Microbial Risk Assessment of the Contamination Level of Bovine Carcasses Slaughtered in an Abattoir in Maramureş County

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Abstract

Cattle carcasses may be contaminated during the flow diagram process with a wide variety of spoilage and pathogenic microorganisms with different origin, from various sources (soil, water, tools, knives, feces, operators), but only few of them will be able to develop in the new ecosystem conditions, and from these only a small proportion will eventually start the spoilage processes. Taking into account the above mentioned we aimed to perform a microbiological risk evaluation focusing on the aerobic plate count (APC), *E. coli* count, Enterobacteriaceae count and the identification of *Salmonella* spp. The research material was represented by 60 surface samples of refrigerated beef, collected during January-December 2013, in a slaughterhouse in Maramureş County. In order to evaluate the contamination level of the carcasses we used the following standardized methods: SR ISO 4831/2003 for aerobic plate count, SR ISO 21528-2/2007 for Enterobacteriaceae count, SR ISO16649-2/2007 for *Escherichia coli* and SR ISO 6579/AC/2006 for *Salmonella* spp. identification. The statistical data obtained concerning the slaughtering process revealed that the total microbial load was between 2.5–5.67 log cfu/cm². These elevated values indicate non-compliance regarding the good hygiene practices and good manufacturing during the technological flow. From the total collected samples analyzed, the germs belonging to Enterobacteriaceae family, exceeded the maximum admitted level in 10.8%, respectively *E. coli* presented values between 0.5-1.9 log cfu/cm². Samples were negative for *S. enteritidis* and *S. typhimurium*.

Keywords: cattle carcasses, microbial risk assessment, slaughterhouse.

INTRODUCTION

Bacterial microflora from the carcass is largely dependent on the conditions in which the animals were reared, slaughtered and on carcass processing conditions in the processing units. An initial contamination occurs at the time of the first section, later contamination is due to aerosols and the dust generated after skinning, to the contact with the operators hands or by the contact of the skin with the subcutaneous connective tissue (Zweifel *et al.*, 2008). During processing, carcass microbial load increases through germ

contamination from the instruments used: hooks, chains, knives, saws, work surfaces etc. (Gill *et al.*, 1998; Murraz *et al.*, 2001). There is much debate about the possibility of obtaining sterile meat, but in the meat from healthy animals and processed in strict compliance with the rules of hygiene, the microbial load is very low, below 10² cfu / cm², of which 1% or sometimes even 10% can be developed under refrigerated conditions (Ingram et Roberts, 1976). In the case of pork meat, initial microflora differs from that found in cattle, due to different processing steps at these

species. After blanching processes, hair removal, singeing, microbial load is reduced significantly, below 10^3 or even 10^2 cfu / cm², however after further processing steps recontamination occurs. Another major source of carcass surfaces' contamination is the evisceration. Rapid cooling of the carcasses at low temperatures, increased speed of air flow may result in a reduction of bacterial population, while mild cooling will allow the growth and development of germs. As a result, strict compliance with the cooling chain is vital, as it must ensure not only extend validity period, but also that these products are safe for the consumer. Therefore, in this study, we aimed to evaluate the degree of carcasses contamination with germs performing the following determinations: the total number of germs, the germ load of the *Enterobacteriaceae* family, *E. coli* β -glucuronidase-positive and *Salmonella* genus.

MATERIALS AND METHODS

The research material was represented by 60 samples of cattle and pigs carcasses harvested in January-December 2013 from a slaughterhouse in Maramureş County. A number of 5 samples from the surface of the chilled cattle carcasses and 5 samples from pork carcasses surface were taken each month. The samples were analyzed in the laboratory of Food Inspection and Control of the FMV Cluj Napoca. To assess the degree of contamination of carcasses the following standard

microbiological methods were used: determination the total number of germs (APC) SR ISO 4833/2003, determination of the number of bacteria belonging to the *Enterobacteriaceae* family (SR ISO 21528-2/2007), identification of *Salmonella* spp. (SR EN ISO 6579/AC/2006), identification of *Escherichia coli* beta glucuronidase-positive (SR ISO 16649-2/2007). The results were analyzed statistically and mathematically using the Origin 8.5 program, using the ANOVA single factor categorical analysis system.

RESULTS AND DISCUSSION

From the results obtained it was found that the aerobic plate count (APC) was between 3.0–2.5 log cfu/cm² with an average of 02 ± 2.7 , namely the number of bacteria of the *Enterobacteriaceae* family ranged between 1.9–1.0 log cfu/cm² with a mean value between 04 ± 1.4 . All samples were within the maximum allowed (Fig. 1). Regarding *E. coli*, mean values ranged from 0.35–1.54 log cfu/cm², with a mean of 0.95 ± 0.5 log cfu/cm². For the samples collected in January, no bacteria of the *Salmonella* genus were identified.

