PATHOLOGICAL INVESTIGATION OF FOWL TYPHOID IN CHICKENS IN MYMENSINGH

P.K. Paul1, M.G. Haider2, R. Khaton1, S.K. Ghosh3, P.M. Das1 and M.M. Hossain1*

Abstract

The present study was conducted to determine the prevalence of Salmonella Gallinarum in alive and dead chickens in Mymensingh district of Bangladesh. A total of 125 samples which included 65 swab samples (20 from liver, 15 from ovary and 30 from intestine) of dead layer chickens suspected to Fowl typhoid (FT) and 60 from cloacal swabs of alive layer birds were attempted for isolation of bacteria. Routine methods of bacterial cultures in different media, Gram’s staining techniques and different types of biochemical tests were used for the isolation and identification of Salmonella Gallinarum. Routine histopathological method was used for the detection of tissue level alterations in fowl typhoid infected cases. Out of 125 samples, 8 isolates (6.4 %) which included 5 from dead birds (7.69%) and 3 from live birds (5%) were identified as Salmonella Gallinarum. Grossly, the liver was enlarged and congested and liver revealed bronze discoloration with focal necrosis. Old raised hemorrhages were found in the caecal tonsil. Pericarditis with turbid yellow fluid in the pericardial sac and fibrin attached to the surface of the heart. Congested, deformed, and pedunculated ova were other important findings. There was catarrhal inflammation in the intestine. Microscopically, the section of liver showed congestion and multifocal necrosis with infiltration of heterophils and RE cells. In ovary, different shaped ova with severe congestion and inflammation of egg yolk capsules were found. In heart, pericarditis and myocarditis were characterized by infiltration of RE cells, plasma cells and lymphocytes. Caecal tonsil showed severe hemorrhagic inflammation with infiltration of heterophils, RE cells and lymphocytes.

Key words: Fowl typhoid, Pathology, Chickens

Introduction

At present, there are more than 130 hatcheries producing 5.4 million day-old-chick per week, and about 130,000 commercial broiler and layer farms supplying 0.51 million metric tons of poultry meat and 6210 million table eggs per year (BBS, 2011). Among the different constraints, bacterial infections are the major problem; fowl typhoid is one of these. Fowl typhoid is frequently referred to as a disease of adult birds caused by Salmonella enterica subspecies enterica serovar Gallinarum which is now a day referred to simply as Salmonella Gallinarum (CIDRAP, 2006). It is one of the most important bacterial diseases in poultry industry causing heavy economic loss through mortality and reduced production (Hoque...
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et al., 2000). A survey on both breeding flocks of commercial broiler and layer in major poultry raising belts in and around Dhaka and Gazipur districts in Bangladesh was conducted by Saleque et al. (2003) and reported 16.9% Salmonella infections among the infectious diseases in breeding flock, and 23.2% in layer farms. Investigations at the Department of Pathology, Bangladesh Agricultural University, Mymensingh, Salmonella infections were found in 13.12% cases (Talha et al., 2001).

Fowl typhoid is a most significant disease because the causal agent of the disease is transmitted mainly vertically from parents to offspring. Salmonella Gallinarum is potentially responsible for various pathogenic processes in poultry (Freeman, 1985). Fowl typhoid is worldwide distributed and natural outbreaks occur in chickens, turkeys, guinea fowl, pea fowl, duckling, quail and pheasant either in acute or chronic form. The disease may cause varieties of clinical signs from acute systemic disease and gastrointestinal disorders to embryonic problem in hatchery. The disease is cosmopolitan in distribution all over the world (Shivaprasad, 1997).

It is evident from the above review that for the expansion of poultry industry, it requires preventing and controlling this devasting disease like FT. Effective preventive and control measure cannot be undertaken unless the present status of FT is known. Therefore, the present study is undertaken to know the present status of fowl typhoid in poultry farms of Bangladesh.

**Materials and Methods**

The present research work has two parts: the isolation and identification of Salmonella Gallinarum organism and the pathological studies in Salmonella Gallinarum infected tissues of necropsized poultry birds.

