J. Bangladesh Agril. Univ. 10(1): 87–94, 2012 **ISSN 1810-3030**

Experimental pathogenesis of pullorum disease in chicks by local isolate of *Salmonella* Pullorum in Bangladesh

M. G. Haider[1], E. H. Chowdhury[2], A. K. M. Ahmed[2] and M. M. Hossain[2]
[1]Department of Pathobiology, Bangabandhu Shiekh Mujibur Rahman Agricultural University, Gazipur and
[2]Department of Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
E mail : mmhossain04@yahoo.com.au

Abstract

This study was undertaken to observe the experimental pathogenesis of locally isolated *Salmonella enterica* subspecies *enterica* serovar Pullorum in chicks. Fifty chicks were experimentally infected by the oral route with 2 x 10^7 (CFU) units of *Salmonella* Pullorum organisms reconstituted in 0.5 ml of sterile phosphate buffer saline (PBS), PH 7.2 and 50 chicks were given only 0.5 ml of sterile PBS as control. Observations were made on clinical signs, gross pathology, and reisolation of *S*. Pullorum from different organs and blood, histopathological lesions, detection of antibody levels and detection of *S.* Pullorum by PCR at different time intervals of experimental period. Five birds were randomly selected and sacrificed on 6 hrs before inoculation and 6 hrs, 12 hrs, 24 hrs, 2 days, 3 days, 1st week, 2nd, 3rd and 4th weeks of post infection (PI). The clinical signs of infected chicks were depression, loss of appetite, huddled together, loss of feed and water intake, reduced mean body weights, ruffled feathers, diarrhoea, laboured breathing and pasty vent. The highest gross lesion was (84%) unabsorbed and coagulated yolk and the lowest lesion was (32%) pericarditis and necrotic foci/ nodules in heart. Microscopically, the liver showed congestion, focal necrosis with multifocal infiltration of histiocytes in liver parenchyma. *Salmonella* organisms were reisolated from different organs and blood at 12 hrs PI. The antibody titre increased gradually and the highest titer was 7275.717 ± 5087.24 at 4 wks PI. In rapid plate agglutination test, the positive result was found from one wk of PI with the sera of infected birds. At 12hrs PI *Salmonella* was detected by PCR from 20% liver and 20% lung samples of infected birds and no *Salmonella* was isolated from control group. The orally inoculated *Salmonella* Pullorum organisms produced lesions in digestive tract, invaded digestive tracts and entered to blood and seeded to different organs in different time intervals and ultimately produced clinical signs, gross and microscopic tissue lesions with immunological response.

Keywords: Pathogenesis, Pullorum disease, ELISA, PCR, Chicks

Introduction

Salmonellosis in poultry cause heavy economic loss through mortality and reduced production (Khan *et al.,* 1998). With great expansion of the poultry rearing and farming, PD has become wide spread problem in Bangladesh (Das *et al.,* 2005; Rahman, 2007). For the treatment, prevention and control of a particular disease, the pathogenesis must be known clearly. Some investigations on pullorum disease have been performed on the natural cases, but a very few work on experimental pathogenesis and pathology of pullorum disease has been reported in pullet (semi-mature birds) in Bangladesh by locally isolated *Salmonella* Pullorum (Haider *et al.*, 2008). Pathogenesis of a disease also varies with the age of the host. Therefore, the present study was undertaken to study the experimental pathogenesis and pathology in chicks (immature birds) with locally isolated *Salmonella* Pullorum.

Materials and Methods

Chicks: 100 day-old (*Salmonella* Pullorum seronegative) chicks of Isa Brown breed were purchased from Nourish Hatchery Ltd. for this experimental inoculation. The birds were divided into 2 groups. One group was used as experimental infection and other was kept as control.

Experimental infection: Chicks were reared in experimental shed with optimum brooding temperature and they were given commercial feed and water *ad libitum* throughout the study period. Fifty chicks were experimentally infected by the oral route with 2 x 10^7 (CFU) unit of *Salmonella enterica* sub. *enterica* serovar Pullorum organisms in 0.5 ml of sterile phosphate buffer saline (PBS), pH 7.2 (Roy *et al.,* 2001). The control birds received only 0.5 ml PBS without bacteria.

Clinical signs: Clinical signs of chicks after experimental infection were observed and recorded up to the end of the experiment.

Sample collection: Five birds were randomly selected and sacrificed on 6 hrs before inoculation and 6 hrs, 12 hrs, 24 hrs, 2 days, 3 days, 1st week, 2nd, 3rd and 4th weeks of post infection. A total of 50 birds were used for the control group, and were necropsized on scheduled dates as in the infected groups.

