

# Influence of Evisceration Delay on Microbial Count in Bovine Carcasses under Tropical Conditions

## *Influência do Atraso na Evisceração na Contagem Microbiana em Carcaças Bovinas em Condições Tropicais*

Alexandre Lopes Junqueira<sup>a</sup>; Marina de Nadai Bonin<sup>a</sup>; Marjorie Toledo Duarte<sup>b</sup>; Luciana Miyagusku<sup>c</sup>; Aline Gomes da Silva<sup>a</sup>; Luis Carlos Vinhas Ítavo<sup>a</sup>; Marília Williani Filgueira Pereira<sup>a</sup>; Welligton Conceição da Silva<sup>c,d,e</sup>; Raimundo Nonato Colares Camargo Júnior<sup>\*cf</sup>

<sup>a</sup>Universidade Federal do Mato Grosso do Sul, Meat Technology and Processing Laboratory of the College of Veterinary Medicine and Animal Science. MS, Brasil.

<sup>b</sup>Universidade Federal do Mato Grosso do Sul. MS, Brasil.

<sup>c</sup>Universidade Federal do Mato Grosso do Sul, Food Technology and Public Health Unit of the College of Pharmaceutical Sciences, Food and Nutrition. MS, Brasil.

<sup>d</sup>Universidade Federal do Pará, Postgraduate Program in Animal Science, PA, Brasil.

<sup>e</sup>Universidade Federal Rural da Amazônia.

<sup>f</sup>Centro Universitario da Amazônia. AM, Brasil.

<sup>†</sup>Instituto Federal de Educação, Ciência e Tecnologia do Pará. PA, Brasil.

\*E-mail: [camargojunior@gmail.com](mailto:camargojunior@gmail.com)

### Abstract

The study of the influence of delay in evisceration on microbial count in bovine carcasses under tropical conditions is of utmost importance for the food industry and public health. Evisceration is a critical stage in meat processing, and any delay in this process can lead to favorable conditions for the growth and multiplication of pathogenic microorganisms. The precautionary principle is employed to ensure a high standard of protection for consumer health in the food sector, especially when a strong scientific basis for legislation is lacking. The objective was to evaluate the influence of delayed evisceration on the microbial count of bovine carcasses. 90 carcasses were sampled, eviscerated at 30 (control), 60, 90, 120, 150 and 180 minutes after bleeding, at an average temperature of 26.8 °C (or 80.24 °F). External and internal surface swabs were collected to count mesophiles and generic *Escherichia coli*. No sample exceeded safe limits for fresh consumption. The internal and external surface mesophilic count was higher compared to the control only with 180 min. The highest frequency of *E. coli* also occurred at 180 min. and was higher on the inner surface than on the outer surface, indicating the importance of sampling this surface in cases of delayed evisceration. The dilatation of the rumen by gases markedly made evisceration difficult after 150 min. Microbiological analyzes are important to evaluate each case, but it is not recommended to exceed 180 min. for removal of the viscera, under the mentioned temperature.

**Keywords:** Rumen Dilatation. *E. coli*. Mesophylls. Translocation.

### Resumo

*O estudo da influência do atraso na evisceração na contagem microbiana em carcaças bovinas em condições tropicais é de extrema importância para a indústria alimentícia e a saúde pública. A evisceração é uma etapa crítica no processamento da carne, e qualquer atraso nesse processo pode criar condições favoráveis para o crescimento e multiplicação de microrganismos patogênicos. O princípio da precaução é empregado para garantir um alto padrão de proteção para a saúde dos consumidores no setor alimentício, especialmente quando a legislação carece de uma sólida base científica. O objetivo foi avaliar a influência da evisceração retardada na contagem microbiana de carcaças bovinas. Foram amostradas 90 carcaças, evisceradas aos 30 (controle), 60, 90, 120, 150 e 180 minutos após a sangria, em temperatura média de 26,8 °C (ou 80,24 °F). Swabs da superfície externa e interna foram coletados para contagem de mesófilos e *Escherichia coli* genérica. Nenhuma amostra excedeu os limites seguros para consumo fresco. A contagem de mesófilos de superfície interna e externa foi maior em comparação com o controle apenas com 180 min. A maior frequência de *E. coli* também ocorreu aos 180 min. e foi maior na superfície interna do que na externa, indicando a importância da amostragem dessa superfície em casos de evisceração tardia. A dilatação do rúmen por gases dificultou acentuadamente a evisceração após 150 min. As análises microbiológicas são importantes para avaliar cada caso, mas não é recomendado ultrapassar 180 min. para retirada das vísceras, sob a temperatura mencionada.*

**Palavras-chave:** Dilatação Ruminal. *E. coli*. Mesófilos. Translocação.

## 1 Introduction

Beef consumers are increasingly demanding (ABREU et al., 2021; SILVA et al., 2020, 2021). In this way, the international food trade has been growing, providing important social and psychological benefits. In the routine of slaughterhouses/meat processing industries, several factors can lead to the stoppage of the slaughter, such as mechanical errors (CANADA, 2019; SILVA et al., 2023), lack of electrical energy and others, increasing the time of removal of the viscera and of exposing the skinned parts of the carcass to the environment without refrigeration.

