ISOLATION AND CHARACTERIZATION OF INTEROBACTERIA ASSOCIATED WITH HEALTH AND DISEASE IN SOMALI CHICKENS

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ABSTRACT

Bacteriological examination of intestinal swabs of 30 apparently healthy and 30 sick/died Somali chickens (Fayoumi hen × IR cock) aged between 25 to 60 weeks were carried out to determine the enterobacteria associated with health and disease. During the period from March to October 2003. These birds of either sex and mixed breed were reared in multi-brooding system under the SLDP-2 project area in the district of Feni. The 60 swabs were collected at slaughter (nonotory in cause natural and unnatural broods). In addition, the gross tissue changes of the sick and dead birds were recorded. The prevalent bacterial flora in intestinal swabs were Salmonella (33.33%), E. coli (95.19%), Shigella flexneri (4.09%), S. typhimurium (55.69%), Salmonella typhi (5.69%) and E. coli (36.60%) were colonized with marked pathological lesions. The isolated enterobacteria and their associated gross and histopathological changes are described and discussed. It may be concluded from this study that the enteric bacteria usually remain as clinically overt infection and do not produce clinical disease unless local or other factors are involved.

Key words: Enterobacteria, apparently healthy chickens, sick/ dead chickens, pathology

INTRODUCTION

Bangladesh is one of the poorest countries of the third world in terms of material resources. Its economy mainly depends on agriculture. Poultry industry is an emerging agribusiness starting practically during eighties in Bangladesh. Poultry rearing may play a vital role for the poverty alleviation. It fulfills one of the important sources of animal protein. Diseases caused by enterobacteria hamper the profitable poultry production. Somali chickens (Fayoumi hen × IR cock) have been rearing in Smallholder Livestock Development Project-2 (SLDP-2) area as well in rural area as a poverty candidate in Bangladesh. Island reports on the occurrence of enterobacteria associated with health and disease in the area are very limited (Samed, 2000). This paper describes the isolation and characterization of enterobacteria of apparently healthy and sick/died Somali chickens and their association in clinical disease production.

MATERIALS AND METHODS

Collection of swabs

Intestinal swabs were collected from 30 samples from healthy and 30 samples from sick/ dead chickens aged between 25 to 60 weeks at slaughterhouse and necropsy in major tea estates containing white, brown and intermediate broods by using sterilized cotton tipped swab stick from SLDP-2 area, Feni and Government Central Poultry Farm, Mirpur, Dhaka, during the period from March to October 2003.

Culture of bacteria

The brood containing swabs in the test tubes were incubated at 37°C for 24 hours for the growth of bacteria. Then they were plated on different culture media and incubated following standard procedures for the isolation of specific bacterial colonies (Merchant and Packer, 1967; Cowen, 1974 and Buxon and Fraser, 1977).

Stooling of enterobacteria

Modified Gram's and Leichmann's stain were used for the staining of isolated bacteria as described by Merchant and Packer (1967) and Cheesbrough (2000) for their morphotype.

Biochemical tests

The isolated bacteria were subjected to different biochemical tests. Permanence of different sugars, Methyl Red, Voges-Proskauer, Sato's, Cephalin and Coagulase were performed for identification of the organisms following the procedures described by Merchant and Packer (1967) and Cheesbrough (2000).

Modility test

New broth cultures of the organisms were inoculated at 37°C or below the optimum temperature (22°C) was examined in 'hanging drop' preparation, using a high power oil immersion objective with reduced illumination (Carr, 1979).
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Genus pathology
The birds were necropsied within five hours of death or collection. At necropsy, the organs were examined carefully, and gross tissue changes were recorded. The lesions containing representative tissues samples from suspected bacterial disease cases were collected in 10% neutral buffered formalin for histopathological studies (Stubbs, 1954).

Histopathology
The formol-fixed tissues were trimmed, processed, sectioned and finally stained with hematoxylin and eosin (Luna, 1968).

RESULTS AND DISCUSSION
The bacteriological methods were used to isolate and identify the enterobacteria in 30 apparently healthy and 30 sick / dead chickens, and the results are presented in Table 1. Salmonella spp. (33.3%), E. coli (95.0%), Shigella flexneri spp. (51.66%), S. typhimurium spp. (40.0%), P. multocida (33.3%) were isolated from the intestinal swabs of 60 birds, but Salmonella spp. (36.66%) and E. coli (26.66%) organisms were found to be associated with marked pathological lesions and diseases.

