Hygienic Quality Assessment Of Pork Carcasses Obtained In Industrial And Traditional Slaughtering Units

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Abstract

In March 2016, the microbiology laboratory of the Faculty of Veterinary Medicine Cluj-Napoca, 3 type of antiDespite the fact that many types of foods can be sources of food borne illnesses, meat and meat products are very important sources of infection in people with a wide variety of pathogens that cause food poisoning.

The aim of this study was to conduct an assessment of meat hygienic quality and configuration by assessing the microbial load and the presence of pathogens.

The material under investigation was represented by 40 samples of muscle tissue from pork carcasses collected in stages, between 2015-2016, from slaughtering units both from the traditional system and the industrial system. Samples were analyzed at the Department of Food Safety and Control of Animal Origin Products.

Total viable count in samples taken from the carcasses, in 2016 presented values similar to those in 2015, ranging from a minimum of $2.74 \pm 0.36 \log \text{ cfu/cm}^2$ in January and December and a maximum $3.73 \pm 1.13 \log \text{ cfu / cm}^2$ recorded in February, without showing significant differences between the samples processed in the studied period ($p>0.05$). The results regarding contamination of swine carcasses (surface) with germs from *Enterobacteriaceae* family were non-compliant, exceeding the maximum permissible limit of $3.0 \log \text{ cfu/cm}^2$ in all 12 samples examined (30%). In what regards the molecular testing for the confirmation of *E. coli*, a total of 6 samples were positive, but none of this tested positive for the presence of the toxicity genes (*Stx1*, *Stx2*).

In the case of the samples taken from pork carcasses the results regarding germs from *Enterobacteriaceae* family were non-compliant, 29.16% of the samples examined during the period under study were found to exceed the maximum limit, which showed deficiencies regarding the hygiene requirements.

Keywords: *hygienic quality, pork, slaughterhouse*

INTRODUCTION

Food pathogens annually cause millions of food born diseases, most of them resulting in cronic diseases or even death of the consumer. The globalization of the food chain also led to increased risks of foodborne illnesses spreading beyond international barriers. The main food that can pose life-threatening in consumer

are: meat, meat products, fish, eggs and dairy products. Among this there are: *Salmonella spp.*, *Campylobacter jejuni/coli*, *Yersinia enterocolica*, *E. coli verotoxigenic* *Listeria monocytogenes* (Algino *et al.*, 2009). The most common chain of events leading to diseases that are caused by meat consumption involves meat supplying animals that are healthy but carrying pathogens that are

Tab. 1. The primers used in the detection of Verotoxigenic *E.coli*.

Gene	Primer sequence (5'- 3')	Lenght (bp)	Alin temp. (°C)	Reference
23S <i>E.coli</i>	F: AAGGAATCACCTTGCAGATAAACTC R: TTTCCGAGTACATTGGCATCGT	736	56	Malorny <i>et al.</i> , 2004
Stx1	F: CGC TGA ATG TCA TTC GCT CTG C R: CGT GGT ATA GCT ACT GTC ACC	302	60	Blanco <i>et al.</i> , 2004
Stx2	F: CTT CGG TAT CCT ATT CCC GG R: CTG CTG TGA CAG TGA CAA AAC GC	516	60	Blanco <i>et al.</i> , 2004

subsequently transferred to humans through production, handling and consumption of meat and meat products (Bonardi *et al.*, 2008).

Universal methods for assessing the microbiological hazards posed by total viable count and main pathogenic bacteria (*E. coli*, *Salmonella spp.*, psihrotrofic bacteria) they are still less developed in our country.

MATERIALS AND METHODS

The material under investigation was represented by 40 samples of muscle tissue, taken from carcasses (as it will be described further) harvested in stages, between 2015-2016, from meat processing units both from the traditional system and industrial system. From the pig carcasses samples were taken from four different areas: chest, flank, cervical region, thigh. Sampling was done randomly, given that they come from carcasses obtained both at the beginning and at the end of slaughtering line, on different days of the week, respecting the norms.