Bacterial sample: The bacteriological samples (crop, liver, lung, heart, cecum, blood, bile and yolk or spleen) were collected at different time interval described in the experimental design with pre-enriched in buffered peptone water in sterile poly bags.

ELISA and rapid plate agglutination (RPA) sample: The serum samples were collected during necropsy at different time interval described in the experimental design for ELISA and Rapid Plate Agglutination (RPA) test (Islam *et al*., 2006; Haider *et al.,* 2007a)

Histopathological sample: Liver, lungs, heart, spleen, intestine, etc. were collected in 10% buffered-formalin at different time interval described in the experimental design for histopathological examination.

PCR sample: Liver and lungs were collected in 50% buffered- glycerol in sterile poly bags at different time interval described in the experimental design and preserved in - 80^0C for PCR (Haider *et al.,* 2007b).

Gross pathology: The postmortem examination of all the birds performed as per schedule. At necropsy, gross tissue changes were observed and recorded carefully following method described by Wray and Davis, 2001.

Reisolation of *S*. Pullorum from different organs and blood: *S*. Pullorum was reisolated from different organs (one gm) and blood (one ml) samples by standard methods (Cheesbrough, 2000).

Detection of antibody levels by ELISA: An enzyme–linked immunosorbent assay (ELISA) with antigen coated ELISA kits (FLOCKSCREEN™ Guild Hay Ltd. UK) was used (Solano *et al*., 2000). The obtained optical density (OD) with sera was measured at 550 nm with an ELISA reader (Toso Ltd. MRP-A4i, Tokyo, Japan).

Detection of antibody by rapid plate agglutination (RPA): Rapid plate agglutination (RPA) test was done with *Salmonella* Pullorum antigen (Yamane *et al.,* 2000). Two drop sera samples of different time interval were mixed with an equal volume of antigen on a plate, and typical agglutination occurred within 2 min. was taken as a positive result.

Histopathological study: The representative formalin–fixed tissue samples were trimmed, processed, sectioned and stained performed by standard procedure (Luna, 1968).

Detection of *S.* Pullorum by PCR: The preserved liver and lung tissue samples were used for the detection of *S.* Pullorum by PCR according to standard procedure (Haider *et. al*., 2007b).

Statistical analysis: Significance of different groups was determined by using MSTAT computer software program. The differences in the increase or decrease of the ELISA antibody titre of chicks of different groups at different week were analyzed by analysis of variance in a Completely Randomized Design (CRD) using an MState computer package program. Significant differences between means were identified by Least Significant Differences (LSD).

Results and Discussion

Clinical signs: The chicks infected with *S.* Pullorum showed depression and loss of appetite after 12 hrs of PI. They were huddled together after 24 hours of PI. Feed and water intake dramatically reduced in infected group. The mean body weights differed significantly ($p<0.01$ and $p<0.05$) between infected and control group. Cumulative clinical sings were 70% loss of appetite, 76% depression, 72% ruffled feathers, 58% labored breathing, 48% diarrhoea and 52% pasty vent (Fig.1).The morbidity (clinical signs) was 100% in experimental infection group. The mortality was 8% after experimental infection from 3 day to 7 days. Recovery from clinical signs began after 2 wk PI. No clinical signs and mortality were seen in the control group.

Gross pathology: The haemorrhagic and congested liver and lung were observed at 12hr of PI and increased severely. The gross lesions reduced gradually at 3 wks of PI. The gross findings were 54% haemorrhagic and congested liver, 38% necrotic foci in the liver, 32% pericarditis and necrotic foci/ nodules in heart, 52% congested, edematous and brown colour lungs, 46% semi-solid, cheesy material in ceca, 84% unabsorbed and coagulated yolk, 42% spleen swollen and congested and 40% kidney enlarged (Table 1 and Fig. 2). The most frequent lesion was (84%) unabsorbed and coagulated yolk and the less frequent lesion was (32%) pericarditis and necrotic foci/ nodules in heart.

