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BACTERIO-PATHOLOGICAL STUDIES ON SALMONELLOSIS, COLIBACILLOSIS AND PASTEURELLOSIS IN NATURAL AND EXPERIMENTAL INFECTIONS IN CHICKENS

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ABSTRACT

Bacterio-pathological investigation on 1751 dead chickens during one year period from January to December 2002 at the BRAC Poultry Disease Diagnostic Centre, Gazipur showed that 39.81% (n = 697) cases with seven types of different bacteriological diseases of which salmonellosis (n = 385), colibacillosis (n = 147) and fowl cholera (n = 114) were found significantly higher rate of prevalence then staphylococcosis (n = 6), gangrenous dermatitis (n = 17), necrotic enteritis (n = 24) and infectious coryza (n = 4). Accordingly, avian salmonellosis, colibacillosis and pasteurellosis were selected for detailed investigation. Age wise prevalence of avian salmonellosis showed highest infection rate in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens. The avian colibacillosis was found widely prevalent in all age groups of chickens (9.52 to 36.73%) with specially high prevalence rate in adult layer birds (36.73%). Fowl cholera was recorded in chickens more than two weeks of age with significantly ($p < 0.01$) highest occurrence in adult chickens. Seasonal influence showed significantly ($p < 0.01$) highest proportionate prevalence of salmonellosis during summer (48.05%) in comparison to rainy (28.31%) and winter (23.66%) seasons. Colibacillosis was recorded more or less uniformly in all the three seasons of the year with significantly ($p < 0.01$) higher rate during summer (40.82%) season. Similarly, the prevalence of fowl cholera was also found significantly ($p < 0.01$) highest during summer (49.12%) in comparison to rainy (26.32%) and winter (24.56%) seasons. The isolated causative agents of avian salmonellosis (*Salmonella pullorum*), avian colibacillosis (*Escherichia coli*) and avian pasteurellosis (*Pasteurella multocida*) were characterized by bacteriological methods which were also subjected to pathogenicity study in 52-day old broiler chickens. Pathogenicity study showed that the incubation period of these three bacterial diseases were recorded as 96 hours and clinical signs appeared on 4th day of inoculation and observed that *S. pullorum*, *E. coli* and *P. multocida* resulted 100% morbidity in chickens.

Key words : Characterization, pathogenicity, salmonellosis, colibacillosis, pasteurellosis, chickens

INTRODUCTION

Avian colibacillosis, avian salmonellosis and avian pasteurellosis have been reported to be the major bacterial disease problem in poultry industry worldwide including Bangladesh. (Calnek *et al.*, 1997; Samad, 2000). Although some research works on avian pasteurellosis (Kamal *et al.*, 1988; Choudhury *et al.*, 1985, 1989; Khan *et al.*, 1997; Hossain *et al.*, 1998, 1999) and avian salmonellosis (Khan *et al.*, 1998; Hoque *et al.*, 1997) have done from Bangladesh but published reports on avian colibacillosis are very limited (Saleque *et al.*, 2003). This paper describes the characterization of the isolated causative organisms of these diseases and their pathogenicity in experimentally infected broiler chickens.

MATERIALS AND METHODS

Moribund and dead birds presented at the Bangladesh Rural Advancement Committee (BRAC) Poultry Disease Diagnostic Centre (PDDC), Nagapara, Gazipur for diagnosis of the diseases during one year period from January to December 2002 formed the material for this study. Specimens were collected from heart, liver, intestine and other organs of the dead birds had characteristic signs and gross lesions (Calnek *et al.*, 1997) belonging to different age groups of broiler, layer and parent stock chickens. These bacterial diseases were routinely diagnosed at the PDDC primarily on necropsy and finally by the isolation and identification of the causative organisms as described by Cowan (1985).

