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THE IMPORTANCE OF CONTAMINATION WITH SALMONELLA SPP. IN ORDER TO OBTAIN SAFE PRODUCTS

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Abstract

This paper aims to present the prevention and control of the bacteria from the Salmonella spp. genus in an intensive farm type, with growing ground technology for laying hens. The work is based on studies made at the farm S.C. Avicola Găeşti S.R.L. The bacterias belonging to the Salmonella genus cause various symptoms, from asymptomatic to "typhoid-like" syndromes in children or animals with high susceptibility. In adults Salmonella spp. are mostly responsible for food-borne diseases. For this reason salmonellosis are considered specific zoonoses. The World Health Organization (WHO) considers salmonellosis as one of the most important diseases caused by consumption of contaminated food. Regarding the species, Salmonella typhimurium and Salmonella enteridis are incriminated as the most important causes of food-borne diseases with serious risks in terms of human health. The basic characteristic of Salmonella contamination is that there are no organoleptic changes in order to draw the attention at the possible presence of germs, the eggs have the appearance color, smell and taste unchanged. In this study was used the official RENAR method, more specific the Horizontal method for the detection of bacteria of the Salmonella spp. genus for food offered for public consumption - SR EN ISO 6579: 2003 / AC 2009. Measurements were done for both shell and egg contents, loads being formed of 10 consumption eggs A category, Class L (63-73 g). The results revealed no bacteria of the Salmonella spp. genus regarding both determinations carried out on the shell as well as those that incurred the content (Absent / 25 g shell Absent / 25 g content). Therefore, the compliance of biosecurity measures in farm, bacteriological control of feed, manure and eggs are fundamental requirements in preventing the occurrence of bacteria of the Salmonella spp. genus which may jeopardize the safety and wholesomeness of the products obtained in the poultry sector.

Key words: *biosecurity, sanitation, safety, food-borne disease, zoonosis.*

INTRODUCTION

Salmonellosis are infectious diseases caused by bacteria of the *Salmonella* genus, which produce human and animal illness, most commonly encountered being enteric disease and septicemia, abortion, arthritis and respiratory infections, sometimes resulting a state of carriers and excreted over a long period of time. Salmonellosis have a universal diffusion and are specific zoonoses, some strains are ubiquitous, and others having regional character. Most strains are pathogenic, requiring only 15-20 cells to cause infection, but this is influenced by the age of the person concerned, the physiological state, some pre-existing infections and the strain involved. In terms of food poisoning, caused by Salmonella, we can say that, in terms of frequency and implications hygienic - health, they occupy first place in most countries (CDC, 2007). Food poisoning caused by Salmonella, occure more frequently in warmer seasons (spring - autumn) when there are more human and animal carriers, the temperature being one of the predisposing factors for the development and multiplication of bacteria. The possibility of human infection is increased in the context of the circulation through contaminated food or raw materials during the technological flow, storage or distribution (CDC, 2010). Due to the high resistance of Salmonella in the environment and food, the disease can be triggered even by eating food in the form of powder with shelf life of years. Also,

increased incidence of Salmonella infections in gallinaceous due to the ubiquitous presence of germs and the existence of the carriers, but also in humans. Salmonella is one of the most important veterinary problems due to economic losses and their implications in human health by triggering food poisoning from eating contaminated food (CDC, 2008**).**

