

## Pathology of Spontaneously Occurring Salmonellosis in Commercial Broiler Chickens of Kashmir Valley

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### ABSTRACT

A study was conducted on spontaneous cases of Salmonellosis in different commercial broiler farms of Srinagar district and adjoining areas within the period from April 2008 to Oct 2009. A total of 42 outbreaks of Salmonellosis were recorded which included 24 outbreaks of fowl typhoid, 8 of paratyphoid, 3 of pullorum disease and 7 due to untypable serotypes. Clinical signs generally included ruffled feathers, huddling near the source of heat and light, anorexia, increased thirst, reluctance to move, pasted vent, diarrhoea, prostration, reduced growth rate and rarely lameness. The gross lesions comprised of hepatomegaly, bronze discoloration of liver, splenomegaly, congestion and necrotic foci on liver and spleen and pericarditis along with greyish white nodular lesions on heart. The lungs revealed varying degrees of congestion and haemorrhage. Bursa was atrophied and caeca revealed presence of caecal core. Histopathological alterations were observed principally in the liver, spleen, heart, kidney and bursa. Changes in liver and spleen comprised of congestion, haemorrhages, areas of necrosis, reticular endothelial hyperplasia along with mononuclear cell and heterophilic infiltration. Heart revealed congestion, focal and extensive infiltration of mononuclear cell and heterophils which occasionally resulted in atrophy, necrosis and replacement of muscles fibres by infiltrating cells. Depletion of lymphocytes was observed in both the spleen and bursa. Intestinal changes comprised of congestion of mucosal vessels along with marked hyperplasia of goblet cells and infiltration of heterophils and mononuclear cells in the lamina propria of villi.

**Key words:** Salmonellosis, pathology, broiler, chicken

### INTRODUCTION

Avian salmonellosis represents a group of acute or chronic diseases caused by one or more members of genus *Salmonella* (Lutfal Kabir, 2010). The most important pathogenic members of avian salmonellosis include the non-motile, *Salmonella enterica* subsp. *enterica* serovar Gallinarum and *Salmonella enterica* subsp. *enterica* Pullorum. These are host specific and represent a major concern for the poultry industry causing fowl typhoid and pullorum disease respectively (Rosu et al. 2007). These serotypes can be responsible for disease outbreaks leading to severe economic losses. However *S. Gallinarum* also, presents a rare risk of zoonotic transmission to man (Wigley et al. 2005). *Salmonella* infections currently constitute a hindrance to the poultry industry worldwide. Fowl typhoid is the leading cause of mortality and morbidity in poultry and is responsible for significant economic loss of poultry

industry in India (Prakash et al. 2005). The aim of present investigation was to study pathology of natural outbreaks of *Salmonella* infections in commercial broiler farms of Srinagar district and its adjoining areas so as to evolve appropriate therapeutic measures in future.

### MATERIALS AND METHODS

A total of 500 disease outbreaks in 265 broiler farms were recorded in Srinagar district and its adjoining areas between the period April 2008 and Oct, 2009. The birds and carcasses from the affected flocks were screened for salmonellosis, primarily on the basis of clinical signs and postmortem examination. Suspected cases were confirmed following isolation and biochemical characterization (ISO 6579, 1993). Serotyping of *Salmonella* isolates was carried out at

“National Salmonella and Escherichia Coli Central Research Institute”, Kasauli, HP India.

The clinical signs were recorded on the basis of information from the farmers and personal farm visits. Detailed systemic postmortem was conducted on all the birds and the gross lesions recorded. Representative tissue samples from different internal organs like liver, spleen, lungs, heart, bursa of Fabricius, kidneys and intestine were collected in 10 per cent formol saline for histopathological studies. Samples from confirmed cases of salmonellosis were processed for routine paraffin embedding technique employing alcohol as a dehydrating agent and benzene as clearing agent. The sections were cut at 4-5 µm thickness and stained by Harris hematoxylin and eosin method (Luna, 1968).