Bacteriological specimens suspected for colibacillosis (n = 147), salmonellosis (n = 385) and pasteurellosis (n = 114) were collected at necropsy examination in sterilized cotton swabs which were kept into the sterilized test tube containing nutrient broth. These test tubes were then transported via thermo-flask containing ice to the bacteriological laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. Then the swab containing test tubes were incubated for growth of the causative organisms at 37°C for overnight. Blood agar, Nutrient agar, EMB agar, MacConkey agar, SS agar, Nutrient broth and five basic sugars (dextrose, maltose, lactose, sucrose and mannitol) were used for isolation and identification of these causative agents as described by Cheesbrough (1985) Parker and Collier (1990) and Swayne *et al.* (1998).

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Pathogenicity study

Day-old broiler chicks (Vencobb broiler strain) purchased locally from Goalundo Hatchery, Faridpur, and maintained in the poultry sheds of the Department of Veterinary Medicine in deep litter system by using rice husk as litter. The birds were supplied with commercial feed (Quality Feed Ltd., Dhaka) and water *ad libitum*, supported with vitamin-mineral premix (Magavit WS®, Navartis). Twelve healthy broiler birds at the age of 52 days, were divided into three groups (A, B and C), each consisting of four birds.

Preparation of inoculum

For colony forming unit (CFU) count, the organisms were grown in nutrient broth with yeast extract for overnight. Then 10 fold dilution was made and 0.5 ml of each 10 fold dilution was transferred aseptically to the nutrient agar plate using a fresh pipette for each dilution. The diluted samples were spread on the plate with sterile L-shaped glass spreader. One sterile glass spreader was used for each plate. The plates were then incubated at 37°C for 24 to 48 hours. Following incubation only those plates exhibiting 30 to 300 colonies were counted. For each dilution three plates were used and the mean of the three plates were calculated. The number of bacteria per ml of original sample was obtained by multiplying the number of colonies by diluting factor. CFU was calculated according to ISO (1995). The results of CFU were expressed as the number of organism per ml of sample.

Each bird of group A, B and C was injected with 1.0 ml suspension of *Salmonella pullorum* (5.75×10^6 CFU), *Escherichia coli* (4.5×10^7 CFU) and *Pasteurella multocida* (6.25×10^6 CFU) orally. All the birds were allowed to rear on same feed and environmental condition and were observed for clinical signs at every six hours interval. The findings were recorded as normal, sick or dead and any signs of sickness or death of the birds during the period was considered as their susceptibility.

Re-isolation and identification

Faecal samples from each of infected birds were collected directly from the cloaca by using sterilized cotton swabs which were kept in nutrient broth for further growth and multiplication at 37°C for overnight in the laboratory. Each faecal sample was divided and inoculated separately in Nutrient agar (NA) and Blood agar (BA) to promote growth of bacteria. Each group of these media was incubated at 37°C for overnight. The colonies on primary cultures were repeatedly subcultured by streak plate method (Cheesbrough, 1985) until the pure culture with homogenous colonies were obtained. Media such as blood agar, Nutrient agar, Eosine Methylene blue (EMB) agar, *Salmonella-Shigella* (SS) agar etc. were used for sub-cultures and were incubated at 37°C for 24 hours for growth. Cultures revealing typical reactions of organisms were further screened on the basis of morphological, cultural and biochemical character as described by Cowan (1985).

RESULTS AND DISCUSSION

Necropsy and bacteriological methods were mainly used to determine the bacterial diseases in commercial chickens. During the one year period of the 1751 dead cases examined, of which 697 (39.81%) cases diagnosed as bacterial infection. Of the 697 bacterial cases, of which salmonellosis (n = 385), colibacillosis (n = 147), fowl cholera (n= 114), staphylococcosis (n = 6), gangrenous dermatitis (n = 17), necrotic enteritis (n = 24) and infectious coryza (n = 04) were diagnosed. Thus, the prevalence of salmonellosis, colibacillosis and pasteurellosis were found significantly high in comparison to other bacterial diseases. Accordingly, the characterization and pathogenicity of the isolated causative agents of these most important bacterial diseases were studied.