Table 1. Enterobacteria isolated from intestinal swabs of apparently healthy and sick / dead chickens.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Isolated Bacteria</th>
<th>Apparently healthy chickens (n = 30)</th>
<th>Sick / dead chickens (n = 30)</th>
<th>Total positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>0</td>
<td>Salmonella spp.</td>
<td>09 50.00</td>
<td>11 36.66</td>
<td>20 33.33</td>
</tr>
<tr>
<td>0</td>
<td>Escherichia coli</td>
<td>28 93.33</td>
<td>*29 *36.66</td>
<td>57 95.00</td>
</tr>
<tr>
<td>0</td>
<td>Shigella flexneri spp.</td>
<td>17 56.66</td>
<td>14 46.66</td>
<td>31 51.66</td>
</tr>
<tr>
<td>0</td>
<td>Salmonella typhimurium</td>
<td>13 43.33</td>
<td>11 36.66</td>
<td>24 40.00</td>
</tr>
<tr>
<td>0</td>
<td>Pasteurella multocida</td>
<td>01 03.33</td>
<td>01 03.33</td>
<td>02 03.33</td>
</tr>
</tbody>
</table>

*A total of 8 (26.66%) cases was associated with marked pathological lesions and disease, although 20 (33.33%) E. coli were isolated from sick / dead chickens.

Occurrence of enterobacteria

Salmonella
The organisms produced black, translucent, round, raised and smooth colonies on SS agar. The organisms were rod shaped and formed short to long chains and were gram negative with Gram's method (Fig. 1). The organisms fermented glucose, maltose, mannitol, xylose and dulcitol. The organisms were positive to Methyl Red and negative to Voges-Proskauer and Indole test. The organisms were identified as Salmonella on the basis of morphology, staining and biochemical test. In this study, the colony characters, black colonies on SS agar due to production of hydrogen sulfide (reducing), staining characters and biochemical tests were corresponded with the finding of others (Sturms and Klaus, 1996). The present prevalence of Salmonella in intestinal swabs was lower than the reports of other authors (Jordan and Parsons, 1996; Jones et al., 2002; Tavechieh et al., 2002). In this study the lower prevalence of Salmonella organisms might be due to the age (around 25 weeks) and breeds of the birds and also for the resistant power of the scavenging poultry.

Escherichia coli
E. coli formed smooth circular colonies with dark centers and metallic sheen on EMB agar and pink colonies on MacCubrey's agar. E. coli appeared gram negative cocoid isosceles-robicunulate (Fig. 2) and was motile in 'hanging drop' preparation. The organisms fermented glucose, lactose, maltose, dulcitol, mannitol and saccharose. The organisms were Methyl Red positive, Voges-Proskauer negative and produced Indole. On the basis of the cultural, morphological and biochemical characters, the organisms were identified as E. coli. The present prevalence of E. coli in intestinal swabs was higher than the findings of Deralhaut and Ghaitha (2002) and El-Siddik et al. (2002). The cultural and staining characters and biochemical properties of the identified organisms were similar to the finding of Jones et al. (1997), Ali et al. (1998) and Mchesa et al. (2002). The highest prevalence of the organisms in this study may be speculated to be the breeds and setti scavenging rearing system of the birds.

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Fig. 1. Isolated Salmonella showing rod shape, about to form short or long bacillary bacteria (Modified Gram’s stain, X 400).

Fig. 2. Isolated E. coli showing short rod, varying from coccoid to bipolar shape (Modified Gram’s stain, X 830).

Fig. 3. Isolated Staphylococcus showing coccoid bacteria and arranged in grape-like clusters (Modified Gram’s stain, X 830).

Fig. 4. Isolated Streptococcus organisms arranged in pairs and short to long chains (Modified Gram’s stain, X 830).

Fig. 5. Isolated Pseudomonas multocida showing small coccoid rods to coccobacilli with a bipolar appearance (Leishman’s stain, X 830).

Fig. 6. Section of liver showing congestion, haemorrhagic, focal degeneration, focal necrosis with infiltration of mononuclear and round cells, and congestion of the central vein in salmonellosis (H & E, X 400).
Isolation and characterization of enterobacteria in Salmoniella chickens

Fig. 7. Section of heart showing scatteredly distributed degenerated muscle fibers with few infiltrations of heterophilis and mononuclear cells with round nucleus in salmonellosis (H & E, X 335).

Fig. 8. Section of spleen showing focal degeneration and necrosis of lymphocytes in salmonellosis (H & E, X 335).

Fig. 9. Section of intestines showing congestion, hemorrhage and infiltration of inflammatory cells in salmonellosis (H & E, X 335).

Fig. 10. Section of ovary showing detached ova with mild to moderate hemorrhage in salmonellosis (H & E, X 225).

Fig. 11. Section of lung showing infiltration of heterophilis, Spaces of exudation and pink mottled foci around the blood vessels in colibacillosis (H & E, X 225).

Fig. 12. Section of liver showing infiltration of heterophilis, lymphocytes and macrophages mainly in portal areas in colibacillosis (H & E, X 335).
Staphylococcus
The colony of *Staphylococcus* was round, smooth, glistening, opaque, and golden yellow color. In this study the organisms were gram positive cocci and arranged in grape like clusters with Gram's method of staining (Fig. 3) and fermented lactose, maltose, dextrose, sucrose and saccharose. These organisms were found positive to catalase and coagulase which identified as *Staphylococcus*. The morphologic, staining and biochemical finding of *Staphylococcus* are agreed with the findings of others (Cower, 1979; Brooks et al., 2002.) This study recorded an overall 51.66% occurrence of *Staphylococcus* organisms is the integumental swabs of chickens which is comparatively lower than Nagase et al. (1992) who reported 90.1% occurrence of *Staphylococcus* organisms in animal and human skin. The lowest prevalence of *Staphylococcus* organisms may not be well explained in this study.

