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

M. A. Rahaman1, M. A. Samad, M. B. Rahaman2 and S. M. L. Kojc2
Department of Medicine, Department of Microbiology and Hygiene2, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2222, Bangladesh.

ABSTRACT
Bacteriological investigations on 1753 dead chickens during one year period from January to December 2002 in the BRAC Poultry Disease Diagnostic Centre, Gazipur showed that 29.8% (n = 60/0) cases with seven types of different bacteriological diseases of which salmonellosis (n = 385), colibacillosis (n = 147) and Pasteurellosis (n = 114) were found significantly higher during the period. The mortality rate was 4.9% (n = 85). The disease was higher in the age group of 4-13 weeks (12.3%) as compared to broiler (14.5%) and growing (6.10%) and pullet (16.10%) chickens. The avian colibacillosis was widely prevalent in all age groups of chickens (6.52-36.7%) with specially high prevalence rate in adult layer birds (36.7%). Fewer chickens were recorded in chickens more than two weeks of age with significantly (p < 0.01) highest occurrence in skylark.

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 pathomorphosis (Karmel et al., 1988, Chandrawya, 1985, 1986, Khan et al., 1997, Hossar et al., 1998, 1999 and avian salmonellosis (Khan et al., 1998; Hossar et al., 1997) have done from Bangladesh. But published reports on avian colibacillosis has been very limited (Sadeque et al., 2007) This paper describes the characterisation of the isolated causative organisms of these diseases and their pathogenicity in experimentally infected broiler chickens.

MATERIALS AND METHODS
Marihu and dead birds presented at the Bangladesh Rural Advancement Committee (BRAC) Poultry Disease Diagnostic Centre (PDDC), Nagarpur, 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 laboratory 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 specified 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 with incubated for 24 hours at 37°C for growth. Blood agar, nutrient agar, EMB agar, MacConkey agar, SS agar, Nutrient broth and five basic selective agents (deoxycholate, motility, hemolyte, sucrose and mannitol) were used for isolation and identification of these causative agents as described by Cheesbrough (1985) Parker and Collier (1940) and Swanson et al. (1998).

Present address: Department of Medicine and Surgery, Barisal Government Veterinary College, Khapun, Barisal, Bangladesh.

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Pathogenicity study

Day-old broiler chicks (Vaccin broiler strain) purchased locally from Goulburn Veterinary, 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 (Bran Fatt Feed Ltd., Dhaka) and were fed with water supplemented with vitamin-mineral premix (Magnivet WSP, Navarin). Twelve healthy broiler broods at the age of 52 days, were divided into three groups (A, B and C), each consisting of four birds.

Proportion of inoculation

For colony formation 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 separately in the nutrient agar plate using a fresh pipette for each dilution. The diluted sample was 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 inoculation 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 dilution factor. CFU was calculated according to ISO (1995). The results of CFU were expressed as log number of organisms per ml of sample.

Each bird of group A, B and C was injected with 1.0 ml suspension of Salmonella pullorum (7.9 x 10^8 CFU), Enterococcus faecalis (4.5 x 10^7 CFU), and E. coli (1.25 x 10^8 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 suspect mortality.

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 three media was incubated at 37°C for overnight. The colonies on primary cultures were repeatedly subcultured by streak plate method (Chestroough, 1985) and pure culture with homogenous colonies were observed. Media such as blood agar, Nutrient agar, Littoral 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. Cultural resembling typical reactions of organisms were further screened on the basis of morphological, cultural and biochemical characteristics as described by Cowan (1995).

RESULTS AND DISCUSSION

Neuropath and bacteriological methods were mainly used to determine the bacterial diseases in commercial chickens. During the one year data collection, of which 897 (39.61%) cases diagnosed as bacterial infection. Of these 697 bacterial cases, of which salmonellosis (n = 382), colibacillosis (n = 147), food poisons (= 114), staphylococcus (n = 6), gram negative, the enteritis (n = 24) and infections (n = 40) were diagnosed. Thus, the prevalence of salmonellosis, colibacillosis and gastroenteritis were found significantly high in comparison to other bacterial diseases. Accordingly, the characterization and pathogenicity of these important bacterial diseases were studied.

Avian salmonellosis

Neuropath examination of salmonellosis affected dead bird showed enlarged and purulent foci on liver and spleen, congested liver and lung, mucus and haemorrhages in intestines, petechial haemorrhages in heart, greenish to brown colour liver (Fig. 1). In chronic cases lesions were defined and ulcers developed on glassy or thick, discoloured and cystic ovarian follicles in the laying hen. There was chronic enteritis, peritonitis and pericarditis.

Salmonella organisms were isolated from liver and spleen of dead birds from histopathological studies. On maragac agar small round translucent smooth colony, on blood agar no haemolysis, on EMB agar no growth, on SS agar pinkish colony were observed (Table 1 and Fig. 2). Microscopic examination of Gram's stained wet mount prepared from colony showed Gram-negative, very short rods, arranged as single or paired organisms (Fig. 3). Salmonella organisms were identified as biochemical tests with five basic sugars, in which showed fermentation of glucose and mannose but did not ferment lactose, sucrose and maltose (Table 2 and Fig. 4).