Brazilian legislation and other countries list evisceration as an important step among operational sanitary procedures to prevent the contents of the gastrointestinal tract (GIT) from contaminating the carcass (BRAZIL, 2017; DASMACHENO et al., 2021; USA, 2018). In addition to the search to avoid rupture and/or leakage of its contents, it is recommended that the viscera be removed as quickly as possible from the interior of the carcass (European Union, 2004), as there is a risk that at some point after death, dissemination of bacteria may occur from the GIT (PAULSEN et al., 2011).

The scientific literature is scarce on the subject, with more

frequent studies being found with game animals, since, due to the conditions in which they are slaughtered, the delay in the removal of the viscera is common (AVAGNINA et al., 2012; SORIANO et al., 2016; VAN DER MERWE et al., 2014). However, hunting is a very different situation from that of an industrial slaughterhouses or meat processing industries, the condition that the present study proposes to investigate.

In the absence of a robust scientific basis for the legislation of the food sector, the precautionary principle is used to guarantee a high level of protection to the health of consumers. Adoption of this principle explains the more stringent tolerable time recommendations for evisceration. However, such measures should be re-examined within a reasonable time frame to clarify the scientific uncertainty and carry out a more thorough risk analysis, so as not to impose unnecessary restrictions (EUROPEAN UNION, 2002; TODD, 2004). Brazilian legislation lacks objective regulation for this situation since the first version of the Regulation for Sanitary and Industrial Inspection of Products of Animal Origin of 1952 and the same is observed in other countries (BRAZIL, 1952, 2017; EUROPEAN UNION, 2004).

This research aims to answer a part of the risk analysis in evisceration delay, the microbiological risk evaluation, with the objective of understand the influence of different evisceration delays periods, under the tropical climate, on the microbial count of the internal and external surfaces of bovine carcasses.

## 2 Material e Methods

### 2.1 Animals and slaughter conditions

Nellore or Nellore-like animals were used. No other criteria were used in the selection of carcasses participating in this study. Slaughter and sampling were carried out under the ordinary conditions of a slaughterhouses/meat processing industries with a daily slaughter capacity of 700 animals, from April to August 2019, in the state of Mato Grosso do Sul, Brazil, under federal inspection regime, according to legislation for animal welfare, ante- and post-mortem inspections, industrial and operational hygiene, sanitary procedures in operations and others (Brazil, 2000; Brazil, 2017).

### 2.2 Experimental delimitation

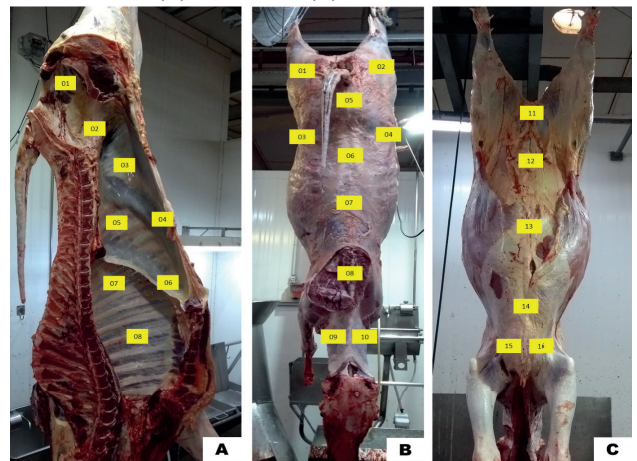
Separate samples were collected from the internal and external surface of 90 carcasses, divided into six groups according to the time between the removal of the viscera and bleeding, as follows: 30 ( $\pm 2$ ), 60 ( $\pm 3$ ), 90 ( $\pm 4$ ), 120 ( $\pm 4$ ), 150 ( $\pm 7$ ) and 180 ( $\pm 10$ ) min. The first group (30 min) was the control group, since it is the average time in which evisceration normally takes place at the slaughterhouse. Each of these groups was composed of 15 carcasses, collected on three different days, five each day, starting with the control group until totaling 15 carcasses in each group, in the order of evisceration delay times. This study system was planned

to replicate the practical conditions in the slaughterhouses and so that the samples were sent to the laboratory in the shortest possible time after collection (maximum of 4 hours), minimizing injuries to microorganisms and offering more fidelity in the results.

