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Research Note

The Impact of an Extended Bleed-to-evisceration Interval on the Microbiological Quality of On-farm Slaughtered Cattle Carcasses

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ABSTRACT

The uncertainties surrounding the microbiological risks of an extended exsanguination-to-evisceration interval have limited the implementation of on-farm slaughter in Europe. On-farm slaughter is increasingly advocated by farmers, consumers, and policymakers as a humane alternative to traditional slaughterhouse operations. However, concerns about hygiene and food safety, particularly bacterial contamination, have led to stringent time limits imposed by Member States on the interval between bleeding and evisceration. Microbiological standards for bovine carcasses in the European Union are governed by Commission Regulation (EC) No 2073/2005, which sets process hygiene criteria for aerobic colony count and *Enterobacteriaceae*. To investigate whether extending the bleed-to-evisceration interval compromises meat safety, five Holstein dairy cattle were slaughtered on-farm, with samples collected from the internal paralumbar area in contact with the intestines for up to 4 h postmortem. The samples were analyzed for *Enterobacteriaceae*, aerobic colony count, and *Escherichia coli*. None of the samples exceeded the established thresholds of 1.5 and 3.5 log CFU/cm^2 for aerobic colony count and *Enterobacteriaceae*, respectively, as defined by Belgian health authorities for the nondestructive sampling method. These preliminary findings suggest that on-farm slaughter with evisceration occurring up to 4 h post-mortem does not pose increased microbiological risks to human health. However, further research is necessary, particularly under warmer environmental conditions and with a larger sample size, to confirm these results and to explore additional factors that may influence bacterial translocation and digestive tract wall integrity.

There is a growing demand from the European Parliament, farmers, and consumers for on-farm slaughter to mitigate various sources of stress related to the handling and transportation of production animals (Struna, 2024). The 2021 and 2024 amendments to Annex III of Regulation (EC) no. 853/2004 allow the slaughter of a limited number of bovine, porcine, caprine, and equine animals on-site at the holding of provenance (European Commission, 2021, 2024). These texts mandate the use of an approved mobile slaughter unit, which transports stunned and exsanguinated animals to a fixed slaughterhouse for the remaining dressing operations. However, no indications are given regarding any specific timeframes to be adhered to, except that evisceration must take place “without undue delay”. As a precautionary measure, European Member States often impose more stringent time limits, which are not always justified. In the context of on-farm slaughter, a permitted time frame of less than one hour is difficult to adhere if one wishes for evisceration to be performed at the slaughterhouse under optimal conditions (Maldague et al., 2022). Extending the interval between bleeding and evisceration of animals raises several concerns, such as intestinal gas production complicating hygienic evisceration, changes in meat organoleptic characteristics, and bacterial growth in the intestines that can spread to other organs (Nagel-Alne et al., 2022). Despite these concerns, there is limited research on the microbiological consequences of prolonged delays between slaughter and evisceration. Most studies focused on wild game, where evisceration times are highly variable. For example, Soriano et al. (2016) found that eviscerating deer 4 h postmortem, compared to 30 min, did not significantly affect the aerobic mesophilic bacterial load in *longissimus dorsi* muscle samples. However, slaughter techniques and

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carcass storage conditions for wild game differ significantly from those in regulated slaughterhouses.

In the 1970s, a group of researchers worked on the subject by analyzing the carcasses of domestic sheep (Gill et al., 1976, 1978). In their most notable study, no bacterial growth (*E. coli*, *C. perfringens*, *B. cereus*, and *P. fluorescens*) was observed on the hind leg and back muscles of 30 uneviscerated sheep carcasses stored for 24 h at 20 °C. According to the authors, only a breach in the digestive system would allow the release of enterobacteria into the abdominal cavity. They add that the processes responsible for its degradation (enzymatic proteolysis, bloating) would not lead to rupture before two days postmortem in healthy animals. In the context of on-farm slaughter, the time between bleeding and evisceration should never reach such intervals. In this framework, our study aims to provide new, missing, or outdated information on the microbiological consequences of extending the time between bleeding and evisceration. Specifically, it focuses on monitoring potential bacterial dissemination from the intestines to a sensitive area of the carcass due to its proximity to the digestive tract, by testing the theory of Gill et al. (1978) within a time frame of 4 h postmortem.

