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Incidence and Pathology of Paratyphoid Infection in Poultry

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ABSTRACT

Background: Paratyphoid infection of poultry is caused by non-host adapted motile salmonellae and are responsible for numerous cases of food borne illness worldwide. The present study was carried out from July 2019 to February 2020 in Jabalpur to know the occurrence and pathology of paratyphoid bacteria in poultry.

Methods: Whole blood agglutination test was performed to know the prevalence of salmonellosis in and areas surrounding Jabalpur region and pooled fecal samples were collected from poultry farms to perform microbe culture and biochemical characterization. Serotyping of *Salmonella* isolates was done using polyvalent antisera. Necropsy examination was conducted to observe gross and histopathological lesions.

Conclusion: Rapid whole blood agglutination test determined the percent prevalence of *Salmonella* as 28.0% from 25 private poultry outlets. The percent prevalence of salmonellosis by collecting pooled fecal samples from 15 broiler and 11 layer farms was recorded as 20.0% and 45.4% respectively. Salmonellosis was recorded in 1.58% of total necropsy cases of birds examined for gross and histopathological studies. Polyvalent antisera diagnosed 27.27% motile paratyphoid salmonellae, out of which 18.18% tested positive for *Salmonella* Enteritidis while 9.09% tested positive for *Salmonella* Typhimurium. Birds with paratyphoid infection showed hepatomegaly, discoloration, hemorrhagic and necrotic foci in liver and various grades of hemorrhagic to catarrhal enteritis were recorded.

Key words: Paratyphoid, Poultry, *Salmonella*, S. Enteritidis.

INTRODUCTION

Indian poultry industry is one of the world's largest and fastest growing industry ranking third in egg production and the fourth largest chicken meat producer in the world (Prabakaran, 2014). Modernization of livestock farms and globalization of bird breeding trade also helps in transboundary spreading of food-borne bacteria such as *Salmonella* (Chakraborty *et al.,* 2020). Although, the poultry sector contributes its great share to the country's economy, the production is hampered by several disease conditions. *Salmonella* infection is one of the most important bacterial diseases in poultry causing heavy economic loss through mortality and reduced production (Haider *et al*., 2004). NTS (Non-Typhoidal *Salmonella*) is one of the most prevalent foodborne infections around the world, causing diarrhea, fever, vomiting and sometimes even death. WHO has estimated that the NTS is responsible for an average number of 78.7 million foodborne diseases with more than 59000 deaths annually (Havelaar *et al*., 2015). There is relatively fewer number of reports of salmonellosis from India despite its high prevalence, which can be attributed to limited diagnostic facilities under field conditions and underreporting (Rajagopal and Mini, 2013). Paratyphoid infections of fowl may also reach the human population via contamination and mishandling of poultry products causing serious health issues.

Keeping the following facts in mind, the present study was conducted to determine the occurrence and pathology of paratyphoid infection in poultry in Jabalpur region of Madhya Pradesh.

MATERIALS AND METHODS

The present research work was conducted for a period of eight months from July 2019 to February 2020, at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur (M.P.) to determine the occurrence and pathology of paratyphoid *Salmonella* infection in poultry.

Seroprevalence of *Salmonella* using whole blood agglutination test

The seroprevalence of salmonellosis was recorded in 25 retail poultry outlets using *Salmonella* coloured antigen. Blood was collected from birds at the time of slaughter. A drop of coloured antigen was taken and then a drop of blood was added to the antigen and mixed and rocked gently for one minute and observed for agglutination reaction.

Isolation of organism from poultry farms

A total of 26 farms including 15 broiler farms and 11 layer farms were visited covering approximately 100 km radius area in and around Jabalpur region. Proper data collection

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was performed through interaction with farm manager and several aspects regarding health status of birds, biosecurity measures and other prevailing management practices.