## RESULTS

Out of a total of 500 outbreaks, 42 outbreaks of salmonellosis were confirmed by NSERI, Kasauli H.P. Out of 42 outbreaks, 24 were of fowl typhoid (*Salmonella enterica* subsp. *enterica* serovar Gallinarum), 8 were Paratyphoid (*Salmonella enterica* subsp. *enterica* serovar Typhimurium), 3 Pullorum disease (*Salmonella enterica* subsp. *enterica* serovar Pullorum) and 7 due to untypable *Salmonella*.

### Clinical observations

Clinical signs observed in broilers in the spontaneous outbreaks of salmonellosis were of variable nature and as such could not be considered of major diagnostic significance. In general the diseased birds showed dullness, severe depression, anorexia, appeared listless, stood motionless about with head sunk onto the chest and with both eyes closed. Most of

the infected chicks developed progressive weakness, complete inappetance, increased thirst and droopy wings with ruffled feathers. Watery to mucoid greenish yellow diarrhoea was the most characteristic clinical sign in acute cases. Lameness was also recorded in birds in a few outbreaks. In few cases mild respiratory distress was also observed. The clinical signs were less severe in broilers of above 25 days of age. In peracute cases of paratyphoid, birds mostly died without showing any premonitory signs. However in most of the other outbreaks of paratyphoid, birds showed depression with closed eyes, ruffled feathers, fecal-soaked vent feathers, unabsorbed yolk and profuse diarrhoea.

### Gross pathology

The lesions in chicks affected with fowl typhoid were indistinguishable from those associated with pullorum disease. In typical cases of fowl typhoid, appearance of bronze coloured liver was characteristic and prominent lesion. The bronze discolouration of the liver (Fig 1) was observed more frequently at 7 to 15 days of age. Livers of affected chicks of this age group also revealed numerous greyish necrotic foci or necrotic patches (Fig 2), reddish haemorrhagic foci (Fig 3) which were distributed uniformly on their surfaces. The chicks which died in early age group also showed pale discolouration, enlargement and congestion of liver along with mild distension of gall bladder. In few of these cases diffused areas of necrosis were also observed. The proportionate distribution of gross lesions in fowl typhoid, pullorum disease and paratyphoid observed in naturally infected broiler chicken is given in Table 1.

**Table 1.** Proportionate distribution of gross lesions in fowl typhoid, pullorum disease and paratyphoid observed in naturally infected broiler chicken

S. No.	Organ	Characteristic lesion	Fowl typhoid (%)	Pullorum disease (%)	Paratyphoid (%)
1	Liver	Discolouration	54.6	31.2	35.7
		Enlargement	35.3	24.5	23.8
		Necrotic foci	29.2	18.9	26.4
		Haemorrhagic foci	34.0	36.3	7.7
		Perihepatitis	4.88	1.4	16.4
2	Heart	Thickening of pericardium	4.6	1.4	14.8
		Small elevated greyish white nodular areas on the ventricular region	Rare	Rare	3.2
3	Spleen	Enlargement	26.2	11.6	29.5
		Necrotic foci	35.4	24.8	24.1
		Mottling	32.8	16.2	11.3
		Haemorrhagic foci	16.1	12.3	2.8
4	Lungs	Congestion	11.5	6.8	6.2
5	Kidneys	Congested and slightly swollen	19.8	9.24	14.4
		Congested with haemorrhages	24.2	16.8	21.4
6	Intestines	Impaction of cloaca	-	3.10	-
		Caecal core	-	-	4.8
7	Bursa	Mildly atrophied	8	6.9	3.7
8	Yolk	Unabsorbed yolk	2.5	5.4	6.7
		Yolk sac infection	1.6	6.2	18.3



