Scientific Works. Series C. Veterinary Medicine. Vol. LXVII (2), 2021
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

SOME INDICES OF CONTAMINATION OF POULTRY PRODUCTS WITH BACTERIA OF THE GENUS *Salmonella* spp. AND *Listeria* spp.

Olga JUNCU, Nicolae STARCIUC, Tatiana ANTOHIEV, Natalia OSADCI

State Agrarian University of Moldova, str. Mircesti 44, Chisinau,republica Moldova

Corresponding author Nicolae STARCIUC email: n.starciuc@uasm.md

Abstract

The scope of the proposed research was to establish the presence of bacteria of the genus Salmonella spp. and Listeria spp. in the samples of poultry meat and eggs sold in the commercial food network of the Central Agricultural Market from m. Chisinau. The bacteriological investigations were performed in the laboratory of Microbiology of the Department II of the Faculty of Veterinary Medicine, SAUM. The serotyping of the isolated bacteria forms was performed at the Republican Center for Veterinary Diagnosis. The following culture media were used for isolation and identification: RVS broth, MKTTn broth, XLD agar, BSA, semi-Fraser broth, ALOA agar, Ottaviani and Agosti agar, Oxford agar. In total, 120 samples including 60 egg samples, 20 samples of frozen poultry carcasses and 40 samples from refrigerated broiler carcasses were collected and examined. As a result, the bacteriological investigation on the eggs of current consumption has established the presence of bacteria of the genus Salmonella typhimurium. Also, in the samples of frozen meat were isolated the serotypes Salmonella enteritidis and Listeria monocytogenes and finally, in the samples of refrigerated poultry carcasses was established the presence of serotypes Salmonella typhimurium and Listeria monocytogenes. The bacteriological investigations of poultry products (eggs and meat) showed the presence of their contamination with bacteria of the genus Listeria monocytogenes in 2.5% of the examined samples and with Salmonella bacteria in 8.3%, which may lead to the transmission of these bacteria to consumers by means of contaminated products.

Key words*: samples, contamination, poultry products, Salmonella, Listeria.*

INTRODUCTION

In recent years, poultry production as a branch of the livestock sector in the Republic of Moldova remains one of the most developed branch with a high priority share in the consumption of animal products at the national level. Therefore, obtaining safe poultry products in terms of microbial contaminants is an essential component of commercial poultry production.

The protection of poultry flocks against contamination with undesirable micro-organisms requires constant monitoring by the veterinary health service, as well as the maintenance of high-level biosecurity measures for bird populations. Nonetheless, the application of daily biosecurity procedures can contribute to reduce the possibility of contacting zoonotic microbial infections with an important impact on public health such as: salmonellosis, campylobacteriosis, listeriosis, etc. (Sexton, 2018; Youn, 2017) Compared to other bacteria, the genus *Salmonella* spp.bacteria has been determined as a frequent cause of food poisoning. In addition, some *Salmonella* serotypes (*S. gallinarum, S. pullorum, S. enteritidis*, etc.) which have been detected in chickens raised for meat, or in laying hens can cause serious clinical symptoms in birds; thus, providing an increased risk of poultry meat and eggs contamination with these bacteria (Hardie, 2019; Nidaullah, 2017).

Currently, at European level, approximately 200 *Salmonella* serotypes are associated with foodborne infections in humans, of which two serotypes are considered more dangerous for public health: *Salmonella enteritidis* and *Salmonella typhimurium.* However, these two serotypes have successfully been reduced in many countries due to the introduction of strict biosecurity measures, effective surveillance of poultry flocks and vaccinations against salmonellosis (Asma, 2018; Webber, 2019).

There are various mediums where the horizontal transmission of the above-mentioned infections occurs: in the contaminated feed, in the incubation stations' equipment, in the buildings for birds raising, in the process of slaughtering and storage of poultry products.

Another, important route of contamination with the bacteria of the genus *Salmonella* spp., *Listeria* spp. etc. remains the elements of the poultry product marketing chain. Hence, good management of production processes and bio-safety of technological processes, as well as systematized control within different segments of the production chain are critical aspects that can minimize the risk of contact and persistence of infections (Bourassa, 2019; Li, 2018).

The goal of our research was to establish the degree of presence and the diversity of the genera *Salmonella* spp. and *Listeria* spp. in poultry meat and eggs for common consumption, given the highly negative health impact that these bacteria can have over humans and poultry flocks.

