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Gross/Histopathological Impact of *Salmonella* Gallinarum Isolated from Layer Chickens in Jaipur and their Antibiogram Assay

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Abstract The study was conducted from April 2009 to March 2010 to assess the antimicrobial sensitivity pattern of eight *Salmonella* Gallinarum strains recovered from various sources in Jaipur of India by testing with eight different antimicrobials by disc diffusion method where the isolates showed least sensitive to tetracycline. Gross lesions incriminated to the pathogen, were comprised of caseous enteritis, hepatic necrosis, necrotizing myocarditis and oophoritis. Histopathological investigation revealed passive congestion of the visceral organs. Degenerative changes in hepatic lobules and consequential destruction of architectural arrangement of hepatocytes, necrotic foci in the enteric mucosa and in hepatic tissues were seen. Ovaries showed fibrinosuppurative to pyogranulomatous inflammation of ovarian follicles characterised by necrosis and fibrinosuppurative inflammation mixed with bacteria within the ovules to chronic pyogranulomatous inflammation. Heart characterised by locally extensive foci of myofibre necrosis with infiltration of heterophils mixed with few lymphocytes and plasma cells.
Keywords *Salmonella Gallinarum; Antibiogram; Histopathology; Layer Chickens*

1. Introduction

In poultry industry, salmonellosis creates intense economic losses along with reduction in productivity and high mortality (Khan et al., 1998). Out of different serovers, *Salmonella* Gallinarum produces Fowl Typhoid; a septicemic disease in poultry which may be characterized by anorexia, greenish-yellow diarrhoea with considerable mortality, also causes drop in egg production. *Salmonella* Gallinarum vertical transmission through eggs leads to higher mortality or producing weak chicks.

In recent times, antibiotic resistance in livestock becomes alarming in India, blaming the easy availability of antibiotics in markets and indiscriminate use of them. Research workers like Mir et al. (2010) and Taddele et al. (2012) reported isolation of *Salmonella* Gallinarum strains showing multiple drug resistance pattern in poultry in India.

In Jaipur, India, there is little systematic study on incidence and antibiotic resistance pattern, histopathological studies of Salmonella Gallinarum serovar. Therefore, the present study was conducted to isolate and identify the serotype *Salmonella* Gallinarum in the fresh carcasses of layer chickens in Jaipur area, their antimicrobial susceptibility pattern and documentation of associated gross and histopathological lesions.

2. Materials and Methods

2.1. Sample

In the department of pathology, Apollo College of Veterinary Medicine, seventy three necropsy tissue samples i.e. lungs, liver, intestine, heart, spleen and kidney from dead layer chickens with gross apparent lesions were subjected to microbiological as well as histopathological investigations for a duration of one year i.e. 2009-10.

2.2. Isolation of Salmonellae

For isolation of Salmonallae, one gram of minced tissue sample was aseptically transferred into sterile tube having 10 ml Tetrathionate Broth and/or Rappaport Vassiliadis Salmonella Enrichment Broth and inoculated at 42˚C for 24 hours aerobically. Then a loopful of broth culture was streaked onto Brilliant Green Agar plates (BGA), kept at 37˚C for 48 hours aerobically and Mac Conkeys Lactose Agar (MLA) plate were used to inoculate the suspected pinkish colonies on BGA. The plates were incubated at 37˚C for 24 hours aerobically for isolation of the salmonellae. Non lactose fermenter (pale) colonies on MLA, suspected for *Salmonella* were obtained in pure culture on nutrient agar slants by conventional method.

2.3. Gram's staining

The isolated bacteria were stained by Gram's Method to determine their staining characteristics and purity of the culture. Identification was done by IMViC reaction, TSI reaction, Nitrate reduction test, urease test, H_2S production test, and other fermentative or non-fermentative sugar tests (Edwards and Ewing, 1972; Cruickshank, et al., 1975). For Serotyping, the isolates were referred to National *Salmonella* and *Escherichia* centre, Central Research Institute, Kasauli (H.P.).

2.4. Antibiogram

In-vitro antibiotic sensitivity of the isolates were assessed using disc diffusion method (Bauer et al., 1966) while using disc coated with 12 antibacterial drugs (Hi-Media) viz. Ampicillin 10 mcg (A 10), Cephalexin 30 mcg (Cp 30), Ciprofloxacin 5 mcg (Cf 5), Enrofloxacin 5 mcg (Ex 5), Gentamicin 10 mcg (G 10), Kanamycin 30 mcg (K30), Ofloxacin 5 mcg (Of 5) and Tetracycline 30 mcg (T 30).

2.5. Histopathology

Tissue samples with grossly visible lesion were preserved in 10 per cent formalin. Histopathological investigation was done using standard protocol (Luna, 1968). The paraffin embedded tissues were cut into sections of 4 to 5 µ thickness. Sections were stained with Haematoxyline and Eosin (H&E) stain and microscopic lesions in different organ were documented.