Avian colibacillosis

Neuropath examination of chicken died of colibacillosis showed peritonitis, pericarditis, petechial haemorrhages in the spleen, liver and mesenteric haemorrhages in the mucosa intestine, emaciated resulted unshaded yolk sac.
Fig. 1. Enlarged bronze coloured liver of a 12-day-old broiler chicken due to Pullorum disease showing infiltration, small white necrotic foci throughout the liver.

Fig. 2. A pinkish coloured characteristic colonies of Salmonella enteritidis on SS agar isolated from a 20-day-old broiler chicken.

Fig. 3. Microscopic features of Salmonella pullorum organism isolated from a dead broiler showing Gram negative rod and occur singly, few in pairs in smudged form.

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

Fig. 5. Pure culture colonies of Escherichia coli on EMB agar showing characteristic dark with a metallic sheen.

Fig. 6. Gram negative bacillus coli showing various shape and size and shape arranged in singly, paired and short chain.
Bacterial infections in chickens

Fig. 7. Biochemical tests of Escherichia coli organism showing fermentation of all the five basic sugars with production of acid and gas.

Fig. 8. Microscopic features of Methylene blue stained impression smears of a heart of a dead broiler showing bipolar flagellated monosaccharide singly and in groups.

Fig. 9. A pure culture colony of Proteus vulgaris on nutrient agar showing characteristic glistening and black discoloration centrally.

Fig. 10. Microscopic features of Gram's stained smears of P. vulgaris showing Gram's negative rods, arranged in singly, parallel or in short chains (X1000).

Fig. 11. Biochemical tests of Pseudomonas multocida isolated from endocardium of 72-day-old chicken showing fermentation of xylose, rhamnose and mannitol but not maltose and lactose.

Fig. 12. Experimentally induced Fowl cholera affected swollen foot of a 62-day-old broiler chicken showing multiple necrosis led with coagulative necrosis.
Bacteriological smears collected from entrees, beet and liver lesions were streaked on nutrient agar where produced smooth, white to greyish white colony and peculiar foetid odour on blood agar where haemolysis occurred, on EMB agar where produced characteristic duo with metallic sheen (Fig. 2).

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

<table>
<thead>
<tr>
<th>Nutrient Broth</th>
<th>Nutrient agar</th>
<th>Blood agar</th>
<th>EMB agar</th>
<th>SS agar</th>
<th>Shape</th>
<th>Arrangement</th>
<th>Gram's Staining</th>
<th>Isolated Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey growth with heavy occultum sediment</td>
<td>Small round transparent smooth colony</td>
<td>No haemolysis</td>
<td>No growth</td>
<td>Pinkish colour colony</td>
<td>Very short rods</td>
<td>Single or paired</td>
<td>-ve</td>
<td>Salmonella pullorum</td>
</tr>
<tr>
<td>Turkey growth</td>
<td>Smooth, white to greyish white colony, peculiar foetid odour</td>
<td>Produced haemolysis</td>
<td>Dark with metallic sheen</td>
<td>Slight pinkish colour colony</td>
<td>Short rods, paired or in short chain</td>
<td>-ve</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>Cloudy growth and in a few days a sticky sediment</td>
<td>Colony with bluish colour and smooth, convex, translucent, glinting</td>
<td>No haemolysis</td>
<td>No growth</td>
<td>Rod shaped and bipolar organism</td>
<td>Single, paired or in short chain</td>
<td>-ve</td>
<td>Pasteurella multocida</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SN</th>
<th>Isolated Organism</th>
<th>Fermentable properties with carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salmonella pullorum</td>
<td>+AG +AG +AG +AG +AG +AG</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td>+AG +AG +AG +AG +AG +AG</td>
</tr>
<tr>
<td>3</td>
<td>Pasteurella multocida</td>
<td>+A +A +A +A +A +A</td>
</tr>
</tbody>
</table>

A = Acid, AG = Acid and gas.

Microscopic examination of Gram's stained 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 (glucose, and produced acid and gas (Fig. 7)).
Bacterial-pathological studies in chicks

Avian salmonellosis (Foot-claws)

 Necropsy examination of dead chickens, caused by Salmonella pullorum in 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 185 (19.9%) chickens, of which 69.0% were single, 29.0% in two types and 1.8% as three types mixed infection. Higher infection rate was recorded in adult layers (33.2%) in comparison to brooding (13.5%), growing (16.1%) and pullet (16.1%) chickens. The 21.99% proportionate prevalence of salmonellosis recorded in this study support the report of Mira et al. (1997) that reported 21.4% incidence of salmonellosis following infectious bursal disease in poultry from India. However, Sarker (1976) reported 15%, Ramlal and Hossain (1992) reported 4.82%. Bhatnagar et al. (1990) reported 9.28%, Talha et al. (2001) reported 13.12% and Giasuddin et al. (2002) reported 4.9 mortality of chickens due to salmonellosis from Bangladesh.