MATERIALS AND METHODS

The researches were conducted at the Department II of the Faculty of Veterinary Medicine of State Agrarian University of Moldova. Serotyping of isolated bacteria *Salmonella* spp. and *Listeria* spp. were performed at the Republican Center for Veterinary Diagnostic and at the National Agency for Public Health. As research materials served the samples from the poultry carcasses and eggs of current consumption from some units for the production of poultry meat and eggs such as: SRL "Silver Bird", v. Ciorescu, mun. Chisinau, SRL "Codim Com", v. Sadaclia, district Basarabeasca, "IM PB Nord" SRL, district Edinet, v. Blesteni, SRL "Intervetcom", district Cimisliea, SRL "Redi Agro", district Dondușeni, v. Tirnova, SRL "Dant Agro", district Ungheni, v. Pirlița, SRL "Solar Nord" district Edinet, v. Gordinesti, Avicola "Riscani", district Riscani, v. Corlateni.

The isolation and identification of bacteria of the genera Salmonella spp. was performed according to the methodology SM EN ISO 6579-1: 2017 - Microbiology of the food chain. The horizontal method was used for the detection, counting and serological typing of bacteria such *Salmonella*, as well as bacteria of the genera *Listeria* spp., according to the methodology SM EN ISO 11290-2: 2017 - Microbiology of the food chain, horizontal method was used for the detection and enumeration of Listeria spp. The samples were subjected to classical microbiological tests using the national standards methods and the confirmation was made using "Microbact" tests according to the manufacturer's instruction for Listeria spp. - ATCC 19118 and for *Salmonella* spp. ATCC 14028. For the isolation and identification of the bacterial forms were used ordinary, selective and special culture media (peptone water buffered, XLD agar (Xyloze Lysine Deoxycholate), BSA (Brilliance Salmonella Aga), Semi-Fraser broth, ALOA agar, Agar Oxford), monoreceptor sera for serotyping. Totally for examinations were taken 60 samples of eggs and 60 poultry carcasses samples (40 samples from refrigerated carcasses and 20 samples from frozen carcasses).

RESULTS AND DISCUSSIONS

Some investigations of the incidence of salmonellosis in laying birds have been performed based on pathomorphological observations specific for avian salmonellosis. Attention was drawn to the presence of enterocolitis, vetellin peritonitis, salpingitis, and ovaries. The incidence of mortality caused by this changes ranged from 2 to 4%. In broiler chickens, symptoms and pathomorphological changes specific for salmonellosis (diarrhea, enterocolitis and liver miliar necrosis) ranged from 3 to 5% of growing chickens. No specific clinical symptoms and macroscopic changes were detected for listeriosis in flocks of birds. In order to establish the presence of pathogenic serotypes of bacteria of the genera *Salmonella* spp. and *Listeria* spp., samples of eggs and meat were taken from the poultry enterprises mentioned in the material and methods.

The results of bacteriological investigations showed an increased number of *Salmonella* spp. colonies in over 75% of the samples taken from bird carcasses, which demonstrates the presence of *Salmonella* serotypes on objects that come in contact with poultry products.

For the isolation and identification of bacteria of the genera *Salmonella* spp., samples were taken from poultry carcasses with a weight of 25 g, which were subsequently inoculated into 225 ml APT and incubated. After incubation, the culture obtained in RVS broth was inoculated on the surface of the Petri dish with

the selective environment XLD (Xyloze Lysine Deoxycholate) and BSA (Brilliance Salmonella Aga) to obtain isolated colonies. Colonies with a typical morphological structure of Salmonella grown on the XLD medium have a black center and a bright transparent area of red color (Figure 1), and on the Brilliance Salmonella Agar medium, the *Salmonella* spp. colonies are purple (Figure 2).

Salmonella H2S serotypes negative on XLD medium are dark pink with a dark center. Lactose-positive *Salmonella* on XLD are yellow with or without a black center.

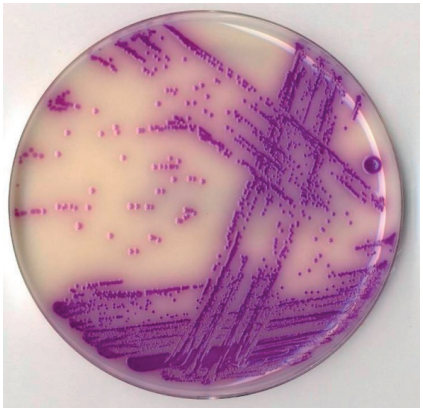

Figure 1. Brilliance Salmonella Agar, (purpury colonies of *Salmonella*)