Avian salmonellosis

Necropsy examination of salmonellosis affected dead bird showed enlarged and necrotic foci on liver and spleen, congested liver and lungs, mucus and haemorrhages in intestine, petechial haemorrhages in heart base, greenish to bronze colour liver (Fig. 1). In chronic cases resulted in deformed and under developed ova attached with stalk, discoloured and cystic ovarian follicles in the laying hens. There was catarrhal enteritis, peritonitis and pericarditis.

Salmonella organisms were isolated from liver and /or spleen of dead birds on bacteriological media. On nutrient agar small round translucent smooth colony, on blood agar no haemolysis, on EMB agar no growth, on SS agar pinkish colour colony were observed. (Table 1 and Fig. 2). Microscopic examination of Gram's stained smears prepared from colony showed Gram negative, very short rods, arranged as single or paired organisms (Fig. 3). *Salmonella* organisms were identified on biochemical tests with five basic sugars, in which showed fermentation of dextrose and mannitol but it did not ferment lactose, sucrose and maltose (Table 2 and Fig. 4).

Avian colibacillosis

Necropsy examination of chickens died of colibacillosis showed perihepatitis, pericarditis, petechial haemorrhages in the spleen, heart and liver, enteritis, haemorrhage and mucus in intestine, omphalitis resulted unabsorbed yolk sac.

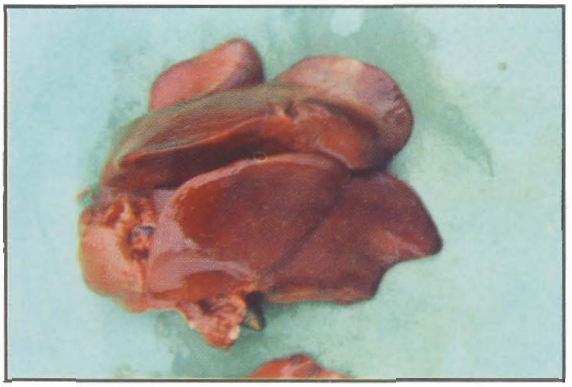

Fig. 1. Enlarged bronze coloured liver of a 35-day-old broiler chicken died due to Pullorum disease showing discrete, small white necrotic foci throughout the liver.

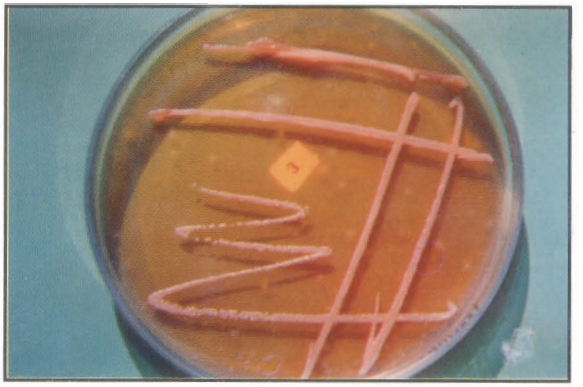

Fig. 2. A pinkish coloured characteristic colonies of *Salmonella* organism on SS agar isolated from a 35-day-old broiler chicken.

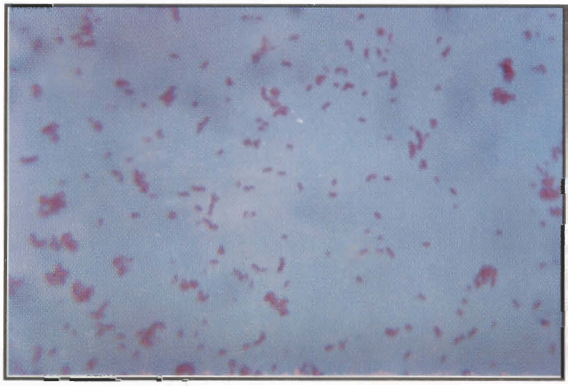

Fig. 3. Microscopic features of *Salmonella pullorum* organism isolated from a dead broiler showing Gram negative short rods, occur singly, two or more in united form.

