



# Process hygiene of cattle carcasses in one slaughterhouse in Serbia during 36 months

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## ABSTRACT

Process hygiene in slaughterhouses plays a key role in ensuring product safety and protecting the health of both workers and final consumers. A three-year monitoring was conducted in one slaughterhouse in Serbia, using non-destructive sampling and testing according to microbiological criteria for total viable count (TVC), *Enterobacteriaceae* (EC), and the presence of *Salmonella* spp. The results showed that all TVC and EC values were below the prescribed m and M limits, while *Salmonella* spp. was detected in two samples. These findings highlight the need for continuous monitoring. Only consistent implementation of self-control measures, application of the HACCP system, and ongoing staff training can ensure meat safety and protect public health. This study emphasizes that properly applied hygiene measures can significantly reduce the risk of microbiological contamination of meat.

## 1. Introduction

The demand for beef as a protein source is increasing globally, although in most countries it represents less than half of total meat consumption, while offering a high sensory quality in both developed and, increasingly, developing countries (Smith *et al.*, 2018). A major global challenge in maintaining the hygiene and safety of meat products is the steady growth of the population, which puts extra pressure on production systems and control measures. It is generally accepted that the primary sources of microorganisms on processed carcasses are contaminated hides and possible rupture of the gastrointestinal tract, with additional contamination arising from unclean tools, as well as workers' hands and clothing (Diyantoro and Wardhana, 2019). The lev-

el of animal cleanliness at the time of entry into the slaughterhouse plays a crucial role. Animals in inadequate hygienic condition are a significant source of microbial contamination in slaughterhouses. Microorganisms present on their skin, hair, and hooves can easily be transferred to equipment, working surfaces, and the meat itself during slaughter operations (Hong *et al.*, 2024). These facts show how important it is to keep track of everything from the farm to the slaughterhouse, to ensure hygiene and safety are really being followed all the way through.

In most countries, including Serbia, a scientifically-based preventive food safety system is implemented, founded on the principles of hazard analysis and critical control points (HACCP). The implementation of HACCP is not feasible without the

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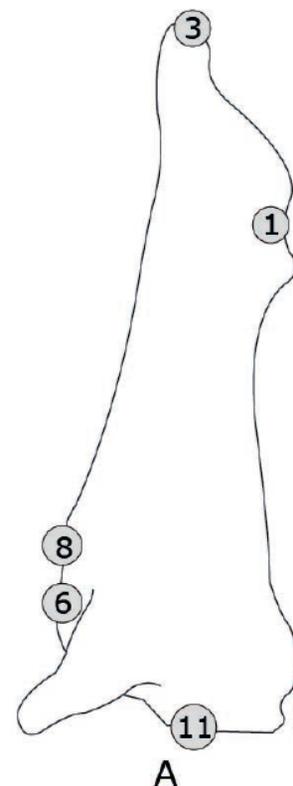
application of good manufacturing practice (GMP) and good hygiene practice (GHP). These control systems are often supported by microbiological food criteria, which include safety parameters focused on foodborne pathogens and hygiene indicators (*Serbian Regulation*, 2019). The number of total viable counts (TVC) and *Enterobacteriaceae* (EC) and the presence or absence of *Salmonella* spp. are the process hygiene criteria for cattle carcasses, as specified in the Serbian Regulation on General and Specific Conditions of Food Hygiene and Microbiological Criteria for Food (*Republic of Serbia*, 2024). As the *World Health Organization* (2024) states, bacterial pathogens are among the most significant causes of foodborne diseases. Each year, they cause illnesses that range from mild gastrointestinal disorders to severe and fatal outcomes. Contaminated food threatens both public health and nutrition security. It also generates major socioeconomic consequences. These challenges highlight the need for governments to prioritize food safety. Evidence-based policies and effective control systems are essential to safeguard health. In 2023, *Salmonella* spp. remain one of the most important bacterial zoonotic agents transmitted through food in Europe, with 77,486 reported human cases (*EFSA and ECDC*, 2024).

The highest levels of *Salmonella* spp. contamination on cattle hides are typically found on the brisket and distal leg areas, likely due to prolonged contact with floors contaminated with feces while the animals are standing or lying down during the pre-slaughter phase, including time spent in the stun-box. This contamination pattern is reflected on the carcass, with higher microbial loads at skin-opening sites (brisket, distal leg) and hide-meat contact areas (rump, flank) (*Buncic and Sofos*, 2012). The detection on carcasses of *Enterobacteriaceae* (EC), a bacterial family widely distributed in the environment, may indicate fecal contamination. Total viable count (TVC) clearly reflects the general hygiene level in slaughterhouses (equipment, tools, etc.) (*Mrdović et al.*, 2017). This highlights the importance of microbiological monitoring of hygiene indicators in the assessment of hygiene levels in slaughterhouses and the control of carcass contamination to protect the health of both working staff and final consumers.

