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Experimental Pathogenesis of Pullorum Disease with the Local Isolate of *Salmonella enterica* serovar. *enterica* subspecies Pullorum in Pullets in Bangladesh

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ABSTRACT The research work was carried out to study the pathogenesis covering the clinical signs, gross and histopathological lesions in different organs, and reisolation and identification of the organisms after experimental infection with the local isolate of *Salmonella enterica* serovar. *enterica* subspecies (*S.*) Pullorum at different time interval of the experiment during the period February 2006 to December 2006. One hundred pullets (seronegative to *S.* Pullorum of 12 weeks age were purchased and divided into 5 (A, B, C, D and E) groups and each group consisted of 20 birds. Four groups (A, B, C and D) were infected orally with a dose of 10^6 CFU, 10^7 CFU, 2×10^7 CFU, 10^8 CFU of *S.* Pullorum, respectively, and one group (E) was treated as uninfected control. The used methods were necropsy and histopathology, culture of bacteria, staining and biochemical test of *Salmonella.* Five birds from each group were randomly selected and sacrificed 1st week, 2nd, 3rd and 4th weeks of post infection (PI). From all the groups, the bacteriological samples (crop, liver, lung, heart, spleen, bile duodenum, ceca and blood) were collected with pre enriched in buffered peptone water in sterile poly bags. Liver, lungs, heart, spleen, intestine, etc. were collected in 10% buffered-formalin for histopathological examination. No clinical signs, gross and histopathological lesions were found in control group and no *S.* Pullorum was reisolated. Clinical sign of experimentally infected with *S.* Pullorum in pullets were loss of appetite (100%), slight depression (75%), ruffled feathers (85%), diarrhea (60%) and loss of weight (100%) in chickens. The feed intake and body weight at different weeks after PI differed significantly ($p<0.01$) among the groups. Grossly, the highest recorded lesion was button-like ulcer in the ceca (80%) and the lowest was white nodules in lungs (1.25%). *S.* Pullorum were reisolated from crop (91.25%), liver (91.25%), lung (83.75%), heart (71.25%), spleen (87.75%), bile (33.25%), duodenum (92.50%), ceca (97.50%) and from different group of infection (61.25%). The highest microscopic findings were intestinal and cecal mucosa and submucosa exhibited infiltration of mononuclear cells and congestion (96.25%), and the lowest finding was nodule formation in the lungs (3.75%). The pattern of the disease production by local isolate of *S.* Pullorum in Bangladesh is almost similar with other isolates in different countries.

(Key words : pullorum disease, pathogenesis, microbiological and histopathological finding)

Introduction

Pullorum disease (PD) is an acute, infectious, and fatal disease of chicks causing much loss during the first 2～3 weeks of age. Adult fowls, especially laying hens, act as a carrier and transmit infection through eggs to the chicks by transovarian transmission. PD is also called bacillary white diarrhea (Wary and Davies, 2001). PD is caused by the *Salmonella enterica* subspecies *enterica* serovar (*S.*) Pullorum (Office International Des Epizooties, 2004). Rettger described the etiologic agent of PD in 1900 and the disease was called fatal septicemia of young chicks (Rettger, 1900). It is septicemic disease affecting primarily chickens and turkeys, other birds such as quail, pheasants, ducks, peacocks, and guinea fowl are susceptible. Mortality associated with the disease in chicks ranged from 0 to 100%, seriously threatening the poultry industry (Shivoprasad, 1997). Death occurs within 4 days of exposure, but usually occurs after 5～10 days in chicks. Anorexia, diarrhoea, depression, and dehydration are the prominent signs (Chauhan and Roy, 1996). With great expansion of the poultry rearing and

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farming, PD has become wide spread problem in Bangladesh (Rahman, 2007). For the treatment, prevention and control of a particular disease, the pathogenesis must be known clearly. Some investigations on PD have been performed on the natural cases, but experimental pathogenesis and pathology are not known in Bangladesh by locally isolated *S.* Pullorum. Therefore, the present study was undertaken to study the experimental pathogenesis and pathology of PD in pullets with locally isolated *S.* Pullorum in Bangladesh.

Materials and Methods

1. Procurement of Chicks

One hundred Isa brown pullets (Seronegative to *Salmonella* Pullorum) at 12 weeks of age were purchased and divided into one of 5 groups with 20 birds each. Four groups (A, B, C and D) were used for the experimental infection while the rest (E) served as uninfected control.