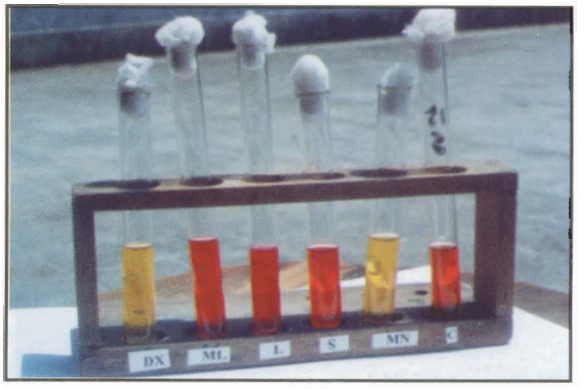

Fig. 4. Biochemical tests of *Salmonella pullorum* showing fermentation of dextrose and mannitol but not maltose, lactose and sucrose.

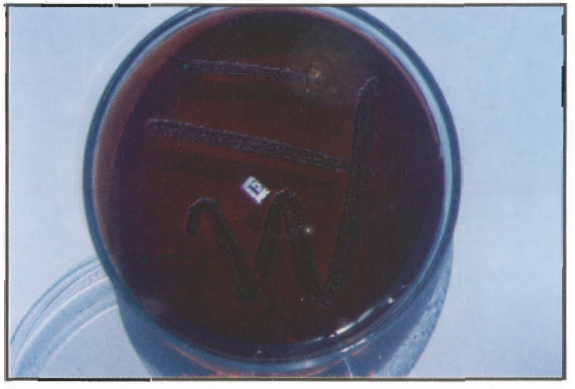

Fig. 5. A pure culture colonies of *Escherichia coli* on EMB agar showing characteristic dark with a metalic sheen.

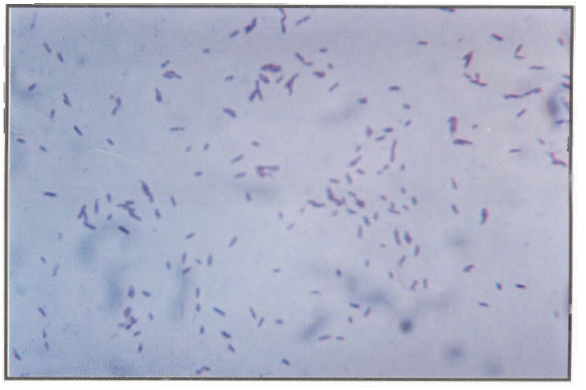

Fig. 6. Gram negative *Escherichia coli* showing variable size and shape arranged in singly, paired and short chain.

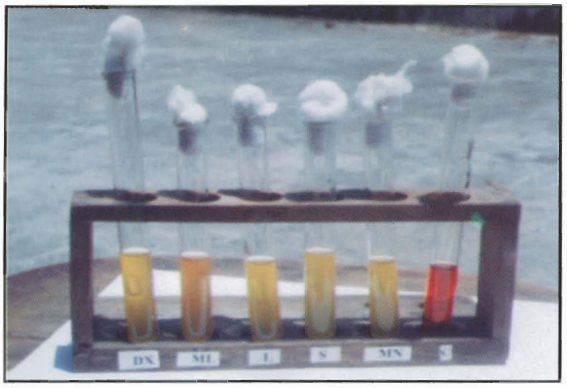

Fig. 7. Biochemical tests of *Escherichia coli* organism showing fermentation of all the five basic sugars with produciton of acids and gas.