### 2.3 Sampling and collection points

For sampling, a surface smear was performed. ISO 17604:2015 advocates sampling a total area of 400 cm<sup>2</sup> on external surfaces of bovine carcasses. This parameter was also followed for the internal surface, in which 16 collection points with 25 cm<sup>2</sup> each were defined, eight in each half carcass, contemplating sites contiguous to different segments of the (GIT) in the abdominal cavity and organs of the thoracic cavity, which would be possible sources of contamination by bacterial translocation. In order to maintain the number of points on the internal surface and contemplate different areas of the external surface exposed to the environment, the same number of points was defined for sampling this surface, including those recommended by the aforementioned ISO (Figure 1).

**Figure 1** - Smear points of the internal surfaces (A), external surface, dorsal (B) and ventral (C) views



Internal Surface: 1A. Muscle covering the obturator foramen in the pelvic floor. Adjacent structures: rectum, vagina, ureters; 2A. Middle portion of body of ilium. Adjacent structures: rectum, vagina, ureters and cecum; 3A. Internal portion of the carcass at the level of the flanks, formed by the region close to the insertion of the internal abdominal oblique muscle. Adjacent structures: dorsal sac of the rumen on the left side and duodenum and colon on the right side; 4A. Middle portion of the 12th rib. Adjacent structures: dorsal sac of the rumen on the left side and liver/gallbladder on the right side; 5A. Umbilical region inside the linea alba. Adjacent structures: ventral rumen sac and small intestine; 6A. Cranioventral portion of the diaphragm. Adjacent structures: reticulum on the left and abomasum on the right; 7A. Internal thoracic surface at the proximal height of the ninth rib. Adjacent structures: right and left lungs; 8A. Internal thoracic surface at the midpoint of the fifth rib. Adjacent structures: lungs and esophagus. Dorsal View: 1B. Right side gluteal biceps muscle (rump region), 2B. Left side gluteal biceps muscle (rump region), 3B. Right flank, 4B. Left flank, 5B. Middle portion of the sacrum in the line of the median crest, 6B. Middle portion of the lumbar spine in the medial line (caudal portion of the sirloin steak), 7B. Middle region of the thoracic spine in the medial line (middle portion of the sirloin steak), 8B. Initial region of the thoracic spine after remove the hump and exposure the muscles below (cranial portion of the sirloin steak), 9B.

Right side neck, 10B. Left side neck. Ventral View: 11C. Perineal area, 12C. Scrotal area in males or udder area in females, 13C. Linea alba at the level of the navel, 14C. Linea alba at the height of the xiphoid cartilage, 15C. Left side of the sternum (chest), 16C. Right side of the sternum (chest).

Source: the authors.

The carcasses of the groups that suffered an intentional delay in evisceration were diverted to the Department of Final Inspection (DFI), located inside and under the same conditions as the slaughter room, remaining in this location for the time stipulated for each group. After the delay times, they returned the main line to the external surface sampling location, then eviscerated with the paralyzed line and then sawed off to sample the internal points.

Saws, knives and other utensils were sanitized with water at least 82.2 °C between collections, following standard operating sanitary procedures. The ambient temperature was recorded on all collection days with a properly calibrated thermometer. All collections were carried out in the slaughter room of the slaughterhouse, at the end of the day, maintaining the conditions found in industrial practice.

As a safety measure, the viscera of the carcasses that suffered the purposeful delay in evisceration were condemned and the carcasses sequestered until the result of the microbiological analyzes that, verifying accordance, supported their release.

#### 2.4 Sample packaging and analytical technique

Samples were made with the swabs of the 16 internal apart from the 16 external points, stored under refrigeration in sterile packages, properly identified and placed in an isothermal box with ice, not allowing direct contact with the packages, avoiding injury to the micro-organisms.

The samples were transported to the food microbiology laboratory of the College of Pharmaceutical Sciences, Food and Nutrition of the Federal University of Mato Grosso do Sul and immediately processed, totaling an average period between the end of the collections and the beginning of the analyzes of 3.5 hours.

Microbiological analyzes were performed using the in-depth inoculation technique (pour plate), using Petrifilm™ culture medium, and the AOAC 990.12 methodology

(Petrifilm™ AC 6406 - 3M Company, St. Paul, MN, USA) and for generic *Escherichia coli* (*E. coli*) counting the methodology AOAC 998.08 (Petrifilm™ EC 6414 - 3M Company, St. Paul, MN, USA).

The analyzes were carried out with the previous preparation of serial dilutions, where 1 milliliter aliquots from the diluted sample were plated on Petrifilm™ for aerobic mesophilic bacteria count and *E. coli* count. Incubation was carried out at a temperature of 35 ± 1 °C, for a period, respectively, of 48 ± 3h and 24h.