Materials & methods

Slaughters. This study was conducted between April and May 2023 in eastern Belgium. Five cull Holstein dairy cattle were slaughtered at the educational and experimental farm of the University of Liège. The health status of the cows was previously assessed by a veterinarian. They were slaughtered according to best practices (Nielsen et al., 2020), stunned with a captive bolt pistol and then hung using a front loader after verifying signs of unconsciousness (Terlouw et al., 2016). Exsanguination was performed within a minute by a trained veterinarian, using a single knife to sever the skin in the neck region, both carotid arteries, and jugular veins simultaneously.

Sampling for microbiological analysis. After positioning the carcass on its left flank on a table located in a dedicated surgical room at the farm, the area forming the hollow of the right flank was cleaned and disinfected according to standard surgical protocols (Trent et al., 2017). After placing a sterile operative field, a laparotomy of the paralumbar fossa was performed through a skin incision, approximately 15 cm below the sacrum, until the visceral peritoneum of the large intestine was exposed. The incision, whose length was equivalent to the base of the triangle formed by the flank cavity, extended between the hip angle and the last rib.

Nondestructive sampling technique, the “swabbing method”, was used as detailed in various publications and regularly applied during official inspections in the meat sector in Europe (Ghafir & Daube, 2008; Korsak et al., 2017; Martínez et al., 2010). The deep abdominal muscles in contact with the intestines were swabbed on the accessible internal surface, using a moistened swab followed by a dry swab (Fig. 1). The moistened swab was an abrasive sponge (3M Cellulose Sponge Stick 3.8 × 7.6 cm), presoaked with 10 ml of Letheen broth diluent. The dry swab was a sterile cotton pad. The first swabbing was performed immediately after opening the right flank, and subsequent swabbings were conducted on the same area every 45 min thereafter, up to 3 h and 45 min after the first sampling. Additionally, after these six samplings, two out of the five slaughtered cows were turned onto their right flank. Their left flank abdominal muscles were also swabbed, but only once, using the same procedures. After each sampling, the sponges and cotton swabs were sealed in their original bags and placed in a refrigerated container.

In parallel with the samples collected from the carcass, the surrounding air was sampled using a portable air sampler (Spin Air basic, Iul, Spain) with a speed of 4 revolutions per min. Air volumes of 10 L, 100 L, 500 L, and 1000 L were directly spread on Plate Count Agar (PCA). Incubation and enumeration followed the same procedures as described above.

Bacterial enumeration. The samples were transported to the laboratory immediately after the last swabbing. Ten milliliters of 0.09% physiological saline solution were added to each bag, before individually mixing them with a stomacher for 2 min. The resulting suspensions were spread on Plate Count Agar (PCA), Violet Red Bile Glucose (VRBG), and Tryptone-bile-glucoronate (TBX) culture media and incubated aerobically for 72 h at 30 °C (PCA) or for 24 h at 37 °C (VRBG, TBX). Manual counting was performed following ISO 4833-2:2013/Cor1:2014 for aerobic colony count (ACC), ISO 21528-2:2004 for *Enterobacteriaceae* enumeration, and ISO 7251:2005 for *E. coli* enumeration.

Calculation and analysis. The enumeration results were analyzed in two forms. The first, categorized as “individual data points”, corresponds to the results obtained at each sampling time. The second, “compiled data points”, considers the results from the preceding time point and corresponds to the contamination at time T(x) added to the result at time T(x − 1). This second category corrects potential bias and avoids underestimation of colony numbers. Indeed, it was assumed that, as a result of the sequential swabbing of the same carcass area, the abrasive action of the sponge would detach certain surface-associated bacteria during each successive swabbing.

Colony counts were converted to log CFU (colony forming units)/cm^2 to meet the assumption of normality. For samples below the detection limit (no detectable colonies), an arbitrary value equal to half of the detection limit value was used (Korsak et al., 2017).