Aerobic plate count (APC) in February was between 3.7–2.9 log cfu cm² with an average of 0.3 ± 3.1 log cfu/cm². The level of contamination with bacteria from the *Enterobacteriaceae* family ranged from 2.1–1.2 log cfu/cm² with an average of 0.34 ± 1.65 and *E. coli* ranged between 0.35–1.54 log cfu/cm², with a mean of 0.98 ± 0.5 log cfu/

Fig. 1. Aerobic plate count, Enterobacteriaceae, *E. coli* and *Salmonella* spp. medium load at the surface of bovine carcasses during January

cm². Because they complied with good hygiene practices (GHP) and good manufacturing practices (GMP) on all processes it can be observed that the mean values of surface contamination of cattle carcasses with microorganisms are lower, fitting within the maximum limits.

Studies published by Zweifel *et al.* (2008) highlighted the values of surface contamination of cattle carcasses with microorganisms from the *Enterobacteriaceae* family between 3.8 log cfu/cm², noticing an increased value that exceeded the maximum allowed limit of 2 log cfu/cm².

The mean value of aerobic plate count (APC) on the surface of cattle carcasses, in March showed slightly higher values than in February, falling between 3.6–5.5 log cfu/cm², with a mean of 4.44 ± 0.85 log cfu/cm² ($p > 0.05$). Analyzing the results, we found that 2 samples exceeded the maximum allowed of 5.0 log cfu/cm² (Fig. 2). These high values denote non-compliance regarding the code of good manufacturing and hygiene practices (GHP, GMP), along the processing flow of slaughtering in cattle. The level of contamination with *Enterobacteriaceae* was between 1.6–2.5 log cfu/cm², with a mean of 0.33 ± 1.95 log cfu/cm² being in the limit, while the load of *E. coli* ranged between 0.6–1.6 log cfu/cm², with an average of 1.03 ± 0.5 log cfu/cm².

Analyzing the results it was found that aerobic plate count (APC) in April was 5.2–2.8 log cfu/

cm² with an average range of between 0.95 ± 4.02 log cfu/cm². From the total amount of samples analyzed, one of them was not within the limit, which shows that did not complied with good hygiene practices and have taken corrective measures for reducing microbial contamination, the same problem as in March where two of the samples analyzed exceeded the maximum limit. The level of contamination with bacteria from the *Enterobacteriaceae* family was between 2.1–1.5 log cfu/cm², with a mean of 0.26 ± 1.7 log cfu/cm². Regarding *E. coli*, mean values ranged from 1.2–0.4 log cfu/cm², with a mean of 0.79 ± 0.39 log cfu/cm².

The aerobic plate count (APC) on the surface of cattle carcasses in June showed slightly higher values than in May ($p > 0.05$), falling between 5.67–4.5 log cfu/cm² average of 0.54 ± 5.11 log cfu/cm², 3 samples exceed the maximum permissible limit of 5.0 log cfu/cm². The load of germs from *Enterobacteriaceae* family showed values between 3.48–2.3 log cfu/cm² average of 0.46 ± 2.77 log cfu/cm², 3 of the samples analyzed exceeded the maximum permissible limit of 2.5 log cfu/cm². These elevated values show that the slaughterhouse did not respect the strict requirements of GMP and GHP program implementation. In case the limits of the parameters regarding technological process hygiene criteria, the measures aimed at improving hygiene in the unit and increase the

Fig. 2. Aerobic plate count, *Enterobacteriaceae*, *E. coli* and *Salmonella* spp. medium load at the surface of bovine carcasses during March

frequency of sampling until their classification in the appropriate values. The mean load of *E. coli* were higher than in previous months, ranging between 1.9–1.2 log cfu/cm² with an average of 0.38 ± 1.6 log cfu/cm² (Fig. 3).

In July it was found that the aerobic plate count (APC) ranged between 4.0–3.0 log cfu/cm² with an average of 0.37 ± 3.5 log cfu/cm², respectively the number of bacteria from the *Enterobacteriaceae* family ranged between 1.0–2.3 log cfu/cm² with an average of 0.63 ± 1.46 log cfu/cm². Regarding *E. coli*, the mean values ranged between 0.3–0.45 log cfu/

cm², with a mean of 0.61 ± 0.27 log cfu/cm². For the samples analyzed in July there were no identified bacteria from the *Salmonella* genus. It can be seen in the results presented above that in July they took corrective action, thus avoiding the growth of higher values of microbiological contamination of cattle carcasses, observing strict hygiene standards throughout the technological flow. All samples analyzed were within the maximum allowed. Studies by Murraz et Gadden (2001) showed values of carcasses contamination in bovine with *Enterobacteriaceae* between 0.70 ± 2.80 log cfu/

Fig. 3. Aerobic plate count, Enterobacteriaceae, *E. coli* and *Salmonella* spp. medium load at the surface of bovine carcasses during June