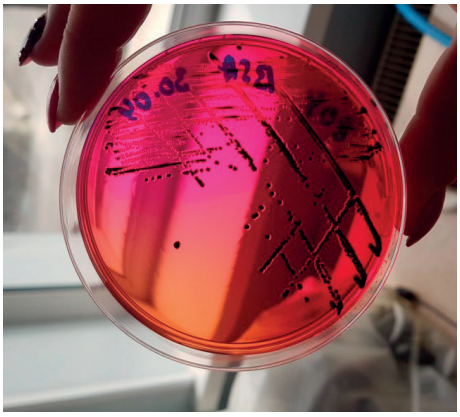

Figure 2. Typical *Salmonella* colonies on XLD medium with black center and transparent area and red color

The level of contamination with *Salmonella* bacteria was determined by counting *Salmonella* colonies on the surface of Petri dishes. Inoculations performed on Brilliance Salmonella Agar medium from samples taken from frozen meat carcasses ranged from 54 to 127 colonies. In the case of inoculations from refrigerated carcass samples, the number of *Salmonella* colonies ranged from 89 to 187.

In the case of inoculations from lavages taken from the surface of egg samples, the number of *Salmonella* colonies varied from 69 to 114 colonies, but in the case of inoculations performed from egg contents, the number of colonies varied from 6 to 17.

In the case of inoculations performed on XLD agar from samples taken from frozen carcasses, the number of *Salmonella* colonies ranged from 31 to 135, and from refrigerated carcasses the number of colonies ranged from 77 to 205.

Inoculations from egg surface washings confirmed the presence of *Salmonella* colonies with variations from 43 to 127 colonies, and in the case of inoculations from egg contents the number of colonies ranged from 8 to 25.

For the isolation and identification of bacteria of the genera *Listeria* spp., a primary enrichment was performed with 25 g from the meat sample in 225 ml of selective liquid of enrichment medium, with low concentration of selective agents (Semi-Fraser broth) and subsequent incubation according to the established method. From the culture obtained by enrichment by striation, it was applied on the surface of the first selective medium - ALOA agar to obtain isolated colonies, and later on the second selective striation medium - Oxford agar.

On the nutrient medium Agar ALOA, the typical colonies of *Listeria* spp. are green-blue surrounded by an opaque halo (Figure 3). On the nutrient medium Agar Oxford, the typical colonies of *Listeria* spp. after 24 h incubation are small (1 mm), gray, surrounded by a black halo. After 48 hours they become darker in color, with a possible greenish tint, with a diameter of 2 mm, with a black halo and a concave center (Figure 4).

In the case of inoculations taken from frozen poultry carcasses, there was one positive sample out of 20 examined, but in the case of inputs taken from samples from refrigerated carcasses, from 40 examined samples two samples were contaminated with *Listeria* (Table 1). The number of *Listeria* colonies in the case of inoculations performed from

samples of frozen carcasses varied from 4 to 28, and from refrigerated samples the number of colonies varied from 33 to 62.

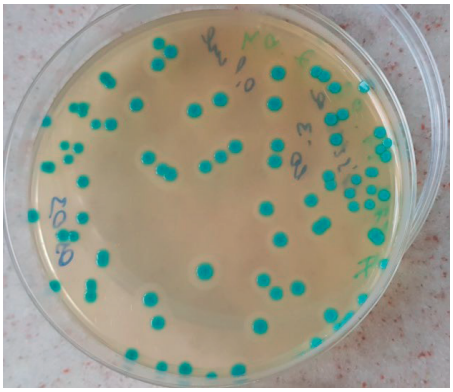

Figure 3. ALOA agar - the tipical colonies of *Listeria monocytogenes*

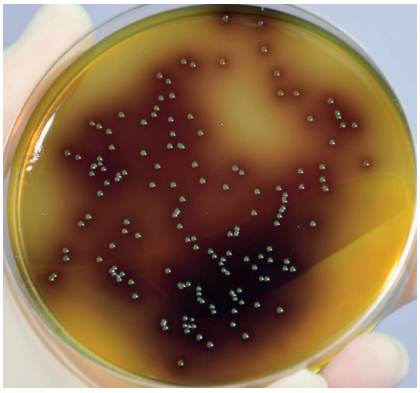

Figure 4. Oxford agar - the tipical colonies of *Listeria* spp.

Subsequently, smears were prepared from colony cultures and examined under a microscope (ob.10x100). Figure 5 shows the morphological structure of bacteria of the genus *Salmonella* spp. which is placed in the field of the microscope in separate rods or in piles with oval heads. The microscopic examination of the smears prepared from the colonies of bacteria from the genus *Listeria* (Figure 6) are represented by sticks having the shape of short thin form or boomerang shape.