From the surface of the muscle tissue were taken superficial flaps with a thickness of 2-3 mm, totaling an area of 100 cm². Samples were placed in sterile petri dishes with a diameter of 15 cm and transported at 4°C to the laboratory of Inspection and Control of Animal Origin Products discipline from the Faculty of Veterinary Medicine Cluj-Napoca, where they were introduced in work immediately.

The isolation of the pathogens present on the carcasses surface was made on account of the standardize methods in conformity with the Reg. (EC) 1441/2007: the *Enterobacteriaceae* identification respecting SR ISO 21528/2007, the total viable count (TVC) following SR ISO 4833/2003 the identification of *E.coli* respecting SR ISO 16649/2007.

Bacterial DNA extraction, necessary to perform the PCR technique, followed the basic steps described by Yang *et al.* (2008), with some particularities. Briefly, 150 µl of Chelex (10%) reagent (BioRad) was addes in Eppendorf tubes (1,5 ml) (RatioLab). The tubes were subjected to UV sterilization in a laminar flow microbiological hood -class II, to remove any possible contaminants from previously handling. 1-2 colonies were collected with a sterile microbiological loop and immersed in the Chelex reagent. They were used following extraction temperatures: 57°C - 30 minutes, 94°C - 5 seconds using a thermoblock.

The last step included a high speed centrifugation (14,000 rot / min.) for one minute.

The amplification was conducted using a Thermocycler which has the ability to heat and cool samples in repetitive steps. PCR MasterMix(25 µl) included: 1X PCR DNA dye, 2,5 mM MgCl₂, 5 pmol from each primer, dNTP each at 200 uM, 2,5 U of Taq DNA polymerase (Promega, Madison, WI, USA) și 100 ng genomic DNA.

PCR reaction in the termocycler was made in the following conditions: 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 secondes, 50° - 68 °C for 30 sec, 72°C for 1 minute and the final step elongation at 72°C for 7 min. The same protocol was used to identify the toxicity genes of verotoxigenic *E.coli*.

The PCR used to identify *Escherichia coli* are presented in Table 1.

The agarose gel used for the migration of the amplified DNA samples had a concentration of 2,5% si 2 %, for coloring and visualization of the products EvaGreen (1.7µl – 35 ml/gel; 7µl – 140 ml/gel) was used. The migration of the samples in the gel was done by using an electrophoresis device programmed with a power of 70 V and 100V

Fig. 1. The total viable count in the analyzed swine carcasses in 2015

Fig. 2. The total viable count in the analyzed swine carcasses in 2016

for one hour. Reading and interpreting the results was performed using a BioRad transiluminator.

The results were analyzed statistical and mathematical using the Origin 8.5 program, using the ANOVA test.

RESULTS AND DISCUSSION

The results obtained on microbial load and configuration on the surface and depth of carcasses is largely dependent on strict compliance with the rules of hygiene throughout the processing flow of carcasses.

Analyzing the graphic in Fig. 1 shows that the average logarithmic of the total viable count in meat samples taken in 2015 from the carcasses show relatively similar values between 3.18 ± 1.23

log cfu / cm² in May and 4.44 ± 0.63 log cfu / cm² recorded in August of the same year.

The value for TVC in samples taken from the carcasses, in 2016 were similar to those in 2015, ranging from a minimum of 2.74 ± 0.36 log cfu / cm² in January and December and a maximum 3.73 ± 1.13 log cfu / cm² recorded in February, without showing significant differences between the samples processed in the studied period ($p > 0.05$) (Fig. 2).

By analyzing graph from Fig. 1 and 2 it can be noticed that none of the examined samples exceeded the maximum limit allowed of 5.0 log cfu / cm² (Reg. (CE) 1441/2007).