The obtained compiled data points were then compared to the corresponding microbiological criteria. Regulation (EC) no. 2073/2005 specifies European microbiological criteria for carcasses at slaughterhouses

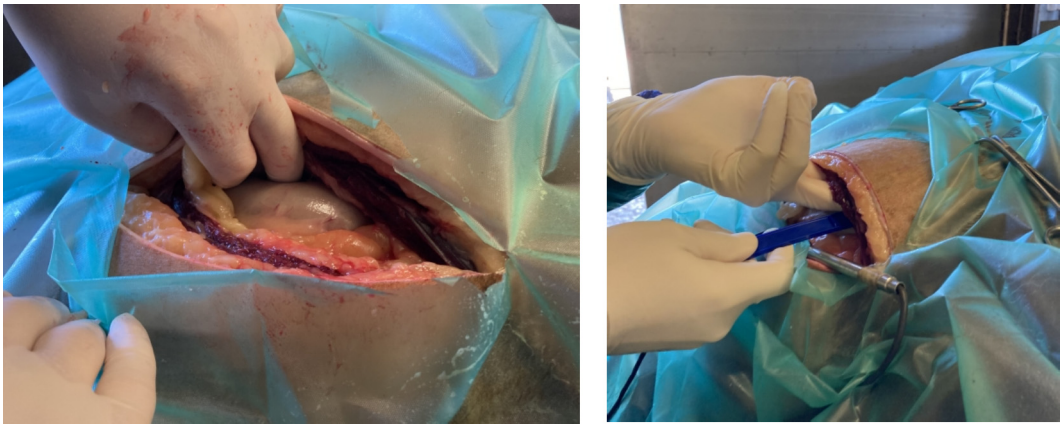

Figure 1. Swabbing method of the internal surface of abdominal muscles, after opening the abdominal cavity and exposing the intestines.

(European Commission, 2020). Satisfactory values are situated below 3.5 and 1.5 log CFU/cm^2, but results remain acceptable up to 5 and 2.5 log CFU/cm^2 for ACC and *Enterobacteriaceae*, respectively. In Belgium, health authorities have specified threshold values considering the sampling technique specifically. The nondestructive swabbing method recovers fewer colonies compared to the destructive method (ISO 17604: 2015), prompting Belgian health authorities to implement a compensatory reduction in acceptable thresholds for *Enterobacteriaceae* and ACC compared to European regulations. Therefore, the compiled results of our study were compared to Belgian criteria, where all thresholds are reduced by half a log compared to the established European criteria for the destructive method (AFSCA, 2020). Since there are no specific microbiological threshold directives for *E. coli*, the same values as for *Enterobacteriaceae* were arbitrarily used for comparison.

Results and discussion

Cattle slaughtered and sampled area. Five cull Holstein dairy cows were slaughtered between April and May 2023. Their ages ranged from 2 to 10 years. The slaughters were carried out in late morning or early afternoon (between 11:00 a.m. and 1:30 p.m.), and the average time between exsanguination and the first sampling was 24.2 min (minimum: 19 min, maximum: 31 min). The portion comprising the right flank of the internal oblique muscle of three carcasses was sampled over an area of 240 cm^2, and of two carcasses over an area of 400 cm^2 (Table 1).

Microbiological analysis. The average compiled results by swabbing time, compared to the Belgian satisfactory and acceptable limits, are presented in Fig. 2. None of the carcasses exceeded the prescribed thresholds at any time point. Colonies of *E. coli* and *Enterobacteriaceae* were detected in 3 out of the 30 samples tested (see Supplementary material). For the remaining samples, where no positive cultures were

Table 1
Profile of slaughtered cattle

Sample	Age (years)	BCS[a] (1–5)	Sampled surface (cm^2)	DT[b] (CFU/cm^2)
Cattle 1	10	3.75	400	0.03
Cattle 2	4.5	3.75	400	0.03
Cattle 3	3	3.5	240	0.04
Cattle 4	2	1.75	240[c]	0.04
Cattle 5	4.5	2.25	240[c]	0.04

[a] BCS = Body Condition Score. A BCS of 1 corresponds to a very thin cow, while 5 corresponds to a very fat cow (Edmonson et al., 1989).
[b] DT = Detection Threshold, corresponding to detection limit value arbitrarily defined based on the swabbed surface area, when no colonies were detected in the sample.
[c] This value applies to both the right and left flanks.