Isolation of *Salmonella* was performed according to the protocol followed by ISO (2017). Pooled faecal samples (100g) were collected from different sheds of individual poultry farms in sterile sample bottles containing 100 ml of buffered peptone water (BPW) and then transported in iceboxes at 4°C and then the samples were incubated overnight at 37°C. Liver and intestinal swab samples were also collected at the time of necropsy. Both samples were then inoculated into Tetrathionate broth and Rappaport Vassiliadis Medium and incubated at 37°C and 42°C respectively, for 18-24 hours for selective enrichment of the organism. Organisms from the broth medium were then streaked on to selective media XLD and BGA and incubated at 37°C for overnight for isolation of organism.

Identification of organism

Identification of organism was done based on study of characteristics of colony morphology and further Gram's staining was performed using pure colonies. Biochemical characterization of bacterial isolates was done using IMViC test and Triple Sugar Iron (TSI) agar test and metabolic changes that were interpreted visually.

Identification of motile *Salmonella* organisms by use of polyvalent antisera

A presumptive identification of *Salmonella* was done by using commercially available antisera (SSI Diagnostica, Denmark) specific for S. Enteritidis and S. Typhimurium. A small drop of antiserum (20 µl) was added on a glass slide. A small amount of positive *Salmonella* culture was taken and then mixed with the antiserum. The slide was then tilted for 5-10 seconds. A positive reaction was seen as visible agglutination, whereas a negative reaction was observed as homogeneous milky turbidity.

Pathological examination

Examination of carcasses of birds (approximately >200) received at Department of Veterinary Pathology, C.O.V.Sc. and A.H., NDVSU, Jabalpur, was conducted for the presence of gross gastrointestinal lesions associated with paratyphoid infection during post mortem. For histopathology, the formalin fixed tissues of liver spleen, intestine and ovary from birds found positive for *Salmonella* infection were further processed, trimmed, sectioned and stained using routine Haematoxylin and Eosin Staining following standard procedure and presence of the bacteria in intestinal sections was determined by MacCallum Goodpasture staining as described by Gridley (1960).

Statistical analysis

Data gathered during the study was utilized for the analysis of the presence of disease within layers and broilers of various farms using Chi-square test as described by Snedecor and Cochran (1994).

RESULT AND DISCUSSION

The present work was conducted to determine the occurrence and pathology of paratyphoid infection in poultry farms and from the necropsy cases.

Seroprevalence of *Salmonella* using whole blood agglutination test

In the whole blood agglutination test, the positive agglutinates were of variable degrees in the outlets. On the basis of the whole blood agglutination test the seroprevalence of *Salmonella* in Jabalpur was determined as 28.0%. In India, there is a practice of procuring the chicken meat from small retail outlets with a general observation of severe lack of hygiene and cleanliness.

Similar grading was used by Habib-ur-Rehman *et al.* (2003) and Rahman *et al.* (2011). However, this rapid detection test alone cannot give the accurate prevalence of salmonellosis infection. Due to the high sensitivity and low specificity of the whole blood agglutination test, it is widely considered to be of importance only as a screening test (Gast, 1997).

Prevalence of *Salmonella* at poultry farms

All the broiler farms followed deep litter system of housing for birds while cage system was followed in the layer farms. Based on the cultural and biochemical characteristics, salmonellosis was recorded in 1.58% (30/189) of necropsy cases (isolates named P1, P2 and P3). The *Salmonella* infection was confirmed in 20.0% (3/15) in broiler farms (B1, B2 and B3) and 45.45% (5/11) in layer farms (L1, L2, L3, L4 and L5).

Colony morphology

Isolates of *Salmonella* sp. were observed as 2-3 mm pinkish red colonies with black centre on XLD Agar along with change of colour of media showing reddish appearance as observed by Ranjbar *et al.* (2020). In BGA, the *Salmonella* isolates appeared as pinkish white colonies with change of the colour of agar medium from green to pink similar to observations of Sannat *et al.* (2017).

Gram staining of pure colonies

All the suspected isolates showed the presence of pink coloured, small rod-shaped bacteria present alone or in groups from the pure colonies after observing under oil immersion in microscope. *Salmonella* was confirmed by presence of Gram-negative pink coloured rods after analysing Gram-stained smears (Tille, 2017).

