ISOLATION AND IDENTIFICATION OF *SALMONELLA* IN A LOHMANN BROWN CHICKEN LOT, RAISED IN A TRADITIONAL SYSTEM

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Abstract

Infections produced by pathogenic strains of Salmonella are called salmonellosis and have an important economic and health impact. The major risk is represented by the transovarian transmission and the consumption of infected eggs which may cause severe food poisonings in humans.

These microorganisms can be found in the intestins, but manifest tropism for the gallbladder where they may be found in large numbers and where they usually remain defining the carrier condition. In birds, most Salmonella strains don't cause clinical signs. Even so, virulent serotypes that produce the disease exist and have specific evolution paterns. Salmonella enteritidis and Salmonella typhimurium are the most often isolated serotypes that are involved in public heath issues.

In 2018, a salmonellosis outbreak was identified, produced by Salmonella sp. group D, in a laying hens lot, raised in a traditional system. Clinical manifestations were seen only in some individuals and consisted in low egg production, severe weight loss, sleepiness, lack of appetite, polydipsia and death. The forensic exam showed egg yolk peritonitis, increased volume of the spleen and liver, brittle liver, fibrinous exudate in the thoraco-abdominal cavity, light cattharal enteritis. Microbiological exam was done by making bacterial cultures from different organs extracted from the bird cadavers (heart, gallbladder, bone marrow, gastro-intestinal mass and the eggs in different stages of development). Identification of the etiological agent was done based on phenotypic aspects (cultural and morphological), biochemical (MIU, TSI, API-20E) and serology (Siglepath-Salmonella, Serum Anti-Salmonella D) .

After establishing a laboratory diagnostic, the laying hens lot was eliminated and after the depopulation, severe cleaning and disinfection actions were taken.

Key words: hen, microbiology, diagnostic

INTRODUCTION

The salmonellosis are produced by bacteria belonging to the genus *Salmonella*, mainly two species: *Salmonella enterica* and *Salmonella bongori* (Grimont P.A.D., Weill F.X., 2007, Perianu T., 2011).

Salmonellas are present in the digestive tract of humans, animals, birds and cold blooded animals. Their presence is not automatically linked to the carrier state or signifies the outbreak of the disease (Răducănescu H, Bica Popii V, 1986). The salmonella circuit in nature is maintained through the feces that contaminate the environment and especially the water and the food, which leads to a higher incidence of the disease in some species (Răducănescu H, Bica Popii V, 1986, Răpuntean S., 2005, Perianu T., 2011). The lack of host specificity in most salmonellas increases the significance of the links and factors involved in their natural circuit (Perianu T., 2011). Chickens and other bird species are hosts for a number of pathogenic Salmonellas like: *Salmonella*: *Salmonella pullorum, Salmonella gallinarum, Salmonella eteritidis, Salmonella arizonae,*

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Salmonella typhimurium, etc. (Răducănescu H., Bica Popii V., 1986, Shivaprasad H.L., 2000; Perianu T. 2011; Carp Cărare C., Guguianu E., Rîmbu C., 2014).

Salmonellas have a complex antigenic structure, represented by the somatic antigens O, flagelar antigens H and the suface ones Vi. Diferentiation of these germs can be done based on these antigens. The serologic groups are formed based on the presence of the major somatic antigens and are indicated using capital letters from A to Z. Their pathogenicity differs based on the serotype to which they belong, and the pathogen ones have the ability to multiply themselves intracellularly or extracellularly in the affected organisms (Răducănescu H., Bica Popii V., 1986). In group D the following *Salmonella* species are included: *Salmonella typi, Salmonella enteritidis, Salmonella dublin, Salmonella gallinarum, Salmonella pullorum* (Răducănescu H, Bica Popii V, 1986, Lin FY, 1988, H. L. Shivaprasad, 1990). In birds, pathogenic Salmonellas have been characterized based also on the H antigen, and included in the mobile or imobile salmonellas (paratyphosis), (Perianu T., 2011).

Salmonellic infections in birds pose a major health risk, due to the high probability of transmission to humans through infected meat and egg consumption resulting in severe food poisoning outbreaks. Economically speaking, salmonellosis produces high losses through mortality and the control measures that need to be taken.

MATERIAL AND METHOD

Eight Plymouth Rock hen cadavers, coming from individual holdings and raised in a traditional system, were subjected to the necropsy.

To establish etiological diagnostic, biological samples were taken from the heart, bone marrow, gallbladder, gastro-intestinal mass and eggs in different stages of development.

The microbiological exam followed the clasic steps for setting a diagnostic to which specific isolating methods for salmonellas were added. The identification of the etiologicala gent was done based on the cultural, morphological, biochemical and serological aspects. Starting with the salmonellosis suspicion, the samples were grown in an enriched liquid growing media called Rapaport Vassiliadis and incubated at 42^0C. After 24 hours, the samples were passed on usual growing media (nutritional agar and broth) and on selectiv media for *Enterobacteriaceae* (agar XLD *Xylose Lysine Deoxycholate agar*, mediul Levine)

The testing for the biochemical and metabolic characteristics was done on polytropic media MIU (mobility, indol, urea), TSI (*triple sugar iron*), Simmons and API 20E well tests. For the serological confirmation immunochromatographic tests were used: GLISA-Rapid -Siglepath-*Salmonella* and Anti-*Salmonella* group D serum.