## 2. Materials and methods

Slaughterhouses are required to have a self-control plan and establish a food safety assurance system at all stages, in accordance with the princi-

ples of good manufacturing and hygiene practice and hazard analysis and critical control points (HACCP), according to the local food safety law (*Serbian Regulation*, 2019). Sample collection was undertaken from January 2022 to December 2024 (Table 1). During this period, 180 samples were collected from one slaughterhouse in Serbia and the number of microorganisms on the carcasses were determined using standard methods (*ISO*, 2015). At each visit to the slaughterhouse five carcasses were randomly selected and sampled at step of the slaughtering process – after the final washout before chilling. The non-destructive swabs were taken at four sites on each carcass (rump, flank, brisket and neck; Figure 1). In this study a non-destructive sampling method was applied in order to avoid damaging the musculature. This approach followed the recommendations of the Guide for the Application of Microbiological Criteria for Food (*Ministry of Agriculture, Trade, Forestry and Water Management of the Republic of Serbia*, 2011) and, therefore, required a stricter evaluation of the results than would have been the case



**Figure 1.** Microbiology of the food chain — Carcass sampling for microbiological analysis according to the ISO 17604:2015 standard (*ISO*, 2015). The circle indicate the sampling sites on the cattle carcass surface, and the numbers correspond to defined sampling positions: (1) Rump, (3) Flank, (6 & 8) Brisket, (11) Neck.

if the limit prescribed for the destructive method by Serbian regulation (*Serbian Regulation, 2024*) had been used. Process hygiene was monitored and evaluated in accordance with the local law (*Serbian Regulation, 2024*). The samples collected were conditionally stored in a hand refrigerator and transported to the Institute of Meat Hygiene and Technology in Belgrade. Process hygiene criteria for cattle were: TVC, EC count and presence/absence of *Salmonella* spp. In addition, the evaluation of the presence of *Salmonella* spp. on carcasses was conducted using the rolling window sampling approach. This method ensures systematic and continuous monitoring, with results assessed through overlapping “windows” of data that shift forward as sampling progresses. By applying this principle, contamination patterns can be more precisely detected and the effectiveness of

hygienic measures evaluated. Sampling for *Salmonella* spp. was carried out with the abrasive sponge method, covering a minimum surface area of 400 cm<sup>2</sup>, and the number and locations for sampling for the presence of *Salmonella* spp. were the same as those previously specified. According to the Regulations, results are evaluated in series of 50 samples (10 consecutive series with 5 samples each). The acceptable process hygiene level is defined as a maximum of 2 positive samples out of 50 per series, in accordance with the domestic legislation (*Serbian Regulation, 2024*) and the Guide for the Application of Microbiological Criteria for Food (*Ministry of Agriculture, Trade, Forestry and Water Management of the Republic of Serbia, 2011*). In Serbia, the regulation prescribes limits for process hygiene test results for cattle carcasses (Table 1).

**Table 1.** Process hygiene criteria for cattle – non destructive method.

Microorganisms	Sampling plan		Limits		Analytical reference method	Stage where criterion is applied	Action in the case of unsatisfactory results
	n	c	m	M			
Total Viable Count			2.8 log CFU/cm <sup>2</sup>	4.0 log CFU/cm <sup>2</sup>	SRPS ISO 4833-1:2014	Carcasses after final washing, before chilling	Improvement of slaughter hygiene and re-evaluation of the process
<i>Enterobacteriaceae</i>			1,2 log CFU/cm <sup>2</sup>	2 log CFU/cm <sup>2</sup>	SRPS ISO 21528-2:2009		
<i>Salmonella</i>	50	2 (2)*	Absence in the area tested per carcass.		SRPS ISO 6579:2008		Improvement of slaughter hygiene, re-evaluation of process control, determination of animal origin, and implementation of biosecurity measures on farms

### 2.1 Statistical analysis

Statistical analysis of the results was performed using Microsoft Office Excel 2016. The average logarithmic TVCs and ECs for each carcass were calculated, based on the previously transformed log values from four corresponding sampling sites per carcass. Subsequently, the average daily logarithmic

values were computed. The average daily logarithmic count for *Salmonella* spp. cannot be calculated due to regulatory requirements, which specify only the presence or absence of *Salmonella* spp. Data on *Salmonella* presence were analyzed using Microsoft Excel. The prevalence was calculated as the proportion of positive samples relative to the total number

of tested carcasses, expressed as a percentage. This calculation involved dividing the number of positive samples by the total number of samples and multiplying by 100.