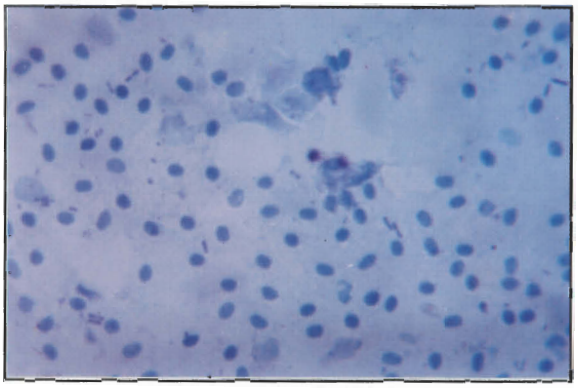

Fig. 8. Microscopic features of Methylene blue stained impression smear of a heart of a dead broiler showing bipolar *Pasteulla multocida* singly and in pairs.

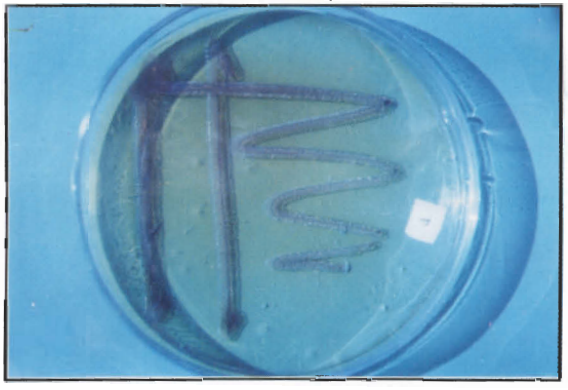

Fig. 9. A pure culture colony of *Pasteurella multocida* on nutrient agar showing characteristic glistening and bluish discoloration centrally.

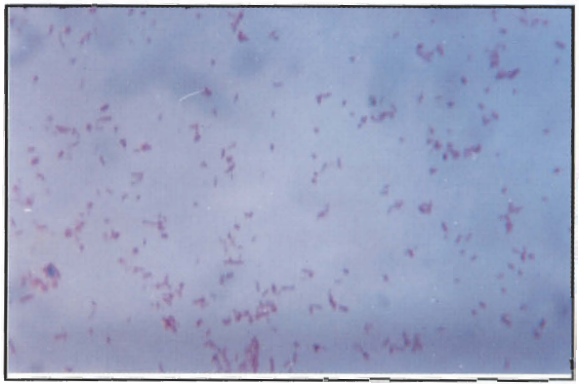

Fig. 10. Microscopic features of Gram's stained smears of *P. multocida* showing Gram's negative rods, arranged in singly, paired or in short chain (X 1000).

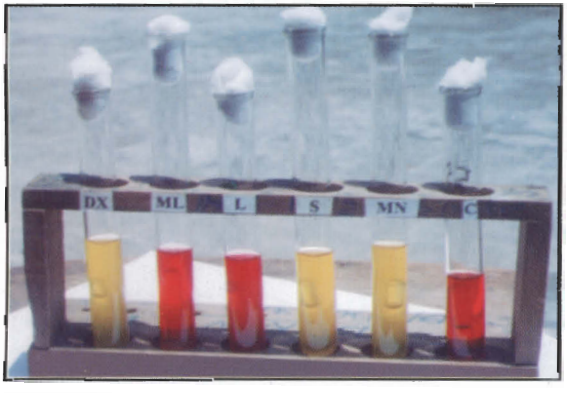

Fig. 11. Biochemical tests of *Pasteurella multocida* isolated from endocardium of 72-day-old chicken showing fermentation of dextrose, sucrose and mannitol but not maltose and lactose.