**Figure 1.** Broiler bird affected with fowl typhoid showing bronze discolouration of liver.



**Figure 2.** Broiler chick affected with salmonellosis showing prominent necrotic foci on liver.



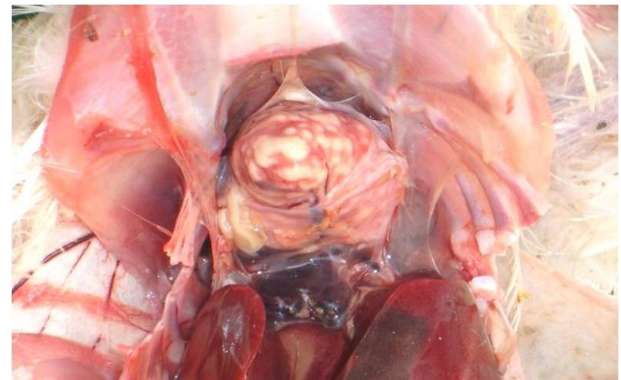
**Figure 3.** Broiler chick affected with salmonellosis showing necrotic and haemorrhagic foci on liver.

The heart generally revealed congestion and thickening of pericardium (4.6%) along with few indistinct necrotic foci in some cases. In a few birds of over 2 weeks of age, small elevated greyish white nodular areas were observed on the myocardium, which were more predominant on the ventricular region (Fig 4). Spleen revealed congestion and enlargement in chicks at early age (1 to 7 days). Presence of hemorrhagic or whitish diffused or multiple necrotic foci were the consistent lesions in the spleen of birds older than this age group. The lungs in general were congested in 11 per cent of affected birds. At early age, there was mild congestion in lungs whereas chicks

older than 1 week, revealed moderate congestion and in a few birds focal areas of consolidation were also observed. The kidneys in general were congested and slightly swollen in 19.8 per cent affected birds. Grossly, intestines appeared congested with hemorrhages mostly on the mucosal side in 24.2 per cent of the affected birds. Dropping were thin and occasionally blood stained. Bursa showed mild to moderate atrophy in 8 per cent of affected birds.

In acute cases of Pullorum disease in birds having history of diarrhoea, pasting of vent with loose whitish faecal material, impaction of cloaca was observed in 3.1 per cent of affected birds. In addition to above changes in Pullorum disease, unabsorbed yolk and yolk sac infection were observed in 5.4 and 6.2 per cent of affected birds respectively during first week of early age.

The grossly observed lesions in Paratyphoid infection included necrotic foci on liver (26.4%), pericarditis with the presence of fibrinous exudates in pericardial sac (14.8%), perihepatitis (16.4%), presence of greyish white nodules on ventricular region of heart (very rare), congestion of intestines (21.4%) and kidneys (14.4%), yolk sac infection (18.3%). The lesions in caeca were more prominent and severe in cases of Paratyphoid wherein the caeca were inflamed and swollen and in chronic cases revealed presence of cheesy, dry, necrotic material in their lumen (4.8%) (Fig 5).



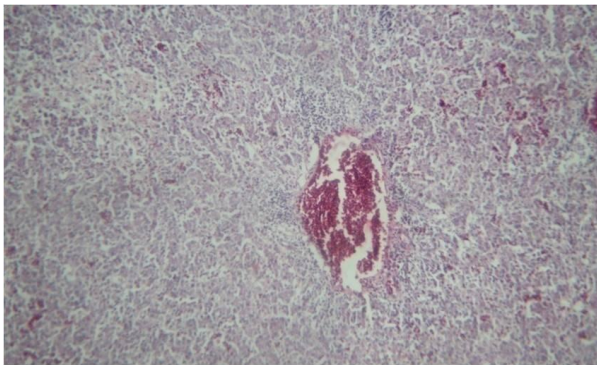
**Figure 4.** Broiler birds affected with salmonellosis showing small elevated greyish white nodular lesions on ventricular region of heart.



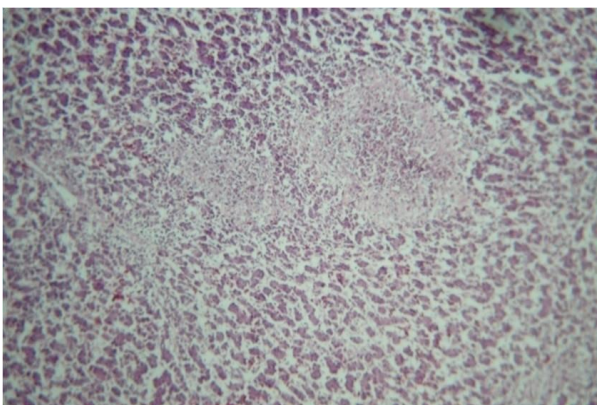
**Figure 5.** Caeca of a bird affected with paratyphoid showing presence of cheesy yellowish caecal core.

