STUDY ON N.T.G. AND ESCHERICHIA COLI CONTAMINATION OF CATTLE AND PIG CARCASSES

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ABSTRACT

The total number of germs (NTG), determined during the technological flow of processing of cattle in the slaughterhouse, provided values between 4.8 - 6.8 log CFU/cm^2 after the skinning stage and 3.1-5.2 log CFU/cm^2 after the cooling stage.

The highest values were recorded after the skinning stage in the inner thigh region, and the lowest after the cooling stage in the chest region .

In pigs, NTG recorded higher values, between 4.2 - 5.8 log CFU/m^2 after the scalding stage and 4.2 -7.1 log CFU/cm^2 after evisceration. The highest values were obtained after the evisceration stage in the external region of the thigh, and the lowest after the scalding stage in the chest region.

INTRODUCTION

The toxicity of some pesticides and other pollutants directly affects the plants but also the animals that consume those plants. If not recognized and treated properly, the toxicity of these substances can lead to morbidity and mortality to animals of the livestock farms. At the same time, the quality of the raw material can be severely affected (Bonciu, 2016, 2018)**.** Contamination of carcasses with fecal matters is an important and pursued aspect in pig and cattle slaughterhouses. The current HACCP system reduces the risk of microbial contamination of meat during the technological flow. The technological flow can be monitored by establishing critical control points, thus reducing or eliminating risks (Cola M.,2010).

The stages of the technological flow include operations to reduce the number of pathogenic bacteria but do not include complementary operations to eliminate them (Rivas et al., 2000).*Salmonella spp*. colonizes the large intestines of pigs, so they are considered asymptomatic carriers of the bacterium.

The most important sources of carcass contamination are fecal matters, gastric content, pharynx or the environment through contact with various surfaces or the hands of workers (Borch et al. 1996).

Contamination of cattle carcasses with *E. coli* may occur during the technological flow, the most important sources of contamination being represented by fecal matters. Thus, the carcasses can be contaminated during storage, by contact between them or indirectly through equipment, workers' hands, or air (Barros et al. 2007). Cattle and pig slaughterhouses must implement a HACCP system, through which to establish and reduce the risks of microbiological contamination, by obtaining quality carcasses (Veloso, Reis, 2003).

MATERIAL AND METHOD

The study was conducted in the ANSVSA food quality analysis laboratories for NTG and for *E. coli*., in animals slaughtered at the ELISIRIA Craiova slaughterhouse, Dolj and the Potcoava Olt slaughterhouse.

128 samples were taken from the carcass surface, 64 from cattle and 64 from pigs. Whole carcasses were used, chosen from the technological flow, both from the primary and from the final processing stage, each sample was collected by

wiping an area of 100 cm^2 using a sterile swab. The surface was delimited with the help of a sterile template. After sampling, the swabs were placed in tubes with peptone water.

From the pigs, 32 samples were collected, after scalding, from the region of the neck, chest and the outer part of the other thigh, 32 samples were collected after evisceration, from the same regions.

From cattle, 32 samples were collected after skinning, from the chest, flank and inner thigh. Another 32 samples were collected after cooling from the same regions.

From the initial samples, serial dilutions up to 10-6 with sterile saline were performed. The total number of aerobic mesophilic germs was determined for dilutions 10-4 and 10-5 on nutrient agar by incorporating 0.1 ml of inoculum. For each dilution, two plates were made. The seeded plates were incubated at 37 C for 24 hours.

Specific selective media were used to determine the indicator microorganisms. To determine the probable number of *E. coli,* the ANSVSA standard 78323/1998 was used. According to this standard, a series of three test tubes, containing selective enrichment medium and Durham tubes, with the sample to be analyzed and its decimal dilutions, are seeded. This medium is represented by broth with tryptone and lauryl sulfate. The tubes thus prepared are incubated at 37°C for 24 hours. After this interval, the second selective enrichment medium, namely the EC broth, is seeded from each test tube in which gas evolution has been observed.

The tubes are incubated at 45°C for 24 hours. From each test tube considered positive for *E. coli*, culture was taken and inoculated on a selective medium, respectively Levine. On this medium, the presence of *E. coli* species was confirmed by the appearance of slightly domed colonies, dark purple with metallic polish and golden-green reflections. At the same time, one test tube with tryptone water is incubated in each test tube with *E.c*. medium which showed a gas release and incubated at 45°C for 48 h. After incubating the test tubes with tryptone water, 0.5 ml of reagent Kovacs is added to each to identify the presence of indole.

Positive tubes were examined at UV light and fluorescence tubes were positive and confirmed for *E. coli.* It is considered a positive reaction if a red ring appears on the surface of the culture.