MATERIALS AND METHODS

Research has been carried out during 2014, the determinations being carried out on a total of 5 lots. Each lot has corresponded to a total of 10 samples that were analyzed in a total number of 50 samples. A sample was represented by egg consumption from the A category (63-73 g). The determinations were both on the content and the shell of the eggs. Studies of this scientific work were held in the holding S.C. Avicola Găeşti S.R.L., a farm with intensive technology type of growth for laying hens. Sampling was an important step for laboratory examination results are largely contingent on how to harvest and transport them. Sampling was done in strict aseptic conditions, avoiding possible external contamination. Samples corresponding to each lot, harvested and individualized by serial number, were quickly dispatched to the laboratory where they were subjected to bacteriological investigations regarding the isolation, identification and serotyping germs, according to standards. The samples were analyzed using the methodology in *Horizontal method for the detection of bacteria of the genus Salmonella spp. for food offered to public consumption - SR EN ISO 6579: 2003 / AC 2009 and accredited RENAR official method*. The principle of the method consists in the isolation and identification of bacteria, on the basis of cultural, morphological, biochemical, serological, and in the case of widespread and important serovars in relation to epidemiology, phage sensitivity is set, bacteriocinotipia and antibiotipia. Important is the initial stage, the isolation and identification of the genus, which involves the differentiation of other members from the *Enterobacteriaceae* family. Examination methods include, firstly, bacteriological exam, which involves bacterioscopic examination, cultivation, isolation and identification of Salmonella. Bacteriological methods ensure the insolation of bacteria and identification of different morphological and cultural characters on which the classification may be done in strains of species and genera (CDC, 2011). In general, Salmonella can be isolated by a variety of techniques that can use or not pre-enrichmed media to revive Salmonella with reduced viability, enrichment media that contain inhibitory substances for contaminating germs. Selective media is used for differential diagnosis allowing their differentiation from other enteric bacteria. Because Enterobacteriaceae are Gram - negative and medium size like many other bacteria of other genera and families, direct microscopy is not usually useful in identifying salmonella. However, an examination of cultures obtained from samples investigated, germs with cocobacillary and bacillary form can be observed, Gram-negative, non-encapsulated, unsporulated but with cilia, which can be divided into Salmonella group. Bacteriological examination is aimed at isolating and respectively identifying species that has the highest diagnostic value, which is why it was done in the shortest time after the receipt of samples. Thus, for the isolation of Salmonella were used at the same time simple and usual media, pre-enrichmed and enrichmed media, then the culture was transferred to selective media for the isolation and purification of cultures. Bacteriological methods ensure the insulation of bacteria, and explore the different features by which it can be stated with certainty the nature of the contamination and to properly orient measures against possible pathological states (Chai et al., 2012). Salmonella can be isolated by a variety of methods that use pre-enrichment or not to revive bacteria with reduced viability, enrichment media that contain inhibitory substances contaminating germs and selective media for differential diagnosis that can be distinguished from other enteric bacteria. Cultural aspects of strains of *Salmonella spp*., isolated on different media, can take various expressions. On the surface of *nutrient agar, Salmonella spp.* develop

