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The Evaluation of the Pathogen Germ Load and Configuration at the Bovine Carcasses Destined for Public Consumption

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Abstract. Taking into consideration the fact that at the surface of the meat and even in depth there might be developing a variety of bacteria, some of them pathogen and that "preventing is more efficient than treating", it is necessary to pay a special attention to the hygienic requirements for obtaining and processing meat, as well as to the strict supervision in the cooling chain, to prolong the shelf life of the product as well as to ensure safe products for the consumer. The material subjected to research was represented by 72 samples of beef collected in the period of 2009-2010, from slaughtering units in the center of Transylvania, taken from four different regions of the carcasses: chest, flank, cervical area, thigh. From the samples collected there were the following determinations made: the aerobic plate count, the Enterobacteriaceae plate count, the identification of the germ from the genres: *Salmonella, Listeria, Campylobacter, Yersinia* and *Escherichia*. The logarithmic average of the total germ load from the bovine carcasses surface in the year 2009 has shown different values, ranging between 2.32±0.71 and 5.24±0.05 log ufc/cm^2. The total Enterobacteriaceae load from the bovine carcasses surface in the year 2009 has revealed values between 0.67±0.12 log ufc/cm^2 and 2.34±0.23 log ufc/cm^2. In the case of the bovine samples there were germs from *Salmonella* and *Listeria* genus identified.

Key words: contamination, pathogen microorganisms, beef, food safety

INTRODUCTION

Taking into account the fact that one of the most requested food type in the majority of the countries is meat, it is necessary for this product to be obtained in the most hygienic conditions, to have the best quality and not to jeopardize the consumer's health. Because of the meat's chemical composition it represents an ideal culture media for raising and multiplying the microorganisms, mainly the bacteria, whose growth can rapidly occur in the case of improper monitoring in the critical control points (CCP) on the animal slaughtering technological flux (Gill et al., 2001; Gill 1986; Gill and Jones, 1997; Dan et al., 2003, 2007, 2008). The microbial contamination can determine on one hand the start of the meat's altering process, the reducing of its shelf life and even greater a risk on public's health through the appearance of some food poisoning episodes in the consumer's population. In the slaughtering units, the carcasses' contamination with pathogens can occur mostly in the steps of skinning and eviscerating, respectively in the cases of meat manipulation by the operators in the cooling and delivering steps, if the good manufacturing practices (GMP) and good

hygiene practices (GHP) are not strictly followed (Gill, 1986; Rao, 1992; Abdalla and Siham, 2009). In slaughtering units, the pathogen bacteria, can determine the indirect contamination of the carcasses, due to the improper hygiene operation performed by the operators (not making the esophagus constriction, the anus ligation, sectioning the large vessels, through hands and tools not properly disinfected) (Yen, 2003).

Not following the good manufacturing practices (GMP) and good hygiene practices (GHP) in some situations, may determine the spreading of pathogens that belong to the genres *Salmonella, Listeria Campylobacter, Yersinia and Escherichia* at the surface as well as in the depth of the carcasses, leading to the food poisoning episodes (Butterorth-Heinemann, 2000; Borch and Arnder, 2002; Neil and Ormel, 2002). In conformity with the common law, the TGN parameters and *Enterobacteriaceae* are considered hygiene indices in the technological process (Warriner et al., 2002; Nel and Lues, 2004, Zweifel and Baltzer, 2005, Reg. (CE) 1441/2007). The presence of the *Enterobacteriaceae* family germs denotes the possibility of contamination with faces of the carcasses, which represents the main source for the pathogens like: *Salmonella, Campylobacter, Yersinia* and *Escherichia* (WHO, 1995).

Taking into account the facts mentioned above, in our research we tried to make a n analysis of the microbiological hazards present at the bovine, swine carcasses, processed in some slaughtering units in Transylvania. In this paper we evaluated the total germ number, the *Enterobacteriaceae* and the prevalence of the pathogen microorganisms (*Salmonella* spp., *Listeria* spp, *Yersinia* spp., *Echerichia coli*) at the bovine and swine carcasses.

MATERIALS AND METHODS

The material subjected to research was represented for each species by 72 meat samples of beef collected in the period of 2009-2010, from slaughtering units found in the center of Transylvania. From the bovine carcasses there were samples collected from four different areas of the carcass: chest, flank, cervical region, tight, and from bird the skin from the neck region. The sample collection was made randomly, taking into consideration that they are from the carcasses obtained in the beginning and also from the end of the slaughtering process, in different days of the week, respecting the methodology norms recommended by the National Agency Sanitary Veterinary and for Food Safety.

