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RESEARCH ON THE CONTAMINATION WITH BACTERIA OF THE SALMONELLA SPP. GENUS REGARDING THE FEED OF LAYING BIRDS AND FOOD SAFETY IMPLICATIONS

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Abstract

This study is necesary for preveting feedind the hens with feed that is contaminated with bacteria from the Salmonella genus to prevent the transmission of diseases caused by this kind of bacteria and to monitor the microbiological quality of the rations in order to improve food safety. Due to Salmonella implications in importat economical losses and their implications in human health by triggering food poisoning from eating contaminated products makes this one of the most pressing issues in veterinary medicine. There are infectious diseases caused by pathogens of the genus Salmonella. They are universal spread and specific zoonoses, some strains are ubiquitous, and others with regional character. In terms of food poisoning caused by Salmonella we can say that, in terms of frequency and sanitary implications, they occupy an important place in most countries of the European Community. The possibility of human infection is constantly growing in the context of the circulation of contaminated food. The disease can be triggered by eating powdered products with an increased shelf life. Microorganisms are present in feed, soil and surface water. In birds that carry it, the bacteria are located in the digestive tract, gall bladder, ovary and they can eliminate it in the environment all lifelong. Specific serotypes are represented by S. enteridis and S. typhimurium. Venue of the study was the unit S.C. Avicola Găeşti S.R.L. Measurements were taken on samples of feed and fodder, harvested amount is 1kg / sample, the sample is then divided into 5 units of equal mass. The method of investigation used was the Horizontal method for the detection of Salmonella spp. - SR EN ISO 6579:2003 / AC 2009, a method that is RENAR approved. At the end, measurements found no pathogens in samples of feed and fodder (Absent / 25 g). Using this method, it proved one of the most important aspects, that of the desire to obtain safe and quality products coming from the poultry sector.

Key words: *quality, contamination, fooder, food-borne disease.*

INTRODUCTION

The *Salmonella* genus includes bacteria widespread in nature, being found in the digestive tract of mammals, birds, cold-blooded animals, food, feed, soil and surface waters. Most strains are parasitic to humans and animals that can cause disease or condition generate carriers that excrete the bacteria a long timeIn addition, the importance of such germs in animal and human pathology is resulting from the fact that preventing the infection with Salmonella by interrupting the circuit in nature is very difficult because, with few exceptions, they do not host specificity, their survival and multiplication can be possible even in abiotic environment represented by food, feed, water (Bhunia et al., 2008). One of the main reservoirs for human infection is represented by chickens (Foley et al., 2008). In humans, the presence and severity of symptoms depend on the infecting dose (**Akbar et al., 2013**). Typically, there is a watery diarrhea lasting several days, which results in dehydration, lower abdominal pain and fever. Sepsis and abscess formation are rare. Salmonella are bacteria with a cell membrane with a complex structure and provides them a membrane consisting of external and internal peptidoglycanic structure

containing muramic acid and other components between the layers. The genus *Salmonella* includes Gram negative, asporogenous, most with mobility, the presence of cilia placed peritrih (except the species *Salmonella gallinarum* and *Salmonella pullorum*). Salmonella are enterobacteriaceae pathogenic to man and animals with the following main characters: ferment glucose with gas production, they produce hydrogen sulfide, can use citrate as a sole carbon source, does not ferment lactose and sucrose and do not produce indole, and urease (**Aktas et al., 2007**). There are over 2,700 serotypes of Salmonella, all included in the genus *Salmonella*. Within the genus there serotypes which do not have some of the general features referred to, such as lactose fermentation (slow fermentation - too late) and the production of hydrogen sulfide: these are, however, very rare serotypes. *Salmonella* major groups are represented by: group I - *Salmonella enterica* subsp. *enterica*; group II - *Salmonella enterica* subsp. *salamae;* group III - *Salmonella enterica* subsp. *arizonae;* group III b - *Salmonella enterica* subsp. *diarizonae;* group IV - *Salmonella enterica* subsp. *houtenae;* group V - *Salmonella enterica* subsp. *indica* (**Abid, 2009**). We conclude that salmonellosis causes significant losses in farms due to mortality (which is sometimes considerable), abortion, increasing delays, costs incurred with the treatment and the application of preventive measures (**Matias et al., 2010**). Add to this the importance of health because animal Salmonella are often responsible for the appearance, in humans, of evolving severe food poisoning (**Barnett et al., 2011**). Also, due to the pathogenicity that members of this genus poses to humans and the fact that they frequently contaminate various food, feed, soil, surface water, makes the species of the Salmonella genus of particular interest (**Burkholder et al., 2008**).