Results regarding surface contamination of swine carcasses with germs from *Enterobacteriaceae* fam. are shown in the graphs in Fig. 3 and

Fig. 3. The Enterobacteriaceae load in the swine carcasses examined in 2015

Fig. 4. The Enterobacteriaceae load in the samples examined in 2016 from the swine carcasses

4. From their analysis, it was observed that the results for samples examined in 2015 show have an average value for Enterobacteriaceae count was from 0.87 ± 0.1 log cfu / cm² in January and a maximum of 3.52 ± 0.06 log cfu / cm² in July (Fig. 3). Of the 40 samples examined 12 samples (30 %) were non-compliant, exceeding the maximum limit allowed of 3.0 log cfu / cm². Regarding the obtained results for samples from internal muscles, taken from the carcasses this showed an average for Enterobacteriaceae count slightly lower, ranging from a minimum of 0.62 ± 0.07 log cfu / cm² in January and a maximum of 3.71 ± 0.18 log cfu / cm² in June, 9 samples having a higher value than the maximum limit (25%) (Fig. 4).

It was also found that the noncompliant values for *Enterobacteriaceae* count were detected during the range May to August, which indicates a failure of Good Hygienic Practices (GHPs) and Good Manufacturing Practices (GMPs) by operators from the slaughtering unit taken study, as well as higher values of temperature within the processing spaces that can be associated with ambient temperature.

We can appreciate that the level is lower for internal muscle samples analyzed, the differences ranging from 0.5 log cfu / cm² and 2.7 log cfu / cm². These lower values may be due to more advanced technological processes that reduce the number of germs on the surface of carcasses at very low levels, so in the evisceration and cutting

Fig. 5. The electrophoretic profile of the 736 bp corresponding to the *E.coli* bacteria. 1 – positive control sample, M- ethalon scale (100 pb); 2-7: isolated samples from the identified pork samples

process, contamination of the muscle tissue is carried only through the section tools, respectively contamination with intestinal contents where the digestive tract is sectioned accidentally 3.2-3.7 log cfu / cm² (Bolton și Pearce, 2002; Warriner, 2002). Lower levels of microbes on the pig carcasses as compared to the cattle can be explained by the fact that the head section and therefore the esophagus is not carried out, which in this case is not properly tied can result in massive contamination of the area cervical. Also, the fact that the evisceration of the GI mass is performed in block pigs, while in cattle is performed separately, is necessary to conduct an additional sections at the pylorus, which in the case of non-compliance with GHP can lead to an increased level of microorganisms of the *Enterobacteriaceae* family.

Similar results to those obtained in our study were obtained by Phouth *et al.* (2006), in order to evaluate the level of contamination with microorganisms carcasses of pigs (n = 62), subject to normal slaughter in slaughterhouses in Laos. The authors reported values between 4.4-4.9 log cfu/cm² TVC, with an average of 4.70 log cfu/cm². The total value of *Enterobacteriaceae* was between 2.1 and 3.3 log cfu/cm², with an average of 2.98 log cfu/ cm². Different results have been obtained by Algino *et al.* (2009), in small slaughterhouses from the US, where the values for TVC and *Enterobacteriaceae* were below the 1.0 log cfu/ cm².

Following the analysis of the results was found that from all samples (n=40) examined, 36 were compliant (85.7%) and in 6 samples was identified the presence of *E. coli* (14.28%).

After the isolation using conventional methods, confirmation was performed by the molecular method PCR using pathogen-specific primers, shown in the previous section, for identifying pathogenic strains and toxicity gene. The results confirmed that six of the total isolates belonged to the genus *E.coli* (Fig. 5).

The presence of *E. coli* bacteria in the carcasses investigated it is not alarming, being a bacteria that can be found naturally in the digestive tract. What would cause concern would be the presence of pathogenic strains producing toxin. Molecular testing for the presence of toxicity genes (*Stx1*, *Stx2*) confirmed that none of these isolates showed positivity.

CONCLUSION

In conducted study was concluded that from the carcasses of pigs were obtained non-compliant results regarding germs from *Enterobacteriaceae* fam., the values found exceeded allowable limits for 29.16% of all samples examined during the period under study, which shows deficiencies regarding the hygiene requirements.

In what regards the molecular testing for the confirmation of *E. coli*, a total of 6 samples were positive, but none of this tested positive for the presence of the toxicity genes (*Stx1*, *Stx2*).

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