Table 1. Cumulative gross pathologic findings of chicks infected with *Salmonella* Pullorum

Lesions	BI 6hrs	PI 6hrs	PI 12 hrs	PI 24 hrs	PI 48 hrs	PI 72 hrs	PI 1 wk	PI 2 wks	PI 3 wks	PI 4 wks	Total (%)
1.Haemorrhagic and congested liver	0/5	0/5	1/5	2/5	2/5	4/5	5/5	5/5	5/5	3/5	54
2.Necrotic white foci in the liver	0/5	0/5	0/5	0/5	1/5	2/5	5/5	5/5	4/5	2/5	38
3.Pericarditis and necrotic foci/ nodules in heart	0/5	0/5	0/5	0/5	0/5	1/5	5/5	5/5	3/5	2/5	32
4.Congested, edematous and brown colour lungs	0/5	0/5	1/5	2/5	2/5	4/5	5/5	5/5	4/5	3/5	52
5.Semi-solid, cheesy material in ceca	0/5	0/5	0/5	0/5	1/5	2/5	5/5	5/5	5/5	5/5	46
6.Unabsorbed and coagulated yolk	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	2/5	0/5	84
7.Spleen swollen and congested	0/5	0/5	0/5	0/5	1/5	3/5	5/5	5/5	4/5	3/5	42
8.Kidney enlarged	0/5	0/5	0/5	0/5	1/5	2/5	5/5	5/5	5/5	2/5	40

Note: Percentage calculated from before infection (BI) 6 hrs to 4 wks post infection (PI)

Histopathological lesions: Hepatitis with infiltration of inflammatory cells in 20% liver, and pneumonia and bronchopneumonia in 20% lung were found after 12 hr PI. Most of the histopathological lesions were found after 1 wk PI. The most histological lesion was focal necrosis with infiltration of inflammatory cells in 80% spleen and the less frequent histological lesion was nodule formation in 16% liver (Table 3 and Fig. 4). The 58% liver showed congestion, focal necrosis with multifocal infiltration of histiocytes in liver parenchyma. Lung showed haemorrhage and infiltration of neutrophils and lymphocytes in experimental PD in chicks. Bronchopneumonia with infiltration of heterophils and mononuclear cells in the lung of chicks was found (60%). Infiltration of heterophils was found in 26% heart. Nodule was formed with infiltration of heterophils, macrophage and lymphocytes in the heart of chicks. The spleen exhibited severe congestion, mild hyperplasia of RE cells and fibrinoid necrosis. The intestinal and cecal mucosa exhibited infiltration of mononuclear cells in the submucosa. No lesion was found in control group.

Reisolation of *S*. Pullorum from different organs: Reisolation of *Salmonella* Pullorum from different organs was variable at different time schedule. At 6hr PI all crop were positive for *Salmonella* Pullorum. *Salmonella* Pullorum was isolated from crop, liver, heart, lungs, cecum, bile and yolk or spleen after 12 hr PI. All organs were positive for *S.* Pullorum after 1 wk PI to 4 wk PI (Table 2 and Fig. 3). The highest number of *S*. Pullorum was found (16.14×10^8 CFU) in liver at 72hr PI. Control group was free of *S.* Pullorum in culture during the study period. Colony characteristic of reisolated *Salmonella* Pullorum were pink opaque coloured colonies surrounded by brilliant red zones in BGA.

Table 2. Mean CFU/gm of isolated and identified *Salmonella* Pullorum from different organs of experimentally infected chicks

Organs	BI 6 hrs	PI 6 hrs	PI 12 hrs	PI 24 hrs	PI 48 hrs	PI 72 hrs	PI 1 wk	PI 2 wks	PI 3 wks	PI 4 wks
1.Crop	00 (0/5)	11.56 x 10^{10} (5/5)	52 x10^{9} (5/5)	57.2 x 10^{8} (5/5)	87 x 10^{7} (5/5)	39.2 x 10^{6} (5/5)	49 x 10^{5} (5/5)	84.6 x 10^{4} (5/5)	39 x 10^{4} (3/5)	42 x 10^{4} (2/5)
2.Liver	00 (0/5)	00 (0/5)	14 x 10^{7} (1/5)	50.75 x 10^{7} (3/5)	71.6 x 10^{7} (5/5)	16.14 x 10^{8} (5/5)	13.24 x 10^{8} (5/5)	69 x 10^{7} (5/5)	38.67 x 10^{6} (4/5)	73 x10^{5} (4/5)
3.Heart	00 (0/5)	00 (0/5)	16 x 10^{7} (1/5)	29 x 10^{7} (2/5)	62.33 x 10^{7} (4/5)	91.6 x 10^{7} (4/5)	71.9 x 10^{7} (5/5)	48.22 x 10^{7} (5/5)	30.67 x10^{6} (2/5)	91.6 x 10^{5} (1/5)
4.Lungs	00 (0/5)	00 (0/5)	32.6 x 10^{7} (1/5)	55 x 10^{7} (2/5)	70.8 x 10^{7} (5/5)	67.32 x 10^{7} (5/5)	35.4 x10^{7} (5/5)	10.2 x10^{7} (5/5)	78.5 x10^{5} (2/5)	55.4 x10^{4} (1/5)
5.Cecum	00 (0/5)	00 (0/5)	22 x 10^{7} (2/5)	42.8 x 10^{7} (4/5)	91.4 x 10^{7} (5/5)	71.6 x 10^{7} (5/5)	62.2 x 10^{7} (5/5)	85.4 x 10^{6} (5/5)	11 x 10^{6} (5/5)	52 x 10^{5} (5/5)
7. Bile	00 (0/5)	00 (0/5)	7 x 10^{7} (1/5)	10.25 x 10^{7} (3/5)	83.2 x 10^{7} (5/5)	30.8 x 10^{7} (5/5)	55.4 x 10^{7} (5/5)	53.6 x 10^{6} (5/5)	29.33 x 10^{6} (2/5)	52 x 10^{5} (1/5)
8. Yolk	00 (0/5)	00 (0/5)	78.3 x 10^{6} (2/5)	96.7 x 10^{6} (3/5)	96.7 x 10^{6} (5/5)	19.34 x 10^{7} (5/5)	54.25 x 10^{7} (5/5)	11.4 x 10^{5} (5/5)	37 x 10^{4} (2/5)	-
9.Spleen	00 (0/5)	00 (0/5)	-	-	-	-	-	30.75 x 10^{6} (5/5)	28.5 x10^{5} (3/5)	72.4 x10^{4} (2/5)