From the surface of the muscle tissue there were superficial muscle tissue areas collected with a thickness of 2-3 mm, meaning a surface of 100 cm^2. The collected samples were introduced in sterile Petri plaques, of 15 cm in diameter and transported at 2-4ºC, at the laboratory of Inspection and food control of feed and animal origin products in the Veterinary Medicine Faculty from Cluj – Napoca, were they were subjected to work immediately.

The isolation of the pathogen microorganisms present at the carcasses' surface was made on account of the standardize methods in conformity with the Reg. (EC) 1441/2007: the enterobacteriaceae identification (SR ISO 21528/2007), the identification of *Yersinia enterocolitica* (SR EN ISO 10273/2003), the identification of *Salmonella* spp. (SR EN ISO 6579/2003 AC/2006), the identification of *Echerichia coli* (SR ISO 16649/2007), the identification of *Campylobacter jejuni/coli* (SR EN ISO 10272/2006).

The statistical calculation was made in an operating system Windows XP, program Origin 7.0. The microbial load results were expressed in logarithmic (log_{10}). The statistical test integrated in the program and used for the calculus and interpretation of our results was the mono-factorial analysis system ANOVA. For the evaluation of the contamination degree, there were calculated the average individual values, expressed according to the standard deviation, the p - indices.

RESULTS AND DISCUSSIONS

The evaluation of the total germ load and of the enterobacteriaceae at the level of bovine carcasses

The logarithmic average of the total germ load on the bovine carcasses' surface in year 2009 has shown different values, with a minimum value of 2.32±0.71 log ufc/cm^2 in the month of November and a maximum level of 5.24±0.05 log ufc/cm^2 in the month of August. The most elevated values of the microbial load were noticed during the course of summer months of the year 2009, where we found that there were exceeding in the maximum admissible limit for six of the samples (16.67%), respectively in the months of July and August of the same year. In conformity with the statements of the Reg. (CE) 1441/2007, the standard maximum admissible limit is of 5.0 log ufc/cm^2.

In the case of the samples collected during the year 2010 the average number of the total germ load was between 1.77±0.32 log ufc/cm^2 in the month of December and 4.94±0.1 log ufc/cm^2 in the month of August, without any exceeding of the maximum admissible limit (fig. 1). The statistical analysis using the ANOVA test has shown that there are distinctly significant differences in the total germ load at the samples collected from the surface in the two years taken into study ($p \leq 0,01$).

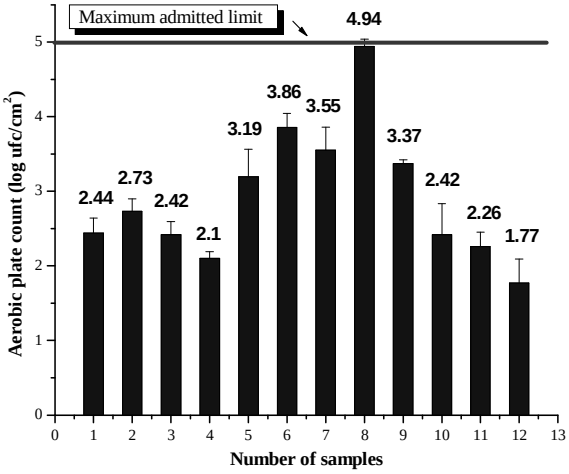

Fig.1. The total germ load from the surface of the bovine carcasses in 2010

In the studies made by Dan et al. (2003) in the bovine slaughtering units from the Cluj County, there were similar values found, between 6.1 x 10^2 ufc/cm^2 and 3.0 x 10^6 ufc/cm^2. Terrence et al. (2004), in the studies concerning the total germ number from bovine slaughtering units in USA, there were very low values obtained in the total germ load, compared to the ones found by us, which were in between 3.7 log ufc/100 cm^2-4.0 log ufc/100 cm^2.

After the statistical analysis of the obtained results at the 36 samples the following results were found: the microbial load with germ from the *Enterobacteriaceae* family found at the surface from the bovine carcasses in the year 2009 has shown values in between 0.67±0.12 log ufc/cm^2 in the month of December and 2.34±0.23 log ufc/cm^2 in the month of August. The highest values of the microbial load has been found in the course of the months June, July, August of the year 2009, without exceeding the admissible limit of 2.5 log ufc/cm^2

stated in the Reg. (CE) 1441/2007. Just like in the total germ load, the highest average values were noticed in the months of June, July and August. As a consequence, we can state that a possible cause of these high values, during the summer, could be due to the improper temperature in the manufacturing areas of the carcasses. The average germ load in the case of the samples collected in the year 2010 was in between a minimum of 0.71±0.02 log ufc/cm^2 in the month of December and a maximum of 2.94±0.05 log ufc/cm^2 in the month of August, noticing an exceeding of the maximum admissible limit in the case of three samples (9.37%), during the month of August 2010 (fig. 6). Similar to the total germ load there was noticed higher values of the *Enterobacteriaceae* at the samples collected in the period were the environment temperature was high. The statistical analysis through the ANOVA test has not shown statistically significant differences during the two years taken into study ($p>0.05$).

