Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2016 Vol. 6 (3) July-September, pp. 45-49/Madadi et al.
Research Article

COMPARATIVE PATHOLOGIC EVALUATION OF ORAL AND VENOUS INOCULATION OF SALMONELLA ENTERITIDIS IN BROILER BREEDER HENS

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ABSTRACT
Laying hens (29 week-old) of broiler breeders were inoculated orally (PO) and intravenously (IV) with 1010 and 106CFU *S. Enteritidis* phage type 4 organisms consequently. Hens were sequentially euthanized at regular intervals throughout the 35-day observation period. Microscopic lesions in IV group hens indicated that liver and kidney congestion, nephritis, Intestines Goblet cell hyperplasia and magnum congestion were more than PO group hens. In contrast to IV group, cholangitis, intestine congestion, hepatitis and myocarditis were more detected in PO group hens.

Keywords: *Salmonella Enteritidis, Laying Hens, Histopathology*

INTRODUCTION
Salmonellosis is one of the most important food-borne diseases. The World Health Organization (WHO) has reported 1.3 billion cases per year of acute gastroenteritis due to non-typhoid salmonellosis with 3 million fatal cases (Gomez *et al.,* 1997). In 2006, a total of 165, 023 confirmed cases of human salmonellosis was reported in the European Union. In this report, the prevalence *S. enteritidis* was identified 62.5% and *S. Typhimurium* was 12.9%. The overall European Union prevalence of *Salmonella* in table eggs was 0.8% in 2006 and >90% of all eggs isolates were *S. Enteritidis* whereas, *S. Enteritidis* was the most common stereotype (52.3%) in the laying flock environment (EFSA, 2007). The persistence of this organism in poultry house environments poses a continuing threat of infection for laying hens (Lapuz *et al.,* 2008). Additionally, there is a suggestion that *S. enteritidis* has some intrinsic characteristics that allow a specific interaction with either the reproductive organs of laying hens or the egg components (Gantois *et al.,* 2009). Microscopic lesions attributable to *S. Enteritidis* of various page types, including gauge type 4, have been de-scribed in chickens, most of these studies have been done with chicks or have been of limited scope in adult chickens. The base of these, aim of study trial was to establish a model infection of *S. Enteritidis* in laying hens in which the internal organs e.g. digestive or reproductive systems could become infected and consequently histopathological effects could be studied. Therefore, hens were inoculated intravenously and orally. Different tissue samples were taken for histopathological analysis.

MATERIALS AND METHODS
Bacterial Strain: *S. Enteritidis* phage type 4, strain NIDO Following of inoculation, productivity decreased to a low 76Sa88 Nalr (parent strain) was used in this experiment, obtained from Ghent University, Belgium. The nalidixic acid resistant strain is well-characterized (Van Immerseel *et al.,* 2002). *Hens:* Fifty 26-week-old broiler breeder hens were selected from an Arian Grand Parent farm that is under strict control of Salmonella and other infectious diseases. They were free of any apparent disease throughout the growing and laying periods. Hens were divided into two groups. Before starting of the experiment cloacal swabs were taken from all hens and checked for Salmonella infection, to confirm that animals were Salmonella-free. Hens randomly divided in two groups of 25 birds. The first group was

inoculated intravenously (IV) with 106 CFU of *S. Enteritidis* 76Sa88 no parent strain bacteria, using 0.1 ml of PBS and second group hens were inoculated by oral (PO) route in the crop, using a plastic tube with 1010 Colony Forming Units (CFU) of the same bacteria in a volume of 1 ml of PBS, as reported previously (Barrow and Lovell, 1991). At days 2, 7, 14, 21 and 35 post-inoculation, two hens per group were euthanized and post-mortem examinations were carried out. For bacterial analysis samples were taken from different parts of digestive (caecal, small intestine, liver- spleen) and reproduction (infundibulum-ovules, magnum, isthmus, cloaca-vagina) systems separately. Cloacal swabs were taken on the same days and examined for S. Enteritidis. Every 10 eggs were pooled and cultured.

