Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XVI/B 2017

Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XVI/B 2017

RESULTS CONCERNING THE DYNAMICS OF CONTAMINATION OF PASSENGER CARCASES DURING THE EMERGENCY PROCESS

Hriscu (Ursu) Elena*, Ismană(Ciobotaru) Irina Elena*, Usturoi Marius Giorgi **

* Sanitary Veterinary and Food Safety Laboratory, Aleea Mihail Sadoveanu no. 10, 700490, Iaşi, Romania, e-mail: dr_ursu_elena@yahoo.com
** University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Aleea Mihail Sadoveanu nr. 8, 700490, Iaşi, Romania

Abstract

The study was carried out in two slaughter units, from which 160 samples (represented by sanitation tests) were taken by 80 samples / unit from four stages of the slaughtering process.

From a total of 160 samples analyzed, 100% of the contaminated samples were tested for NTG and Escherichia coli parameters in both slaughter units.

For the Salmonella spp and Campylobacter spp. Parameters of the total of the 160 samples analyzed, 100% negative samples were obtained.

The highest microbial development was in the first harvest stage for both the total germ count (NTG) with an average of 1.7 x 10 3 ± 19.19 µg / ml (Unit A) and 1.6 x 103 ± 35.89 µg / ml (unit B) with a coefficient of variation of 5.05% and 9.70% and for Escherichia coli obtaining an average value of 1.3 x 103 ± 23.25 µg / ml (unit A) and 1.3 x 10 3 ± 26.63 µg / ml (unit B) and a coefficient of variation of 7.79% - 8.78%.

Key words: *microbial contamination, chicken carcasses, slaughterhouses*

INTRODUCTION

By the term "meat" is meant the edible part of any animal slaughtered in a slaughterhouse (according to Codex Alimentarius), so that a definition of this word, "meat", means the totality of tissues and organs of animals entering into human food regardless of the zoo they belong to (Iurca IM 1998).

The chicken is a remarkable achievement of genetics and nutrition, a realization that could be achieved through scientific and technological advances (Van I. et al., 2003).

For slaughtering, birds are the raw material for processing. Some factors that directly condition meat production include: race and hybrid, body weight, age, sex, growth rate and cutting yield (Jakobsen L et al., 2010).

Modern hygienic conditions, as well as supervised and qualified control, have been created by modern technology applied in slaughterhouses, which has led to the provision and guarantee of

microbiologically safe products (Usturoi M.G., 2008).

At European and world level, food-borne infections caused by pathogenic bacteria have been frequent, with a large extension, with very important economic and social effects (Vacaru-Opriş, I. (coordinator) et al., 2004).

The sanitary quality of food, sanitation, water and air are the basic links to the chain of these guarantees (Banu C., 2007).

From this point of view, we tried to identify the degree of contamination of the chickens during the slaughtering process, from different slaughter units.

The samples were microbiologically tested using standardized and accredited RENAR working methods, the parameters analyzed being: *NTG*, *Escherichia coli, Salmonella spp*. and *Campylobacter* spp.

The detection and enumeration of the microorganisms developed on the special environments of each working method was performed according to the standards in force SR EN ISO 4833/2014; SR ISO 16649-2/2007; SR EN ISO 6579: 2003/AC: 2009; SR EN ISO 10272-1/2006.

For calculation and expression of the results obtained from these samples, calculation formulas according to SR EN ISO 7218/A1:2014, general guide for microbiological examinations were used.

Samples were harvested from two different slaughterhouses of slaughtered broiler chickens.

MATERIAL AND METHOD

The study was performed on a number of 160 samples from two different slaughter units of 80 samples / unit. The period was divided into four stages of harvesting as follows: stage I - live birds at the time of their hanging on the conveyor; stage II chicken carcases before evisceration; stage III - chicken carcases after evisceration; stage IV - chicken carcases after refrigeration.

From each stage, 20 samples were collected on the surface of the chicken carcases of the two slaughterhouses.

The studied material was represented by carcasses from the chicken broiler chickens Ross 308, slaughtered at the age of 40 days.

For enumeration of microorganisms developed at + 30^0C (NTG), it was performed according to standard SR EN ISO 4833-1/2014.

Decimal dilutions 10^{-1} and 10^{-2}were performed. From these dilutions, 1 ml of each dilution was inoculated into Petri dishes over which approximately 15 ml of pre-cooled PCA agar medium was added at + 44 ° C to + 47 ° C in the water bath.

Carefully mix the inoculum with the medium and allow the mixture to solidify by placing the Petri dishes on a cold horizontal surface. The time interval between inoculum distribution and environmental casting should not exceed 15 min.