The study made by other researchers have put into light the germ load of *Enterobacteriaceae* family which was relatively different, because of the existent differences in the slaughtering units, its characteristics, the technology degree, the instruction and qualification of the operators. Thus, the studies made by Terrence et al. (2004), regarding the *Enterobacteriaceae* total number from bovine slaughtering units in the USA, have high lightened very low values, compared to the ones found by us, ranged between 1.3 log ufc/100 cm^2-2.1 log ufc/100 cm^2. In contrary, Adetunji and Isola (2011), in a study concerning the enterobacteria total number and its significance for the public health in Nigeria slaughter houses have revealed very high values ranging between 8.22 log ufc/cm^2 and 8.81 log ufc/cm^2.

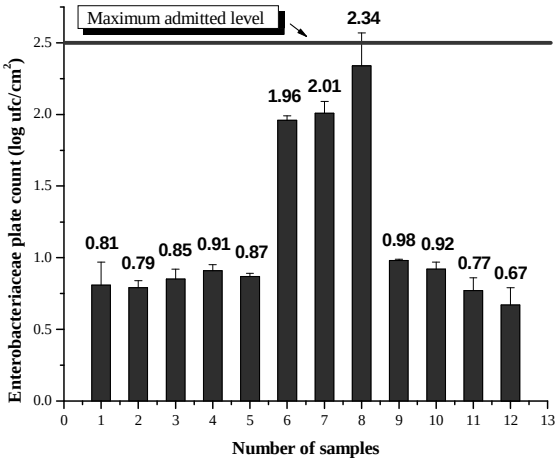

Fig 2.The identification and isolation of the pathogen germs at the bovine carcasses

The food industry operators make all the efforts to evaluate the hazards represented by the pathogens' presence in food destined for public consumption, in order to market only the products that were produced in the maximum food safety conditions. Because of the larger food poisoning episodes on a world level, the veterinary authorities have intensified their efforts to improve the food products' safety.

Along the implementation of the new hygiene pack in the EU, the food chain operators have been obligated to project and apply the management safety system (SMSA), for reducing the food poisonings (Jacxsens and Kussaga, 2009).

It was noticed that only on account of the official inspection before and prior to slaughtering of the good manufacturing practices cannot assure the making and maintaining of some elevated hygiene standards for meat in what concerns the pathogens or altering bacteria. As a consequence, it became obvious that along these ways, uncertain for microbiological risk management, in slaughtering animal units must be implemented food safety assuring systems (HACCP) (Brown and Stringer, 1998; Gill, 2002).

The ecological ways for pathogen germs: *Salmonella enteritidis/typhimurium, Listeria monocytogenes, Campylobacter jejuni/coli* and *Yersinia enterocolitica* is represented by the digestive tract of the meat animals. As a follow up, the risk of carcass contamination can e made during the course of the skinning process but mostly during the evisceration process, in the case of improper manufacturing and hygiene practices (Davies, 2011).

After the bovine carcasses analysis during the year 2009, from the 36 analyzed samples, there were 33 negative ones (91.7%), and three among them (8.3%) were not proper (fig. 15). Among these, in one of them the *Listeria monocitogenes* (2.78%) was found and *Salmonella enteritidis* was isolated in two samples, respectively 5.56%. The characteristic aspects of the *Salmonella* spp. colonies developed on the Rambach media, and that of the *Listeria monocitogenes* colonies on Oxford and ALOA media were seen. We mention that the *Escherichia coli* O_{157}:H_7, *Campylobacter jejuni/coli* and *Yersinia enterocolitica* have not been identified in the analyzed samples in the period of 2009-2010 and in the case of the samples taken during the course of the year 2010, the obtained results have shown the presence of the pathogen germs in the bovine carcasses, all of the samples being proper..