Histopathology: The tissue samples were aseptically collected from every chick in a bottle containing 10 % buffered formalin and kept for 24-36 hours for fixation. Tissues were further processed using standard histological techniques (Islam *et al.,* 2006). Briefly, the tissues were trimmed post fixation with a scalpel blade into about 5mm pieces and were placed into embedding cassettes. The tissues were processed overnight. For processing, the embedding cassettes containing tissues were placed into an automatic tissue processor for dehydration in a graded alcohol (30 %, 40 %, 50 %, 70 %, 90 %, and 100 %) and then cleared by two changes of 100% xylene. Then, the tissue samples were embedded in paraffin wax using semi-automatic tissue embeddor). The paraffin embedded tissues were then trimmed by microtome (Leica RM2155, Germany) at the thickness of 15 µm followed by 5 µm. After trimming the tissues were sectioned at a thickness of 4 µm. The ribbons of sections, tissue were floated on the surface of warm water at 560C in a thermostatically controlled water bath (Leica H1220, Germany). The tissue sections were taken on a glass slide from the water and allowed to dry for 15-20 minutes by placing the slides in vertical position on a simple wooden rack. After drying the slides were placed on a hot plate (the tissue section not touching the plate) at 57 0C for 10-15 minutes for adhesion of the tissue and the de-waxing. The slides were labeled and stained using Haematoxylin and Eosin (HE). After staining, the slides were allowed to dry overnight. Then cover slips were fixed with DPX and slides were examined under the light microscope (Islam *et al.,* 2006).

RESULTS AND DISCUSSION

Results

Liver

In Venous group hens, liver presented more congestion, milder cholangitis, necrosis and large lymphocytic infiltration. Microscopic lesions consisted of acute Multifocal Co-gulative necrosis of hepatocytes with or without inflammation, randomly scattered throughout the liver. When inflammation was present it was primarily heterophilic infiltration in acute stages. In later stages infiltration of a few lymphocytes and plasma cells occurred (Figure 1).

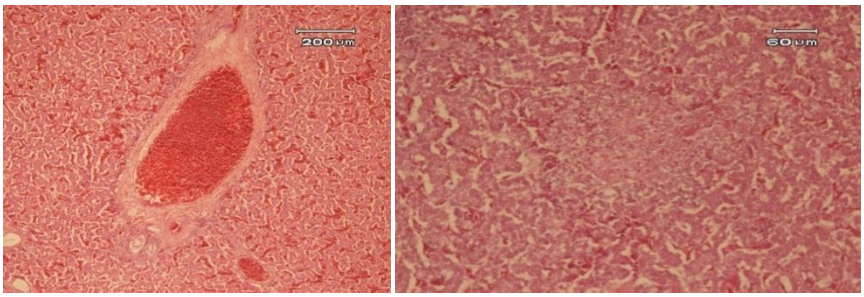

Figure 1: Sever Congestion and Choalangities, Left-Oral Group and Focal Necrosis, Right-Venous Group (H&E, 100x)

Kidney:

The kidneys in general were congested and slightly swollen. The most important microscopic changes were observed in the venous group kidneys was mild congestion, subcapsular hemorrhages, mild nephritis

and mild fatty changes. In oral group hens mild congestion, pyknosis and mononuclear infiltration was observed.

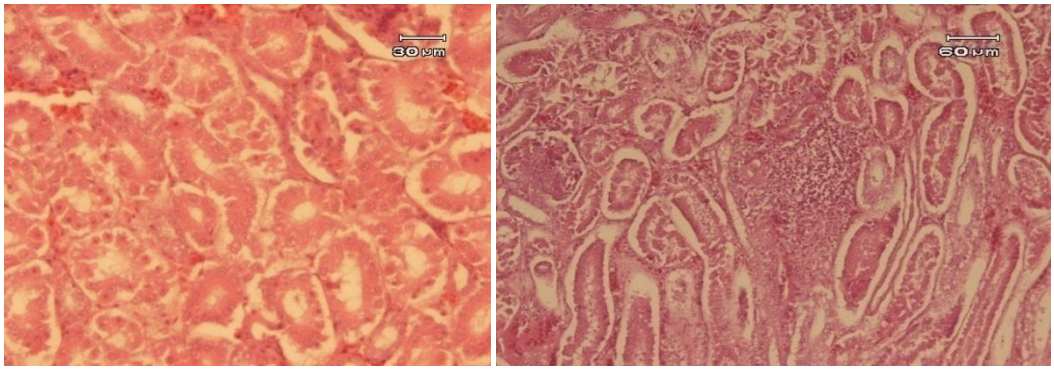

Figure 2: Nuclear Pyknosis, Left-Oral Group and Focal Nephritis, Right-Venous Group (H&E, 100x)

Intestines:

In venous group hens, mild congestion, mono nuclear cell infiltration, mild goblet cell hyperplasia and chronic peritonitis were detected. In oral group hens congestion was more severe than other groups.