2. Experimental Infection

Pullets were kept in experimental shed and they were given commercial feed and water *ad libitum* throughout the study period. Four groups (A, B, C and D) were infected orally with 0.5 ml PBS that contained 10^6 CFU, 10^7 CFU, 2×10^7 CFU, 10^8 CFU of *S.* Pullorum, respectively (Stubbs, 1954), and one group (E) was treated as uninfected control. The control birds (group E) received only 0.5 mL PBS without bacteria.

3. Clinical Signs

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

4. Sample Collection

Five birds from each group were randomly selected and sacrificed 1^{st} week, 2^{nd}, 3^{rd} and 4^{th} weeks of PI. The bacteriological samples (crop, liver, lung, heart, spleen, bile, duodenum, ceca and blood) were collected in sterile poly bags. Liver, lungs, heart, spleen, intestine, etc. were collected in 10% buffered-formalin for histopathological examination.

5. Gross Pathology

The postmortem examination of all the birds was done as per time schedule. At necropsy, gross tissue changes were observed and recorded carefully (Okamura et al., 2000; Roy et al., 2001; Wary and Davies, 2001). The enlargement of liver was determined by the incision on the surface and comparing the apposition of the surface. No complete apposition of two cut surfaces was graded as enlargement of the liver.

6. Reisolation of S. Pullorum from Different Organs

The collected 1 gm tissue samples were weighted and macerated in mortar and pestle, and then the samples were transferred to 10 ml tetrathionate broth and incubated at 37 ℃ overnight. The broth culture was diluted by 10 fold and one ml of dilution was spread on BGA agar plate and incubated at 37 ℃ for overnight. The pink color colonies were counted for *S.* Pullorum. The red or black colonies (non-lactose fermenters) were inoculated on TSI agar slants and incubated overnight at 37 ℃. Tubes with red slants and black or yellow but were identified to be due to *S.* Pullorum (Roy et al., 2001). The *Salmonella* organisms were confirmed by described methods (Carter and Cole, 1990; Cheesbrogh, 2000; Haider et al., 2003a; Mahanta, 1966)

7. Reisolation of *S.* Pullorum from Blood

Collected 1 ml of blood samples was tested for *S.* Pullorum in enriched culture media, and colonies were counted as previously described (Okamura et al., 2000).

8. Histopathology and Photomicrography

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). Photomicrography was taken using a photomicrographic camera (Olympus PM-C 35 Model).

9. Statistical Analysis

Repeated measures analyses were performed with the data of mean feed intake and body weight gain of pullet of different groups at different week in a Completely Randomized Design (CRD) for significant variation using the SPSS package program version 10.0. Pair wise comparison of mean was done by Least Significant Difference (LSD).

Results

1. Clinical Signs

Clinical signs of experimental infection with *S.* Pullorum in pullets appeared after 3 days PI and were 100% loss of appetite, 75% slight depression, 85% ruffled feathers, 60% diarrhea, 100% reduced feed intake (Table 1) and 100% loss of body weight (Tables 2～3). The feed intake in all infected groups reduced significantly ($p<0.01$) compared with control group in 1st week of inoculation. On the contrary, feed intake, and body weight gain in infected groups gradually increased and clinical sign disappeared gradually from second week PI towards the end of experiment. In uninfected control group feed intake and body weight increased significantly ($p<0.01$) at all the time points of the experiments. The least clinical signs were found in group A and the highest clinical signs were found in group D depending on the dose of inoculated bacteria. No clinical signs were seen in control (E) group during study period. No mortality was observed in the study period.

2. Gross Pathology

No gross lesions were found in the experimentally uninfected control (Group-E) birds. During necropsy, gross lesions were seen from 1st week to 4th week of PI. More pathological lesions were

Table 1. The mean feed intake (g)/birds/day of the pullets in grams after experimental post inoculation of *S.* Pullorum

Group	1st week	2nd week	3rd week	4th week	LSD
A	58.76 ± 3.89	66.46 ± 3.76	73.89 ± 1.52	79.01 ± 2.47	B
B	56.47 ± 4.74	63.85 ± 4.73	72.61 ± 1.51	77.58 ± 2.05	CD
C	55.43 ± 6.17	61.99 ± 2.91	70.55 ± 2.94	76.44 ± 1.41	C
D	54.14 ± 7.27	60.12 ± 3.42	69.88 ± 3.30	76.28 ± 1.32	CE
E	67.78 ± 2.35	75.14 ± 1.17	80.44 ± 2.37	87.22 ± 2.78	A

LSD = Least significant difference, Values bearing different letter within the same column differed significantly ($p<0.01$).