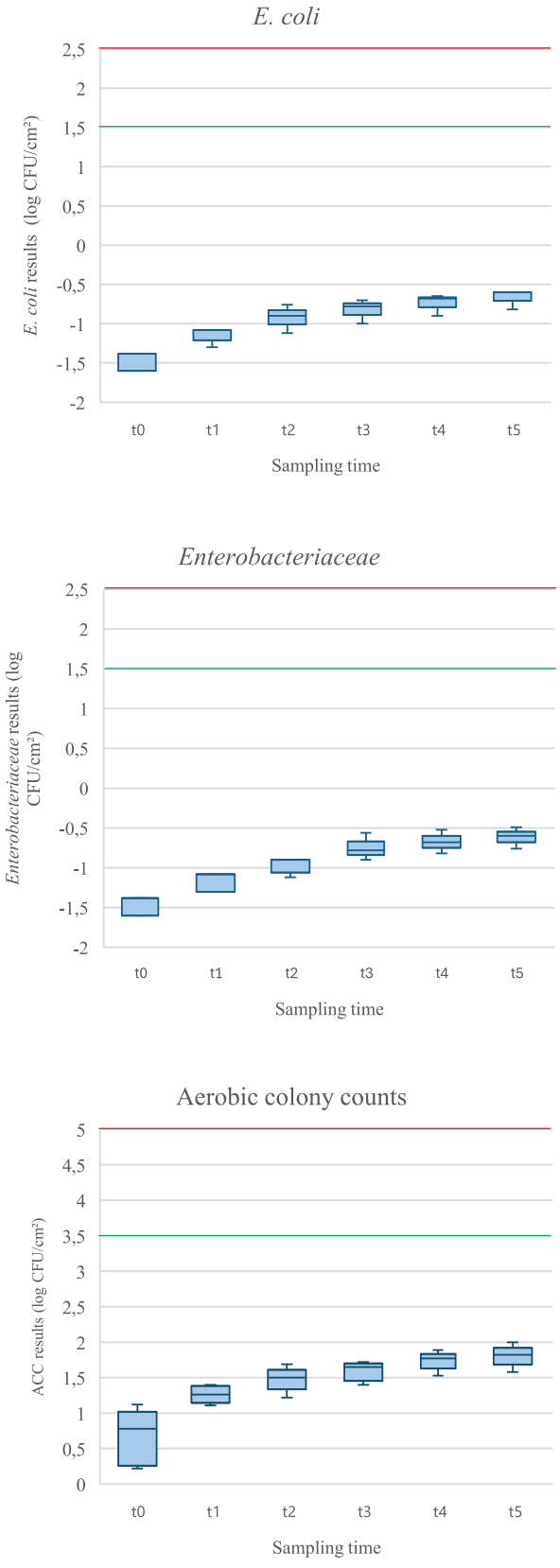

Figure 2. Boxplots and medians of the results obtained for each slaughtered cow, for each bacterial count, at time T0 (corresponding to the first swabbing performed on the right flank immediately after opening the abdominal cavity), T1 (T0 + 45 min), T2 (T0 + 1h 30), T3 (T0 + 2h 15), T4 (T0 + 3 h), T5 (T0 + 3h 45), and T6 (T0 + 4 h). The figure illustrates the distribution of bacterial counts compared to the satisfactory (green) and acceptable (red) limits established by the Belgian health authorities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

found, the arbitrary detection limit value was applied to derive the final results. The aerobic colony counts remained well below the permitted standards and can be easily attributed to the nonsterile ambient environment. This assumption is supported by the portable air sampler data, which indicated a surrounding bacterial load of at least 4.48 log CFU per cubic meter.

These results are consistent with a similar experiment conducted using the destructive method in South Africa, which found that the transfer of *Enterobacteriaceae* to the carcass was absent to negligible in 16 black wildebeest carcasses exposed to evisceration delays of up to 5 h (Vorster van Heerden, 2016).

Swabbing is a standard method used in European slaughterhouses for bacterial monitoring programs. While it provides lower bacterial recovery than destructive methods (Ghafir and Daube, 2008), it was more suitable here, given the healthy condition of the cattle prior to slaughter. This method is particularly valuable for detecting low-prevalence pathogens, such as *E. coli* or *Enterobacteriaceae*, where the small sample size of excision methods would have been a limitation (Martínez et al., 2010).

In this study, the same muscle surface was swabbed five consecutive times. This approach was chosen to minimize openings on the carcasses, which could serve as entry points for environmental bacteria.

This method likely introduces certain biases. However, the aim of the study was not to achieve an accurate bacterial count but rather to identify a potential "breakpoint" that might indicate contamination of intestinal origin, potentially compromising food safety from a specific time point onwards.

