conditions of the transport vehicle (e.g., soil, leaves, blood residues from eviscerated carcasses); evisceration of the carcass lying on the ground (body fluids remain in the body cavity); evisceration of the game in the field; and the game is eviscerated with delay (Figure 5).

Table 3. Environmental factors that may influence the initial microbial load of game carcasses including animal species, sample size, *p*-value and bacterial group examined, reported in the original research articles.

Influencing Factor	Animal Species	*N*	Bacterial Group	Significant Effect	*p*-Value	Reference
Ambient temperature	Moose/ White-tailed deer	100/ 100	Mesophilic aerobic bacteria	Yes	0.023	[13]
	Roe deer	64	Aerobic colony count	No	0.963	[5]
	Ungulates ‡	50	Total aerobic count	Yes	<0.05	[14]
	Wild boar	36	Mesophilic bacteria	No	>0.05	[6]
	Wild boar	120	Aerobic colony count	Yes	<0.05	[8]
	Wild boar	62	Total viable count	Yes	<0.01	[15]
	Moose/ White-tailed deer	100/ 100	*Enterobacteriaceae*	Yes	0.003	[13]
	Roe deer	64	*Enterobacteriaceae*	Yes	0.012	[5]
	Ungulates ‡	50	*Enterobacteriaceae*	Yes	<0.05	[14]
	Wild boar	36	*Enterobacteriaceae*	No	>0.05	[6]
	Wild boar	120	*Enterobacteriaceae*	Yes	<0.05	[8]
	Moose/ White-tailed deer	100/ 100	*E. coli*	Yes	0.011	[13]
	Wild boar	36	*E. coli*	Yes	<0.05	[6]
	Wild boar	62	Pathogens ***	No	-	[15]
	Wild boar	62	*Listeria* spp.	Yes	<0.05	[15]
Rain on the day of hunt	Wild boar	120	Aerobic colony count	No	>0.05	[8]
	Wild boar	120	*Enterobacteriaceae*	No	>0.05	[8]
	Ungulates ‡	50	Total aerobic count	Yes	<0.05	[14]
	Roe deer	119	*Enterobacteriaceae*	No	-	[27]
	Ungulates ‡	50	*Enterobacteriaceae*	Yes	<0.05	[14]

‡ 25 red deer, 18 roe deer, 3 chamois, 1 mouflon, 3 wild boar. *** *Campylobacter* spp., Salmonella and *L. monocytogenes;* - indicates lack of specified *p*-value.

Table 4. Animal-related factors that may influence the initial microbial load of game carcasses including animal species, sample size, *p*-value and bacterial group examined, reported in the original research articles.

Influencing Factor	Animal Species	*n*	Bacterial Group	Significant Effect	*p*-Value	Reference
Body weight after evisceration	Roe deer •	64	Aerobic colony	Yes	-	[5]
	Wild boar	36	Mesophilic bacteria	No	>0.05	[6]
	Wild boar	37	Aerobic colony count	Yes	0.014	[7]
	Wild boar	120	Aerobic colony count	Yes	<0.05	[8]
	Wild boar	62	Total viable count	No	-	[15]
	Roe deer •	64	*Enterobacteriaceae*	No	-	[5]
	Wild boar	62	*Enterobacteriaceae*	Yes	0.03	[15]
	Wild boar	36	*Enterobacteriaceae*	No	>0.05	[6]
	Wild boar	37	*Enterobacteriaceae*	No	-	[7]
	Wild boar	120	*Enterobacteriaceae*	Yes	<0.05	[8]
	Wild boar	36	*E. coli*	No	>0.05	[6]
	Wild boar	62	*E. coli*	Yes	0.04	[15]
	Roe deer •	64	Pathogens *	No	-	[5]
	Wild boar	36	Pathogens **	No	>0.05	[6]
	Wild boar	62	Pathogens ***	No	-	[15]
	Wild boar	153	Pathogens ****	No	0.3071	[28]

Table 4. *Cont.*

Influencing Factor	Animal Species	*N*	Bacterial Group	Significant Effect	*p*-Value	Reference
Animal sex	Moose/ White-tailed deer	100/ 100	Mesophilic aerobic bacteria	No	0.06	[13]
	Wild boar	36	Mesophilic bacteria	No	>0.05	[6]
	Wild boar	120	Aerobic colony count	No	>0.05	[8]
	Wild boar	62	Total viable count	No	-	[15]
	Moose/ White-tailed deer	100/ 100	*Enterobacteriaceae*	No	0.20	[13]
	Wild boar	36	*Enterobacteriaceae*	No	>0.05	[6]
	Wild boar	120	*Enterobacteriaceae*	No	>0.05	[8]
	Wild boar	62	*Enterobacteriaceae*	Yes	0.02	[15]
	Moose/ White-tailed deer	100/ 100	*E. coli*	Yes	0.03	[13]
	Wild boar	36	*E. coli*	No	>0.05	[6]
	Wild boar	62	*E. coli*	Yes	<0.01	[15]
	Wild boar	36	Pathogens **	No	>0.05	[6]
	Wild boar	62	Pathogens ***	No	-	[15]

• Body weight before evisceration. * *Salmonella* spp., *L. monocytogenes*; ** *Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter* spp. and *pathogenic E. coli*; *** *Campylobacter* spp., *Salmonella*, *Listeria* spp. and *L. monocytogenes*; **** *Salmonella* spp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, STEC, *L. monocytogenes*; - indicates lack of specified *p*-value.

Table 5. Ammunition and shooting, hunting and handling factors that may influence the initial microbial load of game carcasses including animal species, sample size, *p*-value and bacterial group examined, reported in the original research articles.

Influencing Factor	Animal Species	*N*	Bacterial Group	Significant Effect	*p*-Value	Reference
Ammunition construction	Roe deer	64	Aerobic colony count	No	0.969	[5]
	Roe deer	64	*Enterobacteriaceae*	No	0.641	[5]
Damage to the gastrointestinal tract	Moose/ White-tailed deer	100/ 100	Mesophilic aerobic bacteria	No	≥ 0.20	[13]
	Roe deer	50	Aerobic colony count	Yes	-	[16]
	Roe deer	78	Aerobic Viable Count	Yes	-	[18]
	Wild boar	47	Aerobic colony count	Yes	-	[16]
	Wild boar	72	Aerobic Viable Count	Yes	-	[18]
	Wild boar	36	Mesophilic bacteria	No	>0.05	[6]
	Wild boar	210	Aerobic colony counts	No	-	[9]
	Wild boar	125	Total Viable Count	No	-	[29]
	Moose/ White-tailed deer	100/ 100	*Enterobacteriaceae*	Yes	0.009	[13]
	Wild boar	36	*Enterobacteriaceae*	No	>0.05	[6]
	Wild boar	210	*Enterobacteriaceae*	No	-	[9]
	Wild boar	125	*Enterobacteriaceae*	No	-	[29]
	Moose/ White-tailed deer	100/ 100	*E. coli*	No	-	[13]
Escape distance	Roe deer	50	Aerobic colony count	No	-	[16]
	Wild boar	47	Aerobic colony count	No	-	[16]
Duration between killing and evisceration	Roe deer	64	Aerobic colony count	Yes	0.049	[5]
	Wild boar	36	Mesophilic bacteria	No	>0.05	[6]
	Wild boar	37	Aerobic colony count	No	-	[7]
	Wild boar	120	Aerobic colony count	No	0.565	[8]
	Roe deer	64	*Enterobacteriaceae*	No	0.840	[5]

Table 5. *Cont.*

Influencing Factor	Animal Species	*n*	Bacterial Group	Significant Effect	*p*-Value	Reference
Duration between killing and evisceration	Wild boar	36	*Enterobacteriaceae*	No	>0.05	[6]
	Wild boar	37	*Enterobacteriaceae*	No	-	[7]
	Wild boar	120	*Enterobacteriaceae*	No	0.082	[8]
	Wild boar	36	*E. coli*	No	>0.05	[6]
Evisceration location: field vs. game-handling establishment	Wild boar	210	Aerobic colony counts	Yes	<0.05	[9]
	Wild boar	210	Aerobic colony counts	Yes	<0.05	[9]
Evisceration: hanging	Wild boar	210	Aerobic colony counts	Yes	<0.05	[9]
	Wild boar	210	*Enterobacteriaceae*	Yes	<0.05	[9]
Visible soiling of body cavity with gastrointestinal content	Roe deer	119	Aerobic mesophilic bacteria	No	-	[27]
	Ungulates‡	50	Total aerobic count	Yes	<0.05	[14]
	Roe deer	119	*Enterobacteriaceae*	No	-	[27]
	Ungulates‡	50	*Enterobacteriaceae*	Yes	<0.05	[14]

‡ 25 red deer, 18 roe deer, 3 chamois, 1 mouflon, 3 wild boar; - indicates lack of specified *p*-value.

The RPNs of the FMEA based on the authors' expertise and defined stepwise search differed mainly due to the different definitions used to evaluate the probability of detection. High risk RPNs were obtained more often in the FMEA based on authors´ expertise than using the defined stepwise search. However, there is a similarity in the assessed RPNs of handling failures (Figure 5), i.e., cross-contamination of carcasses by other animals (high risk RPN), improper shooting accuracy causing damage to the gastrointestinal tract (medium risk RPN) or the musculature of the game animal being highly damaged due to too high impact energy (low risk RPN). The assessment of some other RPNs varied between the experts. An impact of delayed evisceration on IML was ranked similarly by the experts (Figure 6).