Fig. 4. Aerobic plate count, Enterobacteriaceae, *E. coli* and *Salmonella* spp. medium load at the surface of bovine carcasses during September

cm², these elevated values reveals a breach of good hygiene practices (GHP) and good manufacturing practices (GMP) throughout the technological flow. From the statistical processing of the results obtained in August, the value of TC from cattle carcasses surface have ranged between 3.6–2.0 log cfu/cm² with an average of 0.66±3.1 log cfu/cm². The average level of *Enterobacteriaceae* load was between 1.48–1.0 log cfu/cm² with an average value of 0.66±3.1 log cfu/cm². Regarding *E. coli* the mean values ranged from 0.9–0.3 log cfu/cm² with an average of 0.21±0.55. Following the results, we found that all the samples analyzed were within the limit, which means that they have complied with hygiene practices in all processes. Higher values of *E. coli*, on the level of contamination of the surface in cattle carcasses were reported by Sumner *et al.*, 2003, which presented an average of 0.23±1.82 log cfu/cm².

Following the results obtained in September we found that the aerobic plate count (APC) was located between 5.7–3.0 log cfu/cm² with a mean value between 1.03±4.28 log cfu/cm². From the results obtained, one of the samples exceeded the maximum permissible limit being 5.0 log cfu/cm². The load level of *Enterobacteriaceae* was between 2.6–1.3 log cfu/cm² with an average of 0.58–2.04 log cfu/cm² (fig. 4). It was found that the maximum allowed, 2.5 log cfu/cm², was exceeded in two samples. In accordance with Reg. 1441 (EC) / 2007 in the case of hygiene criteria

parameters exceeding within the technological process, measures are taken to improve hygiene in the unit and increase the frequency of sampling to their classification in the corresponding values. Regarding *E. coli* load, values ranged between 1.2–0.5 log cfu/cm² with an average of 0.41±1.0 log cfu/cm².

In October the aerobic plate count (APC) was between 3.67–3 log cfu/cm² with an average of 0.31±3.31 log cfu/cm². The level of contamination with bacteria from *Enterobacteriaceae* family was in between 2.0–1.3 log cfu/cm² with an average of 0.36±1.48, while in the case of *E. coli*, mean values ranged from 0.4–0.6 log cfu/cm² with an average of 0.52 ± 0.08 log cfu/cm². As they complied with good hygiene practices (GHP) and good manufacturing practices (GMP) for all processes, all samples analyzed were within the maximum allowed. In the case of the samples taken in October no *Salmonella* germs were identified. Higher values of microbial contamination were published by Ahmad *et al.* (2013), which in the case of *E. coli* showed values ranging between 2.81–3.51 log cfu/cm². Also 26% of all samples analyzed were positive for *Salmonella*, compared to samples examined by us, where all the samples analyzed were negative.

Medium aerobic plate count (APC) on the surface of cattle carcasses in December showed higher values than in November ($p > 0.05$), ranging from 2.7–4.7 log cfu/cm² with an average value

Fig. 5. Aerobic plate count, Enterobacteriaceae, *E. coli* and *Salmonella* spp. medium load at the surface of bovine carcasses during December

of 1.43 ± 4.0 log cfu/cm², 2 samples exceeded the maximum allowed. The load of *Enterobacteriaceae* germs showed values between 2.6–1.3 log cfu/cm² with an average of 0.52 ± 1.67 log cfu/cm², one of the samples analyzed exceeded the maximum allowed limit of 2.5 log cfu/cm². Elevated values regarding aerobic plate count and *Enterobacteriaceae* count were reported by Dan *et al.*, (2003), in a research regarding psychrotrophic microbial count. These elevated values show that the slaughterhouse did not respect the strict requirements of GMP and GHP program implementation throughout the flow diagram of cattle slaughtering, and at that time in Romania and EU it was a different food legislation, different by the new EU food legislation (e.g. Reg. CE 853/2004, Reg. CE 1441/2007). The average load with *E. coli* ranged between 0.8–0.4 log cfu/cm² with a mean of 0.14 ± 0.58 / log cfu/cm² (Fig. 5). Among the samples gathered in December no bacteria from the *Salmonella* genus were identified.

CONCLUSION

From the total collected samples analyzed aerobic plate count exceeded the maximum admitted level in case of 4.2%, 10.8% of germs belonging *Enterobacteriaceae* family exceeded the maximum admitted level, respectively *E. coli* presented values between 0.5–1.9 log cfu/cm². These results indicate that good hygiene practices and good manufacturing practices were not respected all the time during the flow diagram of cattle slaughtering. We mention that germs belonging to *Salmonella* were not identified. The results are different from those published by Dan *et al.* (2011), regarding evaluation on pathogen load on bovine carcasses, when 5.56% of analyzed samples (n=36), 2 were positive for *Salmonella enteritidis*.

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