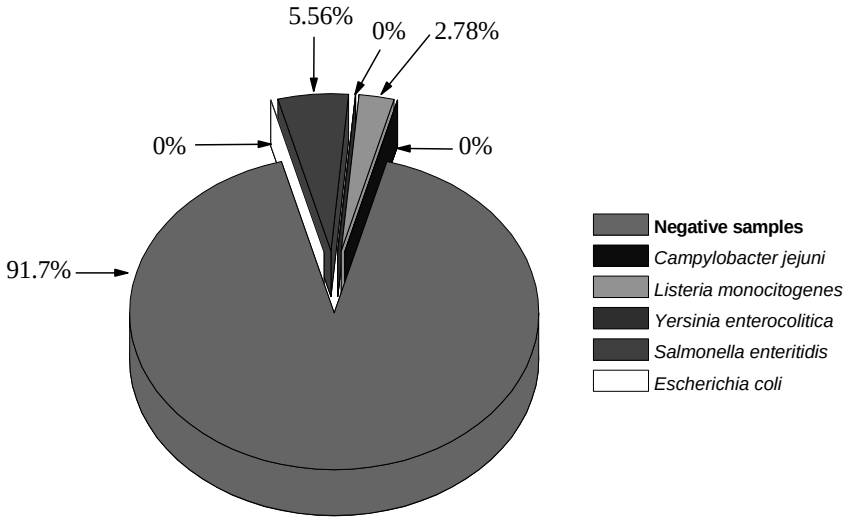

Fig. 3. The prevalence of the pathogen germs at the bovine carcasses in the year 2009

The lowest values regarding the prevalence of *Salmonella enteritidis* in the commercial slaughterhouses in U.S.A, have been presented by Sofos et al. (1999), on a number of 630 samples, taken from 30 bovine carcasses taken from seven slaughter house units. According to the shown results, this was in between the 0.8-1.7% in the case of the

collected samples during the rainy season (spring – autumn), while in the warm season (summer) there were no *Salmonella enteritidis* germs identified.

Similar results were obtained by McEnvoy et al. (2003), in a study regarding the prevalence of the *Salmonella* spp., made on 250 samples taken from bovine carcasses, in a slaughterhouse in Ireland. The prevalence of *Salmonella* spp. was of 7.6%, among which the *Salmonella typhimurium* has represented only 1.06%, compared to the total examined samples. Also, Fegan et el. 2004, in a study regarding the prevalence of *Salmonella* spp., made on 310 faces samples taken from 310 bovines raised in Australia, states that the contamination risk of the meat with *Salmonella typhimurium* is very low, taking into consideration the low number of positive samples from which this microorganism was identified.

CONCLUSIONS

The logarithmic average of the germ load on the bovine carcasses surface has shown improper values in the case of 16.67% from the total examined number of samples only in during the course of the year 2009 and that of the *Enterobacteriaceae* has exceeded the maximum admissible limit in a percent of 9.37% from the total examined samples during the year 2010. At all the species from which the samples were taken, the improper values regarding the indices which concerns the hygienic criteria from the technological process, were high lightened in the course of the warm seasons, respectively the months of May-August, which denotes the deficiencies related to the cooling systems from the units studied. The configuration of the pathogen germs at the bovine carcasses was represented by *Salmonella enteritidis* and *Listeria monocitogenes.*

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REFERENCES

1. Abdalla A.G.A., E. Siham, V.V.H. Suliman, A. Alian. (2009). Microbial contamination of sheep carcasses at EL Kadero slaughterhouse, Khartoum state. *Sudan J. Vet. Sci. Anim. Husb., 48:* 1-2.
2. Adetunji Olusol Victoria, Tajudeen Opeyemi Isola. (2011). Enumeration of *Listeria* and Enteric Bacteria of Public Health Significance on Meat Tables Before and After Sales of Meat in Ibadan Municipal Abattoir, Nigeria. *Pakistan Journal of Nutrition 10 (3):* 224-228.
3. Borch F., P. Arnder. (2002). Bacteriological safety issues in beef and ready to eat meat products as well as control measures. *Meat Sci., 62*: 381-390.
4. Brown M., M. Stringer. (1998). *Microbiological risk assessment in food processing*, Woodhead Publishing Limited, Cambridge, UK.
5. Butterorth-Heineinann. (2000). *The science of food hygiene*. 3rd Edn., Reed Educational and professional publishing ltd London, U.K.