colonies of small, smooth or rough, semi-transparent. On blood agar, the majority of the colonies developed are species of the Enterobacteriaceae family. They are usually quite large, 2-3 mm respectively, after 24 hours in the thermostat, non-hemolytic, shiny, round and gray. Among the pre-enrichment media, buffered peptone water (BPW) is a vital medium for the isolation of Salmonella in eggs. The presence of bacteria, regardless of serovar seeded in this environment, increases the turbidity of the environment that acquires an opalescent appearance (Clarkson et al., 2010). The *Rappaport – Vasiliadis broth* is one of the commonly used enrichment media for the isolation of Salmonella in food of animal origin respectively eggs. If the presence and development of germs after 48 hours of incubation, the media changed in color from dark blue to light blue. Sowing on *agar with red phenol and brilliant green (Edel and Kampelmacher)* strains of *Salmonella spp*. cause transfer of the media color to red and colonies are large, white with transparent edges. Instead, *Escherichia coli* turns the environment yellow-green and the colonies are small, yellowish-white, smooth and glossy. Regarding the development of salmonella in *selective media and differentiation*, most of salmonella do not ferment lactose and produce pale colonies on *MacConkey agar* and an alkaline reaction in the environment (Clogher et al., 2012). However, it should be noted that some strains of *S. arizonae* are lactozo-positive and some strains of *S. typhimurium* were reported plasmids with genes encoding fermentation of lactose. Most Salmonella however give an alkaline reaction in the *brilliant green agar* and develop red colonies. It is worth mentioning that on the environment *Mac Conkey Salmonella spp*. grow translucent colonies, colored, medium size and smooth or rough compared to colonies of *Escherichia coli* which have a white color (Henao et al., 2010). On the *Levine environment*, Salmonella genus forms medium-sized, semi-transparent, tinted colonies, unlike *Escherichia coli* that formes metallic tint, black colonies. On the *Istrati-Meitert environment*, a moderately selective medium for enterobacteriaceae, *Salmonella spp*. develop blue-green colored colonies with dark blue to black center (due to H_2S production), while Escherichia coli colony forming is small, pink-reddish (Clogher et al., 2012). *XLD medium (xylose-lysine-deoxycholate)* is a moderately selective medium for *Enterobacteriaceae*, which was used in all samples studied. On this medium the strains of Salmonella grows H_2S positive colonies with black center and pink edges and transparent. The negative H_2S colonies are translucent reddish pink, and the E. coli colonies are yellow. On *XLD*, most Salmonella serotypes produce hydrogen sulfide and pink-red colonies with a black center. Note that, on *XLD environment*, some strains of *Proteus* can mimic Salmonella colonies with a black center (production of H2S), but the colony periphery tends to have a yellowish tint (Cronquist et al., 2012). *The Rambach medium is a solid medium, which we used to differentiate between Salmonella spp. and other members of the Enterobacteriaceae family. This environment provides the research of a new character phenotype of the Salmonella spp., respectively the formation of the acid from propylene glycol, feature that can be used in combination with a color indicator for beta-galactosidase (the use of lactose L+), in order to distinguish Proteus spp. and other members of the Enterobacteriaceae family. As inhibitors of Gram positive environment we included the deoxycholate.* Except for *S. typhi* which appears particularly bright red colonies on this medium allowing an easy differentiation of *Salmonella* species, from those of the genus *Proteus*. On this medium, cultures of *Salmonella spp*. colonies formed a bright red color. A visual examination of *Rambach agar* plates and observing red colonies provide unambiguous detection of *Salmonella* strains (Crump et al., 2008). Making an appropriate selection to ensure specificity close to 100% is possible by combining two characteristics: distinctive red coloring colonies that metabolize propylene (local acidification causing precipitation of neutral red in the colony) and an intense blue staining of galactosidase producting colonies. Examination of a single colony is sufficient,

in contrast to conventional media which needs five colonies, because among colonies of Salmonella there are colonies from the species *Citrobacter* and *Proteus*. This simple selection reduces the number of examinations by excluding those who ought to be made for false positive colonies (Henao et al., 2012).

RESULTS AND DISCUSSIONS

The results of tests for Salmonella spp. *Horizontal method for the detection performed by bacteria of the genus Salmonella spp. for food offered to public consumption - SR EN ISO 6579: 2003 / AC 2009* is provided in the following five tables.

As can be observed, no positive samples were detected for either of the 5 groups analyzed respectively did not reveal *Salmonella spp*. by tests carried out on 50 samples collected, 10 samples for each batch of analysis.

Table 1. The results for Lot 1

Sample number	Content	Shell
Sample 1	Negative	Negative
Sample 2	Negative	Negative
Sample 3	Negative	Negative
Sample 4	Negative	Negative
Sample 5	Negative	Negative
Sample 6	Negative	Negative
Sample 7	Negative	Negative
Sample 8	Negative	Negative
Sample 9	Negative	Negative
Sample 10	Negative	Negative

Table 2. The results for Lot 2

Sample number	Content	Shell
Sample 1	Negative	Negative
Sample 2	Negative	Negative
Sample 3	Negative	Negative
Sample 4	Negative	Negative
Sample 5	Negative	Negative
Sample 6	Negative	Negative
Sample 7	Negative	Negative
Sample 8	Negative	Negative
Sample 9	Negative	Negative
Sample 10	Negative	Negative