3. Results and Discussion

Out of seventy three samples screened from layer birds, 8 (10.96 %) *Salmonella* could be recovered. All the isolated strains belong to Salmonella Gallinarum; antigenic structure 9, 12,--,--.

3.1. Antibiogram of the Isolates

In-vitro antibiotic sensitivity of the isolates revealed as (Table 1)

***Table 1**: Antibiogram of the Isolates*

Sl. No.	Antibiotic	Number Tested	Number Sensitive	(%)
1.	Ampicillin 10 mcg (A 10)	8	5	62.5
2.	Cephalexin 30 mcg (Cp 30)	8	6	75
3.	Ciprofloxacin 5 mcg (Cf 5)	8	8	100
4.	Enrofloxacin 5 mcg (Ex 5)	8	7	87.5
5.	Gentamicin 10 mcg (G 10)	8	7	87.5
6.	Kanamycin 30 mcg (K30)	8	6	75
7.	Ofloxacin 5 mcg (Of 5)	8	6	75
8.	Tetracycline 30 mcg (T 30)	8	1	12.5

The highest sensitivity was seen in Ciprofloxacin followed by Enrofloxacin, Cephalexin, gentamicin, kanamycin and ofloxacin. Tetracycline showed least sensitivity. Similar pattern was also reported by Taddele, et al. (2012). They found kanamycin, tetracycline as least sensitive with higher sensitivity towards gentamicin, amoxicillin/clavulanic acid, ciprofloxacin, ofloxacin and enrofloxacin. However, resistance to kanamycin was not seen in present study.

Knowing the resistance pattern of *Salmonella* Gallinarum strains towards different antimicrobial agents helps to offer a better and more effective treatment against fowl salmonellosis. *Salmonella* Gallinarum strains in the present study have shown higher resistance towards tetracycline. This can be due to reasons like resistance gained due to indiscriminate use of the antibiotic, improper antibiotic treatment time frame, use as growth promoter etc.

Clinical signs showed by death birds were anorectic, ruffled feathers, dullness, depression, dropiness, dehydrated, fall in egg production and vent feathers soil with diarrheic fecal materials.

Gross changes found in post mortem examination were recorded as follows-

Liver showed enlargement with severe congestion and focal haemorrhages in most of the cases, in few cases found focal grayish white necrotic foci in the parenchyma (Figure 4). Heart showed discrete nodular necrotic growth of variable size and shape of grayish white in colour (Figures 1 and 2). Intestine especially small intestine found severe congestion and hemorrhages and cecum of few birds showed caseated necrotic mass (Figure 1). Ovaries of all dead birds showed severe inflammatory changes in likes misshaped, hemorrhagic and discoloured cystic ova (Figure 3). The involved ova may contain oily and caseous material enclosed in a thickened capsule. These degenerative ovarian follicles closely attached to the ovary and few of them pedunculated and detached from the ovarian mass and embedded in the inner lining of the peritoneal cavity. The oviduct contains caseous exudates in the lumen with impacted uterus with degenerated eggs in few cases. Lung, kidneys and spleen also showed severe congestion and haemorrhages and enlargement of spleen in few were recorded. Fibrinous peritonitis and perihepatitis, with or without the involvement of the reproductive tract also recorded in some necropsied birds.

Histopathologically, the liver showed severe congestion in central vein and sinusoidal area with degeneration (Figure 6), focal necrosis with infiltration of mononuclear cells. The intestinal mucosa exhibited congestion and hemorrhages with infiltration of mononuclear cells in the submucosa and caseous necrosis in the centre. These types of histological lesions are supported for *Salmonella* infection by different investigators (Talha, et al., 2001; Habib-ur-Rahman, et al., 2003).

Ovaries showed fibrinosuppurative to pyogranulomatous inflammation of ovarian follicles characterised by necrosis and fibrinosuppurative inflammation mixed with bacteria within the ovules to chronic pyogranulomatous inflammation. In males, necrosis of the epithelial cells lining the seminiferous tubules may be seen, followed by fibrinosuppurative inflammation (Saif, et al., 2003).

Heart characterised by locally extensive foci of myofibre necrosis with infiltration of heterophils mixed with few lymphocytes and plasma cells (Figure 5). In later stages, these cells may be replaced by large numbers of fairly uniform mononuclear cells of the histiocytic type with irregular vesicular nuclei and faintly staining foamy eosinophilic cytoplasm. These cells may be arranged in solid sheets, forming nodules that often protrude from the epicardial surface. Such nodules, both grossly and microscopically, can be confused with certain tumours caused by Marek's disease virus or possibly retroviruses (Shivaprasad, 1997; Saif, et al., 2003)

***Figure 1**: Gross Photograph of Layer Bird Showing Discrete Nodular Necrotic Growth on Heart and in Cecum Having Caseated Necrotic Mass*