6. Dan S.D., O. Rotaru, Gh. Răpuntean, M Mihaiu, G. Zegrean. (2003). The dynamic of psychrotrophic microflora in beef during slaughtering process. *Bul. USAMV-CN Veterinary Medicine 60*: 67-71.
7. Dan S. D., M. Mihaiu, Ioana Dalea. (2007). Microbial changes on the surface of pork carcasses due lactic and acetic acids decontamination. *Bul. USAMV-CN Veterinary Medicine, 64:* 403-408.
8. Dan S.D., M. Mihaiu, O. Rotaru, Ioana Dalea. (2008). Microbiological hazard evaluation represented by the psychrotrophic germs from the level of avian carcasses. *Buletin USAMV Veterinary Medicine, 65(2):* 240-245.
9. Davies P. R. (2011). Intensive Swine Production and Pork Safety. *Foodborne pathogens and disease, 8 (2)*: 189-201.
10. Gill C.O. (1986). The control of microbial spoilage in fresh meats. In *Advances in Meat Reasearch: Meat and Poultry Microbiology*, Pearson A.M., T.R. Dutson (Eds), MacMillan, New York, pp. 49-88.
11. Gill C.O., J.Bryant, M.Badoni. (2001). Effects of hot water pasteurizing treatments on the microbiological condition of manufacturing beef used for hamburger patty manufacture. *International Journal of Food Microbiology 63*: 243–256.
12. Gill C.O. (2002). Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In *The Microbiology of Meat and Poultry*, Davies A., R. Board (Eds), Blackie Academic & Professional, London, UK, ISBN 0 75140398 9 pp. 117-157.
13. Gill C.O., T. Jones. (1997). Assessment of the hygienic characteristics of a process for dressing pasteur- ized pig carcasses. *Food Microbiol, 14:* 81-91.
14. Heuvelink E. Annet**,** G.L. Roessink**,** K. Bosboom**,** E. de Boer. (2001). Zero-tolerance for faecal contamination of carcasses as a tool in the control of O157 VTEC infections. *International Journal of Food Microbiology, 66 (1-2):* 13-20.
15. Jacxsens L., J. Kussaga, P.A. Luning, M. Van der Spiegel, F. Devlieghere, M. Uyttendaele. (2009). A Microbial Assessment Scheme to measure microbial performance of Food Safety Management Systems. *International Journal of Food Microbiology 134*: 113–125.
16. Neil T., P. Ormel, J. L. Garcia de Silas, G. Heins, M. James. (2002). State of meat food in developing countries, p. 115.
17. Rahimi, E., H. Momtaz, F. Hemmatzadeh. (2008). The prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp. on bovine carcasses in Isfahan, Iran. *Iranian Journal of Veterinary Research, 9 (4):* 365-370.
18. Sofos J.N., Kochevar S.L., Bellinger G.R., Buege D.R., Hancock D.D., Ingham S.C., Morgan J.B., Reagan J.O., Smith G.C. (1999).Sources and Extent of Microbiological Contamination of Beef Carcasses in Seven United States Slaughtering Plants. *Journal of Food Protection, 62 (2):* 140-145.
19. Terrance M.A.. (2004). *Escherichia coli* O157 Prevalence and Enumeration of Aerobic Bacteria, Enterobacteriaceae, and *Escherichia coli* O157 at Various Steps in Commercial Beef Processing Plants. *Journal of Food Protection, 67 (4):* 658–665.
20. Yen P.K. (2003). Preventing harm from food borne illness. *Geriatr. Nurs., 24:* 376-377.
21. Zweifel C., D. Baltzer, R. Stephan. (2005). Microbiological contamination of cattle and Pig carcasses at five abattoirs determined by swab sampling in accordance with EU Decision 2001/471/EC. *Meat Sci., 69*: 559-566.
22. *** REGULAMENTUL (CE) NR. 1441/2007 AL COMISIEI din 5 decembrie 2007 de modificare a Regulamentului (CE) nr. 2073/2005 privind criteriile microbiologice pentru produsele alimentare.

23. ***Ordin nr.91/2005 al preşedintelui ANSVSA pentru aprobarea Normei sanitare veterinare ce stabileşte regulile aplicabile controalelor regulate asupra stării generale de igiena, efectuate de operatori în unități, în conformitate cu Norma sanitară veterinara privind condițiile de sănătate pentru producerea şi comercializarea carnii proaspete, aprobată prin Ordinul ministrului agriculturii, alimentației şi pădurilor nr. 401/2002, şi cu Norma sanitară veterinară privind problemele de sănătate care reglementează producerea şi comerțul cu carne proaspăta de pasare, aprobată prin Ordinul ministrului agriculturii, alimentației şi pădurilor nr. 402/2002.
24. *** WHO, 1995. Surveillance Programme. Sixth Report of WHO surveillance Programme for control of some food Infections and intoxications in Europe Berlin: FAO/WHO collaborating centre for Research and Training in food Hygiene and zoonosis.