### 3. Results

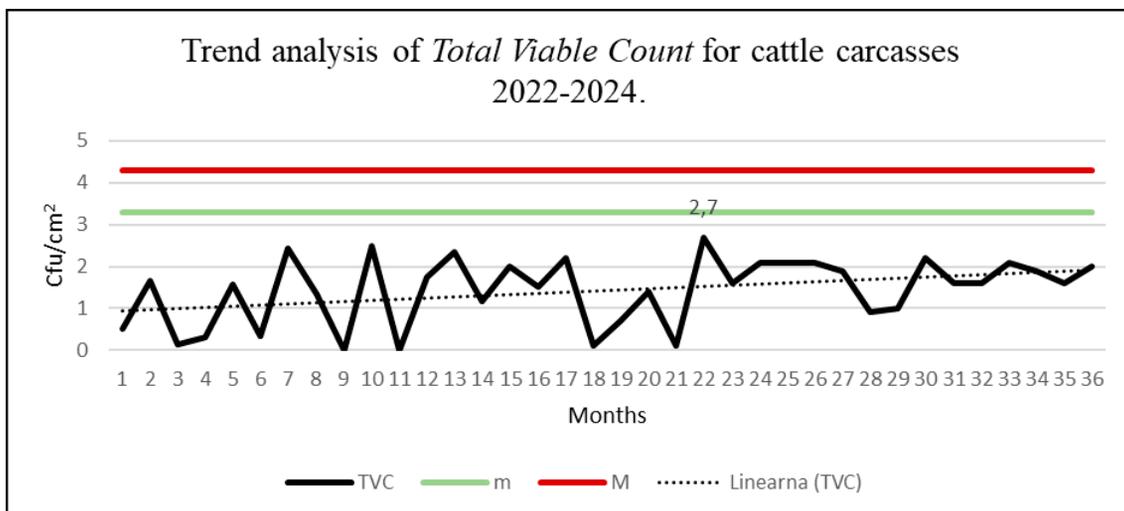
#### 3.1 Total viable count numbers and trends

The mean monthly TVCs on cattle carcasses during the three-year monitoring period ranged from undetected for September and November 2022 to 2.7 log CFU/cm<sup>2</sup> for October 2024 (Table 2). In most months, the TVCs remained below the reference value *m* (2.8 log CFU/cm<sup>2</sup>), and none exceeded the maximum permitted value *M* (4.0 log CFU/cm<sup>2</sup>). Over the three-year period, none of the sam-

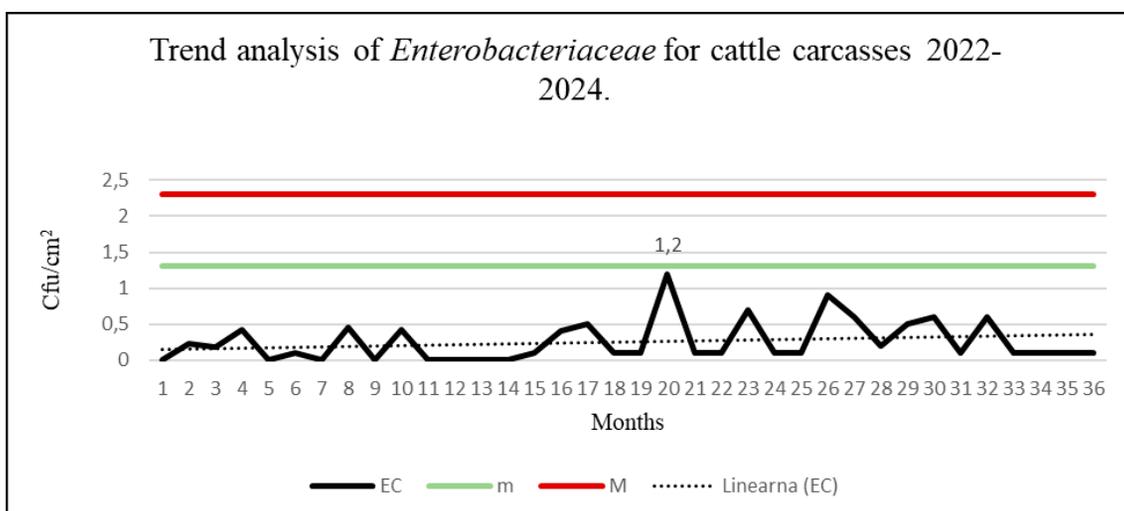
ples exceeded the *m* or *M* values, indicating that 100% complied with the satisfactory criterion.