### Histopathology Liver

Microscopically severe vacuolar degeneration, fatty changes was frequently observed change in the liver in chicks of early age group. Congestion of blood vessels and haemorrhages were observed in chicks of all age groups. The chicks mostly of 7 to 15 days old showed isolated foci of necrosis in hepatic parenchyma along with infiltration of leucocytes predominantly mononuclear cells which were mostly centered around portal triads and perivascular areas (Fig. 6). Generally adjacent to the necrotic areas there was infiltration with mononuclear cells and heterophils (Fig. 7) In a few cases aggregations of mononuclear cell resulted were observed. Sometimes mild reticular cell hyperplasia was observed which resulted in disruption of hepatic cords



**Figure 6.** Section of liver from salmonellosis affected bird showing hepatocellular necrosis along with congestion and diffused perivascular mononuclear cell infiltration HE x240.

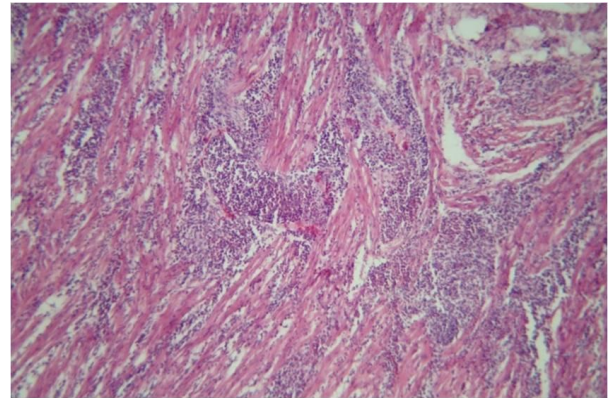


**Figure 7.** Section of liver from salmonellosis affected bird showing large necrotic areas along with infiltration of heterophils. HE x960.

### Heart

Thickening of pericardium along with the mononuclear cell infiltration was generally observed in cases of typhoid and paratyphoid infection. Congestion and haemorrhage especially below the epicardium was most frequently observed change in early age group. Necrosis was also observed focally along with infiltration by mononuclear cells. In a few isolated cases of over 2 weeks of age group with nodular appearance grossly, there was extensive infiltration of mononuclear cells along with few heterophils which at several occasions resulted in atrophy, necrosis and

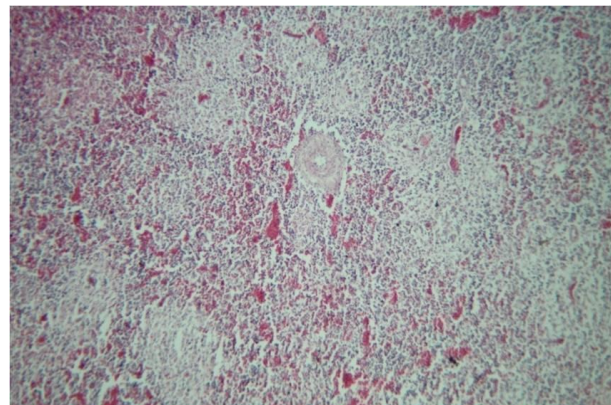
replacement by the infiltrating cells (Fig 8).In certain cases of paratyphoid there was complete replacement of muscle fibres by the infiltrating cells.



**Figure 8.** Section of heart from salmonellosis affected bird showing severe mononuclear cell infiltration in myocardium that resulted in disruption and replacement of cardiac muscle fibres. HE x960.