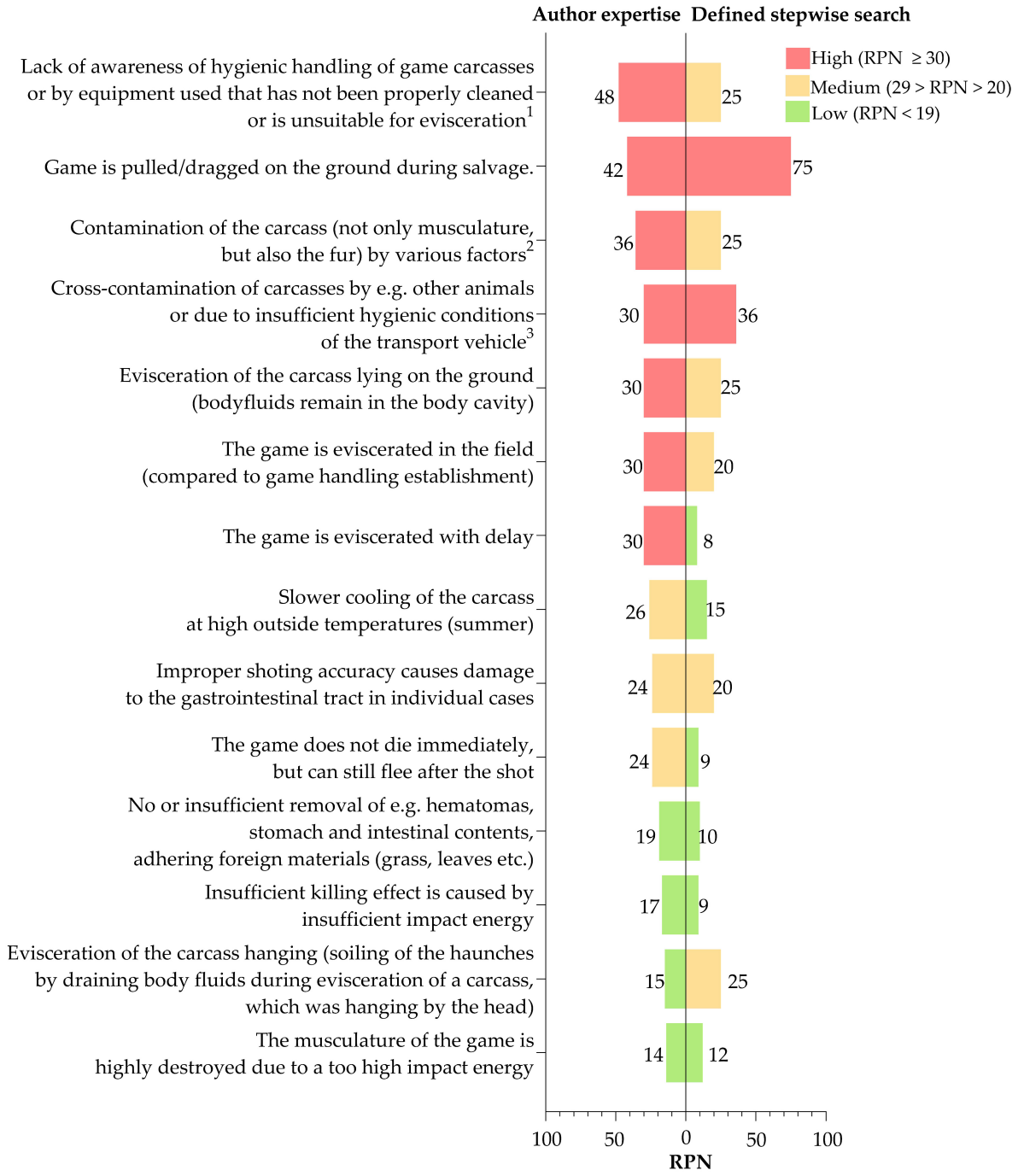

Figure 5. Risk Priority Numbers (RPNs) were compared using the authors' expertise and a defined stepwise search. This search was conducted based on the results of this study, other published original research articles or when there was no published evidence on reported hunters' experiences from the grey literature. The subjects are shown as presented in the (expert) assessment including the footnotes for further specification: (1) contamination of carcass by, e.g., unwashed hands in the absence of running water or improper handling with gloves or unclean or blunt knives; (2) e.g., rain, grass, leaves, surface water, etc. on the ground when the tarpaulin is not in use or when the stomach and intestinal tract of the game is damaged during evisceration and the contents contaminate the carcass; (3) e.g., stacking or too close placement of several killed animals on a transport vehicle that is contaminated with soil, leaves, blood residues from eviscerated carcasses.

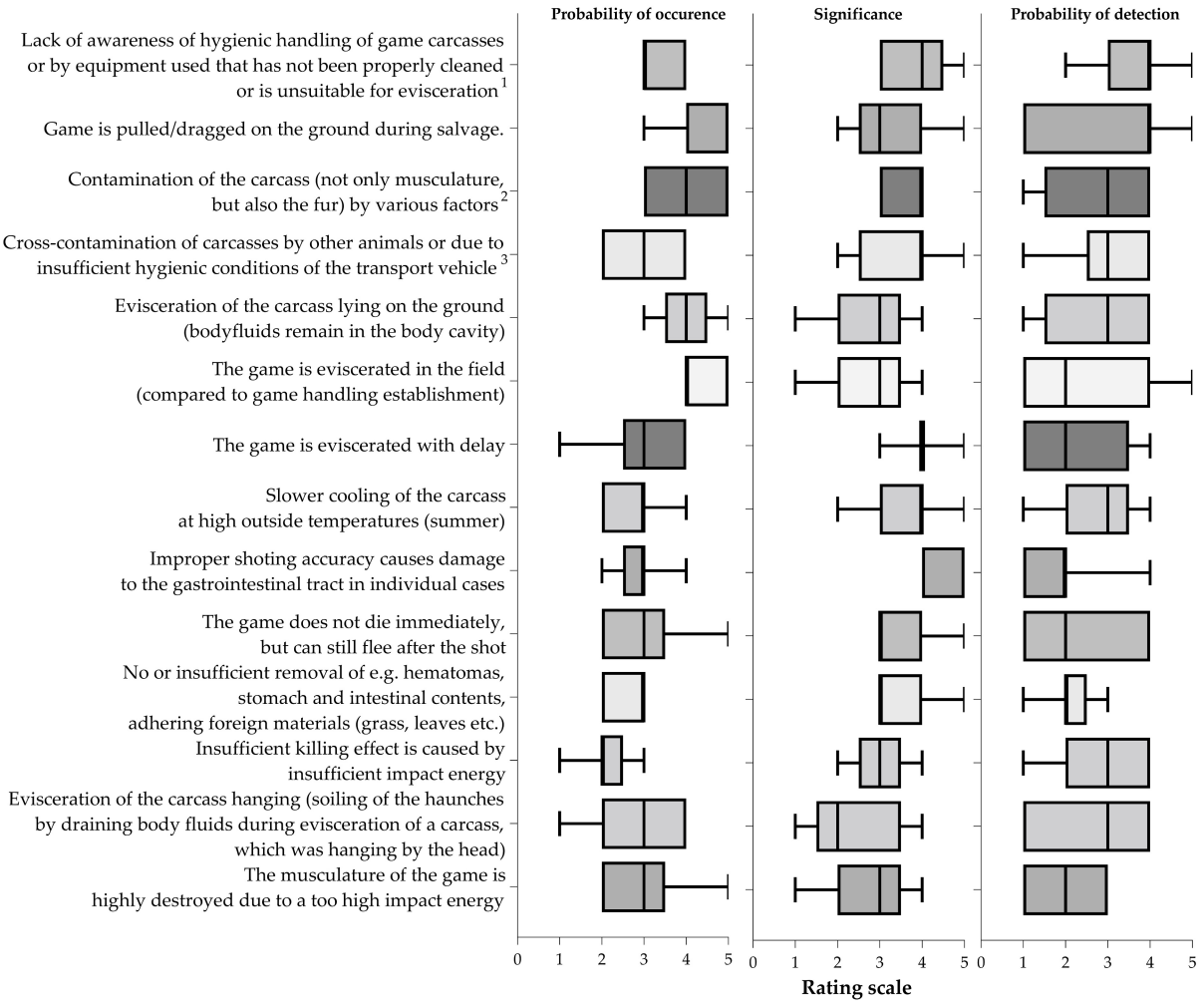

Figure 6. Boxplots show the variability of the probability of occurrence, significance and probability of detection rankings with minimum and maximum values for Failure and Mode and Effect analysis based on authors' expertise. The subjects of the assessments including the footnotes for further specification: (1) contamination of carcass by, e.g., unwashed hands in the absence of running water or improper handling with gloves or unclean or blunt knives; (2) e.g., rain, grass, leaves, surface water, etc. on the ground when the tarpaulin is not in use or when the stomach and intestinal tract of the game are damaged during evisceration and the contents contaminate the carcass; (3) e.g., stacking or too close placement of several killed animals on a transport vehicle that is contaminated with soil, leaves, blood residues from eviscerated carcasses.

4. Discussion

The IML of game carcasses is affected by IFs and provides an indication of the microbial quality of the resulting food product. The IML of hunted carcasses is higher than that of livestock animal carcasses slaughtered under controlled conditions; additionally, game meat obtained in the field is generally more likely to show sensory deviations, faster spoilage and consequently have a reduced shelf-life [31]. However, if appropriate hygienic measures are taken, such as the use of gloves when eviscerating carcasses, game meat with improved microbial quality can be obtained even under field conditions, as shown in this study. Therefore, the initial processing of game meat is very important. Although the bacterial load of consumed game products can be influenced by many other factors later in the value chain, the focus in this study on the early steps of game meat harvesting was made since bacterial

growth is exponential and this period is the most lacking in controlled conditions. This study highlights IFs on the IML of game carcasses processed under field conditions. Due to various IFs at a hunt covering animal-related parameters, environmental factors, hunting and handling practices, the IML of carcasses may vary between animals even during the same hunt. This results in data that can appear very complex between studies and are rarely comparable. In addition, it seemed that still some potentially important IFs have not yet been supported by evidence. Therefore, based on risk analysis of original IML data and a literature search, the present study identified factors that can be described numerically or by categorization and that may have a significant impact on the IML of carcasses. With this study, more evidence is available for the IFs on IML: ambient temperature; the presence of rain during the hunt; the shooting and escape distance of the game or the carcass's BW. The magnitude of these IFs on IML of game carcasses was determined based on a holistic approach combining RRs and FMEA to mirror the relevance of each factor to potential handling failures during the early hunting chain.

The results of this study were discussed along the timeline of the steps of the hunting chain (Figure 1). Hunting begins with the observation of the living game to assess the game animals' appearance and behavior and thus their health condition. Furthermore, the game animals were classified by the species, sex, age or BW. Stella et al. [15] have reported higher bacterial loads for male wild boar carcasses and Branciari et al. [5] found no significant influence of the sex on roe deer carcasses. Another IF could be the animal species, because the IML of ruminants are different from those of wild boars [18]. Wild boars, as monogastric animals, have different gastrointestinal anatomy and microbiome compared to roe deer as ruminants. The results across species were nevertheless used since sample matrices, methods, locations or bacterial groups examined seemed comparable to the present study. Furthermore, the age of the animals was not determined as an appropriate IF, neither in this study based on a risk analysis of the original IML data nor in the study by Stella et al. [15]. The hunters estimated the age classes of animals based on the visible body condition of the animals, e.g., the shape of the antlers of the male animal. Since the age class estimation is imprecise and the reported age depends on the individual experience of the hunter, this parameter seemed unsuitable to be used as an IF for the IML up to now. However, the impact of age class on IML of carcasses could be interacting with the possible effects of sex or BW of the sampled carcasses on the IML, which could be investigated in future research projects with more valid age information and a higher sample size.