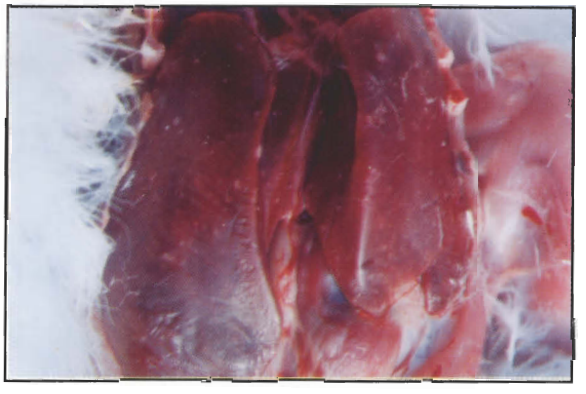

Fig. 12. Experimentally induced Fowl cholera affected swollen liver of a 62-day-old broiler chicken showing multiple necrotic foci with coagulative necrosis.

Bacteriological smears collected from enteritis, heart and liver lesions were streaked on nutrient agar where produced smooth, white to grayish white colony and peculiar foetid odour on blood agar where haemolysis occurred, on EMB agar where produced characteristically dark with metallic sheen (Fig. 5).

Table 1. Cultural colony characteristics and Gram's staining reaction of the organisms isolated from dead and experimentally infected chickens

Nutrient Broth	Nutrient agar	Blood agar	EMB agar	SS agar	Shape	Arrangement	Gram's staining	Isolated
Turbid growth with heavy flocculent sediment	Small round translucent, smooth colony	No haemolysis	No growth	Pinkish colour colony	Very short rods	Single or paired	–ve	*Salmonella pullorum*
Turbid growth	Smooth, white to grayish white colony, peculiar foetid odour	Produced haemolysis	Dark with metallic sheen	Slight pinkish colour colony	Short rods	Single, paired or in short chain	–ve	*Escherichia coli*
Cloudy growth and in a few days a sticky sediments	Colony with bluish colour and smooth, convex, translucent, glistening	No haemolysis	No growth	No growth	Rod shaped and bipolar organism	Single, paired or in short chain	–ve	*Pasteurella multocida*

Table 2. Results of biochemical characteristics of the organisms isolated from dead and experimentally infected chickens

S/N	Isolated organisms	Fermentation properties with carbohydrates				
		Dextrose	Maltose	Lactose	Sucrose	Mannitol
①	*Salmonella pullorum*	+AG	–	–	–	+A
②	*Escherichia coli*	+AG	+AG	+AG	+AG	+AG
③	*Pasteurella multocida*	+A	–	–	+A	+A

A = Acid, AG = Acid and gas.

Microscopic examination of Gram's stained of smears prepared from colony showed Gram negative, short rods and arranged as single, paired or in short chain (Fig. 6). Biochemical characterization of *E. coli* was made on 5 basic sugars were fermented and produced acid and gas (Fig. 7).

Avian pasteurellosis (Fowl cholera)

Necropsy examination of dead chickens, caused by *Pasteurella multocida* showed marked congestion, petechial haemorrhage in heart, lungs, intestinal mucosa, epicardium and endocardium. Fragile liver which apperred to be swollen and showed multiple foci of coagulation necrosis in some cases. In layers, deformed or flaccid ova and occasionally free yolk in the peritoneal cavity were observed. At necropsy, methylene blue stained impression smears of liver and heart blood form acute septicemic cases often revealed bipolar Gram negative organism (Fig. 8) suggestive of *P. multocida* organism. Samples collected from heart blood and liver of dead birds were inoculated on nutrient agar showed bluish colour smooth, convex and translucent colony (Fig. 9) and growth on blood agar with no haemolysis but it did not grow on EMB and SS agar.

Gram's stained smears of cultured materials showed bipolar Gram negative organism (Fig. 10). *P. multocida* organism was also identified on biochemical tests with five basic sugars in which showed fermentation of dextrose, sucrose and mannitol but did not ferment maltose and lactose (Fig. 11).