MATERIALS AND METHODS

The study was conducted during the year 2014 by analyzing the administered compound feed in hens feeding during October, November and December. The determinations of this scientific work took place at the farm S.C. Avicola Găeşti S.R.L., with an intensive type of growth of chickens on the ground for eggs. Fodder presented as batch, for each group being harvested 1 kg sample, which is divided into five equal units and ground, each weighing 200 g. Five samples were collected for each calendar month, making a total of 15 samples. Sampling has posed a significant step for laboratory tests, the results were influenced by the way they were harvesed and transported. Sampling was done under strict conditions, avoiding possible contamination of particles or microorganisms from the external environment. The sample for each lot, harvested and individualized by serial number was quickly sent to the laboratory where it was subjected to bacteriological investigations regarding the isolation, identification and serotyping of germs, as required by law. Samples were analyzed using algorithms provided *in Horizontal method for the detection of bacteria of the genus Salmonella spp. – SR EN ISO 6579:2003 / AC 2009*, official and accredited RENAR 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 genus and differentiation involving other members of the family *Enterobacteriaceae*. Examination methods primarily include classical bacteriological exam, which involves bacterioscopic examination, cultivation, isolation and identification of *Salmonella* in feed (Matias et al., 2010). Classical bacteriological methods ensure the insulation of bacteria and identification of different morphological and cultural characters on which the classification may be strains of species and genera (Carrique-Mas et al., 2008). In general, Salmonella can be

isolated (various backgrounds) through a variety of techniques that can call or not the pre-enrichment of Salmonella with reduced viability, enrichment media that contain inhibitory substances contaminated with germs and selective media and differential diagnosis which can be distinguished from other enteric bacteriaIsolation and identification of Salmonella depends not only on quality of media, from the laboratory, the technical skills of specialists and serovars growth characteristics, especially those adapted to specific hosts (Jennings et al., 2011). Bacterioscopic examination involves using *Gram staining method*. The principle of this method is based on the reaction of the dye solution and the structural components of bacterial cell wall. Thus, if Gram positive bacteria, which have a thicker wall, rich in polysaccharides and ribonucleate magnesium, gentian violet and iodine complex forms that do not fade under the action of alcohol-acetone keeping the blue-violet. In contrast, Gram negative bacteria, in which the cell wall is thinner than that of the Gram-positive, crystal-violet-iodine complex is extracted from the cell, and after recolorării with Fuxin, Gram negative bacteria will be red. Bacteriological examination is conducted in several stages, varying in number and sequence, depending on the quality of feed samples, culture media, and serovars of *Salmonella spp.* suspected. For Salmonella isolation there is a rich assortment of culture media, liquids and solids, used for pre-enrichment, enrichment, selective for their character or for differential diagnosis, media combination the use of which is left to the specialists in laboratory depending of experience and availabilities (Maddadi et al., 2010). In our own determination we used the classical method of isolation and identification using standardized methods for isolation and culture media recommended for the genus *Salmonella* germs. The media are the usual *simple nutrient broth, agar nutrient, agar and blood*. Pre-enrichment media are non-selective liquid media having a role in the revitalization of bacteria. The medium used is buffered peptone water (BPW). Enrichment media are liquid media, the composition of which allows the selective growth of Salmonella, while inhibiting the growth of other bacteria. The media for this purpose must meet two essential characteristics: to stimulate the multiplication of Salmonella, while inhibiting the associated flora. In an attempt to optimize the isolation of these germs were created many such environments, the choice of which is a rather difficult, given the substrate examined and its total microbial load. The media with selenite inhibits a greater degree of association flora, but also presents some degree of toxicity for Salmonella. Selenite broth with basic nutritional substrate is peptone and inhibitory substance for the rest of enteric bacteria, selenite. Cystine supplementation favors, in addition, the development of salmonella. Lactose also present in the environment formula fermented by some species of flora associated acidifying the environment that gets disgenetic for many of the Enterobacteriaceae concurrent and continuous, pH within certain limits, can be favorable to the development of salmonella. If the pH falls below 5.8, all Enterobacteriaceae are inhibited, while, between 5.8 - 6.3, Salmonella are selectively favored. At a pH greater than 6.3 some enterobacteria (*Proteus* and *Enterobacter* especially) multiply rapidly and compete with Salmonella, canceling their multiplication (Klinkenberg et al., 2011). Tetrathionate broth being less an inhibitor nevertheless has the disadvantage of low selectivity. Although it is a peptone medium, the content of bile salts inhibit Gram positive flora, brilliant green inhibits gram-positive lactozo - fermentation and the Gram positive and sodium thiosulfate, although not completely innocuous for Salmonella, is somewhat less toxic than other for enteric bacteria (Coburn et al., 2007). Currently, the major trend is to develop enrichment media for the isolation of several bacterial pathogens simultaneously, for example, enriched broths can be used for the detection of both species of the genus *Salmonella*, and