**Samples**

For the isolation and identification purposes, a total of 125 swab samples were collected from different poultry farms in Mymensingh. Among the total samples, 65 (20 from liver, 15 from ovary and 30 from intestine) tissues and swabs from the same tissues were collected from the necropsized birds and 60 cloacal swab samples were from alive layer birds. The bacteriological samples were collected in tetrathionate broth and transferred to the Bacteriology Laboratory of the Department of Pathology, BAU, Mymensingh. All the tissue samples for histopathological study were preserved in 10% neutral buffered formalin solution.

**Culture media**

Tetrathionate broth (TTB), Nutrient agar, Triple sugar iron (TSI) agar, MacConkey (MC) agar, Salmonella -Shigella (SS) agar, Nutrient broth (NB), Triple Sugar Iron (TSI) agar slant, Methyl-Red solution and MR-VP Medium were used.

**Isolation and identification of the organisms**

Test tubes containing samples in Tetrathionate broth (TTB) were incubated for 24 hours at 37°C. From the Tetrathionate broth (TTB), subcultures were made on Nutrient agar, MacConkey agar, TSI agar and SS agar, and incubated at 37°C for over night. The
identification of the organisms was performed by the tests as described by Brooks et al., (2002) and Muktaruzzaman et al. (2010). On the basis of colony and staining characters, and biochemical tests the organisms were isolated and identified.

**Morphological characterization by Gram’s staining method**
The representative Salmonella colonies were characterized microscopically using Gram’s stain according to the method described by Haider et al. (2012).

Identification of isolated *Salmonella* Gallinarum by using specific biochemical tests For this study, carbohydrate fermentation tests, TSI slant reaction, MR-VP reaction and dulcitol fermentation test were selected for identification of isolated suspected colonies. All of the isolates from different sources were tested for the detection of the organism according to the methods Charlton (2000) and Haider et al. (2012).

**Carbohydrate fermentation test**
The carbohydrate fermentation test was performed according to the method of Haider et al. (2012).

**Methyl red test**
The test was performed according to the method described by Haider et al. (2012).

**Voges-Proskauer (V-P) test**
The VP test was performed according to the method described by Haider et al. (2012).

**Dulcitol fermentation test**
This test was performed as like as carbohydrate fermentation test as described earlier and reaction profiles were recorded.

**Motility test**
The motility test was performed according to the method described by Haider et al. (2012).

**Maintenance of stock culture**
During the experiment the isolated organisms was preserved for longer periods by standard procedure for further study.

**Gross Pathology**
The postmortem examination in all the cases was performed for the dead birds. At necropsy, gross tissue changes were observed and recorded carefully, and representative tissue samples containing lesions were fixed in 10% neutral buffered formalin.

**Histopathology**
The tissues were trimmed, washed, processed in ascending grades of alcohol, cleared in chloroform, embedded in paraffin, sectioned using a microtome and stained as per standard procedure (Luna, 1968). Finally the sections were mounted with cover slip using DPX.

**Photomicrography**
Photomicrography was taken at the Department of Pathology using photo microscopic camera (Olympus PM-C 35 Model).

**Results and Discussion**
To determine the prevalence of *Salmonella* Gallinarum, a total of 60 cloacal swabs were collected for isolation and identification from alive layer birds. On the other hand, 65 swabs and tissue samples were also collected for the isolation and identification of bacteria from dead birds. Histopathological examinations were performed in tissues from where *S. Gallinarum* was isolated, namely liver, ovary and intestine. By all these studies, *Salmonella* Gallinarum was isolated and identified.
Prevalence of isolated and identified organisms
The bacterial floras were isolated from liver, ovary and intestine of dead birds and cloacal swabs of live birds. The results of isolation of *Salmonella* Gallinarum in different samples (liver, ovary, intestine and cloacal swabs) are shown in Table 1.

The prevalence of *Salmonella* Gallinarum infection in liver, ovary and intestinal swabs of dead birds was 7.69% and the prevalence of *Salmonella* Gallinarum in cloacal swabs of live birds was 5%. The prevalence of *Salmonella* Gallinarum infection was higher in ovarian and liver samples than intestinal samples in dead birds.

