

## Microscopic Findings in Replacement Start Chicks from Layers Affected by Salmonellosis

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### Abstract

Salmonellosis is an infectious disease of sharp and chronic course that affects the domestic and wild birds, characterized for high lethality and economic losses. With the aim of publicizing the microscopic findings in replacement starter chicks of egg-laying of White Leghorn L<sub>33</sub>, affected for this pathology. Were sent to bacteriology and pathology department of veterinary diagnostic laboratory of Santiago of Cuba, sample from the heart, liver and intestine of 20 animal, for the isolation of *Salmonella* and the observation of microscopic lesions, to ruler out the presence of enterobacteriosis. Diffuse proliferative enteritis was observed in the moderate to severe cecal tonsil, focal myocarditis in left-handed ventricle with presence of inflammatory cells and alterations of the fibers of the myocardium, focus of inflammatory cells in liver parenchyma, inflammatory cells in the portal space provoking destruction the vein's wall. It is concluded that microscopic lesions characterized by diffuse proliferative enteritis in the moderated to several cecal tonsil, focal myocarditis, foci of necrosis in the liver parenchyma and portal space, which correspond to the presence of enteric infection.

**Keywords:** Injuries; Microscopics; Replacement; Salmonellosis; Chicks and Layer Egg

### Introduction

Birds can be infected by a wide variety of *Salmonella* serotypes [1]. Infections with these bacteria adapted to the avian host, *Gallinarum* and *Pullorum* biovarieties of the *Gallinarum* serotype, cause specific diseases, such as typhosis and pullorosis, while others not specific to birds, may or may not be associated with clinical disease. Within this second group, we can highlight that the affectation by the Typhimurium and Enteritidis serotypes are among the most frequent and that they are generally associated with systemic processes that are accompanied by clinical signs, and can cause the death of a significant number of individuals.

The presence of clinical signs associated with *Salmonella* infection not specifically adapted to the avian host is more frequent in chickens less than two weeks old. These symptoms are nonspecific and include depression, apathy, winging or diarrhea, among others. Morbidity and mortality values are highly variable although usually 10% of casualties are not exceeded in the affected groups [2]. In addition

to the economic impact associated with production losses, infections with this bacterium in poultry are of great relevance since chicken meat can be a source of infection in outbreaks of food poisoning by *Salmonella* in man [3,4].

While in developed countries, infections by bacteria of the *Salmonella* genus in poultry farming are particularly important as a public health problem, due to the role of chicken meat and eggs as a source of outbreaks in man, however in developing states both aspects of infection, as a public health problem that limits the profitability of farms, are relevant.

Different antibiotics and vaccines have been used to reduce the morbidity and mortality associated with *Salmonella* infections in poultry farming [5]. Genetic selection of lines of birds with greater immune response capacity may also have utility in the prophylaxis of this infection. More recently, new control options have been introduced that include the use of probiotics [6] competitive exclusion mixtures [7] prebiotics [8,9] or new products with antimicrobial activity and of natural origin [10].

Several authors have proposed that the receptivity to infection by bacteria of the *Salmonella* genus can be modified, especially in the case of younger chickens, by acting on the intestinal microbiota. Newly hatched chickens have been shown to be more receptive to *Salmonella* infection than somewhat older animals and this resistance has been associated with the establishment of an adequate microbiota capable of raising the concentration of volatile fatty acids in the intestine [11]. Various works have evaluated, over the last 20 - 25 years, the effect of the administration of competitive exclusion mixtures or probiotic products on *Salmonella* infection in first-time chickens [7,9].

Kerr, *et al.* [12] recently carried out a meta-analysis to determine the efficacy of different competitive exclusion products in the control of infections by bacteria of the *Salmonella* genus. They analyzed more than 200 publications and finally used data obtained from more than 150 studies evaluating a total of 14 products. Most of these products were shown to be able to reduce colonization by *Salmonella* after being administered to broiler chickens in the first 24 hours of life.

### Objective of the Study

The objective of the work was to publicize the microscopic findings in replacement start pullets of layers affected by salmonellosis, in a starter replacement farm of White Leghorn L<sub>33</sub>.

### Materials and Methods

In the month of May of the year 2019, a sanitary picture that was characterized was reported in a rearing of start chicks replacing seven-day-old layers, of the White Leghorn L<sub>33</sub> breed, from a farm in the province of Granma, Cuba. Due to the presence of whitish diarrhea, pendulous wings, anal plug, dyspnea and drowsiness. Sample from the heart, liver and intestine of 20 animals was sent to the bacteriology and pathology department of the Santiago de Cuba veterinary diagnostic laboratory, the isolation of *Salmonella*, and the observation of microscopic lesions was performed to rule out the presence of salmonellosis.