Table 2. The mean body weight (g) of the pullets after experimental post inoculation of *S.* Pullorum

Group	1st week	2nd week	3rd week	4th week	LSD
A	861.2 ± 3.53^{b}	934.4 ± 16.93^{b}	1022.4 ± 15.47^{b}	1126.9 ± 10.5^{b}	B
B	834.6 ± 4.28^{e}	904.5 ± 15.92^{e}	991.6 ± 9.88^{e}	1092.0 ± 16.85^{e}	C
C	796.0 ± 16.41^{d}	862.1 ± 15.16^{d}	939.2 ± 9.77^{d}	1039.3 ± 21.77^{d}	D
D	782.4 ± 13.74^{c}	844.4 ± 17.83^{c}	910.8 ± 15.34^{c}	1006.2 ± 8.93^{c}	E
E	902.6 ± 9.18^{a}	989.4 ± 43.34^{a}	1087.0 ± 11.77^{a}	1207.3 ± 20.43^{a}	A

LSD = Least significant difference, Values bearing different letter within the same column differed significantly ($p<0.01$).

Table 3. Different clinical signs of the experimentally infected with *S.* Pullorum in pullets in different infected group A, B, C and D

Time schedule post infection	Loss of appetite (*n*=20)	Slight depression (*n*=20)	Ruffled feathers (*n*=20)	Diarrhoea (*n*=20)	Laboured breathing (*n*=20)	Loss of body weight (*n*=20)
PI 1 wk	20/20	20/20	20/20	20/20	20/20	20/20
PI 2 wk	20/20	16/20	20/20	20/20	12/20	20/20
PI 3 wk	20/20	12/20	16/20	12/20	08/20	20/20
PI 4 wk	20/20	12/20	12/20	0/20	0/20	20/20
Total (%)	100	75	85	60	50	100

found in group-D infected with 10^8 CFU. The gross lesions were shown in the Table 4. Liver (31.25%) was enlarged and 30% liver was congested and hemorrhagic (Fig. 1). White nodule was found in two livers at two weeks PI. 30% lungs were congested, edematous and brown colored. White nodules were found in one lung at three weeks PI. Thirty percent pericarditis and myocarditis and 2.5% white nodule were found in the heart. And 46.25% spleens were swollen and congested and 20% kidneys congested and enlarged. Mild hemorrhagic to catarrhal enteritis was found in intestine and cecum was enlarged and contained yellow creamy or cheesy materials during necropsy. Button like ulcer and hemorrhage were found in cecal tonsils (80%, Fig. 2).

3. Reisolation of *S.* Pullorum from Different Organs

Birds of control group remained *Salmonella* negative throughout the experimental period, as confirmed by bacteriological

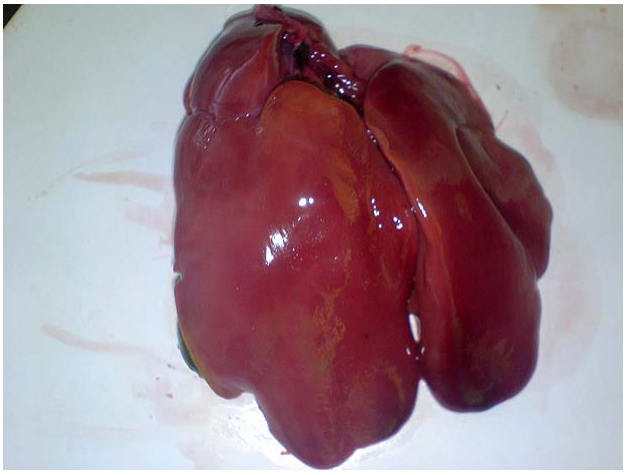

Fig. 1. Enlarged, congested and hemorrhagic liver of pullet at 1st week PI with *S.* Pullorum.