Sarcinae
The organisms formed small white, hard few drop-like colonies on nutrient agar and caused hemolysis on blood agar. The organisms were gram positive cocci, remained in pairs and had short to long chains (Fig. 4.) *Sarcinae* spp. fermented lactose and saccharose with acid production. *Monilis* was not fermented. The *Sarcinae* spp. showed negative to catalase and coagulase tests. The cultural characters, staining and biochemical tests confirmed the organism as *Sarcinae*. The cultural characters, morphological and biochemical properties were similar to the findings of Mulligan (1966) and Chrostine (2000). The lowest prevalence of the organism in intestinal swab may be due to the resistant power of the birds.

Pasteurella multocida
The prevalent *P. multocida* in intestinal swabs of apparently healthy and sick/dead chickens produced moderate size, round and grayish colonies on blood agar. *P. multocida* were small coccioid rod in shape, gram: negative coccochick with a bipolar appearance with Leishaun's stain (Fig. 5.) The bacteria were positive to oxalode and catalase tests. Their cultural, staining characters and biochemical characters identified the organisms as *P. multocida*. The prevalence of fowl cholera was reported 11.33% by Syrip (1976). In this study the cultural, morphological and biochemical characters corresponded the findings Merwey and Packier (1967). Chauhan and Roy (1996). The present study showed the low percentage of fowl cholera organism in Somali chickens and this may be due to proper vaccination with fowl cholera vaccine.

Pathology
Of the five types of enterobacteria isolated from the integumental swabs of chickens, of which only *Salmonella* and *E. coli* were found to be associated with marked pathological lesions.

Salmonellae
*Salmonellae* the investigation recorded a total of 11 (36.66%) cases of *Salmonellae* out of 30 sick and dead chickens. The affected birds exhibited somnolence, weakness, poor growth and inappetence. Chalk white excrescence sometimes stained with greenish brown adhered with the vent. Laboured breathing and gasping were observed. The adult affected birds showed depression, diarrhea, hæmorrhage and dehydration.

Grossly the liver was enlarged and congested and in few cases liver revealed hemorrhages and focal necrosis. Petechial hemorrhages were seen in the spleen, base of the heart and kidneys. In some cases, lungs were pleural.

There was catarrhal inflammation in the intestine. The mucosa were deformed, discolored and sycotic. Microscopically the section of the liver showed congestion, hemorrhages, focal degeneration, focal necrosis with infiltration of mononuclear and neutrophil cells and congestion of the central veins (Fig. 6.) The pulmonary lesions consisted of diffuse congestion and hemorrhages associated with spongy fibrosis. Sections of the liver showed scatteredly distributed degeneration of mitotic fibers with few invasions of heterophils and mononuclear cells with round nucleus (Fig. 7.) The spleen showed the focal degeneration and necrosis of lymphocytes (Fig. 8.) The intestinal mucosa exhibited congestion, hemorrhages and infiltration of inflammatory cells. In many instances alveoli of macrophages was recorded (Fig. 9.) The ovary showed deformed shaped oval with mild to moderate hemorrhages (Fig. 10.)

Caltheliosis
A total of 29 (96.66%) *E. coli* was isolated from 30 sick and dead birds, of which only 8 (26.66%) cases were found to be unconnected with marked pathological lesions and disease (Table 1.) The birds were found lethargic, dehydrated and deformed with poor growth performance. Gross lesions included thickening of the air sacs, cutaneous exudation on the inspiratory surfaces, petechial hemorrhages in the heart, and congestion in the liver and spleen. Microscopically, section of the lungs showed infiltration of heterophils, fibrinous exudation and pink-coloured fluid around the blood vessels (Fig. 11.) There were severe diffuse congestion in liver. Section of the liver showed infiltration of heterophils, lymphocytes and macrophages mainly in portal areas (Fig. 12.)
The recorded cases of salmonellosis (36.66%) and colibacillosis (36.66%) in the present study were relatively lower than the reports of other authors (Wilkins et al., 2002; Saloglu et al., 2003). Salmonellosis and colibacillosis were confirmed by isolating the organisms from infected birds, necropsy findings and histopathological lesions. All the parameters used for the diagnosis of salmonellosis and colibacillosis corresponded with the findings of many authors (Chisti et al., 1989; North and Bell, 1990; Chachi and Roy, 1995; Jordan and Patterson, 1996; Ley and Yoder, 1997; Talha et al., 2001; Phipps and Young, 2000; Shown et al., 2002; Wilkins et al., 2002; and Saloglu et al., 2003).

Therefore, the present investigation reveals that the presence of bacteria in the intestines is not directly related to the production of disease in the Somali chickens. The birds show relatively low susceptibility to bacterial disease. It may be speculated that some other factors are associated with the production of diseases.

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REFERENCES