The inoculated plates were thermostated at + 30 ° C for 72 ± 3 hours (fig 1).

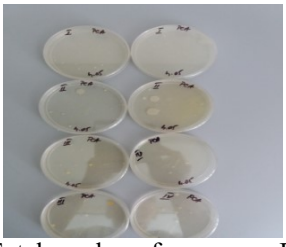

Fig. 1 - Total number of germs per PCA agar

To isolate and identify bacteria of the genus *Escherichia coli*, the ISO 16649-2 / 2007 standard was worked out.

Decimal dilutions 10^{-1} and 10^{-2}were performed, unaware of the microbial load.

With a sterile pipette, one sterile Petri dish was transferred to 1 ml of each dilution, and another pipette was used for each dilution.

Pour approximately 15 ml of TBX agar medium (Tris-Bila-glucuronate) into each Petri plate, pre-cooled to + 44 ° C to + 47 ° C in the water bath. Carefully mix the inoculum with the medium and allow the mixture to solidify by placing the Petri dishes on a cold horizontal surface.

The time interval between inoculum distribution and environmental casting should not exceed 15 min.

The inoculated plates were returned and introduced into a thermostat set at + 44 ° C for 18 hours to 24 hours (fig. 2).

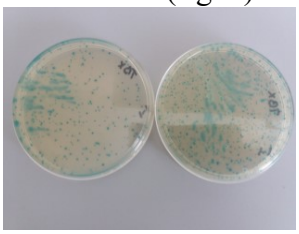

Fig. 2 - Typical colonies of E. coli on TBX agar

The working method for isolation and identification of bacteria of the genus *Salmonella spp* was performed according to the standard SR EN ISO 6579: 2003/AC: 2009.

Detection of Salmonella spp. requires four successive phases:

1. Pre-enrichment in non-selective liquid media;
2. Enrichment in liquid selective media;

3. Isolation and identification;

4. Confirmation of identity.

The isolation and identification of bacteria of the genus *Campylobacter spp.* has been performed according to the standard SR EN ISO 10272 -1/2006.

Selective enrichment - from the sample to be analyzed, a known amount was obtained, in our case 10 ml to make a 10/100 dilution using a Bolton broth; was incubated at + 37 ° C for 4-6 h then at + 41.5 ° C for 44h under anaerobiosis conditions.

Isolation and identification

With a sterile loop, the Bolton broth is cut from the surface of a Charcoal Cefoperazone Deoxycholate Modified Agar Base agar plate. Plates thus sown are incubated at + 41.5 ° C for 40-48 hours in a microaerobic atmosphere.

After the incubation period there were no specific microbial growths for *Campylobacter spp.,* and for this reason no biochemical tests were performed.

Expression of results - indicate the presence or absence of germs of the genus *Campylobacter spp*. in the test sample, specifying the mass in grams or the volume in milliliters of the test sample.

RESULTS AND DISCUSSIONS

Following these analyzes, microbial growth was found on broiler chickens in both units, for the microbiological parameters of *Escherichia coli and NTG* (total germ count).

Of the total of 160 analyzed samples, represented by sanitation tests collected from different areas on the surface of the chicken carcasses from the four stages of harvesting the two slaughter units, we obtained the following results:

”UNIT A”

Of the 80 samples analyzed on a lot of chickens brought to slaughter on a farm of this unit, positive samples were obtained for the *NTG and E. coli* parameters in all four harvesting stages of the slaughtering flow (tab 1).

For the *NTG* parameter examined from live birds, an average value of 1700 ± 19,19 ufc/ml was obtained, then the microbial load declining in the next process step to 875 ± 14,66 ufc/ml, because at the end of the harvesting stage, after refrigeration, the mean value reached 394 ± 8,47 ufc / ml.

According to these values obtained for this analyzed parameter, a coefficient of variation is found between 5,05% in the first stage of harvesting and 9,61% in the last stage after refrigeration, which means good

homogeneity; this indicates a very good uniformity of the character being analyzed.

With regard to the results of the microbiological examination for the Escherichia coli parameter, the mean value obtained in the first step of harvesting from live birds is 1335 ± 23,25 ufc/ml and 29,55 ± 1,25 ufc/ml in the last step harvesting after refrigeration.

The values obtained for this analyzed parameter indicate a coefficient of variation between 7,79% and 18,55%, which indicates an average homogeneity of the analyzed character.