Based on the risk analysis of the original IML data from roe deer carcasses in the present study, the BW of the animals was identified as an IF on the total viable count and is in accordance with the findings by Branciari et al. [5]. Stella et al. [15] were able to determine the influence of wild boar BW only for Enterobacteriaceae levels, although total bacterial counts were also examined. Carcass BW can be measured and thus, is less susceptible to reporting bias. Based on a literature search [5,7,8,15], higher BW may result in a higher IML. For example, carcass handling of heavier individuals may impair proper hygienic handling and could result in higher bacterial counts [32]. This could be improved, for instance, by having a second person to assist with the handling of heavier carcasses.

The next step in the hunting chain is to shoot and kill the game, which represents a very individual scenario for each animal, resulting in the differences in identifying the potential impact factor on the IML as stated above. The parameters used in literature to describe the shooting and killing process qualitatively are the shooting accuracy [5,6], number of shots [6], impact energy or caliber of ammunition used [5,6], pre-mortem stress of game [33], shooting distance [11] or escape distance [16,34]. However, it has hardly been confirmed if and how these conditions influence the bacterial load. Based on our risk analysis, the ammunition construction, impact energy of the ammunition, improper shooting accuracy, shooting and

escape distance contributed as IFs on IML and might result in gastrointestinal tract damage or the delayed death of the game animal. However, the effects of these factors described on the killing process of animals depend mainly on the decisions made by the hunter prior to the shot. Since the effects of these qualitative IFs on the IML are difficult to interpret, two FMEA were applied. The FMEA based on the authors´ expertise assessed the escape of shot game that do not die immediately as a medium-risk failure for higher IML (averaged RPN = 24) while FMEA based on a defined stepwise search assessed escape as a low risk (RPN = 9). A higher evaluation of the significance and the probability of detection by the experts led to this difference. Some experts commented that they also considered other IFs in this scenario, such as an incorrect shot accuracy or longer time until chilling. On the contrary, the RPN calculation based on a stepwise literature search was restricted to only one defined IF.

After the game animal is killed, it is salvaged from the place of killing. The hunter could carry the carcass or drag the carcass on the ground, which usually depends on the game's BW. Since the samples in this study were taken after the carcasses had already been salvaged, eviscerated and transported within the field, the impairments by the salvage practices only were impossible to identify. In addition, information on the impact of salvaging on IML in original research articles is also lacking. However, using FMEA based on the authors' expertise and the defined stepwise search, dragging carcasses on the ground during salvage was ranked as a high-risk handling failure due to the probability of occurrence and significance. This handling failure harbors the risk that during dragging, the fur of the carcass might be contaminated with soil or bacteria, which could be transferred to the meat during evisceration or skinning. Bacterial contamination, e.g., of carcass fur, is a major source of cross-contamination on the meat surface [22]. The probability of detection of cross-contamination was ranked comparably high in FMEA, based on the authors' expertise and the defined stepwise search.

Based on the total cause-and-effect analysis, the evisceration process includes several factors that may contribute to a higher IML of carcasses due to handling practice. The place of evisceration was identified as an IF based on the authors' expertise. Depending on the hunting method, environmental circumstances and the shortest possible duration between killing and evisceration, hunters have to decide whether they eviscerate killed game directly at the salvage location, after transport to a collection point or at a game chamber. The roe deer carcasses sampled in this study at drive hunts in Brandenburg were eviscerated on location according to the instructions of the organizer of the hunt. Exposure to environmental conditions may affect the microbial condition of the carcass more frequently, such as the presence of rain. In particular, wet fur can make hygienic handling more difficult. The presence of rain was identified as an IF that can lead to higher IML in this study based on the risk analysis of original data, as was previously reported by Ranucci et al. [8] for wild boar carcasses. On rainy hunting days, it might be more beneficial for lower cross-contamination to transport the game carcass to a place protected from rain before evisceration.

Based on the risk analysis of original IML data, the evisceration technique used and position of roe deer carcass during evisceration were identified as additional IFs. Evisceration can be performed either with or without opening the pelvis on a carcass lying on the ground [9] or hanging from, e.g., a wild gallows [26]. Unexpectedly in this study, the IML of roe deer carcasses eviscerated hanging by the hind legs (n = 3) was higher than the IML of carcasses eviscerated lying on the ground (n = 16) with an opening in the pelvis. In contrast, Mirceta et al. [9] found higher bacterial loads in carcasses that were eviscerated lying under field conditions than in carcasses eviscerated hanging in a game handling establishment. However, opening the pelvis seemed to create a larger surface area in the body cavity that can become contaminated. Performing evisceration without opening the pelvis, the body cavity remains protected from surface contamination; however, this also seems to delay the

cooling compared to carcasses with an open pelvis. This could promote the bacterial growth, especially at higher ambient temperatures [6,15,30,35]. In the current case, sampling was performed during the autumn and winter season in the Northern hemisphere at comparatively low ambient temperatures. Based on the findings of this study, it could also be beneficial for the microbial quality of carcasses to eviscerate heavier carcasses faster than carcasses with low BW and to open the pelvis of all carcasses. This is due to a potential interaction between the slower chill time of heavier carcasses and the ambient temperature, which was determined within the fitting regression model. However, the meat surface needs to be protected from contamination as much as possible during further handling.

Carcasses with damaged gastrointestinal tracts by improper shooting are known to show higher IML as confirmed by the risk analysis of the original IML data and the literature search [28]. In many cases, the microflora of the gastrointestinal content has a big impact on the IML [36]. In this study, the *Lactobacillus* spp. counts were higher in the belly flap and the fillet samples in roe deer carcasses killed with damage to the gastrointestinal tract.

The use of gloves during evisceration was queried. Based on the risk analysis of original IML data, lower bacterial counts were found when gloves were used than when they were not. Therefore, gloves can be used as a hygiene measure to obtain carcasses with a lower IML besides their use as a personal protection measure, e.g., possible infection with hepatitis E [37]. Beyond the direct effect of using gloves during carcass evisceration, lower IML of carcasses handled with gloves might also represent an indirect effect of the hunter's awareness regarding hygiene measures in general. For example, Mirceta et al. [9] reported higher bacterial counts when untrained hunters eviscerated wild boar carcasses than trained hunters [9]. Furthermore, the lack of awareness of the hygienic handling of game carcasses has been determined as a main handling failure based on the FMEA.

The sampling of roe deer carcasses occurred in this study after the carcasses were transported within the field and handed over from the hunters to the sampling personnel. Based on FMEA, taking into account the collective transport of several carcasses at the same time, this transport step was assessed as a handling failure with a high risk of obtaining carcasses with a higher IML. Stacking of multiple animals should be avoided to reduce contamination and delayed cooling, as described before.

This study identified several IFs on IML in the early processing of the game meat chain with a holistic approach. Some factors are extremely difficult to identify because they appear rarely or irregularly in practice. Besides this, other methodological challenges can arise if different studies used other hunting practices or definitions for the same IFs. That is why, in this study, the quantitative original research results regarding factors influencing IML and the associated possible handling failures were combined with two FMEAs. Both FMEAs showed the highest variation in RPN due to the rating of the probability of detection of bacterial effects. This might reflect the fact that the IML is not visually detectable and can also be altered at the following steps of the hunting chain.

The most relevant handling failures are:

1. Lack of awareness of hygienic handling of game carcasses;
2. Pulling/dragging of carcass on the ground;
3. Contamination of the carcass (not only musculature, but also the fur);
4. Cross-contamination of carcasses during transport by e.g., other animal carcasses, delayed chilling or due to insufficient hygienic conditions of the transport vehicle;

5. Evisceration of the game in the field even if there is a possibility to eviscerate the game immediately in a game handling establishment;
6. Delay in the evisceration of the carcass.

5. Conclusions

This study identified factors that may influence the IML during the harvest of game carcasses using data on IML collected from roe deer carcasses as original research and using a literature search. In addition, the magnitude of these IFs on IML of game carcasses was estimated. Potential handling failures and recommendations during the hunting chain were investigated more closely based on the risk analysis of the original data, literature search and FMEA. This combined approach allows for the provision of some recommendations to persons who obtain game carcasses in the field for human consumption and thus participate in the first part of the supply chain for game meat. Visual cleanliness of carcasses does not have to be related to a low bacterial load. This underlines the significance of sensitizing and training the hunters on the importance of their practical contribution to lower the microbial load of game meat.

The study results for handling game carcasses support existing European regulations during harvest and highlight some new aspects, which are summarized hereinafter.

1. Hunters should be trained regarding hygiene including personal protection;
2. Contact of the carcass with the ground and other environmental factors should be reduced, as much as possible;
3. Game carcasses should be eviscerated without delay in a weather-protected place;
4. After the evisceration process of the carcass, the meat surface should be protected from cross-contamination as much as possible during further handling;
5. Special effort should be taken to keep the time after evisceration as short as possible to ensure effective chilling;
6. Multiple carcasses should be transported separately from each other.

Supplementary Materials

The following supporting information can be downloaded at: **https://www.mdpi.com/article/10.3390/foods11223726/s1**, Table S1: Rate Ratios of variables identified as influencing factors of bacterial species in roe deer belly flap (n = 24) by linear regression with backward selection with 95% confidence intervals (95% CI) and p-values. Significance levels of Rate Ratios were highlighted by stars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$); Table S2: Rate Ratios of variables identified as influencing factors of bacterial species in roe deer fillet (n = 23) by linear regression with backward selection with 95% confidence intervals (95% CI) and p-values. Significance levels of Rate Ratios were highlighted by stars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$); Table S3: Evaluation of the extent of failure based on the Risk Priority Number (RPN) calculated by the FMEA based on defined stepwise search. Values of O, S and D were classified based on the effects of IF on IML determined by linear regression and RRs in this study. When the classification of factors affecting IML could not be explained by the results of this study, the original research articles based on the literature search were reviewed for evidence. As a last step, when there was a lack of published evidence, classification was based on experience reported by hunters;

Table S4: Rating of probability of detection (D) for possible handling failures during game carcass obtaining based on defined stepwise search (part 1); Table S5: Rating of probability of detection (D) for possible handling failures during game carcass obtaining based on defined stepwise search (part 2).