- = culture not done

Table 3. Cumulative histopathological findings of chicks infected with *Salmonella* Pullorum

Lesions	BI 6hrs	PI 6hrs	PI 12 hrs	PI 24 hrs	PI 48 hrs	PI 72 hrs	PI 1 wk	PI 2 wks	PI 3 wks	PI 4 wks	Total (%)
1. Hepatitis, infiltration of inflammatory cells	0/5	0/5	1/5	3/5	5/5	5/5	5/5	5/5	3/5	2/5	58
2. Multifocal necrotic foci in the liver	0/5	0/5	0/5	0/5	1/5	2/5	3/5	4/5	1/5	1/5	24
3. Nodule formation in the liver	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5	3/5	2/5	16
4. Pneumonia, bronchopneumonia	0/5	0/5	1/5	2/5	4/5	5/5	5/5	5/5	4/5	4/5	60
5. Nodule formation in the lungs	0/5	0/5	0/5	0/5	0/5	0/5	2/5	3/5	4/5	2/5	22
6.Pericarditis/myocarditis	0/5	0/5	0/5	0/5	1/5	4/5	5/5	4/5	2/5	0/5	32
7. Nodule formation in the heart	0/5	0/5	0/5	0/5	0/5	0/5	3/5	5/5	4/5	2/5	26
8. Focal necrosis and inflammatory cells in the spleen	-	-	-	-	-	-	-	5/5	5/5	2/5	80
9. Infiltration of inflammatory cells in the intestine	0/5	0/5	0/5	0/5	2/5	4/5	5/5	5/5	5/5	3/5	48
10. Ulcer in the cecal tonsils	0/5	0/5	0/5	0/5	0/5	3/5	5/5	5/5	4/5	2/5	38
11. Tyhpilitis, infiltration of inflammatory cells	0/5	0/5	0/5	0/5	2/5	4/5	5/5	5/5	5/5	4/5	50
12. Congestion in the kidneys	0/5	0/5	0/5	0/5	1/5	2/5	5/5	5/5	4/5	2/5	38
13. Infiltration of inflammatory cells in gizzard	0/5	0/5	0/5	0/5	1/5	1/5	5/5	5/5	3/5	1/5	34

Note: Percentage calculated from 6 BI to 4 wks PI

Reisolation of *S.* Pullorum from blood: The mean CFU/ml of *Salmonella* Pullorum was reisolated from blood. The highest number of *S.* Pullorum was reisolated (16.56x 10^8 CFU) at 72hr PI and the lowest number of *Salmonella* Pullorum was reisolated (49.8x 10^5 CFU) at 4 wk PI. No *Salmonella* Pullorum was found in the control group.

Detection of antibody titer by ELISA: Antibody titer (Mean±SD) against *Salmonella* Pullorum was determined in sera collected in different time interval. The antibody titres of infection group were increased gradually and the antibody titres were decreased gradually in basal level in control group (Table 4 and Fig. 5).