Listeria. However, the simultaneous detection of pathogens may be a proliferation of certain bacteria that can lead to false negative results (El-Bassiouny et al., 2008). Media used were the *enrichment broth Muller - Kauffmann tetrathionate - novobiocin, MKTTn, phenol red agar and brilliant green (Edel and Kampelmacher).* Selective differentiation media and seeded with ribbed surface in order to obtain isolated colonies, depending on the composition of the media to present different characters with respect to the taxonomic group to which they belong. The results are read after an incubation for 24-48 hours at 37°C. All the selective media contain a nutritive base insulation, one (usually lactose) or two sugars (lactose and sucrose), a color indicator bacteria which ferment sugars with the production of acid from the medium, and most also have a system of highlighting the production of hydrogen sulfide (Maddadi et al., 2010). There are media that allow a variable extent differentiated growth. They inhibit the growth of other bacteria and provide information on some key biochemical characteristics differentiating Salmonella. Results can be read after incubation for 24-48 hours at 37°C. In such environments, Salmonella colony forming is characteristic which can be distinguished from colonies of other bacteria which have not been inhibited. However, the difference between *Proteus* and *Citrobacter* is particularly difficult. Use of selective media is not recommended if the cell concentration is low. As a result, a cultivation period of non-selective enrichment media is preferred, usually at the start of the analysis. On the other hand, the introduction of selective agent in the culture media enriched non-selectively has been shown to reduce to a minimum the excessive increase in background microflora (Matias et al., 2010). Antibiotics, such as novobiocin or malachite green, can significantly reduce the amount of bacteria background and therefore may contribute to the recovery of Salmonella. However, the addition of antibiotics in the early stage of cultivation in rich media, can also cause stress to Salmonella, in particular sublethally injured cells, and can therefore run the risk of their recovery. The selective media were used *Mac Conkey Agar, Methylene Blue - Eosin (EMB - Levin), Istrati - Meitert, XLD (xylose - lysine deoxycholate) and Rambach Agar*. *XLD media* autoclaves and has a reddish color. Because it offers very good rate of development of salmonella, the environment, moderately selective, has been used consistently for isolating bacteria in compound feed, being included in the standardized methods. Because the ingredients included *XLD Agar* allows differentiation of non-pathogens Salmonella lactozo-fermentation, and the non-lactose or xylose fermentation, while improving recovery rates of salmonella, consecutive absence of inhibitors on their potential toxicity (Maddadi et al., 2010). This environment is extremely effective for primary isolation of these germs. Xylose incorporated into the medium is fermented by enteric germs, except shigels, which makes it possible to distinguish Salmonella from them. Lysine allows differentiation of Salmonella enteric other non-pathogenic bacteria, whereas its absence would degrade rapidly Salmonella xylose, which would make them difficult to distinguish from non-pathogenic (after exhausting xylose, Salmonella lysine degradation, with reversion to an alkaline pH mimicking the shigels behavior). In order to prevent a reversion similar to coliforms that are lysine positive in the media to include lactose and sucrose whose degradation produce excessive acidification. In order to improve the differentiation of the formula a measure was introduced to indicate the production of H2S (ferric ammonium citrate and sodium thiosulfate), resulting in the appearance of colonies with black center, a reaction which takes place in alkaline environment. Non-pathogens produce H_2S, but do not decarboxylate lysine and therefore acidification reaction produced by them prevents the blackening of the colonies. They are therefore considerations which *XLD agar* is a selective medium for the differential diagnosis and properties conferred by sodium deoxycholate, which