Author Contributions

Conceptualization, B.K., J.S.-W., D.M., A.M., N.B., H.A.S. and C.G.; methodology, B.K., F.R., T.A., J.S.-W., D.M., K.N., T.A., I.R., A.M. and C.G.; software, B.K., J.S.-W., D.M. and R.H.M.-V.; validation, B.K., F.R., J.S.-W., D.M. and I.R.; formal analysis, B.K., F.R., J.S.-W. and D.M.; investigation, B.K., A.H., J.S.-W., D.M., A.M., R.H.M.-V. and C.G.; resources, M.L.-W., H.A.S., N.B. and K.N.; data curation, B.K. and I.R.; writing—original draft preparation, B.K., J.S.-W., A.H., D.M., F.R. and R.H.M.-V.; writing—review and editing, B.K., J.S.-W., F.R., D.M., T.A., C.G., R.H.M.-V., A.H., A.M., H.A.S., N.B., K.N., I.R. and M.L.-W.; visualization, B.K., J.S.-W., D.M., R.H.M.-V., A.H. and F.R.; supervision, F.R., T.A., N.B., J.S.-W. and M.L.-W.; project administration, B.K., J.S.-W., F.R., T.A. and M.L.-W.; funding acquisition, M.L.-W., H.A.S. and K.N. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this article are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest.

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Table S1. Rate Ratios of variables identified as influencing factors of bacterial species in roe deer belly flap (n = 24) by linear regression with backward selection with 95% confidence intervals (95% CI) and p-values. Significance levels of Rate Ratios were highlighted by stars (*p < 0.05, **p < 0.01, ***p < 0.001)

Influencing factor	Bacterial species	Rate Ratio	95% CI	p-value
Body weight after evisceration +1 kg	Total viable count	0.9	0.8 – 1.0	0.1592
	E. coli	1.1	1.0 – 1.2	0.1975
Animal sex: female	*Enterobacteriaceae*	2.0	0.8 – 5.2	0.1316
	E. coli	1.5	0.8 – 2.7	0.1941
Ambient temperature on the day of hunt +10 °C	Total viable count	3.2	1.3 – 7.7	0.0128*
	Pseudomonas spp.	4.1	1.8 – 8.9	0.0013**
	Enterobacteriaceae	3.2	0.9 – 11. 6	0.0747
	E. coli	1.6	0.6 – 4.0	0.2847
Rain on the day of hunt: yes	*Pseudomonas* spp.	1.6	0.9 – 3.0	0.1209
	Enterobacteriaceae	2.2	0.9 – 5.5	0.0911
	E. coli	1.9	0.9 – 4.0	0.0759
Ammunition contraction: deforming	*Enterobacteriaceae**	2.5	1.0 – 6.1	0.0467
	*E. coli***	2.6	1.4 – 4.7	0.0059
Damage to the gastrointestinal tract: yes	Total viable count	5.1	2.1 – 12.3	0.0011**
	Pseudomonas spp.	2.3	1.1 – 5.0	0.0332*
	Lactobacillus spp.	8.4	2.9 – 24.2	0.0004***
Shooting distance +10 m	*Enterobacteriaceae*	1.3	1.0 – 1.8	0.0324*
	E. coli	1.7	1.4 – 2.0	0.0001***
Escape distance +10 m	*Enterobacteriaceae*	0.8	0.6 – 1.2	0.2825
Duration between killing and evisceration +10 min	*E. coli*	1.1	1.0 – 1.1	0.0604
Evisceration: hanging	*Enterobacteriaceae*	2.7	0.6 – 11.4	0.1692
	E. coli	12.1	4.6 – 31.6	0.0001***
Evisceration: without opening pelvis	*Enterobacteriaceae*	0.5	0.2 – 1.5	0.2214
	E. coli	1.1	0.5 – 2.5	0.7271
Use of gloves during evisceration: yes	*Lactobacillus* spp.	0.4	0.2 – 1.0	0.0472*
	Enterobacteriaceae	0.2	0.1 – 0.6	0.0070**
	E. coli	0.2	0.1 – 0.5	0.0015**
Visible soiling of body cavity with gastrointestinal content: yes	Total viable count	0.5	0.3 – 1.1	0.0843
	Lactobacillus spp.	0.5	0.2 – 1.2	0.1010
	E. coli	0.5	0.2 – 1.0	0.0604

Table S2. Rate Ratios of variables identified as influencing factors of bacterial species in roe deer fillet (n = 23) by linear regression with backward selection with 95% confidence intervals (95% CI) and p-values. Significance levels of Rate Ratios were highlighted by stars (*p < 0.05, **p < 0.01, ***p < 0.001)

Influencing factor	Bacterial species	Rate Ratio	95% CI	p-value
Body weight after evisceration +1 kg	Total viable count	0.9	0.7 – 1.1	0.1618
	Lactobacillus spp.	0.9	0.7 – 1.1	0.2206
Animal sex: female	Total viable count	0.6	0.2 – 1.4	0.1986
	Enterobacteriaceae	2.5	0.7 – 8.8	0.1376
	E. coli	2.0	0.8 – 4.8	0.1207
Ambient temperature on the day of hunt +10 °C	Total viable count	2.4	0.8 – 7.5	0.1113
	Pseudomonas spp.	3.4	1.5 – 7.7	0.0069**
Rain on the day of hunt: yes	*Lactobacillus* spp.	1.8	0.7 – 4.5	0.2021
Ammunition contraction: deforming	*E. coli*	3.1	1.3 – 7.6	0.0172*
Damage to the gastrointestinal tract: yes	Total viable count	3.4	1.1 – 10.1	0.0299*
	Lactobacillus spp.	5.7	1.8 – 18.1	0.0056**
Shooting distance +10 m	Total viable count	1.3	0.9 – 1.7	0.1054
	Pseudomonas spp.	1.1	0.9 – 1.4	0.1769
	Lactobacillus spp.	1.3	1.0 – 1.8	0.0894
	Enterobacteriaceae	1.5	1.1 – 2.2	0.0156*
	E. coli	1.7	1.3 – 2.2	0.0003***
Escape distance +10 m	Total viable count	1.3	0.9 – 1.8	0.1745
	Lactobacillus spp.	1.4	1.0 – 2.1	0.0769
Duration between killing and evisceration +10 min	*Pseudomonas* spp.	1.0	0.9 – 1.0	0.0691
Evisceration: hanging	*Pseudomonas* spp.	1.5	0.5 – 4.5	0.4869
	Enterobacteriaceae	11.4	1.4 – 90.1	0.0241**
	E. coli	10.4	2.4 – 44. 4	0.0037**
Evisceration: without opening pelvis	*Pseudomonas* spp.	3.2	1.5 – 6.6	0.0044**
	Enterobacteriaceae	0.6	0.1 – 2.3	0.4193
	E. coli	0.7	0.3 – 2.0	0.5362
Use of gloves during evisceration: yes	*Lactobacillus* spp.	0.4	0.2 – 1.1	0.0858
	Enterobacteriaceae	0.3	0.1 – 1.0	0.0545
	E. coli	0.3	0.1 – 0.9	0.0268*
Visible soiling of body cavity with gastrointestinal content: yes	*E. coli*	0.6	0.2 – 1.4	0.2056

Table S3. Evaluation of the extent of failure based on the Risk Priority Number (RPN) calculated by the FMEA based on defined stepwise search. Values of O, S and D were classified based on the effects of IF on IML determined by linear regression and RRs in this study. When the classification of factors affecting IML could not be explained by the results of this study, the original research articles based on the literature search were reviewed for evidence. As a last step, when there was a lack of published evidence, classification was based on experience reported by hunters

Step of hunting chain	Failure	O	S	D	RPN
Salvage	Game is pulled/dragged on the ground during salvage.	5	5	3	75
Transport	cross-contamination of carcasses by e.g. other animals (stacking or too close placement of several killed animals on a transport vehicle) or due to insufficient hygienic conditions of the transport vehicle (e.g. soil, leaves, blood residues from eviscerated carcasses).	3	3	4	36
Evisceration	Evisceration of the carcass lying on the ground (body fluids remain in the body cavity, when eviscerating the carcass lying on the ground).	5	5	1	25
Evisceration	Evisceration of the carcass hanging (soiling of the haunches by draining body fluids during evisceration of a carcass, which was hanging by the head).	5	5	1	25
Evisceration	Lack of awareness of hygienic handling of game carcasses (contamination of carcass by, e.g., unwashed hands in the absence of running water or improper handling with gloves or by equipment used that has not been properly cleaned or is unsuitable for evisceration, e.g., unclean or blunt knives).	5	5	1	25
Evisceration	Contamination of the carcass (not only musculature, but also the fur) by various factors, e.g. rain, grass, leaves, surface water, etc. on the ground when the tarpaulin is not in use or when the stomach and intestinal tract of the game is damaged during evisceration and the contents contaminate the carcass.	5	5	1	25
Shooting/killing	Improper shooting accuracy causes damage to the gastrointestinal tract in individual cases.	4	5	1	20
Evisceration	The game is eviscerated in the field (compared to game handling establishment).	4	5	1	20
Evisceration	Slower cooling of the carcass at high outside temperatures (summer)	3	5	1	15
Shooting/killing	The musculature of the game is highly destroyed due to a too high impact energy.	3	4	1	12
Evisceration	No or insufficient removal of e.g. hematomas, stomach and intestinal contents, adhering foreign materials (grass, leaves etc.)	2	5	1	10
Shooting/killing	Insufficient killing effect is caused by insufficient impact energy.	3	3	1	9
Shooting/killing	The game does not die immediately, but can still flee after the shot.	3	3	1	9
Evisceration	The game is eviscerated with delay.	2	4	1	8

Table S4. Rating of probability of detection (D) for possible handling failures during game carcass obtaining based on defined stepwise search (part 1)