Table 4. Antibody titre in chicks infected with *Salmonella* Pullorum detected by indirect ELISA

Time schedule of PI	Infection group (Mean±SD)	Control group (Mean±SD)
BI 6 hr	290.83±119.09d*	284.18±142.12a*
PI 6 hr	293.35±132.81d*	275.49±138.84ab*
PI 12 hr	253.552±80.99d*	270.05±138.32ab*
PI 24 hr	244.24±83.26d*	249.46±121.76ab*
PI 48 hr	208.31±67.37d*	238.56±122.17ab*
PI 72 hr	157.93±85.61d*	230.13±126.62ab*
PI 1 wk	562.92±222.31cd*	228.93±100.09b*
PI 2 wk	2668.798±1475.35c*	209.55±98.77b*
PI 3 wk	5128.28±3755.53b*	210.64±115.23b*
PI 4 wk	7275.72±5087.24a*	198.58±102.99b*

*The mean difference is significant at $p<0.01$

Rapid plate agglutination (RPA): 100 % chicks were found positive in infected group after 72 hrs PI. Control group was found negative.

Detection of *S.* Pullorum by PCR: *S.* Pullorum was detected by PCR after PI at different time interval. No *S.* Pullorum was detected by PCR before and after 6 hr PI. At first *S.* Pullorum was found after 12 hr PI. At 24 hr PI 60% liver and 40% lungs were positive for *S.* Pullorum by PCR. Five liver (100%) and five lung (100%) samples were positive from 48hr PI to 2 wk PI (Fig. 6). Detection of *S.* Pullorum by PCR decreased after 2 wk PI. Four liver (80%) and two lung (40%) samples were positive at 3 wk PI, and four livers (80%) and one lung (20%) samples were positive at 4 wk PI by PCR. No *S.* Pullorum was detected in control birds during the study period.

Chickens are the natural host of *Salmonella enterica* subspecies *enterica* serovar Pullorum. Clinical signs appeared in chicks after 12 hour of PI and these were loss of appetite, depression, drowsiness, ruffled feather, diarrhoea, labored breathing, loss of body weight, and pasty vent. The clinical findings in this study were similar to the natural/experimental findings of other authors (Shivaprasad, 1997; Wary and Davies 2001). Mortality observed in this study was 8% which was lower than the findings of others (Chauan and Roy, 1996 and Shivaprasad, 1997). Endotoxins are produced by *S.* Pullorum causing fever. In this investigation, the gross findings described above with the findings corresponded with slight variation to the findings of other the authors (Wary and Davies, 2001).

In this investigation, hepatitis, tyhpilitis, bronchitis and pneumonia were corresponded the finding of Roy *et al.* (2001). In the present study, the histopathological lesions were supported for *Salmonella* infection by different investigators (Chauan and Roy, 1996; Shivaprasad, 1997; Wary and Davies, 2001).

Fig. 1. Chicks showing pasty vent at 2 wks PI with *S*. Pullorum with *S*. Pullorum

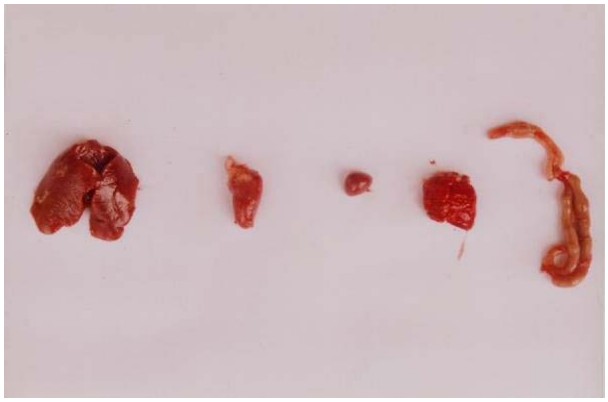

Fig. 2. Liver showing focal necrosis and congestion; heart, spleen and lungs showing congestion; enlarged ceca and contains cheesy materials at 2 wks PI with *S*. Pullorum

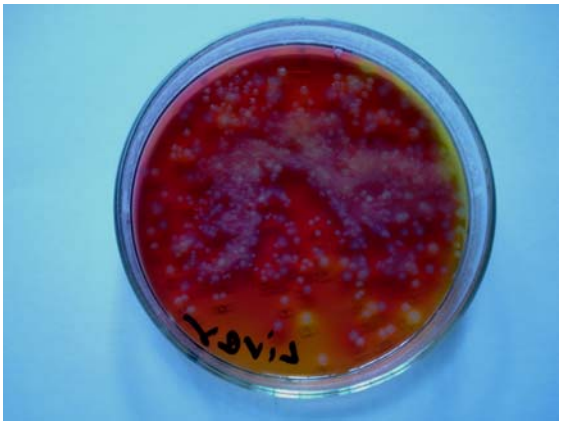

Fig. 3. Experimentally infected with *S*. Pullorum at 1 wk PI showing 263 CFU of *S.* Pullorum / gm tissue of liver at 10^{7} dilution

