Microbiological Hazards Assessment of Psychrotrophic Microflora in Bovine Carcasses Slaughtered in North West Romania

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Abstract

Gram negative bacteria have the greatest capacity to spoil the meat if kept under aerobic conditions, therefore the members of the genera *Pseudomonas*, *Acinetobacter*, *Psychrobacter* and *Moraxella*, will form the dominant microflora. The purpose of this study was to assess the contamination level with psychrotrophic microflora of bovine carcasses in order to prove how the results could be used to improve the slaughter process. The research was carried out between January-December 2015, in two slaughterhouses from North West Romania. The research material was represented by 144 meat samples (slaughterhouse A, n=72, slaughterhouse B, n=72). Weekly, 3 samples were collected from refrigerated carcasses, and examined for total psychrotrophic counts (TPC), *Enterobacteriaceae*, *Pseudomonas*, *Aeromonas* and *Yersinia*. From statistical processing of recorded data was established that mean log TPC from the surface of refrigerated carcasses has presented different values, ranged between 3.70 ± 0.20 log CFU/cm² and 6.90 ± 0.43 log CFU/cm². Initial surface microflora of bovine carcasses was represented by germs from the following genera: *Staphylococcus*, *Micrococcus*, *Aeromonas*, *Acinetobacter*, *Pseudomonas*, *Yersinia*, *Serratia*, *Hafnia*, *Proteus* and *Escherichia*. Microbiological hazard assessment reveals the key role of psychrotrophic microorganisms in the spoilage of meat, if the monitoring system of the slaughtering process is not functioning properly.

Keywords: microbiological hazards, psychrotrophic bacteria, bovine carcasses, hygiene, slaughterhouse.

INTRODUCTION

Taking into account the fact that one of the most requested food in the majority of the countries is meat, it is necessary for this product to be processed in the best sanitary conditions. Meat, because of its chemical composition, is highly susceptible to microbial alteration, being involved also in spreading of some diseases or in the onset of food poisoning episodes. Because of that, the researchers' interest regarding meat and meat products' role in the onset of these food poisoning episodes has begun to increase, instead of decreasing.

The microorganism's presence in meat (microbiological hazard), plays a major role in the

quality, sanitary and freshness state, because the quality of meat can increase or decrease, becoming improper for consumption according to the influence of these particular factors. This outcome is due to their pathogenic actions, or because of the toxic metabolic products resulting after the microorganism's degradation. Bovine carcasses can become contaminated during the technological process with a variety of altering microorganisms or conditionally pathogenic bacteria, which can be found in various sources (e.g. soil, water, equipment, knives, faeces, operators), but only some of these are able to grow in the environments' conditions, and only a few will be able, eventually, to start spoilage processes (Gill, 1997; 1998). The

Gram negative bacteria have the greatest meat and meat products' spoiling capacity. In case they are kept in aerobic conditions, the members of *Pseudomonas*, *Acinetobacter*, *Psychrobacter* and *Moraxella* species, due to their high growing rate, will become the dominant microflora (Molin and Ternstrom, 1982; Molin *et al.*, 1986; Shaw and Latty, 1982, 1984, 1988; Prieto *et al.*, 1992 a, b; Drosinos and Board 1995; Davies and Board, 1998). Certain species of psychrotrophic Enterobacteriaceae commonly occur on chilled meat, but appear to be more prevalent on pork and lamb (Grau, 1981; Dainty and Mackey, 1992). The enumeration of *Enterobacteriaceae* on selective media has shown that certain genera of this family are significant, but not dominant, members of the microbial associations on meats stored aerobically at chill temperatures (Dainty *et al.*, 1989). *Serratia liquefaciens*, together with *Enterobacter aerogenes* and *Citrobacter* spp., were found on lamb chops (Newton *et al.*, 1977). Isolation of *Flavobacterium*, *Alcaligenes*, *Vibrio*, *Aeromonas* and *Alteromonas* is reported less frequently (Patterson and Gibbs, 1977; Nottingham, 1982).

Based on these facts and taking into account that in the beef slaughtering units from the North West region of Romania a research of this kind hasn't been made, we tried to assess the microbiological hazards represented by the psychrotrophic germs, in two slaughtering units.