Identification of the organism by different bacteriological method Cultural characteristics of *Salmonella* Gallinarum in different media (Fig.1, 2 and 3) and staining character (Fig. 4) were shown in table 2.

**Biochemical activities of the isolated organism**

Biochemical activities of the isolated organism were shown in table 3.

The prevalence of *Salmonella* Gallinarum in dead chickens was 7.69% and in live birds was 5%. *Salmonella* Gallinarum bacteria was confirmed by culture in different media, specific colony characters, Gram’s staining techniques, different types of biochemical tests, microscopic examination, gross and histopathological study. In this study, the colony characters of *Salmonella* Gallinarum, the production of hydrogen sulfide gas with black color colonies on TSI agar, whitish

**Table 1. Isolation and identification of Salmonella Gallinarum from different organs of dead and alive birds.**

<table>
<thead>
<tr>
<th>Samples/ Swabs</th>
<th>No. of samples tested (n=125)</th>
<th>No. of Isolates</th>
<th>Prevalence of the different organs</th>
<th>Total Number of Isolates</th>
<th>Overall prevalence among tested cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>20</td>
<td>2</td>
<td>10%</td>
<td>5</td>
<td>6.4%</td>
</tr>
<tr>
<td>Ovary</td>
<td>15</td>
<td>2</td>
<td>13.33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>30</td>
<td>1</td>
<td>3.33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloacal</td>
<td>60</td>
<td>3</td>
<td>5%</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Cultural and staining characteristics of Salmonella Gallinarum in different media.**

<table>
<thead>
<tr>
<th>Media</th>
<th>Cultural characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrathionate broth (TTB)</td>
<td>slight turbidity</td>
</tr>
<tr>
<td>Nutrient Broth (NB)</td>
<td>diffused turbidity</td>
</tr>
<tr>
<td>Nutrient Agar (NA), MacConkey agar</td>
<td>small, round and translucent smooth colony</td>
</tr>
<tr>
<td>Brilliant Green Agar (BGA)</td>
<td>colorless, smooth, pale and transparent colonies</td>
</tr>
<tr>
<td>Triple Sugar Iron (TSI) Agar</td>
<td>whitish opaque color colonies surrounded by a brilliant red zone</td>
</tr>
<tr>
<td>Salmonella-Shigella (SS) Agar</td>
<td>black color colonies and media took reddish color</td>
</tr>
<tr>
<td>Gram’s staining</td>
<td>black colonies and media exhibited yellowish color</td>
</tr>
<tr>
<td></td>
<td>gram-negative, pink color, small rod shaped appearance, arranged in single or paired</td>
</tr>
</tbody>
</table>
Table 3: Biochemical activities of the isolated organism.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Maltose</th>
<th>Sugar media</th>
<th>MR test (Methyl red)</th>
<th>VP test (Voges-proskauer)</th>
<th>Motility test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates 1</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 2</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 3</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 4</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 5</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 6</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 7</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isolates 8</td>
<td>Acid</td>
<td>Acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Legends:
+ = Positive; - = Negative.
opaque color colonies surrounded by a brilliant red zone on BGA and black color colonies on SS agar were corresponded with the findings of other author (Perez et al, 2004). In Gram’s staining, the morphology of the isolated bacteria was small, rod shape, gram negative, single or paired in arrangement which was supported by several authors (Freeman, 1985; Hossain et al, 2006). In the present study, specific biochemical media were used for the confirmation of Salmonella Gallinarum. All of the isolates fermented dulcitol and maltose but did not ferment lactose and all of the isolates were Methyl red positive and Voges-Proskauer negative which were previously suggested by a number of scientists (Haider et al, 2003; Sujatha et al, 2003). The ability or inability of Salmonella Gallinarum to ferment different carbohydrates was used as fundamental basis for their isolation but species identification was difficult (Freeman, 1985). In the present study, eight isolates nonmotile Salmonella Gallinarum fermented dulcitol. On the basis of this test, these eight isolates bacteria were considered as Salmonella Gallinarum (OIE Manual, 2004). The dulcitol fermentation test is performed to confirm their differentiation of S. Pullorum and S. Gallinarum world wide (Robinson et al, 2000). In the present study, eight of the isolates were non motile. Motility test was fundamental basis for the detection of non-motile Salmonella Gallinarum organisms (Buxton and Fraser, 1977; Freeman 1985). Non-motile organisms were considered S. Gallinarum (Goswami et al, 2003).