Biochemical characterization of Salmonella isolates

The TSI test performed using the *Salmonella* isolates obtained showed a yellow coloured butt with a pink slant alkaline reaction and yellow butt showing acid production (Table 1) which indicates that the organism is a dextrose fermenter but unable to ferment lactose and sucrose. Black coloured growth was present indicating H_2S production. Gas production was denoted by presence of bubbles in the butt region. This has been described in concurrent studies done by Mansour *et al.* (2013) and Saravanan *et al.* (2015).

Indole, Methyl red, Voges-Prausker and Citrate utilization (IMViC) Test

The biochemical characteristics of IMViC test of the isolates were in accordance with reactions mentioned for *Salmonella* (-+-+) (OIE, 2012) as mentioned in Table 2.

All the isolates were found to be negative for indole test. Formation of red colour was noted when methyl red added to the MR-VP broth which showed positive methyl red test as observed by Faisal *et al*. (2017). The presented negative Voges-Prausker (VP) reaction. Similar result was found by Sharma and Das (2016). Colour change from green to blue in case of positive citrate utilization test was observed except for one isolate which was found to be indole negative. Negative result for citrate utilization by S. Gallinarum was also noted by Mir *et al.* (2015).

Motility test

Motile *Salmonella* were identified by the development of dark pink growth and movement of bacteria from the inoculated well no. 1 to well no. 2 in the kit unlike the non-motile *Salmonella* (Table 2). A positive result of motility was indicated by the spread of the stab line in the solid media as stated by Aktar *et al.* (2016). Out of the 11 suspected isolates, 27.27% were identified as motile *Salmonella*.

Serogrouping of Salmonella isolates using polyvalent antisera

Salmonella somatic O poly antisera specific for motile organisms S. Enteritidis and S. Typhimurium were used to differentiate the cultures having motile *Salmonella* from the non-motile organisms. Among the 11 isolates obtained, 18.18% tested positive for *Salmonella* Enteritidis antisera. Al Mamun *et al.* (2017) reported prevalence of Paratyphoid *Salmonella* as 27.77% and Long *et al.* (2017) reported it as 4.54%.

In our study, 9.09% tested positive for *Salmonella* Typhimurium antisera. Earlier, the seroprevalence of *Salmonella* Typhimurium reported by Srinivasan *et al.* (2014) and Muna *et al.* (2016) was 2.35% and 1.9% respectively, whereas Mir *et al.* (2015) and Khasa *et al.* (2018) reported the prevalence of *Salmonella* Typhimurium as 15.62% and 81.25% respectively. However, a similar prevalence of 9.09% of *Salmonella* Typhimurium was recorded by Samanta *et al.* (2014) in West Bengal.

Percentage positive samples of Paratyphoid *Salmonella* from broiler farms, layer farms and post-mortem cases with gastrointestinal lesions was recorded as 6.66%, 9.09% and 0.52% respectively. In accordance with our observation, it has been reported earlier also that with increased flock size of birds, *Salmonella* infection rate also increases (Samanta *et al.,* 2014).

Table 1: Biochemical features of *Salmonella* isolates using TSI slant test.

Isolates		Butt	Slant	Gas production	H_2S production
PM cases	P1	Y	P	-	+
	P2	B	B	+	+
	P3	Y	P	+	+
Broiler farms	B1	Y	P	+	+
	B2	Y	P	-	+
	B3	B	P	+	+
Layer farms	L1	Y	P	+	+
	L2	B	B	-	+
	L3	Y	P	+	+
	L4	Y	P	+	+
	L5	B	P	-	+

Y= yellow, B=black, P= Pink, + = present and - = absent.

Pathological examination

• Gross lesions of in birds affected with paratyphoid infection

Out of 189 necropsy cases, 15 broiler birds were found positive for paratyphoid *Salmonella* infection. The birds were received with the history of anorexia, restlessness, dullness, depression and diarrhoea, however, no characteristic clinical signs were noted in the diseased birds from the farms.