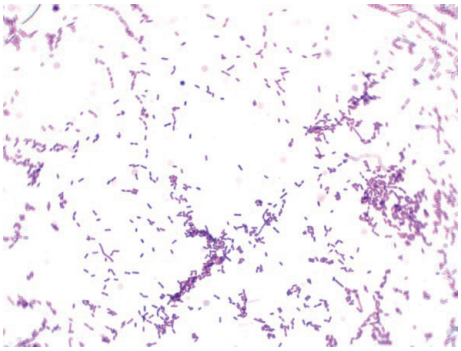

Figure 5. *Salmonella* spp. (separate sticks or ples, gr -)

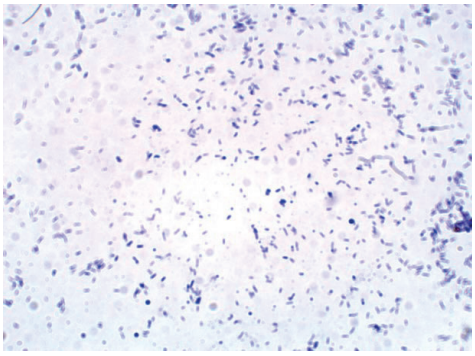

Figure 6. *Listeria* spp. (short, thin sticks, gr +)

Salmonella spp. Colonies were serotyped sing monoreceptor sera (Figure 7), biochemical investigations, commercial putties (Micobact test, Figure 8) with the use of positive controls.

Figure 7. Monoreceptoric sera for serotyping of *Salmonella* spp.

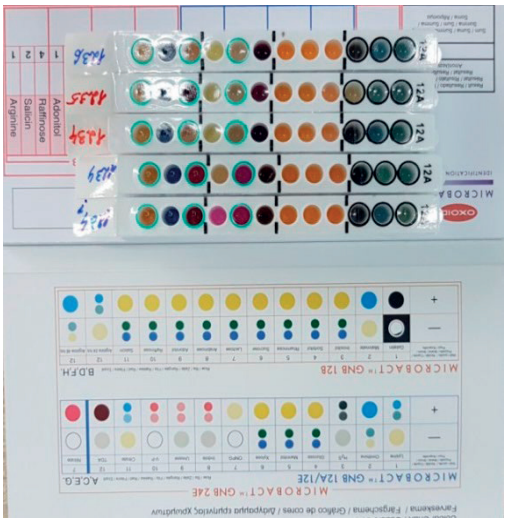

Figure 8. "Micobact" test for serotyping of *Salmonella* spp.

As a result of serotyping the colonies of bacteria of the genera *Salmonella* spp., and *Listeria* spp., were established the results that are presented in the Table 1.

The results of the performed serotyping confirmed the presence of 13 positive samples with pathogenic serotypes of *Listeria* spp. and *Salmonella* spp. Out of 120 examined samples, from the poultry carcasses were isolated and serotyped only 3 positive samples of the bacteria from genera *Listeria* or 2.5% of the examined samples. Positive samples with bacteria of the genus *Salmonella* pathogenic to humans and birds (*S. enteritidis* and *S. typhimurium*) were isolated from 10 samples, which constituting 8.3% of the total number of examined samples of which 4 samples were isolated from eggs for current consumption, and 6 samples from meat carcasses.

Table 1. Results of serotyping of bacteria of the genera *Salmonella* spp. and *Listeria* spp.

Nr.	Product name	Number of examined samples	Bacterial genera			Nr. of positive samples	% of contamination
			L. mono citogenes	*S. enteritidis*	*S. typhi murium*		
1	Eggs for current consumpion	**60**	-	-	4	4	6.6
2	Frosen poultry carcasses	**20**	1	1	-	2	10
3	Refrigelated poultry carcasses	**40**	2	3	2	7	17.5
Total		**120**	**3**	**4**	**6**	**13**	**10.8**

CONCLUSIONS

1. The research on the circulation of pathogenic serotypes of bacteria of the genera *Salmonella* spp. and *Listeria* spp. showed that the veterinary measures currently taken are not sufficient to prevent the incidence of contamination of poultry products with these bacteria; therefore, a perspective program that will have as priority to monitor and analyze the risks of contamination for poultry units and for humans has to be implemented.

2. The pathomorphological analyses confirmed the presence of specific changes for salmonellosis in laying hens such as: enterocolitis, vetellin peritonitis, salpingitis and enterocolitis, liver infarction in broiler chickens, with an incidence from 2 to 4%.

3. Bacteriological investigations of poultry carcasses showed that from the total number of examined samples, the incidence of positive samples with the presence of *L. monocytogenes* was 2.5% and the incidence of pathogenic serotypes of *Salmonella* spp. was detected at 8.3% of examined samples of eggs and carcasses with the predominance of serotypes *S. enteritidis* and *S. typhimurium* which implies a major risk for contamination of consumers of poultry products with these pathogenic bacteria.

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