Age and seasonal influences

Avian salmonellosis

Salmonellosis, caused by *Salmonella pullorum* is one of the most important bacterial disease of poultry causing heavy losses through mortality and reduced production (Khan *et al.*, 1998). Salmonellosis was recorded in 385 (21.99%) chickens, of which 69.09% as single, 29.09% as two types and 1.82% as three types of mixed infection. Highest infection rate was recorded in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens. The 21.99% proportionate prevalence of salmonellosis recorded in this study support the report of Mitra *et al.* (1997) who reported 21.43% incidence of salmonellosis following infectious bursal disease in poultry from India. However, Sarker (1976) reported 15%, Kamal and Hossain (1992) reported 4.82%, Bhattacharjee *et al.* (1996) reported 9.28%, Talha *et al.* (2001) reported 13.12% and Giasuddin *et al.* (2002) reported 6% mortality of chickens due to salmonellosis from Bangladesh.

The necropsy lesions observed in avian salmonellosis are in conformity with the earlier reports of Chishti *et al.* (1985) and Jindal *et al.* (1999). Significantly ($p < 0.01$) highest proportionate prevalence of salmonellosis was recorded during summer (48.05%) in comparison to rainy (28.31%) and winter (23.66%) seasons. These findings support the report of Bhattacharjee *et al.* (1996) who report highest prevalence of salmonellosis during pre-monsoon (13.07%) in comparison to winter (10.40%), monsoon (6.82%) and post-monsoon (6.82%) period. These informations indicate that salmonellosis is still an important disease problem in the poultry industry in Bangladesh. And especially high prevalence rate in adult layer chickens (53.25%) with possible vertical transmission might be the constraint towards the development of poultry industry.

Avian colibacillosis

Colibacillosis, caused by *Escherichia coli* is one of the most common bacterial diseases of poultry, causing syndromes like air sacculitis, cellulitis, omphalitis, peritonitis, salpingitis, synovitis and coligranuloma. This study recorded 8.40% proportionate prevalence rate of colibacillosis in chickens, of which 67.35% recorded as single, 29.93% as two types and 2.72% as triple types of mixed infection with other diseases. These results support the earlier reports of Sarker (1976) who reported 3.75%, Bhattacharjee *et al.* (1996) reported 10.61% and Talha *et al.* (2001) reported 5.51% proportionate prevalence rate of colibacillosis in chickens from Bangladesh. Although the similar reports on the concurrent occurrence of diseases associated with morbidity and mortality of chickens are not available in inland literature but the results recorded in this study supports the reports of Mukherjee and Khanapurkar (1994) who reported *Escherichia coli*, NDV and IBDV in broilers and Tayeb and Hanson (2002) who reported interactions between *E. coli* and NDV in chickens.

Talha *et al.* (2001) reported higher proportionate prevalence rate of colibacillosis in growing chickens in comparison to adults whereas Bhattacharjee *et al.* (1996) reported widely prevalent of colibacillosis in both the brooding (12.82%) and pre-peak-post production layer chickens (5.49 to 8.78%), and this study also recorded widely prevalent of *E. coli* infection in all age groups of chickens (9.52 to 36.73%) with specially high prevalence rate in adult layer birds (36.73%). As the *E. coli* organism has been attributed to vertical transmission and to egg shell contamination followed by penetration, and high rate of *E. coli* infection in layer chickens again need keen attention towards its control to save the poultry industry in Bangladesh. Colibacillosis was recorded more or less uniformly in all the three seasons of the year with significantly higher rate during summer (40.82%) seasons. Bhattacharjee *et al.* (1996) also reported avian colibacillosis in all the seasons of the year in Bangladesh. Pandey *et al.* (1998) reported outbreaks of *E.coli* infection during November to March, and Lambie *et al.* (2000) reported higher *E. coli* infection during rainy season.