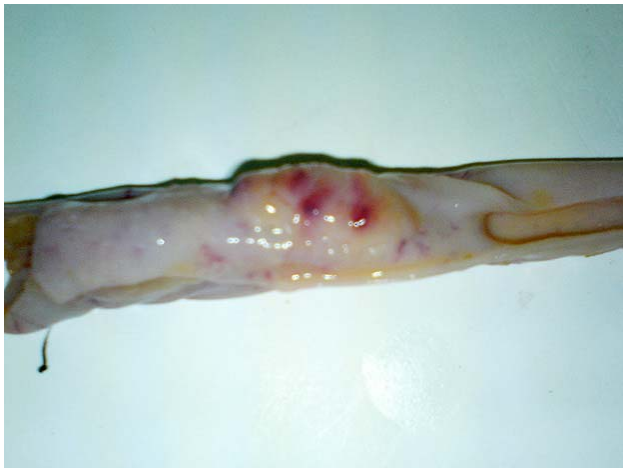

Fig. 2. Button like ulcer and hemorrhages in the cecal tonsils after 1 week of experimental infection.

Table 4. Cumulative gross pathologic findings of pullets experimentally infected with *S.* Pullorum from 1st week to 4th week PI

Cumulative gross lesions	Group A (*n*=20)	Group B (*n*=20)	Group C (*n*=20)	Group D (*n*=20)	Total (*n*=80)	%
1. Liver enlarged	02(10%)	06(30%)	09(45%)	11(55%)	25(80)	31.25
2.Congested and hemorrhagic liver	02(10%)	06(30%)	13(65%)	13(65%)	24(80)	30
3.White nodules in the liver	00(00%)	00(00%)	01(05%)	01(05%)	02(80)	2.5
4. Congested, edematous and brown coloured lungs	02(10%)	06(30%)	09(45%)	10(50%)	24(80)	30
5. White nodules in the lungs	00(00%)	00(00%)	01(05%)	03(15%)	01(80)	1.25
6. Pericarditis	02(10%)	06(30%)	09(45%)	11(55%)	24(80)	30
7. Nodules in the heart	00(00%)	00(00%)	02(10%)	05(25%)	02(80)	2.5
8. Spleen swollen and congested	05(25%)	07(35%)	15(75%)	16(80%)	37(80)	46.25
9. Ceca enlarged and contained yellow creamy or cheesy materials	06(30%)	06(30%)	17(85%)	17(85%)	41(80)	51.25
10. Button like ulcer and hemorrhage was found in the cecal tonsils	13(65%)	16(80%)	18(90%)	19(95%)	64(80)	80
11. Congested and enlarged kidneys	02(10%)	03(15%)	06(30%)	07(35%)	16(80)	20

isolation and identification of different organs after sacrificed. At one, two, three and four weeks PI, different organs (crop, liver, lung, heart, spleen, bile, duodenum, ceca, and blood) of experimentally infected pullets were cultured to isolation *S.* Pullorum. Reisolated *S.* Pullorum produced pink colour colonies on BGA, and CFU/mL of blood was counted and recorded (Table 5 and 6). The colony characters of reisolated *S.* Pullorum organisms on different media were shown (Table 7). In Gram's staining, the morphology of the isolated bacteria was small rod shape, gram negative and single or paired in arrangement. The isolated organisms fermented dextrose, manitol and xylose with gas production and did not ferment lactose, sucrose, dulcitol, inositol and maltose. The organisms were positive to MR test and were negative to indole and VP test. Limited movement was observed in the isolated organisms. *S.* Pullorum were reisolated 91.25%, 91.25%, 83.75%, 71.25%, 87.5%, 33.75%, 92.50%, 97.50% and 61.25% from crop, liver, lung, heart, spleen, bile, duodenum, cecum and blood, respectively (Tables 5 and 6). Group D was more positive for *S.* Pullorum than group A in dose dependent manner.

4. Reisolation of *S.* Pullorum from Blood

S. Pullorum were reisolated from blood after PI in different time interval 45%, 55%, 65% and 80% from group A, B, C and D, respectively. *S.* Pullorum was confirmed by described earlier methods. No *S.* Pullorum organisms were reisolated from control group E in different time interval at PI.