### Spleen

In general the spleen showed congestion and thickening of blood vessels and haemorrhages particularly below the splenic capsule in early stages where as at later stages the changes generally included depletion of lymphocytes along focal areas of necrosis (Fig. 9). Depletion of lymphocytes at times was accompanied by reticular cell proliferation.



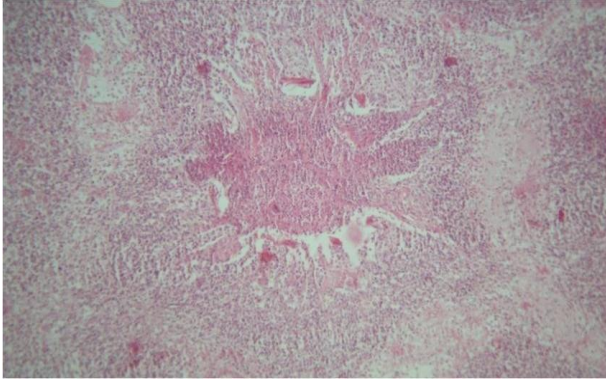
**Figure 9.** Section of spleen in salmonellosis affected birds showing congestion, vascular thickening and necrosis. HE x240.

### Lungs

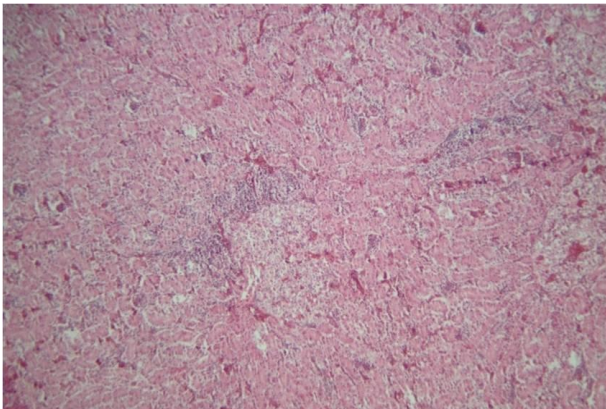
Lungs revealed congestion of the interlobular septae and haemorrhages in the parabronchi. Besides congestion, haemorrhage and mild infiltration of mononuclear cells and suppurative bronchopneumonia characterised by the presence of exudates in the parabronchi comprising of infiltration of heterophils was also observed in few cases (Fig 10).

### Kidneys

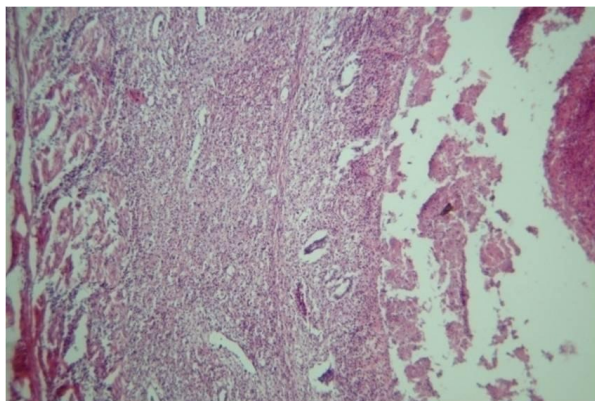
Kidneys showed mild to severe congestion and haemorrhages in the interstitial tissue. In a few isolated cases focal mononuclear cell and heterophilic infiltration was also observed in the interstitial tissue (Fig 11).



**Figure 10.** Section of lung from salmonellosis affected bird showing suppurative bronchopneumonia. HE x240.



**Figure 11.** Section of kidney affected with salmonellosis showing congestion, haemorrhage and infiltration of mononuclear cells in the interstitial tissue. HE x240.



**Figure 12.** Section of caeca from paratyphoid affected bird showing degeneration and desquamation of lining epithelium and mononuclear cell infiltration in the mucosa and submucosa resulting in pressure atrophy of intestinal glands. HE x960

#### **Bursa**

The bursa generally revealed mild to moderate depletion of lymphocytes in bursal follicles and in few cases there was atrophy of bursal follicles. In a few birds only mild degenerative changes were noticed in the bursal follicles.