MATERIALS AND METHODS

Materials

The research material was represented by 144 beef samples, collected during January – December 2015, from two beef slaughtering units located in the north west region of Romania (slaughterhouse A, n=72, slaughterhouse B, n=72). Weekly, 3 samples were collected at four sites from refrigerated carcasses, and examined for total psychrotrophic counts (TPC), Enterobacteriaceae, *Pseudomonas*, *Aeromonas* and *Yersinia*. The day of sampling was changed each week. The samples were obtained by the destructive sampling technique from four different sites stipulated by the Commission Decision 2001/471/CE (e.g. neck, brisket, flank, and rump). Four flaps of superficial muscle tissue from the carcasses surface were collected, each having a thickness of 2-3 mm, and 20 cm². Samples were subsequently placed into cool boxes, and microbiological examinations were

carried out 2–4 h after sampling, in the Animal Food Hygiene and Public Health laboratory from the Faculty of Veterinary Medicine, Cluj-Napoca.

Sample preparation

Samples of each carcass were homogenized together for 120 sec. in 100 ml 0.1% buffered peptone water and 0.85% sodium chloride solution in a stomacher Easymix (AES Chemunex, Bruz, FR). Serial dilutions were aseptically prepared and plated on various selective and differential media.

Microbiological analysis

Suspensions were plated with a spiral plater (Autoplate 4000, Spiral Biotech, Bethesda, MD) on plate count agar (Merck, KGaA, Darmstadt, Germany), violet red bile glucose agar (VRBD agar; Merck, KGaA, Darmstadt, Germany), *Pseudomonas Aeromonas* selective agar (GSP agar; Merck, KGaA, Darmstadt, Germany), *Yersinia* selective agar (CIN agar Merck, KGaA, Darmstadt, Germany). For samples in which bacterial counts were below the detection limit, a log value of zero was used for calculations. Microbiological results were depicted as time trend graphs of trimestrial mean log values. All plates were incubated aerobically for 48-72 h at 20°C (Nottingham, 1982; Greer, 1981, 1982). Manual counting was applied. In order to identify the psychrotrophic germs, from the grown colonies in the dehydrated selective media there were microscopic exams made (Gram staining), followed by biochemical confirmation tests: API 20E and API 20NE (bioMérieux SA Marcy l'Etoile, France).

Statistical analysis

All statistical analysis were performed using the Origine 8.5 software (OriginLab Corporation, Northampton, USA). For all tests, a significance level of $p = 0.05$ was chosen. To evaluate significant differences in TPC between abattoirs, one-way analysis of variance (ANOVA) was performed. Bonferroni post-hoc procedure was used for paired comparisons when ANOVA yielded significant results.

RESULTS AND DISCUSSION

The average psychrotrophic load at the refrigerated carcasses in the samples collected from unit A has shown different values, ranging between 3.70 ± 0.20 log CFU/cm² in the first trimester and 6.90 ± 0.43 log CFU/cm² in the third trimester, with a minimum value of 3.2 ± 0.1 log CFU/cm² in January and a maximum of 7.33 ± 0.20

log CFU/cm² in September (Fig. 1). The highest values of the microbial load was noticed during the second and third trimester, when it was revealed an exceeding in the recommended maximum limit (5.0 log CFU/cm²) at 32 samples (44.44%) (Sutherland *et al.*, 1975; Hanna *et al.*, 1977; Ayres *et al.*, 1980; Cousin, 2000). The psychrotrophic germ load in the case of the samples collected in unit B, ranged between 5.46±0.14 log CFU/cm² in the first trimester and 6.04±0.51 log CFU/cm² in the third trimester with minimum values of 5.30±0.20 log CFU/cm² in the month of January and a maximum of 7.16±0.05 log CFU/cm² in May, noticing that at 17 samples the value was higher than the limit recommended, which means 72%, from the total samples' number (Fig. 1).

Similar values were obtained by Brown (2000), which revealed in the refrigerated bovine carcasses values ranging between 5.54 log CFU/cm² and 8.0 log CFU/cm². Also, Roberts (1980, 1997), in his studies concerning the psychrotrophic load at the refrigerated bovine carcasses, has obtained values between 4.50 log CFU/cm² and 7.0 log CFU/cm².