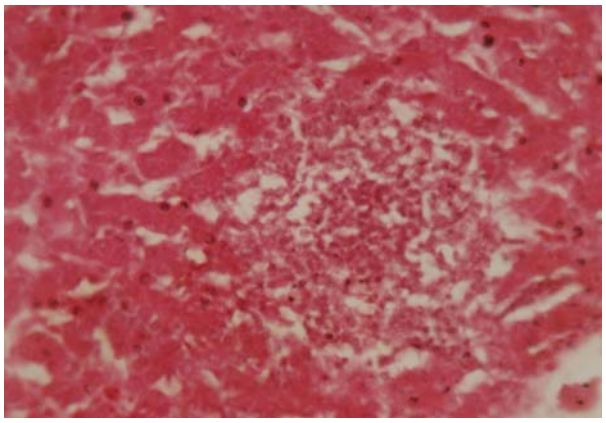

Fig. 4. Liver showing focal necrosis with nodule formation in experimental PD in chicks at 3 wks PI (H&E, X 333)

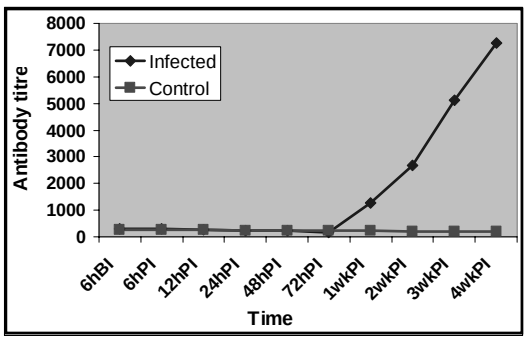

Fig. 5. Mean antibody titer after experimental infection with *S.* Pullorum at different time interval

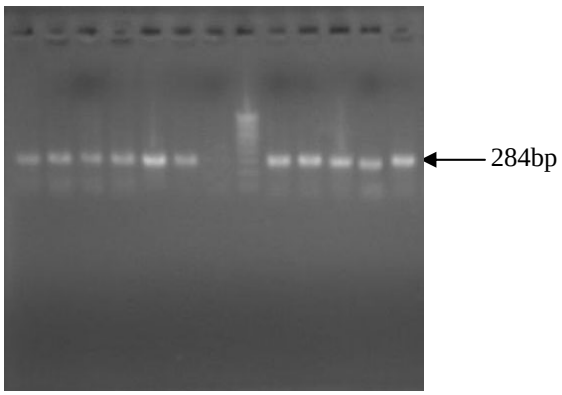

Fig. 6. Electrophoresis on 1.5% agarose gel showing the 284bp PCR product after 2 wk of PI

In the present study, after experimental infection *S.* Pullorum was reisolated from different organs at 12 hr PI. The results of the oral route of inoculation demonstrated that *S.* Pullorum caused bacteremia and colonized in the liver, lungs, heart, kidney, intestine, spleen, and ceca of chicks to various degrees (Wigley, *et al.,* 2005). But the highest number of organisms reisolated from 72hr to 1 wk PI. From 2 wk PI reisolation of *S.* Pullorum organisms reduced gradually. The findings of the reisolation and identification of *Salmonella* Pullorum organisms from different organs were similar with other investigators (Roy *et al.,* 2001; Okamura *et al.,* 2000; Hoop and Pospishil, 1993).The highest percentage of reisolation of S. Pullorum from different organs at 7 days PI corresponded with the reisolation of Roy *et al.* (2001). Wigley *et al.* (2001) also recovered S. Pullorum after experimental inoculation at 7 days PI from all tissues samples which also supported the findings of the present study. The reisolation of *Salmonella* Pullorum in blood culture was highest at 72 hr PI (16.56 X 10^8 CFU) which was similar to Okamura *et al.* (2000).

In this investigation, experimental infection by *S.* Pullorum, the immune response increased gradually from 1wk PI and the highest titre was found at 4 wks PI which was also similar to the findings of Barrow *et al.* (1992) but disagreed with the findings of Hoop and Pospishil (1993). However, other investigators used other *Salmonella* organisms to determine the immune response by ELISA and RPA test. Their findings varied with our findings that might be due to the strain variation, management, age etc. Skov *et al*. (2002) found highest ELISA titre at 3 wks PI with the infection of *S*. Typhimurium in Chickens.