### Isolation and identification of *Salmonella*

For the microbiological study, after the animals were sacrificed, the necropsy was performed using the cervical dislocation technique and a sample was taken from the heart, liver and intestine of each animal. To do this, each sample was placed in a sterile bottle containing 20 mL of peptone water and cut, using sterile scissors, into small fragments. Isolation was carried out following the EN-ISO 6579: 2002/ Amd 1: 2007 standard for the detection of *Salmonella* spp. in faeces and in environmental samples of primary animal production that consists of three stages: pre-enrichment, selective enrichment and culture in selective and differential medium.

In the pre-enrichment stage, the initial suspension of each organ in peptone water was incubated for 20 hours at 37°C. Next, a selective enrichment step was carried out by transferring 0.1 mL of the peptone water to three spots or spots in a Rappaport-Vassiliadis (MSRV) semi-solid medium plate (Merck), incubating at 41°C for 24 and 48 hours. Plates showing migration zones or halos were reseeded, col-

lecting material from the edge of the migration halos and transferring it to plates of two selective and differential media: Xylose-Lysine-Deoxycholate (XLD) agar plates (Merk) and agar plates bright green (BGA) (Merk).

These plates were incubated for 24 hours at 37°C, the positives being recognized by the presence of characteristic colonies in these media: colonies with the black center due to the production of sulfhydryl (SH<sub>2</sub>) in the case of XLD agar and opaque pink colonies in the case of BGA agar. For the confirmation of the isolates, the suspicious colonies were seeded in tryptone soy agar (TSA) (Merk) and confirmed by indole biochemical tests and by a commercial test (MUCAP® test, Biolife). Finally, a certain proportion of the recovered isolates were serotyped following the Kauffmann-White scheme and using commercial antisera (Statens Serum Institut).

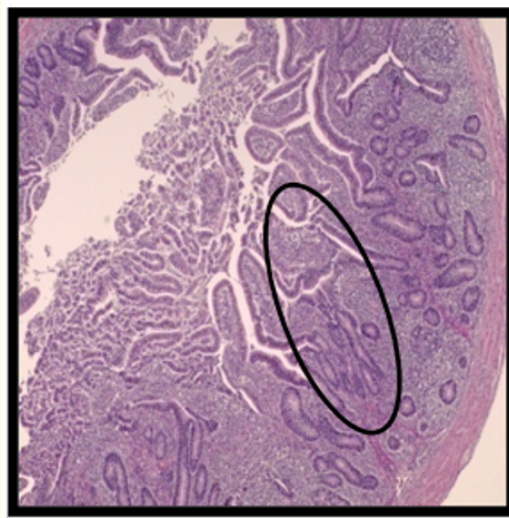
### Histopathological study

For the histopathological study, liver, intestine and heart samples were taken. They were deposited in buffered 10% formalin for fixation. Subsequently they were embedded in paraffin (Myr rotary inclusor), and blocks were made in a Myr AP280 station. 3 µm sections were obtained on a Shandon Finesse rotation microtome.

The sections were mostly stained with hematoxylin and eosin (HE) staining and, in some selected, with Gram staining for histological sections. The preparations were viewed with a Nikon Eclipse E400 microscope and the photomicrographs were taken on the photomicros.

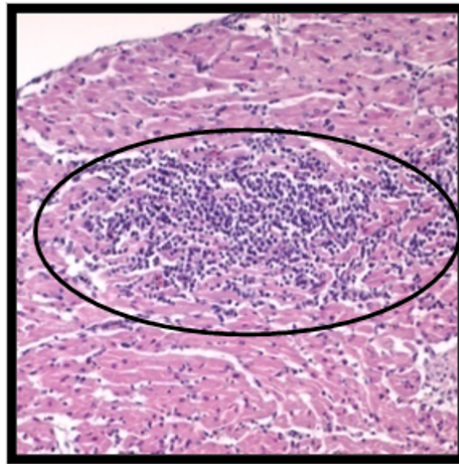
### Results

At necropsy, diffuse proliferative enteritis was observed in the intestinal tissue, from moderate to severe (Figure 1), compatible with clinical or subclinical disease, consisting of an infiltrate of inflammatory cells with macrophages and heterophiles, and some lymphocytes and plasma cells in the Own lamina, with different degrees of intensity, affecting some villi, causing the separation between crypts and the increase in villus thickness.



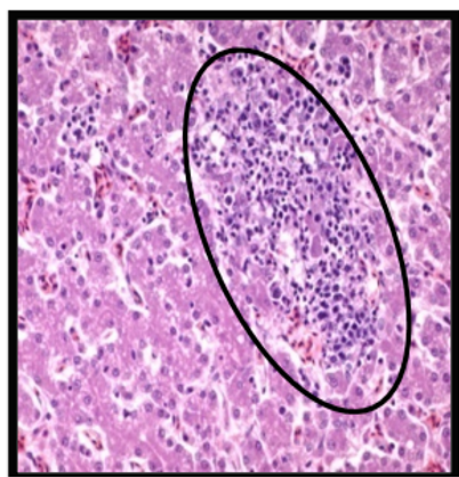
**Figure 1:** Diffuse proliferative enteritis in moderate to severe cecal tonsil (HE 40X).