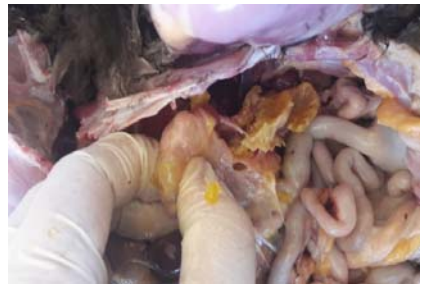
After the samples for the microbiological exams were harvested, necropsy was performed on the cadavers using specific techniques according to the literature.

Salmonellas are pathogenic enterobacterias for humans and animals, shaped like a bacillus, Gram negative, mobile or immobile, that can metabolize glucose, that do not ferment lactose, catalase positive and oxydase negative. Salmonellas produce gas, use the citrate as a sole source of carbon, do not produce indole or urease, produce hydrogen sulphide and have a specific antigen structure showed through serological tests (Carp Cărare C., Guguianu E., Rîmbu C., 2014).

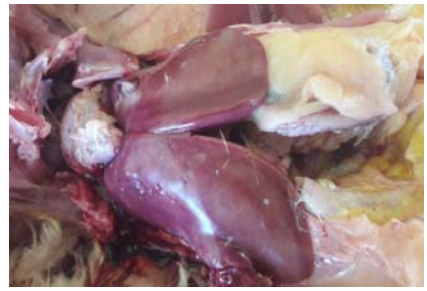
RESULTS AND DISCUSSIONS

The anamnesis showed that part of the hen lot manifested severe weight loss, a decrease in the egg production, sleepiness, lack of appetite, polydipsia and death.

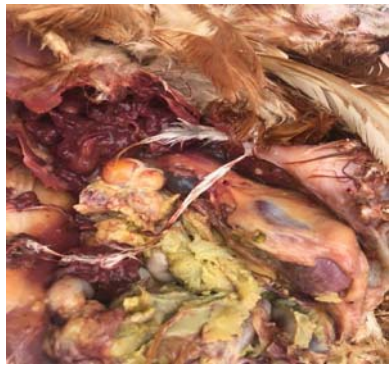
The necropsy showed lesions like: cachexy, egg yolk peritonitis and egg pseudoconcrements (Pic. 1), increased volume of the spleen and liver, greenish-brown liver with necrotic areas, fibrinous exudate localized in the entire thoraco-abdominal cavity (Pic. 2). In the genital apparatus lesions like salpingitis, corelated with ovarian atrophy were observed, manifested through the presence of deformed, crinckled, hemorrhagic folicles with a gray, green, brown or black content (Pic. 3, Pic. 4).



Pic. 1 – Chicken. Cachexy and egg yolk peritonitis



Pic. 2 – Hen. Fowl typhoid - Fibrinoid peritonitis and pericarditis, greenish brown colored liver with necrotic areas



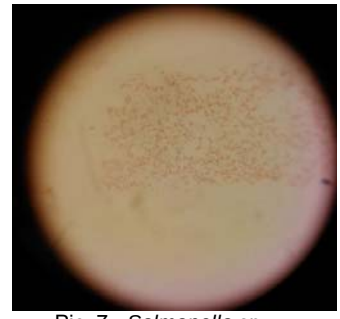
Pic. 3 – Hen. Chronic fowl typhoid - egg yolk from degenerated follicles results in fibrinous adhesive peritonitis

Pic. 4 - Albumin coated fully formed egg with thinned shell

After the inoculation and the incubation of the biological samples on the Rapapport Vasiliadis (RV) liquid media, the samples were inoculated on usual growing media and incubated in anaerobiotic conditions at 37^0 C. Pure bacterial colones developed, with characteristics specific for enterobacterias: colonies of 2-3 mm in diameter, type S, opaque, without pigment (Pic. 5, Pic. 6). Microscopically, we identified coccobacilli and bacilli Gram negative, with an ordered display. (Pic. 7).

Pic. 5 - *Salmonella* sp. - cultural aspects on enriched liquid growing media RV

Pic. 6 - *Salmonella* sp. - cultural aspects on nutritive agar

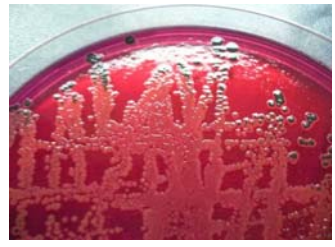


Pic. 7 - *Salmonella* sp. – morphology, Gram col., MO, x 1000

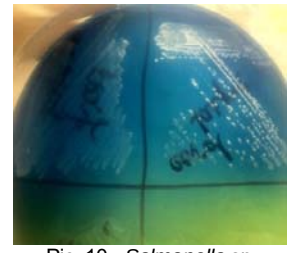
The isolated bacterial strains were simultaneously transplanted on selective media, to verify the capacity to ferment lactose (Levine media (Pic. 8), XLD media (Pic. 9), the capacity to use citrate as a carbon source (Pic. 10), to test mobility, indole and urea fermentation (MIU media), the production of H_2S and the ability to ferment lactose and sucrose (TSI media) (Pic. 11).