Calculation of the most probable number of *E. coli* is performed depending on the number of tubes containing culture considered positive, using the Mac Grady table.

For highlighting *Salmonella spp.,* the SR.EN ISO 6579/2002 standard was observed. According to this standard, 25 ml of sample were inoculated into peptone water (bpw) and were incubated for 24 hours at 37°C. After 24 hours, 2 types of enrichment media were seeded: Rappaport-Vassiliadis (RVS) and Muller-Kaufmann (MKTTN), environments on which other related species develop. Thus, 0.1 bpw in 10 ml RVS, 24 hours at 42°C and 0.1 ml BPW in 10 ml MKTTN 24 hours at 37°C were inoculated.

For selective isolation, the solid enrichment medium was also used: Istrati-Meitert, 24 hours at 37°C. After 24 hours, 5 colonies were taken from each plate and passed on nutrient agar for 24 hours at 37°C. Confirmation was made by M.I.U. and T.S.I. tests

RESULTS AND DISCUSSIONS

The presence of micro-organisms on the carcasses may in certain conditions be dangerous for the consumer. The evaluation of the carcass microflora, as well as the identification of the stages of the technological flow, during which they can be contaminated, are important in establishing the preventive and corrective measures through which the evolution of the microflora can be controlled. The total number of germs (NTG), determined during the technological flow of cattle processing in the slaughterhouse,

provided values between 4.8 - 6.8 log CFU/cm^2 after the peeling stage and 3.1-5.2 log CFU/cm^2 after the cooling stage.
The highest values were recorded after the skinning stage in the inner thigh region, and the lowest after the cooling stage in the chest region (Table 4.1.)
In pigs, NTG recorded higher values, between 4.2 - 5.8 log CFU/cm^2 after the scalding stage and 4.2 -7.1 log CFU/cm^2 after evisceration. The highest values were obtained after the evisceration stage in the outer thigh region, and the lowest after the scalding stage in the chest region (Table 4.2.).
Arruda Pinto (2004) in his study on pig carcasses, determined the NTG, recording a similar value of 5.2 log CFU/cm^2, after scalding and a value of less than 3.6 log CFU/cm^2 after evisceration.
Borch (1996) determined NTG in pig carcasses, finding a value of 3.8 log CFU/cm^2, in the pre-evisceration stages. Rivas (2000) obtains a similar average of 4.3 log CFU/cm^2 after scalding.

Table 4.1

NTG/cm ^{2} values on different regions of cattle carcasses during the technological flow in the slaughterhouse

Technological stage	Carcass region	No. of examined samples	NTG (log UFC/cm ^{2})		
			Minimum value	Maximum value	Average
Skinning	Chest	10	5.3	6.1	5.7
	Flank	10	4.8	5.8	5.3
	The inside of the thigh	12	5.2	6.8	6.0
Cooling	Chest	10	3.1	4.5	3.8
	Flank	10	3.5	5.2	4.3
	The inside of the thigh	12	3.3	4.8	4.0

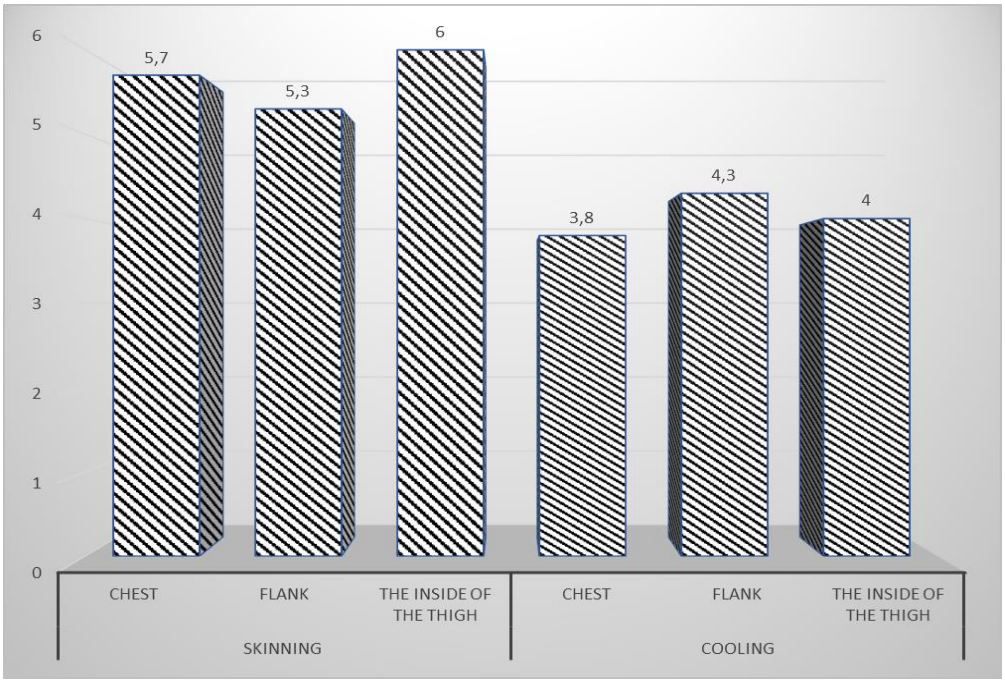

Figure 1 ***Values on different regions of cattle carcasses during the technological flow in the slaughterhouse - NTG (log UFC/cm 2)***