At the end of the incubation period, the counts were carried out in the respective culture medium, and the aerobic mesophiles were quantified by observing the red colored colonies and *E. coli* observing the blue colonies associated with the gas. Results were expressed in logarithm to base 10 of colony forming units per square centimeter (LogCFU/cm<sup>2</sup>).

#### 2.5 Statistical analysis

Data from aerobic mesophilic counts were subjected to Analysis of Variance in the Proc Glimmix of the SAS University Edition. Since the samples were collected on different days, the day of sample collection was entered as a random effect in the statistical model. The evisceration delay time averages were compared to control time by Dunnett's test at a 5% significance level.

The model is:  $y_{ijk} = \mu + t_i + dj(i) + \mathcal{E}(ij)k$

Where,  $y_{ijk}$  = response variable measured in experimental unit  $k$ , at time  $i$ , on day  $j$ ;  $\mu$  = general constant;  $t_i$  = effect of time delay on evisceration  $i$  (fixed effect);  $dj(i)$  = nested effect of day  $j$  when the sample from treatment  $i$  was collected (random effect);  $\mathcal{E}(ij)k$  = unobservable random error.

For *E. coli*, the data were presented in a descriptive way since, due to the low frequency of their findings, the analyzes of trend and dispersion measures were not appropriate for their scrutiny.

### 3 Results and Discussion

#### 3.1 Evisceration times and microbial counts

The result of aerobic mesophilic counts can be seen in Table 1.

**Table 1** - Means of aerobic mesophilic counts on the internal and external surfaces of bovine carcasses, according to the time of evisceration

Microorganism Count	Average Time Delay Evisceration					
	30	60	90	120	150	180
	Internal surface					
LogCFU/cm <sup>2</sup> ± DP	0.76±0.2	0.94±0.4	1.14±0.5	0.85±0.4	0.84±0.3	1.31±0.6 <sup>a</sup>
P value	--	0.717	0.137	0.971	0.980	0.022
	External surface					
LogCFU/cm <sup>2</sup> ± DP	1.07±0.3	1.05±0.5	0.89±0.5	0.73±0.4	0.39±0.3	1.77±0.5 <sup>a</sup>
P value	--	1.000	0.856	0.383	0.073	0.022

(a) Indicates significant difference in relation to the control ( $p < 0.05$ ) by Dunnett's test. SD: standard deviation.

Source: resource data.

On the internal surface, *E. coli* was found in four of the totals of 90 samples, as follows: one with 90 min. (1 LogCFU/cm<sup>2</sup>) and three with 180 min. (1.1 and 2 LogCFU/cm<sup>2</sup>). On the external surface of the carcasses, only one of the 90 samples showed a quantifiable result (0 LogCFU/cm<sup>2</sup> or 01 CFU/cm<sup>2</sup>), within 180 min.

None of the carcasses that suffered a delay in evisceration showed any sensory alteration and none of the 90 samples analyzed exceeded the limit of 3.5 LogCFU/cm<sup>2</sup>, as recommended by the sampling plan for total aerobic mesophilic count of European Regulation No. 2073/2005 (EUROPEAN UNION, 2005).

The literature on delayed evisceration with cattle and other butcher animals under industrial conditions is scarce, and studies with animals slaughtered in sport hunting (Game Meat) can be found, since, under the conditions in which they occur, it is common that the viscera are removed later at slaughter than is commonly practiced in commercial abattoirs. Even under different conditions, the comparison of these results with those obtained in sport hunting can help us to elucidate the problem that is the focus of this study.

In Van Heerden's (2016) research with black wildebeest (*Connochaetes gnu*) killed in winter hunting season in South Africa and subjected to delayed evisceration for up to five hours, samples of rectus abdominis muscle were collected along with the of the peritoneum that covers it. There was no significant growth for Enterobacteriaceae and anaerobic bacteria, and room temperature below 18°C was the explanation found by the authors as a possible cause of lower bacterial growth in the carcass.

Soriano et al. (2016) found no influence on the count of aerobic mesophilic bacteria in the *longissimus* muscle, sampled by excision, of red deer with a period of 210 min. after slaughter to remove the viscera, at an ambient temperature of 20°C. In our study, we found a significant increase in mesophiles with a shorter evisceration delay time (180 min.), however, it is necessary to consider when comparing our results with those of Soriano et al. (2016), since the area sampled in the carcasses (400 cm<sup>2</sup> on the internal and external surfaces *versus longissimus* muscle) and the ambient temperature in the collections (26.8 °C versus 20 °C) were higher in our study, which may help us to understand the occurrence of higher bacterial counts in the carcasses evaluated by us when compared to the results of those authors.