Of the 30 samples analyzed, 27 were entirely free of *E. coli* and *Enterobacteriaceae*. In the few cases where bacteria were detected, they did not persist across subsequent swabs. This suggests, on one hand, that the sponge may have, as expected, detached a bacterial layer with each swabbing. On the other hand, if the bacteria had originated from the digestive tract rather than the environment, their presence would likely have been consistent across all successive swabs. The sporadic presence of *Enterobacteriaceae* and *E. coli* colonies is more plausibly attributable to exogenous contamination or process errors than to any sudden bacterial diffusion from the intestinal tract.

To further address this bias of successive swabbings, two carcasses were flipped to the opposite side and swabbed only once on the left flank at the most critical time point, which was the latest stage of sampling. No *E. coli* or *Enterobacteriaceae* colonies were detected in these samples.

These observations support the idea that within the first 4 h postmortem, there does not appear to have been any rupture, even microscopic, in the intestinal wall as a result of postmortem autolytic processes (McInnes, 2015). It remains possible that intestinal bacteria could have spread indirectly through the blood or lymphatic systems (Mesli et al., 2017). However, the surface analyses conducted in this study did not detect such a phenomenon, which is more typically investigated in organs such as the lungs, heart, lymph nodes and liver (Tuomisto et al., 2013). Recent studies on the subject have not revealed any evidence of postmortem bacterial translocation within the time frame investigated here, extending up to two weeks after death (Gates et al., 2021).

This study was conducted over a limited period with the aim of providing rapid and preliminary evidence that meat from the examined cattle is safe for consumption. This was substantiated by the fact that the studied muscle surfaces displayed bacterial loads below the regulated thresholds for food safety. Quantitative PCR assays on total bacterial flora were performed and confirmed these findings (nonpublished results, Maldague et al., 2023). However, more carcasses should be tested and future studies should also include qualitative microbiological assessments and microscopic analyses of the intestinal mucosa integrity, to elucidate the underlying mechanisms.

Another limitation arising from this limited period is the inability to continue it in warmer weather conditions, as the outside temperatures during the samplings at Liège never exceeded 15 °C. Indeed, it is well established that elevated ambient temperatures lead to an acceleration in the onset and extent of post-mortem changes (Tsokos, 2005). These concerns, however, have already been partially addressed in this context by the study of Junqueira et al. (2024), which focuses on the impact of delayed evisceration on the microbial load of cattle carcasses under tropical conditions (average temperature of 26.8 °C). Their results demonstrated that none of the samples exceeded the European safety thresholds for *E. coli* or aerobic mesophilic bacteria counts, although a significant increase in mesophilic bacteria was observed after 180 min.

The present study was limited to the slaughter of dairy cows. Intrinsic factors such as genetics, species, breed, age, and gender influence microbiota composition (Fan et al., 2021). Extrinsic factors, mainly nutrition, which varies in terms of concentrate/forage ratio in dairy and beef cattle, also significantly influence bacterial populations in the digestive tract (Lin et al., 2023). It would be relevant to consider the potential role of the bacterial composition of the microbiota on the degradation of the intestinal wall and postmortem bacterial translocation.

Finally, this study suggests that meat from cattle not eviscerated within 4 h postmortem is microbiologically safe. However, its organoleptic qualities remain uncertain and should be investigated alongside microbiological characteristics.

Conclusion

The study aimed to investigate the potential microbial contamination of the abdominal muscle surface in contact with the digestive tract over time, using the swabbing method.

One perspective was to offer factual insights and scientific evidence to European Member States considering on-farm slaughter implementation, by providing guidance that will assist them in establishing an appropriate time gap between exsanguination and evisceration, ensuring both field feasibility and food safety standards.

From a microbiological perspective, the various results obtained suggest that consuming meat from cattle slaughtered on the farm should not pose an increased risk to human health, as the microbiological criteria remain well within the limits imposed in Europe throughout this period. However, it would still be necessary to replicate the experiment with more diverse animal profiles and in warmer temperatures.

Ethics approval

The protocol of this study was approved by The Animal Ethics Committee of the University of Liège (case number: 23-2558, delivered on April 4, 2023).

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CRediT authorship contribution statement

A. Maldague: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **G. Daube:** Writing – review & edit-

ing, Validation, Supervision, Resources, Methodology. **L. Martinelle:** Writing – review & editing, Resources, Methodology, Investigation. **C. Lagamme:** Methodology. **S. Crèvecoeur:** Resources. **M. Vandenheede:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **N. Korsak:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.jfp.2024.100392.

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