Avian pasteurellosis

Fowl cholera (FC), caused by *Pasteurella multocida* is an important infectious disease of poultry has been recognized in 6.51% proportionate prevalence rate in chickens, of which 77.19% as single, 21.05% as dual and only 1.75% as mixed with four types of diseases. The 6.51% proportionate prevalence rate of FC recorded in this study is in conformity with the earlier report of Kamal and Hossain (1992) who reported 6.43% prevalence rate of FC in chickens. However, Bhattacharjee *et al.* (1996a) and Talha *et al.* (2001) reported 1.98% and 3.15% proportionate incidence rate of FC in chickens from Bangladesh. In addition, Choudhury *et al.* (1985) reported 25% mortality and Kamal *et al.* (1988) reported 28% mortality in chickens due to FC. This disease was recorded in chickens more than 2 weeks of age with significantly ($p < 0.01$) highest occurrence in adult chickens. The observation is in conformity with the earlier report of Choudhury *et al.* (1985) who reported FC in adult chickens, Kamal *et al.* (1988) who reported FC in chickens aged between 6 to 12 months old and Talha *et al.* (2001) reported FC in chickens from >2 weeks of old with highest incidence in adult (>20 weeks) birds. Seasonal analysis on the occurrence of FC in chickens showed significantly ($p < 0.01$) highest occurrence during summer (49.12%) in comparison to rainy (26.32%) and winter (24.56%) seasons. These results support the reports of Bhattacharjee *et al.* (1996) and Tsai *et al.* (2000) who reported higher infection rate during March to July.

Pathogenicity study

Evaluation of pathogenicity of *Salmonella pullorum*, *Escherichia coli* and *Pasteurella multocida* which were isolated from dead chickens carried out in healthy 52-day old twelve broiler chickens. These broilers were divided into three groups (A to C), each consisting of four birds. Three bird of group was infected with *S. pullorum*, three birds of group B with *E. coli* and three birds of group C with *P. multocida* and the another one bird of each group served as uninfected controls.

***Salmonella pullorum* infection**

The incubation period was found 96 hours in experimentally infected broiler with *Salmonella pullorum* organism and exhibited clinical signs in 50% birds at the 4th day and 100% at the 5th day of infection. The affected birds showed depression, ruffled feather and whitish to greenish diarrhoea whereas all the control birds remained active throughout the course of the experiment. These observations are in conformity with Khan *et al.* (1998) who reported the similar pathogenicity with *Salmonella pullorum* infection in 8 weeks old local birds.

***Escherichia coli* infection**

The pathogenicity of the isolated *E. coli* was studied in broilers of group B and the incubation period vis-à-vis clinical signs appeared at 96 hours after infection. The clinical signs appeared in appetence, watery diarrhoea which in one case was blood tinged, pasting of vent, ruffled feathers and huddling. These observations support the report of Pandey *et al.* (1998).

***Pasteurella multocida* infection**

The pathogenicity of the isolated *P. multocida* organism was studied in broiler birds of group C in which the average incubation period was found 96 hours with clinical signs of dullness, depression, inappetence, rise of body temperature and diarrhoea, initially whitish and finally greenish with mucus. Only one bird showed partial lameness and laid on the sternum. These finding correlates with reports of Kamal *et al.* (1988), Khan *et al.* (1997) and Hossain *et al.* (1999).

Reisolation and identification of the organisms

All the three causative inoculated organisms were reisolated and identified from the respective experimentally infected groups as presented in Table 1 and 2.

Necropsy changes of inoculated birds

All the experimental birds of the three groups (treated and untreated) were slaughtered and examined thoroughly for the presence of any lesion in the internal organs. The *Pasteurella multocida* infected birds which showed lameness and decreased body weight even after treatment with effective drug revealed white nodular granule/foci on the enlarged liver surface extensively (Fig. 12) and haemorrhage in the intestinal mucosa with other lesions. The untreated control bird experimentally infected with *Salmonella pullorum* showed mucus and haemorrhage in the intestine, mild necrotic foci on the liver and spleen. The untreated control bird experimentally infected with *Escherichia coli* showed mucus and haemorrhage in the intestine, necrotic foci on the liver and slight fibrinous covering on the heart. All other treated birds did not show any marked lesions in any of the internal organs.

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