Avagnina et al. (2012), in a study with deer and swine slaughtered in sport hunting in the Italian Alps, used a swab to smear the surface of a total area of 25 cm<sup>2</sup>, only from the medial region of the hind limb, finding no significant difference in the total counts of aerobic mesophiles. and Enterobacteriaceae between groups with less than 30, 30 to 60, 61 to 180 and more than 180 min. between shooting and evisceration.

These works with game animals have conditions and methodological implications inherent to the situation in which they occurred, which have differences worthy of note

in relation to the present study: lower ambient temperature, therefore, less favorable to microbial multiplication and sampling of small portions of tissue or smaller areas of the carcasses, therefore, capable of recovering smaller amounts of microorganisms compared to the sampling carried out in this research.

### 3.2 Bacterial Translocation versus Operational Cross Contamination

Paulsen et al. (2011) consider 180 min. as a critical time in the removal of the viscera of wild ruminants subjected to sport hunting, so that bacterial translocation from the GIT to the carcass does not occur. In our study, the same period caused a significant increase in the count of mesophiles which, even within the safety limit established by European Regulation No. 2073/2005, warns us that this may be the time limit for removing bovine viscera without harming food safety.

In pioneering works carried out by Gill and Nottingham (1976, 1978) and Gill and Penney (1979, 1982), the hypothesis of contamination of carcasses with delay in evisceration as a result of translocation of GIT bacteria was investigated, showing that samples of muscles and lymph nodes inguinal samples of sheep carcasses, without evisceration, for 24 hours at 20 °C, remained free of *E. coli.*, *Pseudomonas fluorescens* and *Clostridium perfringens*, leading them to conclude that this delay was not enough to contaminate them with bacteria from the GIT. These authors suggested that immunological mechanisms remain active for some time after the death of the animal and that there would be no reason to consider the invasion of other tissues by intestinal bacteria as a criterion for rejection of carcasses in the case of delayed evisceration.

Studies on this topic in animal carcasses are scarce, however, in human forensic medicine recent results have been published. Although these studies cannot be extrapolated to cattle, due to obvious anatomical and physiological differences, they reinforce time and temperature as fundamental factors for bacterial translocation from the GIT to other tissues.

Morris et al. (2006, 2007) studying human cadavers concluded that translocation of GI tract bacteria does not alter the results of forensic samples when obtained within 24 hours of death or if the cadaver is properly stored at 4 °C prior to necropsy. In this same field, Palmiere et al. (2016) classified bacterial translocation cases according to the time after the individual's death (< 8, 8-24 and more than 24 h), not verifying a positive correlation between the time after death and the number of cultures.

It is observed, therefore, that the studies with bacterial translocation in animals and humans, evaluating samples collected in times greater than the maximum practiced in this study (180 min.) did not verify a correlation between the microbial load of the samples with the time between the death of the individual and the removal of the viscera. In this way, we have subsidies to suppose that the main cause of the findings of an increase in the mesophilic count in carcasses with 180

min. of delay in evisceration is primarily due to contamination by microorganisms from the operating environment, rather than the translocation of microorganisms from the GIT.

The tropical climate prevailing in Brazil provides relatively high temperatures in the slaughterhouses, as seen in this experiment, with an average of 26.8 ( $\pm 1.7$ ) °C, even though the collections took place in autumn and winter. This is a limiting factor when subjecting carcasses to long periods without evisceration, considering the bacterial growth on the external surface exposed to the environment.

The fluctuations observed in the mesophile counts over time (Table 01) are consistent with the observations made by several authors (SOFOS et al., 1999, 2008; VAN DER MERWE et al., 2014), depending on environmental and operational variables, such as cleanliness of the animals when entering the slaughter room, adequate skinning and evisceration operations, water and air quality in the slaughter room.

In the present study, all carcasses evaluated including those with a longer time for evisceration did not exceed the microbiological safety limits recommended by European Regulation No. 2073/2005 (European Union, 2005), even with carcasses exposed to a temperature favorable to bacterial growth ( $26.8 \pm 1.7$  °C), which can be justified by the low initial microbial load in the slaughter room, due to the hygiene and adequate operational sanitary procedures practiced in the establishment where the research was carried out and emphasizing the importance of operational hygiene practices in the entire slaughter process for the quality and safety of the final product.

### 3.3 Operational obstacles to the delay in the evisceration of bovine carcasses

Although initially not included as a focus of study in this work, it was observed that the progressive dilation of the animals' abdomen with the time of evisceration delay, started to cause considerable difficulty to the operator, mainly after 150 min. At 180 min., with the greatest distension, the ruminal chambers projected out of the abdominal cavity as soon as the operator started to open them, forcing him to contain them with one hand while advancing with the knife through the *linea alba* with the other.