Majority of gross lesions in birds were comprised of congestion of liver (43.33%), congestion of spleen (36.66%) and hemorrhagic enteritis (40.0%) due to bacterial spread. Liver included lesions of hepatomegaly, congestion and hemorrhagic and necrotic foci in liver. Splenomegaly along with congestion and mottling of spleen was observed. The caeca were inflamed and swollen. Severe haemorrhagic gastroenteritis and haemorrhagic typhlitis along with haemorrhagic caecal tonsils was observed.

There is immense scope for contamination in the birds slaughtered in unorganized retail shops due to poor hygiene (Badhe *et al.,* 2013). The two most consistently observed features of paratyphoid infections in mature poultry are intestinal colonization and systemic dissemination to internal organs (Gast, 2013). Crhanova *et al.* (2011) observed that

Table 2: Biochemical characterization of *Salmonella* isolates.

Biochemical Test	Isolates of *Salmonella* from different sources										
	Post-mortem cases			Broiler farms			Layer farms				
	P1	P2	P3	B1	B2	B3	L1	L2	L3	L4	L5
Indole	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+
Voges-prausker	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	-	+
Motility	+	-	-	+	-	-	+	-	-	-	-

infection with S. Enteritidis resulted in significant inflammation which also required a strengthening of epithelial cell resistance and causes dissemination to internal organs.

• Histopathological lesions in birds with Paratyphoid infection

Liver

Microscopic lesions in liver included haemorrhagic foci, hemosiderosis, congestion, dilatation of sinusoids (Fig 1), cellular infiltration and vacuolar degeneration. Kupffer cell hypertrophy was also noted. Coagulative necrosis was also observed causing loss of organ structure in the liver sections (Fig 2). Liver was noted with maximum histopathological alterations in our study.

Presence of multifocal necrosis is an irreversible pathologic alteration (Garcia *et al.* 2013). Hepatic degeneration was also noted by Ogunleye and Carlson (2012). Focal degenerative and infiltrative lesions were observed by Hossain *et al.* (2006) and Dutta *et al.* (2013).

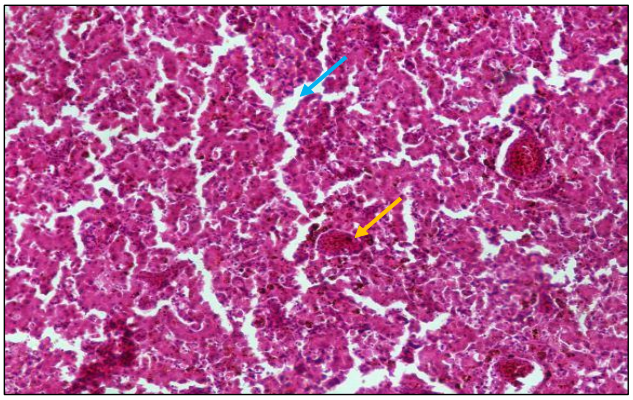

Fig 1: Microscopic section of liver from bird with paratyphoid infection showing congested central vein (yellow arrow) along with dilated and tortuous sinusoids (blue arrow). H & E x 200.

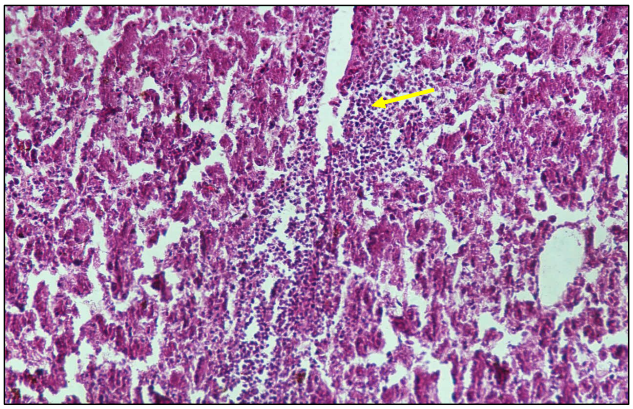

Fig 2: Microscopic section of liver from bird with paratyphoid infection showing large area of polymorph infiltration (yellow arrow) and necrosis. H & E x 200.