The logarithmic average of the pseudomonads load in unit A was in between 4.98±0.45 log CFU/cm² in the third trimester and 6.07±0.33 log CFU/cm² in the second trimester, with a minimum value of 4.53±0.040 log CFU/cm² in August and a maximum value of 6.4±0.26 log CFU/cm² in May. In regard to unit B, the obtained results show that the average germ load belonging to this genus is higher, ranging between 4.62±0.34 log CFU/cm² in the first trimester and 6.27±0.18 log CFU/cm² in

the third trimester, with a minimum of 4.33±0.41 log CFU/cm² in February and a maximum of 7.13±0.15 log CFU/cm² in August (Fig. 2).

From the total of 72 samples collected from the bovine carcasses' surface, in the units studied, at 54 samples (75%) the development of specific *Aeromonas* spp. colonies was noticed.

After evaluating the results, in unit A we found that the average germ load from *Yersinia* genus was in between 4.20±0.26 log CFU/cm², during the first trimester and 5.78±0.44 log CFU/cm² in the third trimester. Regarding the samples collected from unit B, the *Yersinia* average germ load was in between the value of 4.5±0.4 log CFU/cm² during the fourth trimester and 6.13±0.63 during the third trimester, with a minimum value of 4.066±0.30 log CFU/cm² in May and a maximum of 6.8±0.30 log CFU/cm² in the course of September.

The average load of germs belonging to *Enterobacteriaceae* family in unit A was found to range between the values of 2.0±0.03 log CFU/cm² during the fourth trimester and 5.65±0.21 log CFU/cm² during the fourth trimester, with a minimum value in January of 1.96±0.40 log CFU/cm² and a maximum one of 5.86±0.11 log CFU/cm² in August. The results revealed that from the total number of samples, 50 of them have higher values than the maximum one allowed (2.5 log CFU/cm²) (69.44%), the lowest ones being registered in the fourth trimester. Regarding the results found in the samples collected at unit B, we revealed that the average number of *Enterobacteriaceae* was higher than of that found in unit A, ranging between

Fig. 1. Mean log values of total psychrotrophic count at the surface of refrigerated beef carcasses from A (n=72) and B (n=72) slaughterhouses

3.77 ± 0.17 log CFU/cm² during the fourth trimester and 6.07 ± 0.24 log CFU/cm² during the third one, in all the samples the maximum allowed value being crossed over (Fig. 3). These higher values of the *Enterobacteriaceae* load revealed the lack of proper conditions in the carcasses processing on the entire flow diagram and the inconsistency with the good manufacturing practices (GMP), respectively with the good hygiene practices (GHP) especially at the evisceration of the organs from the abdominal and pelvic cavity. On the basis of our observations made on the units taken into study we found that both of the slaughtering units have not performed the double ligatures of

the esophagus, pylorus, and that the plastic bag were not applied at the anus, in order to prevent the germ contamination of the carcasses from the lower digestive tract.

Another major source of carcasses' surface contamination was represented by the beef's tegument, in terms in which the skinning was made manually. In this way, a contamination of the subcutaneous conjunctive tissue has been made with the germs present on the knives and hands, especially if the hygiene operation of the hands and equipment was not done properly. Following these aspects, the results obtained are explainable in what concerns the microbial contamination

Fig. 2. Mean log value of *Pseudomonas* plate count at the surface of refrigerated beef carcasses from A (n=72) and B (n=72) slaughterhouses

Fig. 3. Mean log value of *Enterobacteriaceae* plate count at the surface of refrigerated beef carcasses from A (n=72) and B (n=72) slaughterhouses

with *Enterobacteriaceae* germs at the surface of the refrigerated carcasses.

Comparing the results obtained in the evaluation of the total germ load from the warm carcasses surface samples with the ones taken from the refrigerated ones in unit A we revealed statistically significant differences regarding the psychrotrophic microflora (Tab. 1).

Based on these results we can conclude that by keeping the carcasses at refrigerating temperatures, the spoiling psychrotrophic germs or conditionally pathogenic, have the capacity of multiplying very fast compared to the mesophilic spoiling germs, becoming one of the dominant microorganism in the meats' ecosystem (Jackson *et al.*, 1997, Gill, 1986; Day, 2000). Following this aspect, the psychrotrophic germs will start alterative processes in meat as soon as their number reaches rapidly values of 10^6 – 10^7 CFU/cm² (Ayres, 1980; Gill, 1986).