More than an unlikely bacterial translocation in amounts capable of causing health risks during this short period of time, the distention of the GIT is an operational impediment that deserves attention. As the slaughter line was paralyzed for the evisceration process during this experimental procedure, the employee was able to carry out the operation with the necessary care so that the rupture did not occur and the sampling could be carried out, even so, the greatest difficulty in the operation was evident. Under normal operating conditions, with the carcasses in motion, the rupture of the GIT would be very likely and extensive contamination promoted by its contents

leaking under pressure. This is a factor that must be carefully considered when delaying the evisceration of ruminants in practical situations.

There is a consensus in the literature that rectal occlusion and evisceration operations, if not performed with adequate technique, predispose to fecal contamination, as they involve access to the abdominal cavity and manipulation of its organs (SOFOS et al., 1999; GHAFIR et al., 1999, 2008; CERNICCHIARO et al., 2019), pointing in the direction that the cause of the detection of *E. coli* occurred practically only at 180 min. is of an operational nature, due to the accentuated difficulty imposed in those operations by the distension of the rumen and annexes. On the other hand, the higher frequency of its finding on the internal surface compared to the external surface (four against one) could indicate the occurrence of translocation of the microorganism from the intestine. This study does not make it possible to distinguish the precise source of this contamination, nor does it have this objective, but this finding indicates that the sampling of the internal surface of the carcass, in addition to the external sampling, carried out in the practice of process analyzes (ISO 17604:2015), is useful for investigation of the microbiological safety of carcasses that suffered a delay in evisceration.

In any case, whether due to the time elapsed at room temperature, the difficulty in operations or the translocation of bacteria, the important negative changes in the count and frequency of the microorganisms studied occurred within 180 min. evisceration delay, establishing this period as critical under the conditions of this experiment.

### 3.4 Legal issues

Item 1 of article 118 of Decree 9013/2017, the regulation for industrial and sanitary inspection of products of Brazilian animal origin, provides for the enactment of a supplementary rule for the judgment of carcasses and viscera in cases of delay in evisceration (BRAZIL, 2017), however, such publication has not yet occurred for the bovine species, as in other countries, possibly due to the scarce scientific framework that the topic has.

European Regulation No. 853/2004 in the section on domestic ungulates only says that evisceration must take place without unnecessary delay. The same regulation allows for the slaughter of poultry at the farm of production and that they be kept for up to 15 days without evisceration, provided they are kept at temperatures not exceeding 4 °C (EUROPEAN UNION, 2004).

In the Eurasian Economic Union (Armenia, Belarus, Kazakhstan, Kyrgyzstan and Russia) and Chile, evisceration must be performed within 45 min. maximum for cattle (EURASIAN ECONOMIC UNION, 2013; CHILE, 2009).

New Zealand law allows for a lapse of 120 min. between stunning and termination of evisceration of cattle. If this time is exceeded, both viscera and carcasses must be evaluated for

sensory indicators such as color and odor, unless the delay is such that it exceeds reasonable time (NEW ZEALAND, 2017). without indicating what time is considered reasonable.

The same period is used by Canadian legislation, which recommends microbiological risk assessment in the carcass and its parts, if exceeded, so that the manufacturer can verify that the levels are acceptable and demonstrate that the quality of the products has been preserved before being released to the trade (CANADA, 2019).

The shortest time was found in Argentine Decree No. 4,238, dated 1968, but still in force, which establishes a maximum of 30 minutes. to carry out evisceration after sacrifice and, if, for reasons of force majeure, this period of time is exceeded, a microbiological analysis must be carried out (ARGENTINA, 1968).

Coinciding with the proposition of the legislation of Canada and Argentina and no sensory alteration having been detected, microbiological analyzes were the tools used in this study to estimate the risks involved and take appropriate measures on the use of carcasses for consumption. This is also the approach advocated by Zweifel et al. (2014), who emphasize the importance of specific assessments for each slaughterhouse, since each one is subjected to its own ambient temperatures, as well as its history of hygiene, maintenance and operational sanitary procedures.

#### 4 Conclusion

In up to 180 min. of delay in evisceration, under an average temperature of 26.8°C, there were no microbiological reasons that would motivate the disqualification of carcasses for consumption, however periods longer than this can cause damage to food safety and increase the difficulty operational in the process of evisceration due to distension of the rumen and annexes.

#### References

ARGENTINA, Ministerio de Agricultura, Ganadería y Pesca. Reglamento de Inspección de Productos y Subproductos y Derivados de Origen Animal (Decreto 4.238, de 26 de agosto de 1968).