5. Histopathology

Table 8 describes the cumulative microscopic lesions of different organs from 1st week to 4th week PI. Histologically, 82.5% liver showed congestion. Focal necrosis with multifocal infiltration of heterophils was found in 66.25% liver parenchyma (Fig. 3). Nodular lesions were formed in 39.14% liver with the infiltration of macrophages, lymphocytes, plasma cells and heterophils. In lungs, 79.54% bronchopneumonia was observed

Table 5. Cumulative reisolation of *S.* Pullorum from different organs of experimentally infected pullets at different weeks of PI

Specimen	Group A (*n*=20)	Group B (*n*=20)	Group C (*n*=20)	Group D (*n*=20)	Group E (*n*=20)	Total (*n*=80)	%
Crop	16(80%)	18(90%)	19(95%)	20(100%)	00 (00%)	73(80)	91.25
Liver	16(80%)	17(85%)	20(100%)	20(100%)	00 (00%)	73(80)	91.25
Lung	14(70%)	16(80%)	18(90%)	19(95%)	00 (00%)	67(80)	83.75
Heart	11(55%)	14(70%)	15 (75%)	17(85%)	00 (00%)	57(80)	71.25
Spleen	16(75%)	17(75%)	18(85%)	19(95%)	00 (00%)	70(80)	87.75
Bile	05(25%)	06(30%)	07(35%)	07(35%)	00 (00%)	27(80)	33.75
Duodenum	16(70%)	18(80%)	20(100%)	20(100%)	00 (00%)	74(80)	92.50
Cecum	18(80%)	20(90%)	20(100%)	20(100%)	00 (00%)	78(80)	97.50

Table 6. Mean CFU of reisolated of *S.* Pullorum/ml of blood of experimentally infected pullets

Specimen	1st wk (*n*=5)	2nd wk (*n*=5)	3rd wk (*n*=5)	4th wk (*n*=5)	Cumulative (*n*=20)
Group A	9.12×10^7 (5/5)	12.7×10^3 (3/5)	24.1×10^2 (1/5)	00×10^1 (0/5)	09 (45%)
Group B	15.2×10^7 (5/5)	27.4×10^3 (4/5)	16.4×10^2 (2/5)	00×10^1 (0/5)	11 (55%)
Group C	56.7×10^7 (5/5)	79.1×10^4 (5/5)	61.8×10^2 (3/5)	00×10^1 (0/5)	13 (65%)
Group D	112.5×10^7 (5/5)	84.7×10^4 (5/5)	53.6×10^3 (4/5)	96.0×10^1 (2/5)	16 (80%)
Group E	00×10^1 (0/5)	00×10^1 (0/5)	00×10^1 (0/5)	00×10^1 (0/5)	00 (00%)

Table 7. Colony characters of reisolated *S.* Pullorum in different media

Sl. No.	Name of the media	Colony characters
1	Brilliant Green Agar (BGA)	*S.* Pullorum showed red - pink-white opaque coloured colonies surrounded by brilliant red zones in the agar
2	Triple Sugar Iron (TSI) Agar	*S.* Pullorum showed pale colonies with slight H_2S production
3	Lysine Iron (LI) Agar	*S.* Pullorum showed blackish colonies with slight H_2S production
4	Salmonella-Shigella (SS) Agar	Opaque, transparent, translucent, round, raised and uncoloured smooth colonies with black centers
5	MacConkey Agar	Uncoloured pale and transparent colonies
6	Eosin Methylene Blue (EMB) Agar	Translucent, grayish and colourless colonies
7	Blood Agar	Small, round, discrete, transparent, glistening colonies
8	Nutrient Agar	*S.* Pullorum showed gray, opaque and transparent colonies
9	Luria Bertani Agar	White, opaque, raised and colourless colonies

Table 8. Cumulative histopathological findings of pullets infected with *S.* Pullorum from 1st to 4th week PI