The extensive testing for the biochemical profile of the isolated strains was done using the API 20E well tests (Oxoid, Franta) (Pic. 12). The inoculation and interpretation of results was done in accordance with the reccomandations of the producer.

Pic. 8 - *Salmonella* sp, Levine media, Lactose (-) colonies

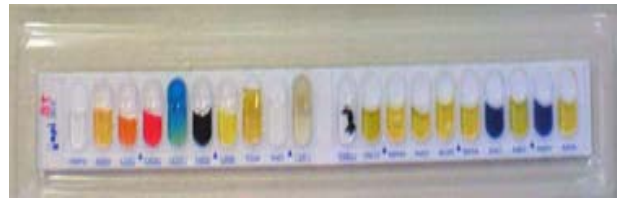


Pic. 9 - *Salmonella* sp, XLD media, Lactoză (-), HS(+) colonies



Pic. 10 - *Salmonella* sp, Simmons media, uses sodium citrate as a carbon source

Pic. 11 - *Salmonella* sp, MIU media: M (-), I (-), U (-);TSI media :L(-), G (+), Z (-)



Pic. 12 - *Salmonella* sp. - biochemical confirmation -API 20E (Oxoid, Franta)

According to the identification code API 20 E, the tested strains belong to the genus *Salmonella*.

The biochemically confirmed isolates were confirmed serologically, using the immunochromatographic fast test Singlepath *Salmonella* and the fast seroagglutination test on glass slides with group serums ABDEL. After these tests, the *Salmonella* sp isolates were confirmed to belong to serogroup D (Pic. 13).

Pic. 13 - *Salmonella* sp. - serological confirmation for genus and group D

The serogoup D includes most of the bipathogenic *Salmonella* species (for humans and animals): *Salmonella enteritidis, Salmonella typhi, Salmonella Dublin, Salmonella gallinarum, Salmonella panama*, etc. (Răducănescu H., Bica Popii V., 1986, Răpuntean S., 2005, Carp Cărare C., Guguianu E., Rîmbu C., 2014). The confirmation of species and serotype may be done definitively using the PCR (*polymerase chain reaction*). Considering the serogroup in which the *Salmonella* isolates were included (group D), the absence of mobility (tested on MIU), the fermentation of glucose with the resulting gas and the production of ornihtin decarboxilase (API 20E) (Răducănescu H., Bica Popii V., 1986), we can conclude that the isolated strain belongs to the *Salmonella enterica subspecies enterica serovars, Gallinarum biovars pullorum* (Răducănescu H., Bica Popii V., 1986, *OIE, 2018).*

This biovariety produces sepsis in young chickens and sometimes in young turkeys, pheasans and peacocks, but the disease may manifest atypicaly in adult birds as well (Shivaprasad H.L., 2000, Perianu T. 2011; OIE, 2018). The most important transmission path is the transovarian one, seen in laying hens. In free range raised poultry, some important vectors, involved in the transmission of the disease, may appear, like: wild birds and red mites (OIE, 2018). The severity of the enzootic disease differs from on outbreak to an other. In the lots from which the carrier birds are not eliminated, the disease manifests every year and with high mortality (Perianu T., 2011). In humans, the infection produced by *Salmonella pullorum* is considered by some authors, as a minor zoonotic risk factor, humans getting infected by consuming contaminated food. Clinicaly, in humans, the disease is characterized by a severe enteritis that may be treated with antibiotics (Shivaprasad H.L., 2000).

In the farm bird lots, the prevention and control measures are well known and applied according to the 60/1974 Law, modified by the 75/1991 Law.

In the lots raised in a traditional way, to avoid any infection risk it is important to find and eliminate all infected birds, to performe a microbiological exam for establishing an etiological diagnostic, when there is a salmonellosis suspicion, to eliminate the entire lot through sacrification and to apply rigurous disinfections.

CONCLUSIONS

1. Salmonellosis is one of the infectious diseases important for bird raising and human health.

2. Based on the phenotypical, biochemical and serological pattern, the strains that were isolated from the bone marrow, heart, gastro-

intestinal mass and ovarian follicles were determined to be highly similar to *Salmonella pullorum*.

3. The affected hens had the following morphopathological lesions: oophoritis (atrophied), salpingitis, peritonitis, enlarged liver with brown colour and necrotic areas, catharral enteritis, degenerated follicles with albumin coated fully formed egg with thinned shell.

4. Chickens raised in an extensive system are frequently exposed to *Salmonella* infections both through vertical transmission when using infected eggs for incubation, taken from carrier birds and through orofecal contamination from wild birds.

5. The hens that were diagnosed with *Salmonella pullorum* were sacrificed, and the location subjected to a rigurous disinfection.

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