does not allow the multiplication Gram positive bacteria. It is used for the isolation and differentiation of enteric bacteria. Allows assessment of several reactions, namely degradation of sugars (xylose, lactose and sucrose), by transferring the pH indicator yellow, thiosulfate and ferric salts are H_2S formation indicators, which is visible due to the precipitation of iron sulphide, black colonies and bacteria that decarboxylate lysine to cadaverine are recognized by the appearance of a red color around the colonies. *Rambach Agar is a propylene glycol-based medium which facilitates the differentiation of Salmonella spp., Proteus spp. and other enteric bacteria. Rambach medium is a solid medium, recently introduced in the identification of bacteria, useful for differentiation of Salmonella spp. and other members of the family Enterobacteriaceae.* This environment provides the research of a new character phenotype of the Salmonella spp., 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 +) to distinguish between *Salmonella spp., Proteus spp.,* and other members of the family *Enterobacteriaceae* (Matias et al., 2010). As inhibitor of Gram-positive may be included in the solid. For general use and speed is recommended seeding concomitant enrichment of both the media and those selective. It is preferable to use at least two liquid enrichment media *(e.g., MKTTn - Muller-Kauffmann-Novobiocin Tetrathionate Broth and RV - Rappaport Vassiliadis broth*) and at least two different selective media with different selective potential: *deoxycholate agar - xylose - lysine (XLD), deoxycholate citrate agar (DCA) brilliant green agar* or another inhibiting character - bismuth sulphite agar, but require a longer incubation for 24 hours. Usually, *Mac Conkey Agar* is used but it is not a selective medium for Salmonella, altough can serve for differential diagnosis from other *enterobacteria*, in combination with one of the above selective solid media and recommended for early sowing (Matias et al., 2010).

RESULTS AND DISCUSSIONS

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

Table 1. The results of the analyzed samples in October

Sample no.	Results				
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5
Sample 1	Negative	Negative	Negative	Negative	Negative
Sample 2	Negative	Negative	Negative	Negative	Negative
Sample 3	Negative	Negative	Negative	Negative	Negative
Sample 4	Negative	Negative	Negative	Negative	Negative
Sample 5	Negative	Negative	Negative	Negative	Negative

Table 2. The results of the analyzed samples in November

Sample no.	Results				
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5
Sample 1	Negative	Negative	Negative	Negative	Negative
Sample 2	Negative	Negative	Negative	Negative	Negative
Sample 3	Negative	Negative	Negative	Negative	Negative
Sample 4	Negative	Negative	Negative	Negative	Negative
Sample 5	Negative	Negative	Negative	Negative	Negative

Table 3. The results of the analyzed samples in December

Sample no.	Results				
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5
Sample 1	Negative	Negative	Negative	Negative	Negative
Sample 2	Negative	Negative	Negative	Negative	Negative
Sample 3	Negative	Negative	Negative	Negative	Negative
Sample 4	Negative	Negative	Negative	Negative	Negative
Sample 5	Negative	Negative	Negative	Negative	Negative

As shown in Tables 1, 2, 3, no positive samples were detected for the 15 groups analyzed respectively not revealed *Salmonella spp*. by tests carried out on the 15 samples collected, one sample per batch analyzed.

CONCLUSIONS

Isolation and identification of bacterial species belonging to the genus Salmonella, to be achieved by a variety of standardized methods, which may call or not for pre-enrichment and enrichment for the resuscitation of bacteria with low viability. Enrichment media that contain inhibitory substances contaminated with germs and selective media and differential diagnosis allows detection of Salmonella in competition with other enteric bacteria.

Samples were analyzed using algorithms provided in *Horizontal method for the detection of bacteria of the genus Salmonella spp. - SR EN ISO 6579: 2003 / AC 2009 and accredited RENAR official method.*

Laboratory diagnosis of fodder harvested samples was done by conventional methods, derived from national and international standards, which has as a principle the isolation and the identification of germs.

Growing on pre-enriched and enriched media allows greater specificity and sensitivity of detection while using inappropriate media may lead to a total failure and thus to increased risks for consumers.

Growing in two stages, which include non-selective broth recovery and selective cultivation under strict conditions, is regarded today as the most feasible procedure for enrichment of Salmonella strains.

Using a wide range of comparative selective media for the isolation of salmonella has proved the superior performance of thr Rambach media, a chromogenic medium that ensures unambiguous detection of Salmonella strains, significantly reducing the time and volume of inquiries, an extremely important goal for promptness diagnosis.

The results revealed no bacteria of the genus *Salmonella spp*., In samples of fodder used to feed hens, as demonstrated by the results presented in the previous section three tables of contents. Bacteriological control of feed is one of the fundamental requirements for prophylaxis occurrence of bacteria of the genus Salmonella spp. Which may jeopardize the safety and wholesomeness of the products obtained in farming sector of chickens for eggs.

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VETERINARY EDUCATION