Histopathological lesions	Group A (*n*=20)	Group B (*n*=20)	Group C (*n*=20)	Group D (*n*=20)	Total (*n*=80)	%
1. Hepatitis, infiltration of inflammatory cells	15 (75%)	17 (85%)	18 (90%)	16 (80%)	66	82.50
2. Multifocal white necrotic foci in the liver	12 (60%)	13 (65%)	15 (75%)	13 (65%)	53	66.25
3. Nodule formation in the liver	03 (15%)	08 (40%)	11 (55%)	09 (45%)	31	39.14
4. Pneumonia, bronchopneumonia	14 (70%)	14 (70%)	18 (90%)	17 (85%)	63	79.54
5. Nodule formation in the lungs	00 (00%)	00 (00%)	02 (10%)	01 (05%)	03	3.75
6. Pericarditis/myocarditis	09 (45%)	13 (65%)	14 (70%)	13 (65%)	39	48.75
7. Nodule formation in the heart	00 (00%)	02 (10%)	06 (30%)	03 (15%)	11	13.75
8. Focal necrosis and inflammatory cells in the spleen	17 (85%)	17 (85%)	20 (100%)	18 (90%)	72	90.00
9. Infiltration of inflammatory cells in the intestine	18 (90%)	19 (95%)	20 (100%)	20 (100%)	77	96.25
10. Ulcer in the cecal tonsils	10 (50%)	16 (80%)	20 (100%)	17 (85%)	63	79.54
11. Tyhpilitis, infiltration of inflammatory cells in cecum	17 (85%)	20 (100%)	20 (100%)	20 (100%)	77	96.25
12. Congestion in the kidneys	12 (60%)	15 (75%)	16 (80%)	14 (70%)	57	71.25
13. Infiltration of inflammatory cells in gizzard	06 (30%)	07 (35%)	11 (55%)	07 (35%)	31	39.14

with consolidation and filling of tertiary bronchi with a mixture of heterophils and mononuclear cells (Fig. 4). Catarrhal bronchitis and nodular formation were also seen in 3.75% lungs. In the heart muscle fiber, 48.75% was infiltration of heterophils in pericardium and myocardium. Macrophages, lymphocytes and plasma cells were found in the nodule in 13.75% heart (Fig. 5). Ninety percent spleens were exhibited severe congestion, mild hyperplasia of RE cells and 2～3% lymphocytic necrosis. In 71.25% kidneys, congestion and infiltration of heterophils were found. There were infiltrations of inflammatory cells mainly heterophils and lymphocytes in 39.14% gizzard. Ulcer and hae morrhage were seen in 79.54% cecal tonsils and 96.25% intestinal and cecal mucosa exhibited infiltration of mononuclear cells in the submucosa and congestion (Fig. 6).

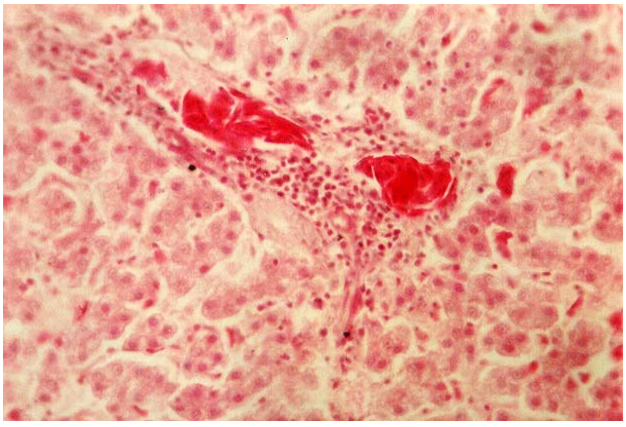

Fig. 3. Focal necrosis, infiltration heterophils and congestion in the portal triad of liver (H&E, X 333).

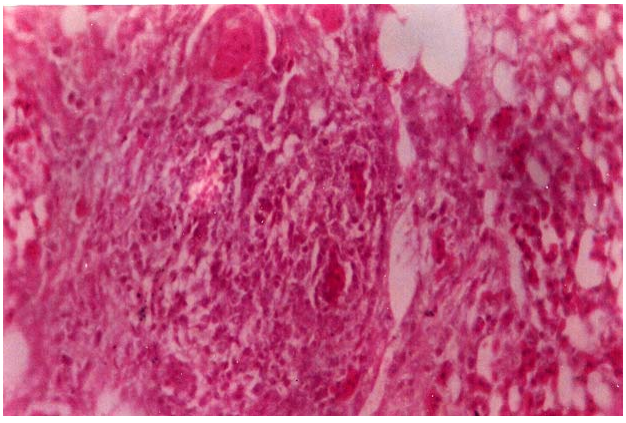

Fig. 4. Bronchopneumonia with consolidation and filling of tertiary bronchi with a mixture of heterophils and mononuclear cells (H&E, X 333).