In the present study, the prevalence of Salmonella Gallinarum in dead and alive chickens were relatively lower than the reports by other authors (Sujatha et al, 2003; Rahman et al, 2004; Hossain et al, 2006). This is probably due to the fact that other authors diagnosed the disease mostly based on the postmortem lesions. In this study, the disease fowl typhoid was confirmed by isolating the organisms from infected birds, necropsy findings and histopathological lesions. Results of all the parameters used for the diagnosis of fowl typhoid corresponded with the findings of others (Shivaprasad, 1997 and Talha et al, 2001). Among the 8 isolates, 2 isolates were isolated from liver, 2 from ovary, 1 from the intestine and 3 from cloacal swabs. These findings revealed that liver and ovary was the main target for the isolation of Salmonella Gallinarum, which are in close agreement with other authors (Sujatha et al, 2003).

**Gross lesions**

In heart, no specific gross lesions were found but there was the presence of pericardial adhesion (Fig.5). The liver was enlarged and congested and in few cases, liver revealed bronze discoloration with focal necrosis (Fig.6). Congested, deformed, discolored and pedunculated ova were other important findings (Fig.7). Old raised hemorrhages were found in the caecal tonsil (Fig.8).

**Microscopic lesions**

The section of liver showed infiltration of mainly lymphocytes, RE cells and heterophils around the central vein in Salmonella Gallinarum infection. (Fig.9). In heart, pericarditis was characterized by thickening of pericardium due to infiltration of RE cells and myocarditis was characterized by infiltration of RE cells, plasma cells and...
Fig. 5. Fowl typhoid affected heart shows congestion (→) with turbid yellow fluid in the pericardial sac.

Fig. 6. Liver shows enlargement, congestion and revealed bronze discoloration (→) with focal necrosis in fowl typhoid.

Fig. 7. *Salmonella Gallinarum* affected chicken shows congestion (→), deformation, discoloration and pedunculated ova with stalk formation.

Fig. 8. *Salmonella Gallinarum* affected chickens show old, raised haemorrhages (→) in caecal tonsil.

Fig. 9. Section of liver showing infiltration of lymphocytes, RE cells and heterophils around the central vein in fowl typhoid, (H & E, X 330).

Fig. 10. Section of heart showing infiltration of RE cells and myocarditis is characterized by infiltration of RE cells associated with mild degeneration of muscle fibers in fowl typhoid (H & E, X 330).
associated with mild degeneration of muscle fibers (Fig.10). Caecal tonsil showed severe hemorrhagic inflammation characterized by infiltration of heterophils, RE cells, lymphocytes and associated with hemorrhages (Fig.11). In Ovary, different shaped ova with severe congestion and inflammation of a egg yolk capsules were found (Fig.12).

In pathological investigation, grossly the liver was enlarged and congested liver with focal necrosis, darker ovary with stalk formation. Microscopically, the liver showed focal degeneration, focal necrosis with infiltration of mononuclear cells. The intestinal wall exhibited congestion with infiltration of mononuclear cells in the submucosa. These types of histological lesions are supported for *Salmonella* Gallinarum infection by different investigators (Rahman, 2004).

In conclusion, liver and ovarian swabs could be the choice for the isolation of *Salmonella* Gallinarum infection. Congestion in ovary with stalk formation, hemorrhage in caecal tonsil and microscopically multi focal necrosis with infiltration of mononuclear cells in the liver could indicate the presence of *Salmonella* Gallinarum infection. However, further studies will be focused on determination of immunogenic variation and development of vaccine candidate.

**References**


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