Possible failure	Rating according to	Parameter used for rating of D	Rating scale of D
Improper shooting accuracy causes damage to the gastrointestinal tract in individual cases	Original IML data	Damage to gastrointestinal tract affect IML	1
Insufficient killing effect is caused by insufficient impact energy	Original IML data	Body weight and ammunition construction affect IML	1
The musculature of the game is highly destroyed due to a too high impact energy	Original IML data	Body weight and ammunition construction affect IML	1
The game does not die immediately, but can still flee after the shot	Original IML data	Escape distance were identified as influence factor on IML	1
The game is eviscerated in the field (compared to game handling establishment)	Literature research	Evisceration location, field vs. game handling establishment affect bacterial load, reported by Mirceta et al. [9]	1
The game is eviscerated with delay	Original IML data	Duration between killing and evisceration affect IML	1
Evisceration of the carcass lying on the ground (body fluids remain in the body cavity, when eviscerating the carcass lying on the ground)	Original IML data	Evisceration position of the carcass affect IML	1
Evisceration of the carcass hanging (soiling of the haunches by draining body fluids during evisceration of a carcass, which was hanging by the head)	Original IML data	Evisceration position of the carcass affect IML	1
Lack of awareness of hygienic handling of game carcasses (contamination of carcass by, e.g., unwashed hands in the absence of running water or improper handling with gloves or by equipment used that has not been properly cleaned or is unsuitable for evisceration, e.g., unclean or blunt knives).	Original IML data	Gloves worn during evisceration affect IML	1

Table S5. Rating of probability of detection (D) for possible handling failures during game carcass obtaining based on defined stepwise search (part 2)

Possible failure	Rating according to	Parameter used for rating of D	Rating scale of D
Contamination of the carcass (not only musculature, but also the fur) by various factors, e.g. rain, grass, leaves, surface water, etc. on the ground when the tarpaulin is not in use or when the stomach and intestinal tract of the game is damaged during evisceration and the contents contaminate the carcass.	Original IML data	Rain at hunting day affect IML	1
Slower cooling of the carcass at high outside temperatures (summer)	Original IML data	Ambient temperature at hunting day and duration between killing and evisceration affect IML	1
No or insufficient removal of e.g. hematomas, stomach and intestinal contents, adhering foreign materials (grass, leaves etc.)	Original IML data	Visible soiling affect IML	1
Cross-contamination of carcasses by e.g. other animals (stacking or too close placement of several killed animals on a transport vehicle) or due to insufficient hygienic conditions of the transport vehicle (e.g. soil, leaves, blood residues from eviscerated carcasses).	Experience reported by grey literature	-	4

4 Discussion

4.1 Key findings of the studies in this thesis

Game meat hygiene is a complex process that begins in the field with the observation of the animal by the hunter as a part of the "farm/forest to fork" principle and needs to be considered holistically. Therefore, game meat hygiene is being discussed from many different perspectives (e.g., political, regulatory, scientific and practical) to ensure the food safety of the final product (BfR 2013). One of the most interesting questions in game meat hygiene is whether the game body cavity should be rinsed after evisceration in all cases or only if it is contaminated with GICs (Bildungs- und Wissenszentrum Aulendorf 2008, Deutz 2012a, Rheinisch-Westfälischer Jäger 2017, Amt für Landschaft und Natur 2019). Water rinsing aims to remove contamination, to reduce the IML (Gill 2004) and thus to improve the microbial condition of the carcass (Chapters 3.2 – 3.3). However, opinions on the rinsing practice of game carcasses are divided. Critics of cleaning the body cavity by rinsing with drinking water argue that this could further distribute bacteria and promote their growth (Mirceta et al. 2017, Orsoni et al. 2020).

For consistent and proper practice in game meat hygiene, hunters need information regarding factors that can influence or increase bacterial growth to take corrective measures. Ultimately, hunters have the most impact on the hygiene status and safety of game carcasses and meat (Chapter 3.4). They put into practice the legal requirements for obtaining game carcasses and meat, apply and implement measures for hygienic production, or assess meat quality after the meat maturation. With the aim of expanding current knowledge, the focus of the studies in this thesis was on the IFs and measures that can affect the IML and subsequent ML of roe deer carcasses and meat, particularly in relation to the rinsing process.

In the first study, very different high IMLs were found in individual carcasses, which were later divided into rinsed and unrinsed carcasses and examined. A significant factor for these different IML levels was the presence of visible soiling with GIC in the roe deer body cavities. The lowest IML was unexpectedly found in the body cavities of carcasses that were visibly soiled with GIC, despite the highest IML being expected in these carcasses. To investigate the effect of visible GIC soiling on the IML of roe deer carcasses in more detail, a second study was performed. In this second study, the uniform distribution of the homogeneous GIC mixture in the body cavity was intended to increase the IML of all carcasses to comparable levels. As expected, the IML increased in the body cavity surfaces after this inoculation. However, variations in subsequent ML after inoculation persisted. It was assumed that the different effects of visible soiling with GIC on the IML of the carcasses depended on the

bacterial level of the GIC and additional factors that may affect the GIC. For example, the feed compositions (Liu et al. 2019, König et al. 2020) or the conditions under which the carcasses were present in the field, such as higher ambient temperatures, may promote bacterial growth (Paulsen and Winkelmayer 2004, Stella et al. 2018).

In neither the first nor the second study did rinsing visible soil from the roe deer body cavities significantly reduce the IML or subsequent ML on the investigated sampling areas (i.e., belly flaps or fillets) with different surface textures (i.e., smooth vs. uneven/fibrous, as the musculature surface was often damaged). However, in the second study, a reduction in the subsequent ML (TVC, *Pseudomonas* spp.) was observed more frequently on smooth surfaces (i.e., belly flaps) than on rough and irregular surfaces (i.e., fillets) after visible soiling had been removed by rinsing on the day of hunting. This may have been found due to the promoted bacterial attachment to irregular surfaces (Delaquis and Mccurdy 1990; Dickson 1988, Firstenberg-Eden 1981). On the other hand, a frequent increase in subsequent ML was observed for both surface textures of the rinsed body cavities after three days of cold storage. This increase, supports the hypothesis that residual moisture may contribute to bacterial growth. However, there was no clear overall trend for the bacterial groups studied. Thus, it was assumed that in addition to the texture of the meat surface other factors have significance for the microbial condition after meat maturation. The fact that storage temperatures, for example, affect the bacterial growth is already known (Maahs 2010).

In addition to the storage temperature (Maahs 2010), the ambient temperature on hunting day also has an effect on the IML of game carcasses (Stella et al. 2018). Moreover, numerous other factors and measures in the early steps of the hunting chain that have or could have an impact on IML were investigated in the third study (Chapter 3.4). How and through what means the microbial contamination of game carcasses can be kept as low as possible is a multifaceted topic. Each process for obtaining game carcass is different. However, it was possible to determine, for example, that the concentration of *Lactobacillus* spp. in the carcasses was lower when gloves were used during the evisceration process. Other critical handling practices related to the distribution or cross-contamination of bacteria are presented in Chapter 3.4.

4.2 Discussion of potential factors that affect the initial microbial load and subsequent microbial load of roe deer carcasses

4.2.1 Effects of visible soiling with gastrointestinal content in roe deer body cavities on the microbial condition of roe deer carcasses are dependent on factors present in the early steps of the hunting chain

For a first assessment of the microbial condition of a game carcass during the early steps of the hunting chain, the visual condition of the carcass is usually considered, particularly those of the body cavity after evisceration. It is generally assumed that compared to visually clean carcasses, the presence of visible GIC in the body cavity is related to the microbial condition of the carcass and could lead to higher IML levels (i.e., at the time of the first sampling of carcasses on hunting day) and promote the spread of fecal bacteria on meat surfaces during further processing (Mackey and Derrick 1979, Gill 2004, Brecheisen 2014). The assumption that there is a significant relationship between the presence of visible soiling and the IML and subsequent ML (i.e., all examined bacterial groups at every sampling after any measure on hunting day or after meat maturation) of carcasses was determined and the results are presented in Study I. When comparing each bacterial group obtained from belly flap samples from rinsed and unrinsed carcasses on hunting day and after meat maturation, soiling with GIC had a significant effect on TAC and levels of *Pseudomonas* spp., *Lactobacillus* spp., *Enterobacteriaceae* and *E. coli*. However, it was not possible to identify a clear trend for all examined bacterial groups, i.e., there was neither a positive or negative relationship between the presence of visible soiling and the microbial condition in body cavities. In contrast, other studies report a positive relationship between the presence of visible fecal or environmental contamination and higher MLs (Slowak 1986, Paulsen and Winkelmayer 2004).

The assumption made in Study II, i.e., that the IMLs of visible soiled body cavities were higher than those of clean body cavities was consistent with the findings of this study. However, the subsequent ML level and dominant type of bacteria examined on the meat surfaces varied 15 min after the GIC mixture application and after three days of meat maturation. After meat maturation of unrinsed carcasses, the ML of visibly soiled body cavities increased, remained the same, or in some cases decreased (Study II). Crucial points for the ML on body cavity surfaces are the transfer or attachment of bacteria to the meat surfaces (Firstenberg-Eden 1981, Farber and Idziak 1984) and the possibility of growth and multiplication. The latter could depend on the required growth temperatures and generation times of the bacteria (BfR 2006), their preferred substrates from meat (Gill 1976b), and atmospheric oxygen conditions (Gill 1983, Lawrie and Ledward 2014, Sofos 2014).

In addition to the relationship between IMLs and visible conditions of roe deer body cavities and storage time, it was hypothesized in Study III that several factors in the early steps of the

hunting chain may affect the IMLs. This was shown by Paulsen et al. (2022), who investigated 352 roe deer carcasses with respect to visible cleanliness and shot wound locations and found a significant relationship between both parameters. Therefore, a cleanliness score with four categories and a categorization of the shot wound locations into four wound types were used (Paulsen et al. 2022). The categories for the cleanliness score are as follows: 1. visually clean; 2. few small green particles; 3. spots of gastric or fecal matter occupying a maximum of 1/10th of the area of the affected body cavities; and 4. larger affected areas or putrefaction. The wound types were classified as follows: 1. both wounds before the 7th rib (cranial thorax); 2. both wounds before the 13th rib, with one or both between the 7th and 13th ribs; 3. one wound in the flank (in the event of more than one hit, the most caudal wounds were considered); and 4. two wounds in the flank (in the event of more than one hit, the most caudal wounds were considered). By following this cleanliness score, the investigated roe deer body cavities examined in the studies of this thesis corresponded to categories 1 (unsoiled) and 3 or 4 (soiled). Even when a small area was contaminated with GIC, the carcass was categorized as soiled for statistical analysis. Soiling with fur, blood or parts of plants was presented descriptively. That the visually contaminated carcasses in Study I had a lower IML than the visually unsoiled carcasses could be due to this cleanliness score classification. In particular, carcasses that contained only small, soiled areas could also have a low IML if those exact areas were not sampled.