”UNIT B”

Of the 80 samples analyzed on a chicken group brought to slaughter, from a chicken broiler farm belonging to this unit, positive samples were obtained for *NTG and Escherichia coli* parameters in all four stages of harvesting on the slaughtering flow (Table 2).

The results obtained for the *NTG* parameter analyzed from live birds resulted in an average of 1655 ± 35.89 ufc/ml, but the microbial load began to decrease in the next process step to 854,50 ± 12,53 ufc/ml for as at the end of the harvesting stage, after the refrigeration the average value reached 382,5 ± 7.35ufc/ml.

As a result of the values obtained for this analyzed parameter, a coefficient of variation between 9,70% in the first stage of harvesting and 8,60% in the last stage after refrigeration results, which means good homogeneity; this indicates a very good uniformity of the character being analyzed.

Also with regard to the results of the microbiological examination for the *Escherichia coli* parameter, the mean value obtained in the first harvesting step on live birds was 1355 ± 26,63ufc/ml and 28,85 ± 1,00ufc/ml in the last step harvesting, ie after refrigeration.

Due to the results obtained for this analyzed parameter, the coefficient of variation was between 8,78% and 15,55%, indicating an average homogeneity of this analyzed lot.

For the parameters of Salmonella spp and *Campylobacter spp*. out of the total of the 160 samples analyzed from the two slaughter units, all were negative.

In order to check the microbial load on the contamination of poultry carcases from the pathogen infestation pathway (*Salmonella spp. and Campylobacter spp.*), all were negative (Lindblad M et al., 2006).

In other research (AS Abu-Ruwaida et al., 2005) the microbial load on the chicken carcasses during the slaughter process had a high contamination (after scalding and depletion) and varied with different types of organisms (*Enterobacteriaceae, coliforms , Escherichia coli*).

Table 1

The results of the microbiological examination carried out in unit "A"

Specification	Estimators of statistics (n=20)			
	$\bar{X} \pm s_{\bar{x}}$(ufc/ml)	v%	minimum	maximum
live birds				
NTG	1700±19,19	5,05	1600	1900
Escherichia coli	1335±23,25	7,79	1200	1500
carcases before evisceration				
NTG	875±14,66	7,49	730	990
Escherichia coli	46,7±1,37	13,14	38	59
carcases after evisceration				
NTG	796,5±13,10	7,35	690	890
Escherichia coli	42,25±1,09	11,56	33	51
housing after refrigeration				
NTG	394±8,47	9,61	330	470
Escherichia coli	29,55±1,25	18,55	21	41

Table 2

The results of the microbiological examination carried out in unit "B"

Specification	Estimators of statistics (n=20)			
	$\bar{X} \pm s_{\bar{x}}$(ufc/ml)	v%	minimum	maximum
live birds				
NTG	1655±35,89	9,70	1300	1900
Escherichia coli	1355±26,63	8,78	1200	1600
carcases before evisceration				
NTG	854,50±12,53	6,56	760	940
Escherichia coli	46,65±1,10	10,57	39	55
carcases after evisceration				
NTG	790±12,22	6,92	670	880
Escherichia coli	38,95±1,13	12,99	32	50
housing after refrigeration				
NTG	382,5±7,35	8,60	330	450
Escherichia coli	28,85±1,00	15,55	21	38

The level of microbial contamination in other slaughter slaughterhouses (Huang Ho, N.Y., and Chen B.J., 2004) decreased after the washing step and before evisceration.

Other researchers (Adriana Morar et al., 2008) identified critical control points from the slaughter flow by analyzing microbiologically regarding contamination of poultry carcases resulting in different microbial loads at each stage of the process.

Pacholewicz Ewa et al., 2016, studied the level of contamination from the slaughterhouse flow in two slaughterhouses on the identification of *Campylobacter spp and Escherichia coli*, the results being variable and identifiable in both units.

The microbial load with Escherichia coli in the first stages of slaughtering of chickens according to the analysis of other researchers (Dan Sorin Daniel et al., 2013) obtained values of 2,0 ± 1,62 log ufc/ml, and 3, 1 ± 2.32 log ufc/ml.

In studies carried out by other researchers (Lindbland M. et al., 2006) on slaughter poultry carcasses, no pathogenic bacteria such as *Campylobacter spp, Salmonella spp*., etc., were detected, but for a total number of total aerobic microorganisms (*NTG*) had an average of values of 3,4 and 4,4 log cfu/ml and for *Escherichia coli* 2,2 and 3,6 log cfu/ml.

CONCLUSIONS

From the analyzes we performed, we found that chicken carcasses were contaminated with the same type of bacteria, decreasing as values obtained after each stage of the process, although the samples come from different growth farms.