On the other hand, the presence of purulent focal myocarditis, with small foci of interstitial infiltrates (miliary and submiliary) of cells, with a predominance of macrophages and with the presence of some heterophile, lymphocyte and plasma cells, was also observed, without altering muscle fibers (Figure 2).

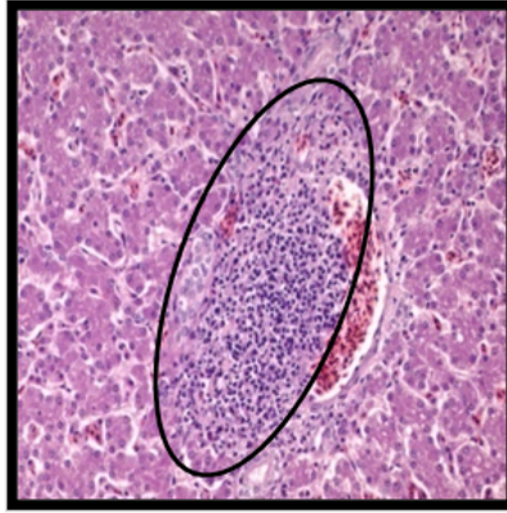


**Figure 2:** Focal myocarditis in the left ventricle with the presence of inflammatory cells and alterations of the myocardial fibers (HE 400X).

In the liver, the presence of purulent multifocal hepatitis was observed, characterized by the existence of small foci in the liver parenchyma, miliary to submiliary, consisting mainly of heterophiles accompanied, to a lesser extent, by macrophages and lymphocytes, in numbers from 10 to 100 cells, and with degeneration, karyoxis and lysis of hepatocytes from the periphery of any of these foci (Figure 3). The appearance of small Gram-bacilli, compatible with *Salmonella* spp., was demonstrated in the necrosis. Inflammatory cells located subendothelially in the portal vein were observed in the portal spaces, altering its wall and with the presence in the lumen of abundant leukocytes and intravascular coagulation (onset of thrombosis) as well as small periportal aggregates of the same cells present in the parenchymal focus (Figure 4).



**Figure 3:** Focus of inflammatory cells within the pale of the hepatic parenchyma with necrosis (HE 200X).



**Figure 4:** Liver with foci of inflammatory cells in the portal space causing destruction of the vein wall. Carioresis within the focus (HE 200X).

## Discussion

In general, the lesion observed were less than those described by other authors in infections for *Salmonella* adapted to the avian host. *Pullorum* and *Gallinarum* biovars. No macroscopic lesions such as those described in natural cases of these infections in the liver, heart or lung were observed. In contrast the type and the location of microscopic lesions was similar to the described in previous studies related to natural infections for *Salmonella Gallinarum* infection with sub-acute or chronic course [13,14].

Other lesions associated with *Salmonella gallinarum* infections include lesions in the cecum, which can enlarge and show a caseous and solid content (cecal nuclei) [15,16]. It is possible to observe necrotic nodules or white foci in the liver, spleen, lungs, heart, pancreas, gizzard and, occasionally, in the cecum; some of these nodules may resemble tumors. Joints may be swollen and contain creamy, viscous fluid.

In addition, liver and lung congestion are observed in pullorosis, as well as grayish-white necrotic areas in the lungs [17]. According to Sánchez [16] the most important lesions in *Pullorum* biobarries infections can include pericarditis, myocarditis and congested kidneys and inflamed tarsi with fluid-viscous and yellowish articular content. In hyperacute cases it can observed that births die suddenly without apparent injuries [18].

In the specific case of *Salmonella enteritidis* infections in chickens and in hens, systemic spread has been described from primary intestinal colonization after experimental challenges defiances using the oral road, observing necrotic foci with heterophiles in the intestinal mucous one belonging to the intestine, in the liver, spleen and oviduct [19] similar to those observed in the present study.

According to Ruiz., *et al.* [20] the experimental challenge with a dose of  $1 \times 10^8$  and  $1 \times 10^9$  *Salmonella enteritidis* UFC (FT 13a) was not associated with apparent pathological changes in organs such as the lung and the heart. In general form, the lesions associated with *Salmonella* enteric infection in the present study were higher than those previously described, specially in the intestine in which the chicks of our study development diffuse and severe enteritis.



### Conclusion

The main microscopic lesions observed were: diffuse proliferative enteritis moderate to severe in cecal tonsils, focal myocarditis, foci of necrosis in liver parenchyma and portal space, which correspond to presence of salmonellosis.

### Conflict of Interest

No conflict of interest.

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