In the Study III, it was found that in addition to the visible soiling of body cavities, the shot position and shot distance were also significant for the IML. When the GIT was damaged or when the shot distance was greater, the IML was higher than in carcasses where the GIT remained intact after the shot. In addition, hunters' performance in hygienic handling of carcasses on hunting day could affect the IML because their actions could promote or prevent the spread of bacteria on meat surfaces, e.g., during evisceration. In Study III, a lack of awareness of hygienic handling of game carcasses or improperly cleaned equipment were described as the major handling failures when harvesting game carcasses. Higher MLs were found when untrained hunters eviscerated wild boar carcasses than when trained hunters carried out the evisceration (Mirceta et al. 2017). Moreover, Orsoni et al. (2020) considered in their study period (from January to April 2015) the eventual training of hunters during previous samplings (i.e., yes/no) and found higher levels of *Enterobacteriaceae* in wild boar carcasses before training of hunters than after training. It can be concluded that to obtain high-quality game carcasses, it is a prerequisite that hunters are up to date with the latest knowledge in hygienic handling of game carcasses and undergo regular practical training.

One aspect of hygienic handling of carcasses is the hunter's awareness of good hand and knife hygiene, as these are important sources of cross-contamination. These sources of cross-contamination have already been investigated in the dressing of beef carcasses, and it

was found that operators´ hands had similar MLs (mean aerobic plate counts were 4.74 ± 0.67 $\log_{10}$ CFU/cm²) as the hides (mean aerobic plate counts of hides investigated at the inside hind leg were 4.63 ± 0.73 $\log_{10}$ CFU/cm²) after making the opening cuts on the carcasses (Bell 1997). Furthermore, knife blades carry approximately one tenth of the contamination found on operators' hands before or after cutting (Bell 1997). To reduce the introduction of bacteria to the meat surfaces of game carcasses via hands or knife blades, the hands and knife blades must be washed and dried after each contact with the carcass fur. However, under field conditions, potable water is not available in every case. In these cases, cross-contamination could be avoided by using gloves during the first opening cut on the carcass and changing gloves before further evisceration. Evisceration of game carcasses when using gloves resulted in lower MLs than when not using gloves (Study III). When the gloves come into contact with the fur of the carcass during further handling, they should be changed again.

In any case, existing visible soiling on roe deer body cavities poses a challenge for the hygienic conditions of carcasses as an indication of adverse effect according to the national regulation LMHV (BfJ 2018) and can lead to higher MLs, as is shown in the findings of other authors (Paulsen and Winkelmayer 2004). Therefore, meat obtained from such carcasses should be handled with special attention, e.g., in terms of immediate refrigeration (Cenci-Goga et al. 2021), continuous cooling (Paulsen and Winkelmayer 2004) and the proper preparation method for the final food (Sofos 2014) that reaches the consumer. At each intermediate step in the processing chain, such as before chilling and after meat maturation, the carcass and meat quality must be verified. In addition to the microbial condition of carcasses or meat, other physical, chemical or sensory properties are to be used for inspection, such as by checking odor as a sensory property using cooking samples in accordance with Annex 4 of the national regulation “AVV Lebensmittelhygiene” (AVV LmH) (BMI and BfJ 2009). Depending on the results of the inspections, the carcass may be released for human consumption or it must be discarded (Paulsen et al. 2003). Furthermore, it could be advantageous from a food safety point of view to avoid using meat obtained from carcasses that exhibit visible soiling, e.g., for raw sausage production. This would apply even when the soiled parts have been trimmed. This is because recontamination by operators´hands and knife blades is possible, and the inactivation of some bacterial groups in this process may be insufficient only when affected by a reduction in pH value (acidification) and drying (reduction in water activity) (Lawrie and Ledward 2014, Sofos 2014). Meat from game carcasses, especially those with visibly soiled body cavities, should be heated to a core temperature of 70 °C for at least 2 minutes before consumption (BfR 2020). Furthermore, it could be beneficial if meat obtained in this manner reached the consumer via the shortest possible distribution channel.

4.2.2 Effects of water rinsing on the microbial condition of roe deer body cavities may be dependent on factors present in the early steps of the hunting chain

In Studies I and II, the effects of rinsing on the IML of roe deer body cavities were examined (before rinsing) in the context of the presence of visible soiling with GIC (i.e., soiled/unsoiled) and different sampling times (subsequent ML after rinsing or meat maturation). The aim was to determine the impact of rinsing on bacterial growth in rinsed carcasses and to interpret these results in comparison to unrinsed carcasses that were obtained under similar hunting, handling and environmental conditions and to determine if there were any differences. The effect of rinsing on the IML for intentionally soiled roe deer body cavities was examined in Study II to determine if the subsequent ML could be reduced from comparatively high MLs of similar inoculated body cavities. The effects of rinsing on the IML and bacterial growth of the bacterial groups studied in the roe deer body cavities were incongruent in Studies I and II for the subsequent ML in each individual carcass after rinsing and after meat maturation. Significant differences between the MLs of rinsed and unrinsed carcasses on hunting day and after meat maturation were not observed on the meat surfaces and in muscle samples (Study I). However, an ML-reducing effect could be estimated in some cases as a short-term effect that occurred directly after rinsing (Study II). In contrast, both Study I and the study by Orsoni et al. (2020) revealed that the subsequent ML in rinsed carcasses (after rinsing) were higher than the IML in unrinsed carcasses. In the study by Orsoni et al. (2020), the IML (aerobic colony counts) of wild boar carcasses were not investigated before rinsing. Since the focus of that study was to examine the microbial condition of hunted wild boar carcasses in relation to their processing in an approved GHE based on, e.g., total weight, meat cut (i.e., fillets or leg quarters), time interval between shooting and evisceration, time, and cleaning with running potable water (i.e., cleaned/uncleaned) (Orsoni et al. 2020). Cleaning the carcasses was only one of these parameters (Orsoni et al. 2020). In Study I, the IML of carcasses intended for rinsing were additionally investigated prior to rinsing, and higher IMLs were also found than in unrinsed carcasses. Therefore, the higher IML in the rinsed carcasses could have already resulted from natural conditions or carcass conditions, independent of the rinsing process. In Study III, for example, it was determined that the body weight of the carcasses can have an influence on their IML, and in Study I, the group of rinsed carcasses had a higher body weight than the unrinsed group (unpublished data). Moreover, the examined meat surface (Study II) may have an impact on the IML and thus on the effectiveness of rinsing. This finding is aligned with the results of the investigation by Orsoni et al. (2020), who determined that the examined meat cut also significantly affected the ML of wild boar carcasses. Here, the ML of meat cut samples taken from fillets were higher than those of samples obtained from leg quarters (Orsoni et al. 2020). A variation in

the ML between the examined sampling surface was also observed in Studies I and II for the rough and irregular surface of fillets and the smooth surface of the belly flap samples. In Study II, higher MLs were determined for fillet samples than for belly flap samples after rinsing the body cavities. Therefore, it is hypothesized that bacteria could be washed into the intermediate or hollow spaces of anatomically deeper parts of the rough and irregular body cavity surface, such as fillets, and could continue to grow over time.

In conclusion, water rinsing could physically remove visible contamination, but its reducing or promoting effect on the IML and subsequent ML of game body cavities may depend on several factors, as described before. Based on the results of Studies I and II, it was not possible to determine whether rinsing could lead to an improvement or a deterioration in the microbiological quality of game carcasses and the resulting game meat. The introduced water can either reduce the IML on hunting days in the short-term or promote bacterial growth during cold storage (Studies I and II). Whereby, Orsoni et al. (2020) found that the subsequent ML increased more slowly over time in rinsed carcasses than in unrinsed carcasses. Furthermore, it appeared that the effectiveness of rinsing individual body cavities of game may depend on the water temperature or the application of rinsing water in the carcass. Effective reduction in ML is achieved by rinsing with warm water (74 °C), and this effect is well known for the surfaces of beef carcasses (Cabedo et al. 1996). However, such rinsing is not practical under field conditions, so the game carcasses examined in Studies I and II were rinsed with water at ambient temperature in winter. Data regarding the water temperature in the study by Orsoni et al. (2020) were not given. During water application, bacterial contamination can spread in the carcass, from the fur to meat surfaces (Mirceta et al. 2017) or be redistributed from posterior to anterior in the body cavity (Bell 1997), resulting in higher MLs. Therefore, the ML-reducing effect of rinsing on the IML and subsequent ML might be masked by the effects of cross-contamination or redistribution. This could be another explanation for the higher ML of the rinsed carcasses in the Study I and in previously reported studies (Mirceta et al. 2017, Orsoni et al. 2020). Finally, the IML of roe deer carcasses were impacted by the effects of other IFs during the early steps of the hunting chain in addition to being affected by rinsing, as described previously in Study III.

4.3 Limitations in the comparability of the microbial loads of hunted game carcasses harvested in field studies

Harvesting of game carcasses can be performed using different hunting and handling practices with similar main steps but with variations in intermediate stages and options or parameters (Chapter 2.3.1). Thus, the major differences among existing studies in sampling hunted carcasses are in the methodology, e.g. sampling location (i.e., field/GHE), various time spans between the intermediate stages of a hunt (e.g., time between killing and evisceration), and sampling time points (i.e., directly after evisceration in the field on the hunting day/at a GHE after several chilling days). It is challenging to compare the MLs of game carcasses or game meat that were examined under different hunting and sampling conditions because the IMLs are affected by these circumstances (Study III). Furthermore, different sampling matrices (i.e., muscle surface/muscle) or bacterial groups (e.g., fecal bacteria/pathogens) have been investigated in various studies (Paulsen and Winkelmayer 2004, Avagnina et al. 2012, Díaz-Sánchez et al. 2012, Paulsen et al. 2012).

In several studies, samples were taken either in the field, for example, at the collection point (Atanassova et al. 2008, Avagnina et al. 2012) or at the GHE (Branciari et al. 2020, Paulsen et al. 2022). It is assumed that different factors during sampling in the field and in the GHE could lead to varying IMLs of carcasses. In the study by Mirceta et al. (2017), samples of wild boar carcasses were collected during different hunts (between October and December 2015 in eight hunting estates) in the field at the collection point or at the GHEs of the hunting estates. When carcasses lying on the ground were eviscerated in the field, higher aerobic colony counts were found on the skin and on the meat surfaces of the carcasses (6.4 ± 1.1 $\log_{10}$ CFU/cm², 6.0 ± 0.9 $\log_{10}$ CFU/cm²) than on the skin and on the meat surface of the carcasses (4.1 ± 0.8 $\log_{10}$ CFU/cm², 4.9 ± 0.8 $\log_{10}$ CFU/cm²) that were eviscerated at a GHE (Mirceta et al. 2017). Therefore, it is expected that when sampling in the field, carcasses are more exposed to environmental conditions, e.g., ground contact during evisceration, and thus are more likely to be cross-contaminated, which would thus affect the IML differently than when sampling at the GHE.