AOAC. Association of Official Analytical Chemists. Official Method 990.12 for Aerobic Plate Count in Foods Dry Rehydratable Film (Petrifilm Aerobic Plate Count Plate Methods). Washington: AOAC International, 2016a.

AOAC. Association of Official Analytical Chemists. Official Method 998.08 for Escherichia coli Counts in Poultry, Meats, and Seafood, Dry Rehydratable Film Method (Petrifilm EC Plate Method). Washington: AOAC International, 2016b.

AVAGNINA, A. et al. The microbiological conditions of carcasses from large game animals in Italy. *Meat Sci.*, v.91, n.3, p.266-271, 2012. doi: <https://doi.org/10.1016/j.meatsci.2012.01.025>

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento. Regulamento de inspeção industrial e sanitária de produtos de origem animal (Decreto nº 30.691, de 29 de março de 1952). Diário Oficial da República Federativa do Brasil. 1952.

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento.

Regulamento técnico de métodos de insensibilização para o abate humanitário de animais de açougue (Instrução Normativa nº 03, de 17 de janeiro de 2000). Diário Oficial da República Federativa do Brasil. 2000.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Regulamento de inspeção industrial e sanitária de produtos de origem animal (Decreto nº 9.013, de 29 de março de 2017). Diário Oficial da República Federativa do Brasil. 2017.

CANADA. Archived food guidance, Chapter 17: Ante and Post-mortem Procedures, Dispositions, Monitoring and Controls - Meat Species, Ostriches, Rheas and Emus. Canadian Food and Inspection Agency. 2019. Retrieved from <https://www.inspection.gc.ca/food/archived-food-guidance/meat-and-poultry-products/manual-of-procedures/chapter-17/eng/1367723343665/1367723573062?chap=7#s26c7>.

CERNICCHIARO, N. et al. Quantification of bacteria indicative of fecal and environmental contamination from hides to carcasses. *Food. Pathogens Dis.*, v.16, n.12, p.844-855, 2019. doi: <https://doi.org/10.1089/fpd.2019.2656>

CHILE. Ministerio de Agricultura. (2009). Aprueba Reglamento sobre Estructura y Funcionamiento de Mataderos, Establecimientos Frigoríficos, Cámaras Frigoríficas y Plantas de Desposte y Fija Equipamiento Mínimo de Tales Establecimientos (Decreto 94, de 02 de junio de 2009).

ESTADOS UNIDOS DA AMÉRICA, Food Safety and Inspection Service, United States Department of Agriculture. Meat and Poultry Hazards and Controls Guide, mar, 2018.

EURASIAN ECONOMIC UNION. Resolution nº 68 of the Council of Eurasian Economic Union Regarding the Technical Regulation of the Customs Union on the Safety of Meat and Meat Products. 9 nov. 2013.

GHAFFIR, Y. et al. Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. *J. Food Protection*, v.71, n.1, p.35-45, 2008. doi: <https://doi.org/10.4315/0362-028x-71.1.35>

GILL, C.O.; PENNEY, N.; NOTTINGHAM, P. Effect of delayed evisceration on the microbial quality of meat. *Appl. Environ. Microbiol.*, v.31, n.4, p.465-468, 1976. doi: <https://doi.org/10.1128/aem.31.4.465-468.1976>

GILL, C.O.; PENNEY, N.; NOTTINGHAM, P. Tissue sterility in uneviscerated carcasses. *Appl. Environ. Microbiol.*, v.36, n.2, p.356-359, 1978. doi: <https://doi.org/10.1128/aem.36.2.356-359.1978>

GILL, C.O.; PENNEY, N. Survival of bacteria in carcasses. *Appl. Environ. Microbiol.*, v.37, n.4, p.667-669, 1979. doi: <https://doi.org/10.1128/aem.37.4.667-669.1979>

GILL, C.O.; PENNEY, N. Microbial contamination of the abdominal cavity in commercial carcasses subject to delays before evisceration. *New Zealand Vet. J.*, v.30, n.10, p.163, 1982. doi: <https://doi.org/10.1080/00480169.1982.34928>

ISO, International Organization for Standardization. ISO 17604:2015. Microbiology of the food chain – Carcass sampling for microbiological analysis. 2015.