After the statistical evaluation of the results shown in figure 4, we have noticed that the Gram negative bacteria represent 78.64%, while the Gram positive ones have a lower occurrence of 21.36%, being represented by staphylococci, micrococci and unidentified Gram positive bacilli. The Gram positive bacteria were identified only on the account of the morphological exam (microscopic), catalase and oxidase tests. From the Gram negative bacteria group, the psychrotrophic germs are predominant, represented by *Pseudomonas* genus (25.79%), *Aeromonas* genus (11.95%), *Psychrobacter* genus (10.14%), *Acinetobacter* genus (7.24%), and *Yersinia* genus (6.51%). The psychrotrophic members from the *Enterobacteriaceae* family were identified using the API 20E kits and were represented only by the alteration germs, like the following ones: *Hafnia alvei* (3.98%), *Serratia lignefaciens* (5.79%) and *Pantoea* spp. 2 (1.09%) (Fig. 4).

Table 1. Significance level of differences between psychrotrophic plate count from the surface of warm bovine carcasses compared with the surface of chilled bovine carcasses in A and B slaughterhouse

PARAMETERS	A slaughterhouse			B slaughterhouse		
	ANOVA	BONFERRONI		ANOVA	BONFERRONI	
	P value	Significance level	Significance level	P value	Significance level	Significance level
Total psychrotrophic count	0.007	p ≤ 0,01	p ≤ 0,01	0.02	p ≤ 0,05	p ≤ 0,05
<i>Pseudomonas</i> spp. count	0.000	p ≤ 0,001	p ≤ 0,001	0.0001	p ≤ 0,001	p ≤ 0,001
<i>Aeromonas</i> spp. count	0.02	p ≤ 0,05	p ≤ 0,05	0.0003	p ≤ 0,001	p ≤ 0,001
<i>Yersinia</i> spp. count	0.03	p ≤ 0,05	p ≤ 0,05	0.01	p ≤ 0,05	p ≤ 0,05
Enterobacteriaceae count	0.002	p ≤ 0,01	p ≤ 0,01	0.0003	p ≤ 0,001	p ≤ 0,001

Fig. 4. The incidence of bacterial species at the surface of refrigerated beef carcasses from B slaughterhouse

Comparing the results concerning the configuration and prevalence of the identified species from the warm bovine carcasses' surface with the ones obtained at the refrigerated samples we revealed that if the initial flora was predominant by the Gram positive (56.51%), the psychrotrophic germs having a lower value (37.17%), after the chilling conditions (48 h), a major change took place in the microbial configuration, because of the new environmental conditions. In this respect, the dominant microflora is in fact the Gram negative one (73.98%), among which the psychrotrophic bacteria is represented in large numbers (71.39%), following these microorganisms' properties of rapidly growing at refrigerating temperatures, compared to the mesophilic microflora. Similar studies made by Sutherland *et al.* (1975), Hanna *et al.* (1977), Ayres *et al.* (1980) and Cousin (2000), have revealed that the initial microflora in the bovine carcasses is dominated by the Gram positive represented by staphylococci, micrococci, streptococci, *Bacillus* and *Lactobacillus* germs, in variable amounts, ranging between 55-75%, while the Gram negative microflora is represented by the genera: *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Aeromonas*, accounting for approximately 25-45%. At refrigerating temperatures, there is a change in the microbial configuration which takes place, so that, the psychrotrophic germs, represented by the microorganisms from the genera *Pseudomonas* (54%), *Moraxella* (9%), *Acinetobacter* (10%), *Aeromonas* (9%), *Psychrobacter* along with a lower quantity of psychrotrophic germs from the *Enterobacteriaceae* family (*Serratia*, *Enterobacter*), become the dominant population (Cousin, 2000).

CONCLUSIONS

The microbiological hazards assessment performed at bovine carcasses proves the key role of the psychrotrophic bacteria in the meat's spoilage processes, when a deficient monitoring of slaughter technology occurs. In order to decrease these high incidences of micro-organisms it is recommended that significant improvements be implemented with regard to compliance with good hygiene practices and good manufacturing practices, within the abattoir and especially in the skinning and evisceration area, where the carcasses are exposed to considerable contamination. From these results it is obvious that the existing food safety assurance system within the abattoir often

fails to produce meat that complies with the Regulation 1441 (CE)/2007. The data obtained during this study will furthermore be used to improve the existing quality assurance program at the specific abattoir and should thus contribute to the delivery of a safe product to the consumer.

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