Due to the hunting conditions, different time spans elapsed between the intermediate stages of a hunt and thus sampling times in Studies I – III. Therefore, the time intervals between killing and evisceration varied for each carcass. Paulsen et al. (2022) found that the time between killing the animal and sampling the carcass had a significant effect on the increase in bacterial load. In that study, sampling at an approved GHE occurred on average after 5 chilling days after killing (range from 1 day to 12 days) (Paulsen et al. 2022). The authors found an indication that the total aerobic counts increased by 0.2 log units per day (Paulsen et al. 2022). Therefore, the timing of sampling of game carcasses for microbiological tests is

a crucial point for the comparability of MLs determined from different studies and should be interpreted from this point of view.

Comparison of the microbial data of game carcasses (Riemer and Reuter 1979, Mirceta et al. 2017) and meat (Membré et al. 2011) from different studies were also challenging due to the sampling matrices used (Bandick and Ring 1995, Orsoni et al. 2020) and the different bacterial groups examined in each study (Atanassova et al. 2008, Avagnina et al. 2012, Ranucci et al. 2021). Highly variable IMLs and subsequent MLs were observed on carcass surfaces and in meat examined in different sampling matrices in Study I (e.g., belly flap and fillet surfaces, leg and back muscles). Orsoni et al. (2020) investigated fillet and leg quarter surfaces obtained from wild boars as sampling matrices and found a significant difference between both meat cuts in terms of *Enterobacteriaceae* levels. The surfaces of fillets are more contaminated with bacteria than those of leg quarters (Orsoni et al. 2020). Therefore, when evaluating the microbial quality of game carcasses or meat, it is difficult to use the microbial data from existing studies for comparison when different sample matrices have been examined. Furthermore, in contrast to the well-studied fecal bacteria in game carcasses, for example, *Pseudomonas* spp. was rarely studied in the literature (Riemer and Reuter 1979). The level of this meat spoilage agent can continue to increase significantly during the cold storage (Study I). However, due to the limited number of data on *Pseudomonas* spp. levels in other studies for game carcasses and meat, it is challenging to assess the extent of the impact of this bacterial group on final game meat quality.

4.4 Future Perspectives

Identifying those factors that influence the microbial quality of hunted game carcasses and thus their meat is becoming increasingly important due to the growing popularity of the game meat market (Marescotti et al. 2019). Therefore, the sampling certificates used in Studies I – III, on which relevant data on possible IFs were recorded, could be further used in the same way by the hunters of the German Federal Forestry Service to establish a database on IML. A large sample size could thus be achieved, which is necessary for statistical investigations of microbial data from game carcasses due to their high variations. This may allow additional IFs to be determined. After additional IFs have been identified, the effects of individual handling practices, such as rinsing game body cavities, can be considered in more detail. In this regard, it may be beneficial for the study design of future studies to reconsider the rinsing process. This is because rinsing game body cavities with water at ambient temperature until the body cavities appeared visually clean was not effective in reducing the IML and subsequent ML. Another option would be to rinse the body cavities with a defined amount of water, regardless of the visible condition. The amounts of water used in Studies I and II varied from 730 to 2400 mL and from 1680 to 7200 mL, respectively, depending on the degree of visible contamination of the body cavities. Due to these variations in water volumes, the efficacy of rinsing may have been compromised and could explain the varying effects of rinsing on the IML and subsequent ML of individual carcasses. Possible effects of water amounts applied on IML levels and subsequent ML levels of carcasses could thus be avoided by using a defined water volume in future studies.

In future studies, more attention should be focused on the properties of GICs, which are obtained on soiled game body cavities. Regarding variable effects of GIC in roe deer body cavities on their IML and subsequent ML, it could be revealing to study their chemical and physical properties. For example, the lactic acid concentration, which could be produced by probiotic *Lactobacillus* spp. (Neal-McKinney et al. 2012) or the pH value of the GIC. In the study by Ritz et al. (2013), total pH values in roe deer forestomach compartments were investigated. Varying pH values ranged from highly acidic at 4.64, to almost neutral at 6.77 were determined. Highly acidic pH values can inhibit bacterial grow of acid-labile bacterial species on meat surfaces (Lawrie and Ledward 2014) and provide an explanation for determining the lowest IML in soiled carcasses (Study I).

Furthermore, the roe deer carcasses examined in the studies were voluntarily provided by hunters and sampled without any categorization, such as by body weight or sex. Nevertheless, to take the aspect of randomness into consideration in the sample distribution, the grouping of roe deer carcasses into carcasses to be rinsed and carcasses not to be rinsed was conducted randomly (Chapters 3.2 – 3.3). However, this randomness could have

played a role in the variations in IMLs of rinsed and unrinsed carcasses. It can be assumed that the effects of hunting and handling practices or environmental conditions on IML levels could be more apparent and the comparability of IML levels could improve when the animal-related IF of carcass body weight would be considered in the sample size of future studies.

Another future perspective is expanding and verifying the cause-effect analyses by using FMEA. This could provide more details on infrequently occurring handling failures if more stakeholders in the game meat chain were interviewed, i.e., hunters and food operators. After verifying the possible handling failures that were observed in Study III, general avoidance strategies for this failures in the early steps of the hunting chain could be developed nationwide.

5 Summary

Influencing factors and reduction measures affecting the microbial load of hunted roe deer carcasses and meat with special emphasis on rinsing

Low IML of hunted roe deer carcasses is an important parameter that contributes to the meat quality and food safety of the final product. Therefore, appropriate measures must be taken in the hygienic handling of carcasses in the field to keep the IML as low as possible. These measures, such as rinsing roe deer body cavities, may not only have short-term effects on the IML of carcasses but may also have longer-term effects on the subsequent ML of the obtained meat, e.g., after meat maturation. The time it takes for the meat from roe deer carcasses to reach consumer´s table varies depending on the distribution channel. As this amount of time increases, the ML of the carcasses or meat may also rise, especially when cold-tolerant bacterial species such as *Pseudomonas* spp. are present. Therefore, appropriate food hygiene, which begins with obtaining primary products during the early steps of the hunting chain, is a prerequisite for producing safe food with the lowest possible IML. Notably, it is a challenge to determine the cause-effect relationship between individual hygiene measures and their effects on the IML and subsequent ML because a large variety of IFs can occur simultaneously during the harvesting process.

The aim of this thesis was to investigate the impact of handling practices and hunting-related factors on the microbial condition of hunted roe deer carcasses. First, the effect of rinsing roe deer body cavities on IML and subsequent ML was investigated, as this is a common but controversial cleaning practice in game meat hygiene. Proponents nevertheless recommend rinsing game body cavities, arguing that rinsing could reduce the IML of carcasses, especially in visibly soiled carcasses (Studies I and II). Additionally, the impact of environmental and hunting conditions, animal-related characteristics, and other handling practices during the early hunting chain on the IML of roe deer carcasses were investigated (Study III).

As expected and presented in the first study, the bacterial counts in the muscle samples obtained from rinsed and unrinsed carcasses were mostly below the limit of detection both on hunting day and after meat maturation. This is an indicator of good microbial quality in game meat. However, higher IMLs with a wide range of variation were found on the meat surfaces of individual carcasses that were sampled directly after handover on hunting day, which could affect the microbial quality of the final product (Chapter 3.2). Highly varying MLs were also found after a putative or known preventive measure was taken, i.e., rinsing roe deer body cavities on hunting day or after a three-day refrigeration (meat maturation). In contrast to the other investigated bacterial groups, only the *Pseudomonas* spp. level on belly

flap samples in Study I showed a significant increase in relation to time and increased after meat maturation in both rinsed and unrinsed carcasses. Since these increases were found in both rinsed and unrinsed carcasses, no differences could be detected between the MLs of the rinsed and unrinsed carcasses. Ultimately, no reduction in IML and thus no improvement in the microbial quality of the carcasses from rinsing the roe deer body cavities could be determined.

Due to hunting and handling conditions, visible soiling was present in some roe deer body cavities, and it was found in the first study that the presence of visible soiling with GIC in body cavities significantly affected the IML. Therefore, in addition to Study I, Study II analyzed the effect of rinsing on the IML of body cavity surfaces that had previously been intentionally soiled with a GIC mixture. Furthermore, in Studies I and II, the subsequent ML of body cavity surfaces were determined after three days of cold storage to investigate the possible effects of time during meat maturation on the IML. Again, no differences in the MLs of rinsed and unrinsed carcasses were observed, whether on hunting day or after cold storage (meat maturation).

Due to great importance of the IML on the quality and shelf life of the final product, a cause and effect analysis was performed in Study III (Chapter 3.4). This approach entailed using data from roe deer carcasses with completed sample certificates from Studies I and II to determine the effects of environmental and animal-related factors, as well as the effects of hunting and handling practices, on IML. For example, it was determined that the visual cleanliness of carcasses might not always be associated with low bacterial loads. However, the use of gloves during evisceration reduced the levels of *Lactobacillus* spp., *Enterobacteriaceae*, and *E. coli* compared to evisceration without the use of gloves.

In summary, the investigations of the three studies resulted in a valuable contribution of knowledge on the crucial aspects that may influence the IML and thus the microbial quality of hunted roe deer carcasses.