In the present study, serum samples showed RPA from 72 hrs PI but all sera were found RPA positive from 2 wk PI. Yamane *et al.* (2000) found all sera were positive at 17 wks PI with the infection of *S.* Enteritidis in layer chickens. The serum rapid plate agglutination was also done in natural infection by Islam *et al.* (2006) and the findings were almost similar to the present findings.

All *Salmonella* strains screened by PCR resulted in visualization of the predicted 457-bp amplified product in ethidium bromide-stained gels (Stone *et al*., 1994) and in the present study *inv*A gene also visualized and produced 284-bp amplicon. In this investigation, *Salmonella* Pullorum was detected from tissue (liver and lungs) samples by PCR with the amplification of *inv*A gene at 12 hr PI. Olivera *et al.* (2003) and Desai *et al.* (2005) also detected 284-bp amplicon from tissue samples in experimental infected chickens.

Conclusion

Salmonella Pullorum causing pullorum disease is a major problem in poultry farms in Bangladesh. It causes great losses in chicks. In this study, 8% mortality was found in chicks from 3 to 7 days of PI. Clinical signs were observed from 24 hrs PI and in all birds. The gross and histopathological lesions were observed from 72 hrs and 2wk PI, respectively to 3 wks PI and the highest lesions were found from 1wk to 2 wks PI and 2-3 wks PI, respectively. Gross and histopathological lesions were variable in different birds at different time intervals. The highest number of *S.* Pullorum was 16.14x10^8 CFU/gm of liver tissues reisolated at 72 hrs PI. Blood was positive for *S.* Pullorum at 12 hrs PI. ELISA titre gradually increased from 1 wk PI and at the same time infection decreased. *S.* Pullorum was detected by PCR from 12 hrs PI in liver and lung. At 2 wks PI, the chicks recovered from clinical signs gradually from experimental infection with *S.* Pullorum. In short, the pathogenesis of experimental pullorum disease in chicks was, after inoculation of *Salmonella* Pullorum by oral administration, the organism localized in digestive tract, from where entered to blood and localized in different tissues at different time intervals producing lesions and immunological response. However, for useful application of the present research findings further studies should be focused on the serotyping and molecular characterization and identification of immunogenic variation and development of vaccine with isolated *Salmonella*.

Acknowledgement

The authors thankfully acknowledge the BAURES authority for providing financial support to conduct this research.

References

Chauhan, H.V.S. and Roy, S. 1996. Poultry Diseases, Diagnosis and Treatment. 2nd edn. New Age International (P) Limited Publishers. New Delhi, India, pp. 23-27.

Cheesbrough, M. 2000. District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press.London, UK.

Barrow, P.A. Berchieri A. Jr. and Al-Haddad, O. 1992. Serological response of chickens to infection with *Salmonella* Gallinarum–*Salmonella* Pullorum detected by enzyme-linked immunosorbent assay. Avian Diseases. 36: 227–236.

Das, P.M., Rajib, D.M.M., Noor, M. and Islam, M.R. 2005. Retrospective analysis on the proportionalincidence of poultry diseases in greater Mymensingh district of Bangladesh. In Proceeding of 4th International Poultry Show and Seminar, from February 28 to March 2, 2003, held in Bangladesh China Friendship Conference Centre, Agargaon. pp. 35-39.

Deasi, A.R., Shah, D.H., Shringi, S., Mi-Jin Lee, Ying- Hua Li, Mae- Rim Cho, Jin-Ho Park, Seong-Akug Eo, John-Hwa Lee, Joon-Seok Chae. 2005. An Allele-Specific PCR Assay for the Rapid and Serotype- Specific Detection of Salmonella Pullorum. *Avian Dis.,* 49:558-561.

Haider, M.G., Islam, M.T., Chowdhury, E.H., Khan, M.A.H.N.A. and Hossain, M.M. 2007a**.** The Serologic Response to Locally Isolated *Salmonella* Pullorum in Experimentally Infected Chickens Followed by an Indirect Enzyme Linked Immunosorbent Assay (ELISA*). Bangladesh Vet. J.*, 41(1-2): 7-16.

Haider, M.G., Ahmedullah, F., Akbor, M., Khan, M.A.H.N.A., Chowdhury, E.H. and Hossain, M.M. 2007b. Standardization of Polymerase Chain Reaction (PCR) for the detection of locally isolated *Salmonella* Pullorum. *Bangladesh Vet. J*., 41(1-2): 27-37.