Spleen

Spleen showed lymphocytic follicle depletion (Fig 3), fibrinoid necrosis and micro haemorrhages. Similar findings reported by Islam *et al.* (2006) and Kumari *et al*. (2013). Presence of severe congestion, haemorrhage and hemosiderosis on the spleen noted by Dutta *et al.* (2013) and Muna *et al.* (2016). Deshmukh *et al.* (2007) reported microscopic changes of congestion, depletions of lymphocytes from white pulp areas with RE cell hyperplasia and scattered infiltration of granulocytes in red pulp of spleen in Japanese quails.

Intestine

Intestine showed haemorrhages, goblet cell hyperplasia and desquamation of epithelium (Fig 4). Intense cellular infiltration in the caeca and intestine was observed. The findings were comparable to the microscopic lesions observed by Muna *et al.* (2016). Haemorrhages with infiltration of mononuclear cells in the intestinal submucosa were also observed in our study similar to the findings of Dutta *et al.* (2015).

Burkholder *et al.* (2008) discussed that acute stressor can lead to significant changes in the normal intestinal

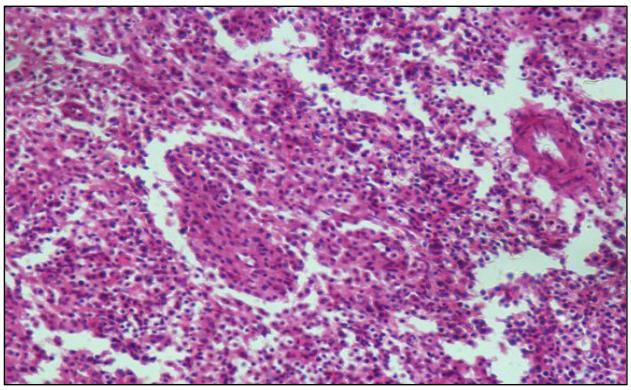

Fig 3: Microscopic section of spleen from bird with paratyphoid infection showing lymphocytic follicle depletion. H & E x 400.

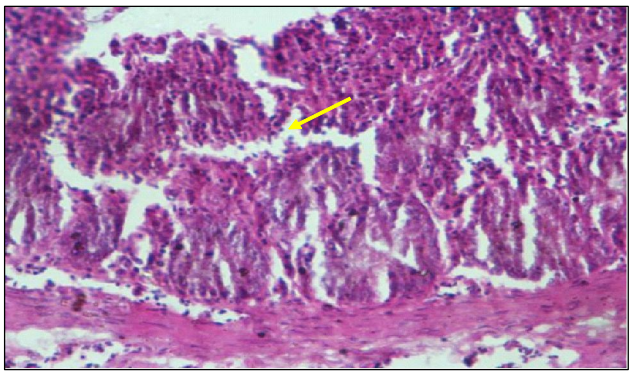

Fig 4: Microscopic section of intestine from bird with paratyphoid infection showing degeneration and desquamation of epithelial lining with denuded mucosa (arrow) and atrophy of intestinal glands. H & E x 400.

microbiota and morphology causing *in vitro* susceptibility for *Salmonella* Enteritidis attachment to the ileum in broilers.

MacCallum goodpasture staining

Intestinal sections which appeared as rod-shaped and pink in colour which confirms the presence of Gram-negative organisms in the intestine.

Hence, we can conclude from our study that even though the salmonellosis infection may not be the primary cause of death in birds, but it certainly contributes to the damage of the organs of alimentary system, therefore, affecting the overall health status of birds.

CONCLUSION

The present study revealed the presence of the paratyphoid bacteria in the poultry farms in Jabalpur region which may be gaining access to the human chain. Clinical signs along with characteristic microscopic lesions confirming paratyphoid infections in birds with gross gastrointestinal lesions were not observed were not considered to be of much significance for the appropriate diagnosis of paratyphoid infection, hence, isolation and identification by following gold standard procedure of culture and biochemical characterization becomes essential. The contaminated fecal samples from *Salmonella* in the poultry sheds and cages can further lead to the contamination of eggs and later chicks and hence control of this bacterial infection within the poultry sheds becomes a challenge.

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