6 Zusammenfassung

Einflussfaktoren und Maßnahmen zur Reduzierung der mikrobiellen Belastung von erlegten Rehkarkassen und -fleisch unter besonderer Berücksichtigung des Spülens

Eine niedrige Anfangskeimzahl von jagdlich erlegten Rehkarkassen ist ein wichtiger Parameter, der zur Fleischqualität und Lebensmittelsicherheit des Endproduktes beiträgt. Daher müssen geeignete Maßnahmen im hygienischen Umgang mit den Karkassen im Feld getroffen werden, damit die Anfangskeimzahl so gering wie möglich gehalten werden kann. Diese Maßnahmen wie z.B. das Spülen von Rehkörperhöhlen können nicht nur kurzzeitige Effekte auf die Anfangskeimzahl der Karkassen haben, sondern auch längerfristige Effekte auf die nachfolgende mikrobielle Belastung des gewonnenen Fleisches, z.B. nach der Fleischreifung. Bis das Fleisch von Rehkarkassen die Teller der Verbraucher erreicht, vergeht je nach Vermarktungsweg unterschiedlich viel Zeit. Mit zunehmender Zeit kann auch die mikrobielle Belastung der Karkassen ansteigen, vor allem, wenn kältetolerante Bakterienspezies wie z.B. *Pseudomonas* spp. vorhanden sind. Daher ist die Lebensmittelhygiene begonnen bei der Gewinnung von Primärerzeugnissen während der anfänglichen Schritte in der Jagdkette eine Voraussetzung für die Herstellung von sicheren Lebensmitteln mit einer möglichst geringen Anfangskeimzahl. Insbesondere ist es eine Herausforderung die Ursache-Wirkungs-Beziehung zwischen den einzelnen Hygienemaßnahmen und deren Effekten auf die Anfangskeimzahl zu bestimmen, da eine Vielzahl von Einflussfaktoren während des Gewinnungsprozesses zeitgleich auftreten können.

Das Ziel dieser Thesis war es, die Beeinflussung des mikrobiellen Zustandes von jagdlich erlegten Rehkarkassen durch Handhabungspraktiken und jagdbedingte Faktoren, zu untersuchen. Als erstes wurde der Effekt des Ausspülens von Rehkörperhöhlen auf dessen Anfangskeimzahl und nachfolgende mikrobielle Belastung untersucht, da es eine gängige, aber kontrovers diskutierte, Reinigungsmaßnahme in der Wildbrethygiene ist. Befürworter sprechen trotzdem die Empfehlung zum Spülen von Wildkörperhöhlen mit dem Argument aus, dass das Spülen die Anfangskeimzahl der Karkassen reduzieren könnte, insbesondere bei sichtbar verunreinigten Karkassen (Studie I und II). Zusätzlich wurde die Beeinflussung der Anfangskeimzahl von Rehkarkassen durch Umwelt- und Jagdbedingungen, tierbezogenen Eigenschaften sowie weiterer Handhabungspraktiken während der anfänglichen Jagdkette untersucht (Studie III).

Wie erwartet und in der ersten Studie dargestellt, lagen die Keimzahlen in den Muskelproben, die aus gespülten und ungespülten Schlachtkörpern gewonnen wurden, sowohl am Jagdtag als auch nach der Fleischreifung meist unter der Nachweisgrenze. Dies

ist ein Indikator für eine gute mikrobielle Qualität des Wildfleisches. Allerdings wurden auf den Fleischoberflächen der einzelnen Karkassen, die direkt nach der Übergabe am Jagdtag beprobt wurden, höhere Anfangskeimzahlen mit einer großen Schwankungsbreite gefunden, die die mikrobielle Qualität des Endproduktes beeinflussen könnten (Kapitel 3.2). Stark schwankende mikrobielle Gehalte wurden auch nach einer vermeintlichen oder bekannten Präventivmaßnahme festgestellt, d.h. nach dem Spülen der Rehkörperhöhlen am Jagdtag oder nach einer dreitägigen Kühlung (Fleischreifung). Im Gegensatz zu den anderen untersuchten Bakteriengruppen zeigten nur der Gehalt an *Pseudomonas* spp. auf den Bauchlappenproben in der Studie I einen signifikanten Anstieg in Abhängigkeit von der Zeit und nahm nach der Fleischreifung sowohl in gespülten als auch in ungespülten Schlachtkörpern zu. Da dieser Anstieg in gespülten und auch ungespülten Schlachtkörpern festgestellt wurde, konnten keine Unterschiede zwischen den Keimzahlen der gespülten und ungespülten Karkassen nachgewiesen werden. Letztendlich konnte keine Verringerung der Anfangskeimzahl und somit keine Verbesserungen der mikrobiellen Qualität der Karkassen durch das Spülen der Rehkörperhöhlen bestimmt werden.

Aufgrund der Jagd- und Handhabungsbedingungen waren in einigen Rehkörperhöhlen sichtbare Verschmutzungen vorhanden und es wurde in der ersten Studie festgestellt, dass das Vorhandensein von sichtbaren Verunreinigungen mit Magen- und Darminhalt in den Körperhöhlen die Anfangskeimzahl signifikant beeinflusst. Daher wurden in der Studie II zusätzlich zur Studie I, die Auswirkung des Spülens auf die Anfangskeimzahl von Körperhöhlenoberflächen analysiert, die zuvor absichtlich mit einem Gemisch aus Magen- und Darminhalt kontaminiert worden waren. Weiterhin wurde in den Studien I und II die nachfolgende mikrobielle Belastung der Körperhöhlenoberflächen nach einer dreitägigen Kühllagerung untersucht, um mögliche Auswirkungen der Zeit während der Fleischreifung auf die Anfangskeimzahl zu bestimmen. Auch hier konnten weder am Erlegungstag noch nach der Kühllagerung Unterschiede in den mikrobiellen Gehalten von gespülten und ungespülten Karkassen beobachtet werden.

Aufgrund der hohen Bedeutung der Anfangskeimzahl für die Qualität und Haltbarkeit des Enderzeugnisses, wurde eine Ursache-Wirkungs-Analyse in der Studie III durchgeführt (Kapitel 3.4). Bei diesem Ansatz wurden die Daten von Rehkarkassen mit ausgefüllten Probenbegleitschein aus den Studien I und II verwendet, um die Auswirkungen von Umweltfaktoren, tierbezogenen Faktoren sowie die Auswirkungen von Jagd- und Handhabungspraktiken auf die Anfangskeimzahl zu ermitteln. Beispielsweise wurde hierbei bestimmt, dass die sichtbare Sauberkeit der Schlachtkörper nicht immer mit geringen bakteriellen Belastungen einhergehen müssen. Aber durch die Verwendung von Handschuhen beim Ausweiden konnten die Gehalte an *Lactobazillen* spp.,

Enterobacteriaceae und *E. coli* im Vergleich zum Ausweiden ohne die Verwendung von Handschuhen reduziert werden.

Zusammenfassend leisten die Untersuchungen der drei Studien einen wertvollen Erkenntnisbeitrag zu den ausschlaggebenden Aspekten, die die Anfangskeimzahl und somit die mikrobielle Qualität von jagdlich erlegten Rehkarkassen beeinflussen können.

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List of Publications

Peer-reviewed and first-author publications

Korkmaz, B., Reich, F., Alter, T., Steinhoff-Wagner, J., Maaz, D., Gremse, C., Haase, A., Mader, A., Schafft, H. A., Bandick, N., Nöckler, K. and Lahrssen-Wiederholt, M. (2022). „Microbial load of rinsed and unrinsed body cavities of roe deer (*Capreolus capreolus*) on the killing day and after cold storage: A preliminary investigation." Food Control **141**: 109141. https://doi.org/10.1016/j.foodcont.2022.109141.

Korkmaz, B., Maaz, D., Reich, F., Gremse, C., Haase, A., Mateus-Vargas, R. H., Mader, A., Rottenberger, I., Schafft, H. A., Bandick, N., Nöckler, K., Alter, T., Lahrssen-Wiederholt, M. and Steinhoff-Wagner, J. (2022). „Cause and Effect Analysis between Influencing Factors Related to Environmental Conditions, Hunting and Handling Practices and the Initial Microbial Load of Game Carcasses." Foods **11**: 3726. https://doi.org/10.3390/foods11223726.

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Congress presentations

Birsen Korkmaz (2019): Ein Beitrag zur Wildbrethygiene: Untersuchungen zum Ausspülen der Körperhöhle jagdlich erlegten Wildes mit Trinkwasser –Pilotstudie-. WALD.WILD.verBUND.FORSCHUNG. Bundesinstitut für Risikobewertung.

Birsen Korkmaz (2020): Pilot study: Microbiological investigations on the influence of rinsing body cavity of hunted game with drinking water on game hygiene. PreDoc Symposium. Bundesinstitut für Risikobewertung.

Birsen Korkmaz (2021): Bestimmung, Bewertung und Kontrolle von Gefahren bei der Gewinnung von Primärerzeugnissen auf der Jagd. Forstwissenschaftlichen Tagung (FowiTa). Online.

Oral presentations

Birsen Korkmaz (2020): Mikrobiologische Untersuchungen des Einflusses der Verwendung von Trinkwasser zum Spülen der Körperhöhle jagdlich erlegten Wildes auf die Wildbrethygiene. Fachgespräch zwischen dem Bayerischen Landesamt für Gesundheit und Lebensmittelsicherheit und dem Bundesinstitut für Risikobewertung.

Birsen Korkmaz (2021): Mikrobiologische Untersuchungen des Einflusses der Verwendung von Trinkwasser zum Spülen der Körperhöhle jagdlich erlegten Wildes auf die Wildbrethygiene. BfR-Seminar 10/2021, Das Studienzentrum 8SZ stellt sich vor. Bundesinstitut für Risikobewertung.

Birsen Korkmaz (2022): Wildbretqualität – Spülen oder nicht?. Wissenstransferveranstaltung „Studien bei lebensmittelliefernden Wildtieren". Online.

Congress posters

Korkmaz, B., Bandick, N., Graubaum, D., Gremse, C., Schafft, H. A. und Lahrssen-Wiederholt, M. (2019). „Das HACCP-Konzept bei der Gewinnung von Primärerzeugnissen auf der Jagd-Bestimmung, Bewertung und Kontrolle von Gefahren.“ WALD.WILD.verBUND.FORSCHUNG. Bundesinstitut für Risikobewertung.

Korkmaz, B., Bandick, N., Graubaum, D., Gremse, C., Schafft, H. A. und Lahrssen-Wiederholt, M. (2021). „Das HACCP-Konzept bei der Gewinnung von Primärerzeugnissen auf der Jagd-Bestimmung, Bewertung und Kontrolle von Gefahren- Ein Beispiel.“ 20. Fachtagung für Fleisch- und Geflügelfleischhygiene.

Congress abstract

Korkmaz, B., Bandick, N., Graubaum, D., Gremse, C., Schafft, H. A. und Lahrssen-Wiederholt, M. (2021). „Das HACCP-Konzept bei der Gewinnung von Primärerzeugnissen auf der Jagd-Bestimmung, Bewertung und Kontrolle von Gefahren- Ein Beispiel.“ 20. Fachtagung für Fleisch- und Geflügelfleischhygiene.

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Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe.
Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

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