MORRIS, J.A., HARRISON, L.M.; PARTRIDGE, S.M. Postmortem bacteriology: a re-evaluation - literature review. *J. Clin. Pathol.*, v.59, p.1-9, 2006. doi: <https://doi.org/10.1136/jcp.2005.028183>

MORRIS, J.A.; HARRISON, L.M.; PARTRIDGE, S.M. Practical and theoretical aspects of postmortem bacteriology. *Curr. Diagn. Pathol.*, v.13, n.1, p.65-74, 2007. doi: <https://doi.org/10.1016/j.cdip.2006.07.005>

- NEW ZEALAND, Ministry for Primary Industries. Operational Code: Slaughter and Dressing - Red Meat Code of Practice Chapter 5, p 2017.
- PALMIERE, C. et al. Bacterial translocation and sample contamination in postmortem microbiological analyses. *J Forensic Sci*, v.61, p.367-374, 2016. doi: <https://doi.org/10.1111/1556-4029.12991>
- PAULSEN, P. et al. *Game meat hygiene in focus. Microbiology, epidemiology, risk analysis and quality assurance*. Wageningen: Wageningen Academic Publishers, 2011.
- EUROPEAN UNION. Regulation (EC) N° 178/2002 of the European Parliament and of the Council of 28 January laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Union, 28 jan. 2002.
- EUROPEAN UNION. Regulation (EC) N° 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union, 25 jun. 2004.
- EUROPEAN UNION. Regulation (EC) N° 2073/2005 of the European Parliament and of the Council of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, 15 nov. 2005.
- SANTOS, E.C.C. et al. *Escherichia coli* O26 and O113:H21 on carcasses and beef from a slaughterhouse located in Mato Grosso, Brazil. *Food. Pathog. Dis.*, v.15, n.10, p.653-659, 2018. doi: <https://doi.org/10.1089/fpd.2018.2431>
- SILVA, W.C.D. et al. Perspectives of economic losses due to condemnation of cattle and buffalo carcasses in the northern region of Brazil. *Plos One*, v.18, n.5, 2023. doi: <https://doi.org/10.1371/journal.pone.0285224>
- SOFOS, J.N. et al. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *J. Food Protec.*, v.62, p.140-145, 1999. doi: <https://doi.org/10.4315/0362-028x-62.2.140>
- SOFOS, J.N. Challenges to meat safety in the 21st century. *Meat Scie.*, v.78, n.1/2, p.3-13, 2008. doi: <https://doi.org/10.1016/j.meatsci.2007.07.027>
- SORIANO, A. et al. Influence of evisceration time and carcass ageing conditions on wild venison quality. Preliminary study. *Meat Scie.*, v.114, p.130-136, 2016. doi: <https://doi.org/10.1016/j.meatsci.2015.12.021>
- TODD, E.C.D. Microbiological safety standards and public health goals to reduce foodborne disease. *Meat Scie.*, v.66, n.1, p.33-43, 2004. doi: [https://doi.org/10.1016/S0309-1740\(03\)00023-8](https://doi.org/10.1016/S0309-1740(03)00023-8)
- VAN DER MERWE, M. et al. The hygienic practices involved in three game meat production systems in South Africa based on environmental and other independent variables. *J. Vet. Sci. Technol.*, v.5, n.3, p.176, 2014. doi: <https://doi.org/10.1016/j.meatsci.2013.01.011>
- VAN HEERDEN, C.V. *The effect of delayed evisceration on the microbiological safety of black wildebeest (Connochaetes gnou) meat* (Thesis for Magister Scientiae). Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa. 2016.
- ZWEIFEL, C.; CAPEK, M.; STEPHAN, R. Microbiological contamination of cattle carcasses at different stages of slaughter in two abattoirs. *Meat Scie.*, v.98, n.2, p.198-202, 2014. doi: <https://doi.org/10.1016/j.meatsci.2014.05.029>
- SILVA, W.C. et al. Percepção do consumidor de proteína animal sobre o bem-estar dos animais de produção em Santarém, Pará, Brasil. *CES Med. Vet. Zootec.*, v.15, n.2, p.64-74, 2020. doi: <https://doi.org/10.21615/cesmvz.15.2.5>
- ABREU, M.L. et al. Percepção dos consumidores de proteína animal sobre o bem-estar dos animais de produção no município de Parauapebas, Pará, Brasil. *Vet. Zootec.*, v.28, p.1-12, 2021. doi: <https://doi.org/10.35172/rvz.2021.v28.484>
- SILVA, C.A.D.S. et al. Animal protein consumer's perception on the welfare of production animals in Belém, Pará State, Brazil. *Acta Scie. Anim. Scie.*, v.43, 2021. doi: <https://doi.org/10.4025/actascianimsci.v43i1.53784>
- DAMASCENO NETO, M.S., CONCEIÇÃO DE LIMA NUNES, E.D.S.; CONCEIÇÃO DA SILVA, W. Análise retrospectiva das causas de condenações de carcaças e vísceras de bovinos abatidos em abatedouros frigoríficos na Região Norte da Amazônia Oriental. *CES Med. Vet. Zootec.*, v.16, n.3, p.28-46, 2021. doi: <https://doi.org/10.21615/cesmvz.6331>