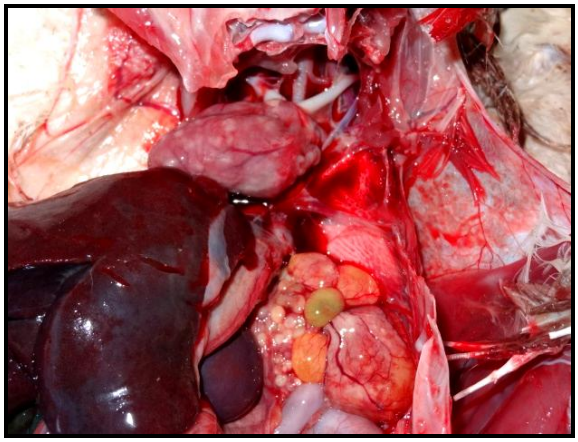

***Figure 2:** Gross Photograph of Layer Bird Showing Discrete Nodular Necrotic Growth on Heart, Severe Inflammatory Changes in Ovaries (Oophoritis), Severe Congestion and Haemorrhages in Liver*

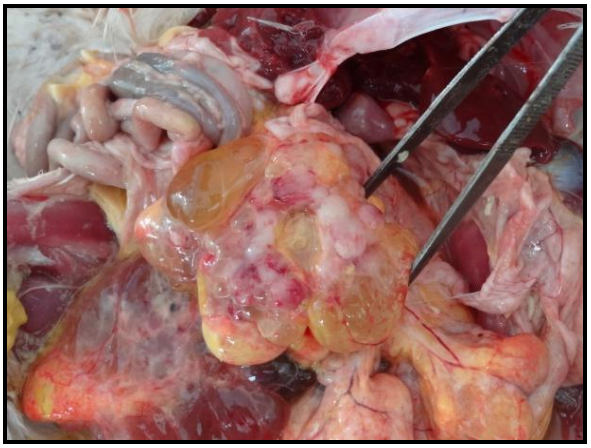

Figure 3: *Gross Photograph of Layer Bird Shows Severe Inflammatory Changes in Ovaries Likes Misshaped, Hemorrhagic and Cystic*

Figure 4: *Gross Photograph of Layer Bird Shows Grayish White Necrotic Foci in Hepatic Parenchyma*

3.2. Histopathological Investigation

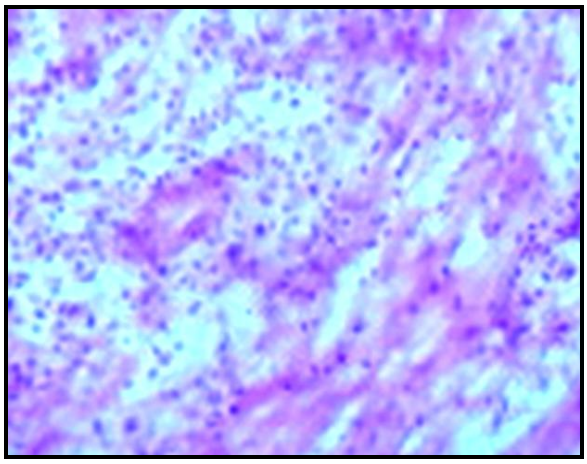

Figure 5: *Photo Micrograph of Heart Showing Fragmented and Necrosed Myocardium Which is Infiltrated by Heterophils and Mononuclear Cells. H&EX400*

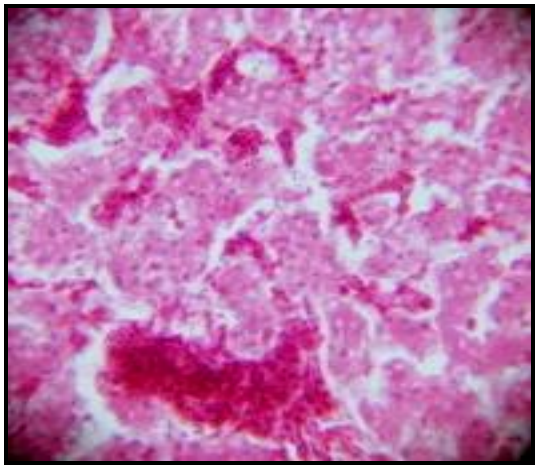

Figure 6: *Photo Micrograph of Liver Shows Severe Congestion in Central Vein and Sinusoidal Area with Degenerative Changes of Hepatocytes. H&EX400*

4. Conclusion

From the present study it was concluded that *Salmonella* spp. were isolated from 8 (10.96 %) samples comprising of different dead layer chicken samples. All the isolates belonged to serotype *Salmonella* Gallinarum. Antibiogram suggested that ciprofloxacin showed the maximum potential to be used as promising whereas least sensitivity towards tetracycline. It may pose potential danger to the poultry industry due to vertical transmission. Moreover, once it is introduced into the farm, become difficult to eliminate infection and becomes clinically evident with the frequent mortality and severe economic losses.

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