In unit "A", the microbial load for the *NTG* parameter decreased from the first step (live birds) to the final harvesting stage (refrigerated carcasses) by 76,83%.

For the *E. coli* parameter. the microbial load dropped from the first step (live birds) to the last step (carcases after refrigeration) by 97,83%.

The results obtained in unit "B" regarding the microbial load for the *NTG* parameter from the first harvesting stage (live birds) to the last stage (carcases after refrigeration) decreased by 76,92%.

For the *E. coli* parameter. the microbial load decreased from the first harvesting stage (live birds) to the last stage (carcases after refrigeration) by 97,88%.

The results show that the degree of contamination between the two slaughter units studied is similar to the obtained values.

With modern slaughtering and processing technologies in poultry slaughterhouses compliance with hygiene rules must not allow the production of contaminated products with pathogenic germs but can not be fully achieved because the sources of contamination of poultry and meat are multiple and difficult to remove .

REFERENCES

1. Abu-Ruwaida A. S. *, w. N. Sawaya**, b. H. Dashti, m. Murad and h.a. Al-othman - Microbiological Quality of Broilers During Processing in a Modern Commercial Slaughterhouse in KuwaitFood Technology Group, Food Resources Division, Kuwait Institute for Scientific Research, Kuwait.
2. Banu C., 2007 –Sensory quality and analysis of food products. Ed. AGIR.
3. Dan Sorin Daniel , Platon Sorin , Mihaiu Marian , Jecan Carmen , Cordea Darius , Cordis Ionut Vald and Lapusan Alexandra * Microbial Risk Assessment in the Main Steps of Poultry Slaughter, Buletin UASVM, Veterinary Medicine, 70(2)/2013.
4. Iurca, I. M. 1998 - Meat and meat products Ed. ICPIAF, Cluj-Napoca.
5. Jakobsen, L., Kurbasic, A., Skjøt-Rasmussen, L., Ejrnæs, K., Porsbo, L.J., Pedersen,K., Jensen, L.B., Emborg, H.D., Agersø, Y., Olsen, K.E., Aarestrup, F.M., FrimodtMøller, N., Hammerum, A.M., 2010. Escherichia coli isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. Foodborne Pathog. Dis. 7 (5), 537–547
6. Jean l. Schoeni, michael p. Dolyle (2003) department of Food Microbiology and Toxicology an Departament of Food Science Volume 60, Pages 2958-2962
7. Lindblad M,*Lindmark H., Thisted Lambertz S., and Lindqvist R. National Food Administration, P.O. Box 622, SE-751 26 Uppsala, Sweden MS 06-043: Received 27 January 2006/Accepted 7 March 2006
8. Morar Adriana , Gh. Milovan, Claudia Sala, I. Stănchescu- Establishing the Bacterial Control Points In Poultry Slaughterhouse; Lucrări Ştiinţifice Medicină Veterinară VOL. XLI, 2008, Timişoara;
9. Pacholewicz Ewa, Arno Swart, jaap a. Wagenaar, Len j. A. Lipman, and Arie H. Havelaar (2016) Explanatory Variables Associated with Campylobacter and Escherichia coli Concentrations on Broiler Chicken Carcasses during Processing in Two Slaughterhouses. Journal of Food Protection: December 2016, Vol. 79, No. 12, pp. 2038-2047;
10. Usturoi, M.G., 2008 –Bird breeding. "Ion Ionescu de la Brad" Publishing House, Iaşi.
11. Vacaru-Opriş, I. (coordonator) et al., 2004 – Treaty of poultry, vol. III. Editura Ceres, Bucureşti.
12. Van, I. et al., 2003 - Growing and industrialization of chickens. Ed. Ceres, Bucuresti.
13. SR EN ISO 6579:2003/AC:2009 - Horizontal method for the detection of Salmonella bacteria
14. SR ISO 16649-2/2007 - Horizontal method for enumeration of ß-glucuronidase-positive Escherichia coli; Part 2: Colony counting technique at 44^0C using 5-bromo-4-chloro-3-indolyl β-D-glucuronate.
15. SR EN ISO 4833-1/2014 –Horizontal method for enumeration of micro-organisms; Part 1: Colony counting technique at 30^0C by plate casting technique.
16. SR EN ISO 10272 -1/2006 - Horizontal method for the detection and enumeration of Campylobacter spp. Part 1: Detection method.
17. SR EN ISO 7218/A1/2014 – Microbiology of food and feed. General requirements and guide for microbiological examinations. Amendment 1.