The necropsy lesions observed in avian salmonellosis are in conformity with the earlier reports of Chibbli (1973) and Judd et al. (1994). Significantly (p > 0.01) highest proportionate prevalence of salmonellosis was recorded during summer (48.05%) in comparison to rainy (22.38%) and winter (22.66%) seasons. These findings support the report of Bhatnagar et al. (1994) who reported highest prevalence of salmonellosis during pre-monsoon (1.07%) in comparison to winter (0.40%), pre-monsoon (6.82%) and post-monsoon (6.82%) period. These estimations indicate that salmonellosis is still an important disease problem in the poultry industry in Bangladesh. And especially high prevalence rate in adult layer chickens (31.25%) with possible vertical transmission might be the cause 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 airsuckling, rethusa, anorexia, peritonitis, septicaemia, synovitis and colicgranuloma. The study recorded 3.04% proportionate prevalence rate of colibacillosis in chickens, of which 67.35% recorded as single, 29.97% as two types and 1.72% as triple types mixed infection with other diseases. These results support the earlier reports of Sarker (1976) who reported 3.72%, Bhatnagar et al. (1996) reported 0.61% and Talha et al. (2001) reported 3.51% 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 it hinted that the results recorded in this study supports the reports of Miahkajol and Khatunpurkar (1994) who reported Escherichia coli, NDV and IBV in broiler and Tarebh and Rahman (2002) who reported interactions between E. coli and NDV in chickens.

Talha et al. (2001) recorded higher proportionate prevalence rate of colibacillosis in growing chickens in comparison in adults whereas Bhatnagar et al. (1996) reported widely prevalent of colibacillosis in both the brooding (12.32%) and pre-peak post production layer chickens (5.49 to 8.7%), 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 various transmission and egg shell contamination followed by generation, and high rate of E. coli infection in layer chickens again raise keen attention towards its efforts 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 (30.42%) seasons. Bhatnagar et al. (1996) also reported avian colibacillosis in all the seasons of the year in Bangladesh. Panov et al. (1998) reported outbreaks of Eschel Infection during November to Minvs, and Lambiot (2000) reported higher E. coli infection during rainy season.
Asian paratuberculosis

Fowl cholera (FC), caused by Pasteurella multocida is an important infectious disease of poultry. It has been recognized in 6.5% of human beings in chickens, of which 37.9% is single, 21.05% is both and only 1.7% is mixed with five types of diseases. The 6.5% proportionate prevalence rate of FC recorded in this study is in conformity with the earlier reports of Kamal and Hussain (1992) who reported 6.45% prevalence rate of FC in chickens. However, Bhattacharjee et al. (1989a) and Talib et al. (2001) reported 1.98% and 3.15% proportionate incidence rate of FC in chickens from Bangladesh. In addition, Chaudhury et al. (1985) reported 25% mortality and Kamal et al. (1988) reported 38% 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 Chaudhury 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 Talib et al. (2001) recorded FC in masses from 2 to 8 weeks of old chicken population in Bangladesh. These results support the reports of Bhattacharjee et al. (1996) and Talib et al. (2000) who reported higher incidence rate during March to July.

Pathogenity 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 birds were divided into three groups (A to C), each consisting of four birds. Three birds of group A were infected with S. pullorum, three birds of group B with E. coli and three birds of group C with P. multocida and the fourth one of each group served as uninfected controls.

Salmonella pullorum infection

The inoculation period was from 96 hours in experimentally infected chickens with Salmonella pullorum organism, with control chicks and in the 7th day of infection. The affected birds showed depression, ruffled feather and weight loss. All the control chicks remained active through out the course of the experiment. These observations are in conformity with Khan et al. (1998) who reported similar pathogenicity with Salmonella pullorum infection in 6 weeks old local birds.

Escherichia coli infection

The pathogenicity of the isolated E. coli was studied in broilers of group B and the inoculation period was 96 hours after infection. Clinical signs appeared 96 hours after infection. The clinical signs appeared in anorexia, diarrhea which in one case was blood stained, pattering of vent, ruffled feather 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 broilers of group C in which the average incubation period was from 96 hours with clinical signs of depression, inappetence, loss of body temperature and diarrhea, initially whitish and finally greenish with mucus. Only one bird showed partial lameness and laid on the ground. These findings correlate with reports of Kamal et al. (1988), Khan et al. (1997) and Hussain et al. (1999).

Resolation and identification of the organisms

All the three causative isolated organisms were resolved 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 slaughted 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 granuloma on the exfoliated liver surface, emphysema (Fig. 12) and hemorrhage in the intestinal mucosa with other lesions. The untreated control birds experimentally infected with Salmonella pullorum showed mucus and hemorrhage in the intestine, mild necrotic foci on the liver and spleen. The untreated control bird experimentally infected with Escherichia coli showed mucus and hemorrhage in the intestine, necrotic foci on the liver and slight fibrotic covering on the heart. All other treated birds did not show any marked lesions in any of the internal organs.

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