Haider, M.G., Chowdhury, E.H., Khan, M.A.H.N.A., Hossain, M.T., Rahman, M.S., Song, H.J. and Hossain, M.M. 2008**.** Experimental pathogenesis of pullorum disease with local isolate of Salmonella *enterica* serovar. *enterica* subspecies Pullorum in pullets in Bangladesh. *Korean J. Poult. Sci.,* 35 (4): 341-350.

Hoop, R.K. and Pospishil, A. 1993. Bacteriological, serological, histological and immunological findings inlaying hens with naturally acquired *Salmonella enteritidis* phage type 4 infections. *Veterinary* Rec., 139:391-393.

Islam M.M., Haider, M.G., Chowdhury, E.H. and Hossain, M.M. 2006. Seroprevalence of Salmonella infections: Isolation of causal agent and Histopathology. Bangladesh Journal of Veterinary Medicine. 4 (2): 79- 85.

Khan, M.A.H.N.A., Bari, A.S.M., Islam, M.R., Das, P.M., Ali, M.Y. 1998. Pullorum disease in semi mature chickens and its experimental pathology. *Bangladesh Vet. J.,* 32: 124-128.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.3rd edn. McGraw Hill Book Co., New York, USA, pp. 1-68.

Okamura, M., Kamijima, Y., Miyamoto, T., Tani, H., Sasai, K. and Baba, E. 2000. Differences Among Six *Salmonella* Serovars in Abilities to Colonize Reproductive Organs and to Contaminate Eggs in Laying Hens. *Avian Dis.,* 45: 61-69.

Olivera, S.D., Roclenbuisch, C.R., Cee, M.C., Rocha, S.L.S., Canal, C.W. 2003. Evaluation ofselective and non-selectiveenrichment PCR procedures for *Salmonella* detection. *Letters in Appl. Microbiol.,*36: 217-221.

Rahman, M. 2007. Protein For All. In: Souvenir of the 5th International Poultry Show and Seminar from 1-3 march, 2007, held in Bangladesh China Friendship Conference Centre (BCFCC), Sher-e- Bangla Nagar, Dhaka, Bangladesh, pp. 212-220.

Roy, H., Dhillion, A.S., Shivoprasad, H.L., Schabarg, D.M., Bandli, D. and Johnson, S. 2001. Pathogenicity of Different Serogroups of Avain Salmonellae in Specific- Pathogen-Free Chickens. *Avian Dis.,* 45: 922-937.

Shivoprasad, H.L. 1997. Pullorum disease Fowl typhoid. In: Diseases of Poultry.Calnek,W.B. (ed.) 10th edn. Iowa State University Press, Iowa State, USA.

Skov, M.N., Feild, N.C., Carstesen, B. and Madsen, M. 2002.The serological response to *Salmonella enteritidis* and *Salmonella typhimurium* in experimentally infected chickens, followed by an indirect lipopolysaccharide enzyme immunosorbent assay and bacteriologic examinations through a one -year period. *Avian Dis.,* 46:265-273.

Solano, C., Galindo, J., Sesna, B., Alurrez, M., Solano, M.J. and Ganazo, C.N. 2000. Sonali Chickens. *Bangladesh J. Vet. Med.,* 2: 15-21.

Stone, G.G., Oberst, R.D., Hays, M.P., McVey, S. and Chengappa, M.M. 1994. Detection of *Salmonella* serovars from clinical samples by enrichment broth cultivation-PCR procedure. J. Clin. Microbiol., 32: 1742-1749.

Wary, C. and Davis, R.H. 2001. Pullorum disease. In: *Poultry Diseases*. Jordan, F., Pattison, M., Alexander, D. and Faragher,T.(ed.). 5th edn. S. B. Saunders, Philadelphia, USA.

Wigley P, Berchieri Jr. A, Page KL, Smith A.L. and Barrow P.A. 2001. *Salmonella* enterica serovar Pullorum persists in spleenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. Infection and Immunity. 69(12): 7873-7879.

Wigley, P., Hulme, S.D., Powders, C., Beal, R. K. Berchieri, Jr. A. Smith, A. and Barrow, P. 2005.Infection of the Reproductive Tract and Eggs with Salmonella enterica Serovar Pullorum in the Chicken is Associated with Suppression of Cellular Immunity at Sexual Maturity. *Infect. and Immun.,* 73: 2986-2990.

Yamane, Y., Leonard, J.D., Kobatake, R., Awamura, N., Toyota, Y., Ohta, H., Otsuki, K. and Inoue, T. 2000. A Case Study on Salmonella enteritidis (SE) Origin at Three Egg- Laying Farms and Its Control with an S. enteritidis Bacterin. *Avian Dis.,* 44: 519-526.