#### **Intestine**

The intestines in affected birds generally

revealed congestion of mucosal vessels, marked goblet cell hyperplasia mild to moderate infiltration of heterophils and mononuclear cells in the lamina propria of the villi, In chronic cases of paratyphoid the lesions in caeca comprised of congestion and haemorrhage with degeneration and desquamation of lining epithelium and mononuclear cell infiltration in the mucosa and submucosa which resulted in atrophy of intestinal glands (Fig 12).

#### **Proventriculus**

No significant lesion were observed except in a few cases there was focal infiltration of mononuclear cells in mucosal layer

#### **DISCUSSION**

Prakesh et al. (2005) also reported *S. Gallinarum* as the predominant serotype which was responsible for 89.5% of *Salmonella* outbreaks in states of Karnataka, Maharashtra and Tamil Nadu from India. Clinical symptoms like dullness, ruffled feather, cyanosed comb, anorexia and greenish yellow diarrhoea was also reported by Bhattacharya et al. (2001) during an outbreak of fowl typhoid in Tripura, India. Bronze discoloration of liver was a prominent lesion in fowl typhoid. This is due to the fact that *Salmonella Gallinarum* organisms have a predilection for bile canaliculi which causes the stasis of bile in the liver. Bronze discolouration was also reported by Basnet et al. (2008) in adult chickens infected with *Salmonella Gallinarum*. Bronze discolouration of liver, mottling, necrotic and haemorrhagic foci on liver were also reported by Chisti et al. (1985). The haemorrhages and caseous nodules on the myocardium affected with *S. Gallinarum* was also reported by Hafeji et al. (2001). Spleen showed mottling, necrotic and haemorrhagic foci which was inconcurrence with the findings of Chisti et al. (1985). Congestion and consolidation of lungs was also reported by Kaura et al. (1990) and Hafeji et al. (2000). Freitas et al. (2007) also reported congestion and slight swelling of kidneys. Intestines in present study showed congestion along with haemorrhages on the mucosal surface. Bursa showed mild to moderate atrophy. Similar changes were also reported by Mohammadi et al. (1976).

Microscopic changes in liver which included severe vacuolar degeneration, fatty changes, Congestion and haemorrhages, isolated necrotic foci in hepatic parenchyma along with infiltration of leucocytes predominantly mononuclear cells and heterophils and mild reticular cell hyperplasia were also reported by Freitas et al. (2007) and Garcia et al. (2010). Perihepatitis and myocarditis observed in broiler chicks affected with fowl typhoid was in accordance with the findings of Kumar et al. (2002). Nodular lesions on heart grossly and extensive infiltration of mononuclear cells along with few heterophils which at several occasions resulted in atrophy, necrosis and replacing the muscle were also reported by Hafeji et al. (2000). Depletion of

lymphocytes and focal necrotic changes accompanied by reticular cell proliferation was also reported by Mohammadi et al. (1976) and Freitas et al. (2007). Microscopic changes in the lungs including congestion, haemorrhage and mild infiltration of mononuclear cells and suppurative bronchopneumonia was also reported by Hafeeji et al. (2000). Congestion and haemorrhages in the interstitial tissue along with focal mononuclear cell and heterophilic infiltration in kidneys and depletion of lymphocytes and atrophy of bursal follicles were consistent with the findings of Desmukh et al. (2007). Prasanna et al. (2001) also reported congestion of mucosal vessels, marked goblet cell hyperplasia, mild to moderate infiltration of heterophils and mononuclear cells in the lamina propria of the villi of intestines.

## CONCLUSION

This research was first attempt undertaken to study pathology of salmonellosis in commercial broiler chickens of Kashmir valley. *Salmonella enterica* subsp. *enterica* serovar Gallinarum was found to be most predominant isolate and was responsible for most of the outbreaks. Although Liver, spleen and heart were the primary targets organs, clinical signs, macroscopic and microscopic lesions varied with the serotype of *salmonella* involved. The lesions in different visceral organs were severe and were observed in more number of birds in fowl typhoid when compared to paratyphoid and pullorum disease.

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