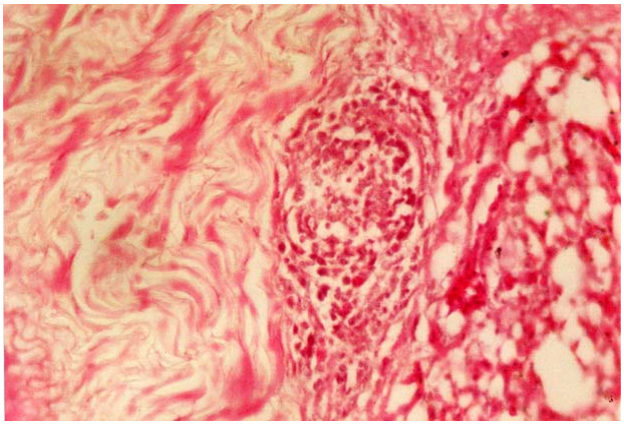

Fig. 5. Nodular lesions with the infiltration of macrophages, lymphocytes, plasma cells and heterophils in heart (333x H&E).

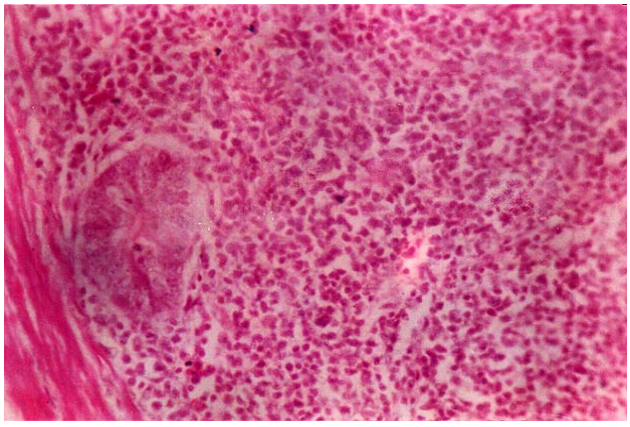

Fig. 6. Infiltration of mononuclear cells in the intestinal mucosa and submucosa, and congestion (H&E, X 333).

Discussion

Chickens are the natural hosts for both *Salmonella enterica* subspecies *enterica* serovar. Pullorum and *Salmonella enterica* subspecies *enterica* serovar Gallinarum and others motile *Salmonellae* organisms. *Salmonellae* organisms may transmit horizontally by infected water, air, food, liter contact, egg eating vectors etc and vertically spread by ovum (Henderson et al., 1999; Shivoprasad, 1997).

Many of the organisms are resistant to acid and proteolytic enzymes present in the intestine causing release of these organisms through feces. *Salmonellae* organisms attach to mannose-like receptor on microvilli of intestinal epithelial cell. The microvilli degenerate locally at the site of attachment, enabling the bacterium to enter the cell, and the breach in the cell surface and then it repairs. After penetration in the epithelium, final pathogenicity depends on bacterial multiplication, spread, toxin production, cell damage and inflammatory responses (Mims, 1990). In the experimental pathogenesis study of pullorum disease through oral route of infection, the pullet showed loss of appetite, ruffled feathers, depression, diarrhea and loss of weight after 3 days of PI which were similar to the findings of other authors (Chauhan and Roy, 1996; Okamura et al., 2000; Roy et al., 2001; Wary and Davies, 2001). The organisms caused bacteremia and colonized in the liver, lungs, heart, spleen, intestine and ceca to various degrees after 1 wk PI. Some other experiments on chicks and layers birds to find out the extract timing

of entrance of *S.* Pullorum in blood and different organs by oral route of inoculation are on-going in our laboratory. In the present study, grossly the liver was enlarged, congested, haemorrhagic and necrotic; the lungs were edematous, congested and brown colored; pericarditis in the heart; swollen and congested spleen; congested and enlarged kidneys; button like ulcer in the ceca and ceca enlarged and contained yellow creamy or cheesy materials in the lumen. These types of necropsy findings corresponded with the findings of others (Hafeji et al., 2001; Hossain et al., 2006; Islam et al., 2006; Khan et al., 1998; Syed et al, 2004). In this study, white nodule formation in the lungs, liver and heart were also found that were almost similar to the results of other investigators of different times interval on experimental birds (Shivoprasad, 1997; Trampel, 2001; Wigley et al., 2005).