Table 3. The results for Lot 3

Sample number	Content	Shell
Sample 1	Negative	Negative
Sample 2	Negative	Negative
Sample 3	Negative	Negative
Sample 4	Negative	Negative
Sample 5	Negative	Negative
Sample 6	Negative	Negative
Sample 7	Negative	Negative
Sample 8	Negative	Negative
Sample 9	Negative	Negative
Sample 10	Negative	Negative

Table 4. The results for Lot 4

Sample number	Content	Shell
Sample 1	Negative	Negative
Sample 2	Negative	Negative
Sample 3	Negative	Negative
Sample 4	Negative	Negative
Sample 5	Negative	Negative
Sample 6	Negative	Negative
Sample 7	Negative	Negative
Sample 8	Negative	Negative
Sample 9	Negative	Negative
Sample 10	Negative	Negative

Table 5. The results for Lot 5

Sample number	Content	Shell
Sample 1	Negative	Negative
Sample 2	Negative	Negative
Sample 3	Negative	Negative
Sample 4	Negative	Negative
Sample 5	Negative	Negative
Sample 6	Negative	Negative
Sample 7	Negative	Negative
Sample 8	Negative	Negative
Sample 9	Negative	Negative
Sample 10	Negative	Negative

Negative results were recorded both through content analysis and by analyzing the shell, the components belonging to the samples considered in this study.

CONCLUSIONS

Isolation of bacterial species, belonging to the genus Salmonella may be achieved by a

variety of methods that may use the pre-enrichment for reviving bacteria with reduced viability or with an enrichment media containing inhibitory substances for contaminated seeds, as well as a number of selective media allowing differential diagnosis of other enteric bacteria.

Bacteriological examination of egg samples was done by conventional methods derived from national and international standards, which have as a principle the isolation and identification of germs.

Laboratory examinations targeting Salmonella deceleration from samples studied were carried out according to international standardized reference method (*SR EN ISO 6579: 2003 / AC 2009*), with the compulsory pre-enrichment and enrichment stages, preliminary isolation of Salmonella.

Growing on media enrichment is an important step in detecting Salmonella that contaminate food. Choosing appropriate enrichment conditions allow for greater specificity and sensitivity of detection, while the use of inappropriate media may lead to a total failure and thus to increased risks to consumers.

Growing in two stages, which include non-selective broth recovery and selective cultivation under strict conditions, is considered today as the most feasible procedure for Salmonella enrichment. Duration of the procedure can theoretically be reduced by shortening the periods of cultivation in enrichment environments, which ensure faster and more efficient recovery and accelerating growth of target bacteria while suppressing background microbial development.

Concomitant use and comparison of a wide range of environments specific to Salmonella isolation proved superior environmental performance of *Rambach*, a chromogenic medium that ensures unambiguous detection of *Salmonella* strains, which greatly reduced the time and volume of investigations, goal extremely important for diagnosis promptness so necessary for the detection and prevention of animal and human salmonellosis episodes.

Rambach environment proved to be extremely useful, allowing exclusion of false positive colonies that before, needed biochemical confirmations; examining a single colony is sufficient, in contrast to conventional media from which was necessary to repick suspect colonies, as among colonies of *Salmonella* may also be colonies of *Citrobacter* and *Proteus*. The results revealed no bacteria of the genus Salmonella spp., both determinations carried out on the shell as well as those incurred when on content as shown in the results presented in the previous section 5 tables of contents. Following the determinations it proves that on the farm biosecurity measures are kept.

Bacteriological control of feed, manure and eggs are fundamental requirements for prophylaxis occurrence of bacteria of the Salmonella spp. genus which may jeopardize the safety and wholesomeness of the products obtained in the poultry sector.

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