Table 4.2

NTG/cm^2 values on different regions of pig carcasses during the technological flow in the slaughterhouse

Technological stage	Carcass region	No. of examined samples	NTG (log UFC/cm^2)		
			Minimum value	Maximum value	Average
Scalding	Neck	10	4.5	5.3	4.9
	Chest	10	4.1	5.2	4.6
	The outer part of the thigh	12	5.1	5.8	5.4
Evisceration	Neck	10	4.8	5.6	5.2
	Chest	10	4.2	5.3	4.7
	The outer part of the thigh	12	5,6	7.1	6,3

The high values of NTG after skinning (cattle) are the consequence of a large load of microorganisms with which they reach the slaughterhouse and after evisceration (pigs) the high values of NTG are explained by the specifics of this operation when the carcass can come into contact with gastrointestinal mass and even with its content, a situation that is more common when the operation is performed incorrectly.

The presence of ***E. coli*** on the surface of the carcasses represents an indicator of sanitation and demonstrates the non-observance of the stages of the technological flow. *E. coli* was more frequently identified in the hindquarters of the carcass in cattle, where 58.3% of the positive samples were obtained from the inner wall of the thigh after skinning.

Kings, Veloso and Wheat, (2003) obtained higher values in cattle (2.8 log CFU/cm^2, after skinning and lower values (1.89 log CFU/cm ^{2}) after washing.

In pigs, 66.6% of positive samples were obtained from the external region of the thigh, after evisceration. Lower values were obtained from the other regions (Table 4.3.).

Arruda Pinto (2004) records a contamination of pig carcasses with *E. coli* 60% after scalding and 16.6% after evisceration.

Present *Salmonella spp.* was not observed in cattle carcasses, but was identified in 8 samples out of the 64 analyzed, from pig carcasses, totaling 12.5%.

The large number of positive samples (carcasses) may be due either to the carrier animals that are slaughtered in the slaughterhouse or to cross-contamination on the technological flow.

Korsak et al. (2003) obtained a value of 11.2% positive samples (17 out of 152 samples) with *Salmonella spp*., compared to McDowel et al. (2007), who obtained a much higher value of 40% positive samples (205 samples out of a total of 513 samples).

Table 4.3

E. coli/cm ^{2} indicator values on different regions of the carcasses of cattle and pigs during the technological flow in the slaughterhouse

Species	Technological stage	Carcass region	No. of samples	Positive samples		Negative samples	
				No.	%	No.	%
Cattle	Skinning	Chest	10	2	20	8	80.0
		Flank	10	3	30	7	70.0
		The inside of the thigh	12	7	58.3	5	41.7
	Cooling	Chest	10	-	-	10	100.0
		Flank	10	-	-	10	100.0
		The inside of the thigh	12	2	16.0	10	84.0
Pigs	Parboiling	Neck	10	4	40.0	6	60.0
		Chest	10	2	20.0	8	80.0
		The outer part of the thigh	12	5	41.0	7	59.0
	Evisceration	Neck	10	5	50.0	5	50.0
		Chest	10	4	40.0	6	60.0
		The outer part of the thigh	12	8	66.6	4	33.4

CONCLUSIONS

The study revealed that from a microbiological point of view, the stages that contribute most to the contamination of carcasses in the slaughterhouse are: skinning (cattle) and scalding and evisceration (pigs), steps that require special attention to reduce their contamination.

Among the body regions analyzed, the inner region of the thigh in cattle carcasses and the outer region of the thigh in pigs are the regions with the greatest deficiencies from a microbiological point of view, most often providing positive results to the examined parameters.

Percentage of carcass contamination with *E. coli*, compared to the total of 64 samples for each species, was 21.8% for cattle and 42.1% for pigs.

Salmonella spp. was identified in the samples collected from pigs, in a percentage of 12.5%.

The establishment of measures, starting from the permanent training of slaughterhouse staff on critical points, the application of advanced technologies and the maintenance of good hygiene and operation of facilities and spaces are absolutely necessary to reduce contamination and improve the microbiological quality of cattle and pig carcasses.

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