#### 3.2 Enterobacteriaceae numbers and trends

During the monitoring period, the average monthly ECs on cattle carcasses ranged from undetected in January, May, July, September, November, December 2022 and January and February 2023 to 1.2 log CFU/cm<sup>2</sup> in August 2023 (Table 3). All monthly values were well below the reference value *m* (1.2 log CFU/cm<sup>2</sup>) and did not exceed the maximum permitted value *M* (2.0 log CFU/cm<sup>2</sup>). Throughout the study period, no sample surpassed the *m* or *M* thresholds, demonstrating 100% compliance with the satisfactory process hygiene criterion for EC.



**Figure 2.** Trend analysis of total viable counts (TVCs) on cattle carcasses, 2022-2024.



**Figure 3.** Trend analysis of Enterobacteriaceae counts (EC) on cattle carcasses, 2022-2024.

### 3.3 *Salmonella* spp. presence/absence

The presence of *Salmonella* spp. was detected on 2 out of 180 cattle carcasses. The detection limit for *Salmonella* spp. is set at two occurrences per 50 carcasses. Since *Salmonella* spp. were detected in the slaughterhouse, it was necessary to verify the origin of the animals and the biosecurity measures on the farms of origin, review process controls, and improve slaughter hygiene.

## 4. Discussion

Overall, all results for process hygiene indicators on cattle carcasses stayed below the threshold values set by *m* and *M*, confirming full compliance with the satisfactory hygiene criteria. In many months, neither TVC nor *Enterobacteriaceae* were detected, and when they were present, their levels were still below the reference limit. *Salmonella* spp. was detected on only 2 of the 180 carcasses tested (1.11%), and satisfactory results were obtained using the rolling window method, confirming the effectiveness of the applied hygiene measures. By contrast, *Mrdović et al.* (2017) reported that *Salmonella* spp. were not detected on any of the 610 cattle carcasses examined in either a large-scale or a small-scale slaughterhouse in Serbia during a five-year monitoring period. In their study, TVC and EC were consistently within the satisfactory or acceptable regulatory ranges, confirming good slaughter hygiene practices. On the other hand, *Gutema et al.* (2021) observed a considerably higher prevalence of *Salmonella* spp. (8.6%) on cattle carcasses in two small-scale slaughterhouses in Ethiopia. Their study also detected *Salmonella* in rectal content (7.1%) and hide samples (7.1%). Interestingly, genetic analysis revealed that direct transfer from feces or hides to carcasses occurred only in a small number of cases. The majority of contamination originated from cross-contamination during the slaughter process, largely linked to poor hygiene practices, such as insufficient cleaning of equipment and limited training of

slaughterhouse workers. Similarly, *Nakamura et al.* (2023) demonstrated that TVC levels were highest immediately after skinning, particularly on the outer carcass surface, but decreased significantly after washing, reaching low levels. *Enterobacteriaceae* were mainly detected on outer surfaces, especially around the anus and splitting site, while inner surfaces showed consistently low levels (*Nakamura et al.*, 2023). These observations are consistent with our findings, where TVC and EC levels remained below regulatory thresholds throughout the monitoring period. Overall, our results correspond well with those of *Mrdović et al.* (2017) in Serbia and *Nakamura et al.* (2023) in Japan, both demonstrating low TVC and EC levels under adequate hygiene practices, whereas the higher prevalence of *Salmonella* spp. reported by *Gutema et al.* (2021) in Ethiopia suggests that differences in slaughterhouse infrastructure, hygiene management, and biosecurity measures may contribute to variation in carcass contamination rates. These findings show that the hygiene measures in place were effective in keeping microbial contamination to a minimum, although the occasional detection of *Salmonella* underlines the importance of ongoing monitoring, checks on biosecurity measures, and consistent application of hygiene procedures to ensure food safety and meet legal requirements.

## 5. Conclusion

Adhering to the HACCP system, along with the prerequisite GMP and GHP, makes it possible to maintain satisfactory microbiological criteria for cattle carcasses at critical points in production. Findings from this three-year study show that the slaughterhouse consistently upheld a high level of process hygiene, with TVC, EC, and *Salmonella* spp. results all remaining within the satisfactory limits set by regulations. Continuous monitoring, together with regular staff training and education, is essential to ensure the ongoing safety of beef and to maintain these high hygiene standards.

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