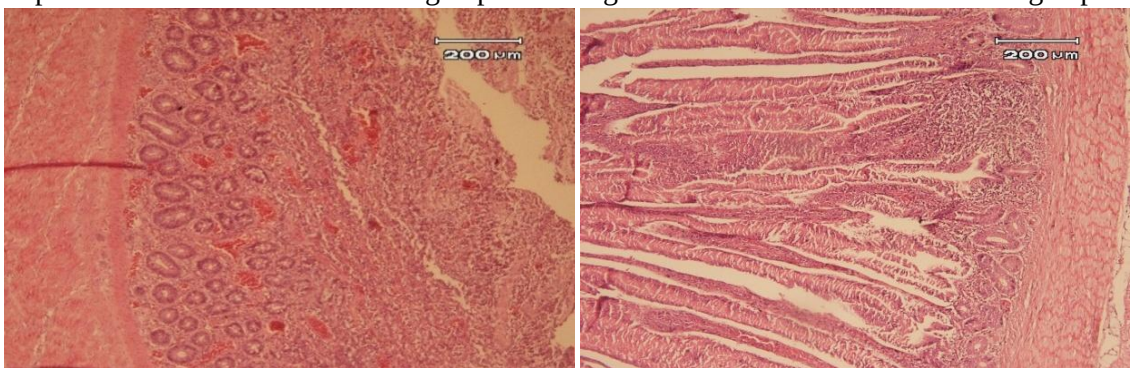

Figure 3: Sever Congestion (Left-Oral Group) and Severe Enteritis (Right-Venous Group) (H&E, 100x)

Reproductive System:

Degenerative changes and serosal hemorrhages were seen in infandibulum of venous and oral group hens consequently. Isthmus of oral group hens had mild congestion and serosal hemorrhages. Severe congestion of magnum and more epithelial hyperplasia and degenerative changes were observed in venous group hens.

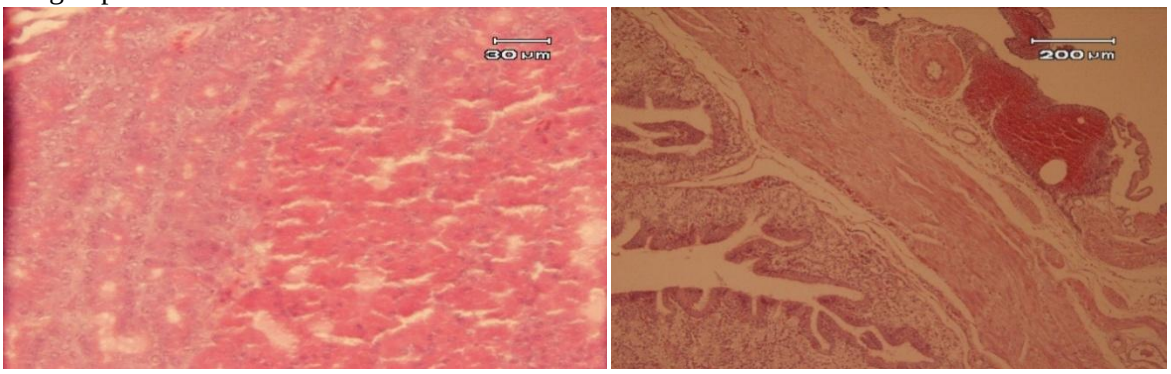

Figure 4: Infandibolum; Serosal Hemorrhages, Left-Oral Group and Degenerative Changes, Right-Venous Group (H&E, 100x)

Discussion

Natural infection of poultry by Salmonella occurs via the oral route and salmonella colonize the intestinal tract with the crop and ceca being the primary sites of colonization (Impey and Mead, 1989). In the present study, contamination of gastrointestinal organs in the PO group was higher than to IV group. Okamura (2001) explained that after IV inoculation, S. Enteritidis could keep bacteremia and remained persistently in the liver and ceca to a high degree. Microscopic alterations in the organs which showed lesions are presented in Figures 1 to 4. The lesions in the liver, reproductive system, caecum and kidney, especially in IV group hens, explain the invasive potential of SE and their sever pathogenicity. The sloughing of superficial layers of villi indicated the damage to the intestinal epithelium and results into translocation of bacteria to other tissues. Microscopic lesions in IV group hens indicated that liver and kidney congestion, nephritis, Intestines Goblet cell hyperplasia and magnum congestion were more than PO group hens. In contrast to IV group, cholangitis, intestine congestion, hepatitis and myocarditis were moredetected in PO group hens. Akhtar *et al.,* (2011) reported that if Salmonella are not cleared by the host immune system the intestinal colonization occurs that can further lead to the invasion and colonization of other organs including the liver and spleen and results in lesions in invaded tissues. The response of the host to S. enteritidis infection is based on genetic characteristics of the birds and the general mechanism of resistance to systemic disease may apply to all serovars of salmonella in chickens (Bumstead & Barrow, 1993). Salmonella inoculation in young chickens leads to severe pathology and more peritonitis, perihepatitis, enteritis, yolk sac infection, and pneumonia. However, in this study histopathological effects of salmonella. Enteritidis in layer hens were detected, lesions resembled those of Lesions observed in the earlier findings and IV inoculation route had severe pathogenicity and leads to moreacute form of illness.

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