The colony character of reisolated *S.* Pullorum, on SS agar was whitish or slight grayish colonies with dark central spot reflecting production of hydrogen sulfide on the media, on LIA was slight blackish color colonies, on TSI agar was black color colonies and on BGA was pink white color colonies which were corresponded with other reports (Carter and Cole, 1990 ; Cheesbrogh, 2000 ; Haider et al., 2004b ; Perez et al., 2004 ; Yuno et al., 1995). In the present study, the results of the sugar fermentation test and biochemical test of the reisolated *S.* Pullorum were similar to the other results (Haider et al., 2004b ; Hossain et al., 2006 ; Islam et al., 2006). Motility of the *S.* Pullorum is supported by the OIE manual in the present study (Office international des epizooties, 2004). In gram's staining, the morphology of the isolated bacteria was small rod shape, gram negative and single or paired in arrangement that was supported by others (Freeman, 1985; Haider et al., 2003a). In the present study, 91.25%, 91.25%, 83.75%, 71.25%, 87.5%, 33.75%, 92.50%, 97.50% and 61.25% *S.* Pullorum were reisolated from crop, liver, lung, heart, spleen, bile, duodenum, cecum and blood, respectively from different group of infection. The findings of the present study were almost similar to the findings of Roy et al (2001). with oral route of infection by *S.* Pullorum.

In the present study, microscopic findings of locally isolated *S.* Pullorum in experimental chickens by oral route appeared strictly higher in 1 week PI and gradually reduced thereafter. The lesions in different organs were infiltration of heterophils, macrophages, lymphocytes and plasma cells. However, nodule formation was found in heart, liver and lungs. These types of histological lesions are supported for *Salmonella* infection by different investigators (Chauhan and Roy, 1996; Hafeji et al., 2001; Refsum et al., 2002; Syed et al, 2004; Wigley et al., 2005).

In short, PD causes retardation of growth. 100% birds showed loss of appetite, reduced feed intake and ultimately body weight loss. Pullet infected with 2×10^7 CFU and 10^8 CFU of *S.* Pullorum by oral route showed more lesions. No mortality was observed in this study. Grossly, congestion and hemorrhage were seen in 31.25% liver of infected pullet. Button like ulcer and hemorrhage was found in 80% cecal tonsils, 91.25% liver and 83.75% lung were positive for the reisolation of *S.* Pullorum. The highest number of *S.* Pullorum was reisolated from different organs after 1 wk PI from different infected group. 56.7×10^7 CFU and 112.5×10^7 CFU of *S* Pullorum were reisolated from blood after 1 wk PI infected with 2×10^7 CFU and 10^8 CFU of *S.* Pullorum, respectively. The distinguishing morphological, cultural, staining and biochemical characters of reisolated *S* Pullorum were described earlier. Microscopically, hepatitis and infiltration of inflammatory cells in 82.5% liver, focal necrosis and inflammatory cells in 90% spleen, and tyhpilitis and infiltration of inflammatory cells in 96.25% ceca were found. In this investigation, the pathogenesis and pathology in pullets by Bangladesh isolates *S.* Pullorum has been studied.

적 요

이 실험은 추백리의 병원성을 연구하고자 2006년 2월부터 12월까지 *Salmonella enterica* serovar. *enterica* subspecies (*S.*) Pullorum 야외주를 분리한 후, 이를 건강한 닭에 실험적으로 감염시킨 다음 임상증상, 여러 기관의 육안 및 조직병리학적 검색과 아울러 공격주의 재분리와 동정을 시도하였다.

S. Pullorum에 혈청학적으로 음성인 12주령의 100수의 암탉을 A～E까지 20수씩 5그룹으로 구분하였다. A～D*S.* Pullorum을 10^6 CFU, 10^7 CFU, 2×10^7 CFU, 10^8 CFU로 각각 경구 감염시켰고, E는 비감염 대조군으로 삼았다.

실험 방법으로는 부검, 조직병리학적 검사, *Salmonella*에 대한 세균 배양, 염색, 생화학적 특성을 조사하고 그 결과를 기술하였다.

(색인어 : 추백리, 병성, 미생물학적 및 병리조직학적 소견)

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