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ISOLATION AND CHARACTERIZATION OF ENTEROBACTERIA ASSOCIATED WITH HEALTH AND DISEASE IN SONALI CHICKENS

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ABSTRACT

Bacteriological examination on intestinal swabs of 30 apparently healthy and 30 sick / dead Sonali chickens (Fayoumi hen × RIR cock), aged between 25 to 60 weeks were carried out to determine the enteropathogens associated with health and disease, during the period from March to October 2003. These birds of either sex and reared under semi-scavenging system under the SLDP-2 project area in the district of Feni. The 60 swabs were collected at slaughter / necropsy in sterile nutrient and tetrathionate broth. In addition, the gross tissue changes of the sick / dead birds were recorded. The prevalent bacterial flora in intestinal swabs were *Salmonella* (33.33%), *E. coli* (95.0%), *Staphylococcus* (51.66%), *Streptococcus* (40%) and *Pasteurella multocida* (3.33%) of which *Salmonella* (36.66%) and *E. coli* (26.66%) were associated with marked pathological lesions. The isolated enteropathogens 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 or until 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. Sonali chickens (Fayoumi hen x RIR cock) have been rearing in Smallholder Livestock Development Project-2 (SLDP-2) area as well as in rural area as a poultry candidate in Bangladesh. Inland reports on the occurrence of enterobacteria associated with health and disease in chickens are very limited (Samad, 2000). This paper describes the isolation and characterization of enterobacteria of apparently healthy and sick / dead Sonali chickens and their association in clinical disease production.

MATERIALS AND METHODS

Collection of swabs

Intestinal swabs were collected from 60 (30 samples from healthy and 30 samples from sick / dead) chickens aged between 25 to 60 weeks at slaughter or necropsy in sterile test tubes containing sterile nutrient and tetrathionate broth 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 broth containing swabs in the test tubes were incubated at 37^0C for 24 hours for the growth of bacteria. Then they were plated on different culture media and subcultured following standard procedure for the isolation of specific bacterial colonies (Merchant and Packer, 1967; Cowen, 1974 and Buxton and Fraser, 1977).

Staining of bacteria

Modified Gram's and Leishman's stains were used for the staining of isolated bacteria as described by Merchant and Packer (1967) and Cheesbrough (2000) for study their morphology.

Biochemical tests

The isolated bacteria were subjected to different biochemical test. Fermentation of different sugars, Methyl Red, Voges-Proskauer, Indole, Catalase and Coagulase were performed for identification of the organisms following the procedures described by Merchant and Packer (1967) and Cheesbrough (2000).

Motility test

New broth cultures of the organisms were incubated at 37^0C or below the optimum temperature (22^0C), was examined in 'hanging drop' preparation, using a high power oil immersion objective with reduced illumination (Carter, 1979).

Gross pathology

The birds were necropsied within few hour of death or collection. At necropsy, the organs were examined carefully and gross tissue changes were recorded. The lesion containing representative tissues samples from suspected bacterial disease cases were collected in 10% neutral buffered formalin for histopathological studies (Stubbs, 1954).

Histopathology

The formalin-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. (33.33), E. coli (95.0%), *Staphylococcus* spp. (51.66%), *Streptococcus* spp. (40.0%) and *P. Multocida* (3.33%) 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 disease.

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

S/N	Isolated Bacteria	Apparently healthy chickens (n = 30)		Sick / dead chickens (n = 30)		Total positive	
		No.	%	No.	%	No.	%
①	*Salmonella* spp.	09	30.00	11	36.66	20	33.33
②	*Escherichia coli*	28	93.33	*29	*96.66	57	95.00
③	*Staphylococcus* spp.	17	56.66	14	46.66	31	51.66
④	*Streptococcus* spp.	13	43.33	11	36.66	24	40.00
⑤	*Pasteurella multocida*	01	03.33	01	03.33	02	03.33

*A total of 8 (26.66%) cases was associated with marked pathological lesions and disease although 29 (96.66%) *E. coli* were isolated from sick / dead chickens.

Occurrence of enterobacteria

Salmonellae

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 was gram negative with Gram's method (Fig. 1). The organisms fermented dextrose, 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 *Salmonellae* 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 (references), staining characters and biochemical tests were corresponded with the finding of others (Sharma and Katock, 1996). The present prevalence of *Salmonellae* in intestinal swabs was lower than the reports of other authors (Jordan and Pattison, 1996; Jones *et al.,* 2002; Tavechio *et al.,* 2002). In this study the lower prevalence of *Salmonellae* 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 MacConkey's agar. *E. coli* appeared gram negative coccoid to short bipolar rod (Fig. 2) and was motile in "hanging drop" preparation. The organisms fermented dextrose, lactose, maltose, dulcitol, mannitol and sucrose. 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 Derabhasantar and Ghanbarpour (2002) and El-Sushon *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 Mishra *et al.* (2002). The highest prevalence of the organisms in this study may be speculated to be the breeds and semi-scavenging rearing system of the birds.

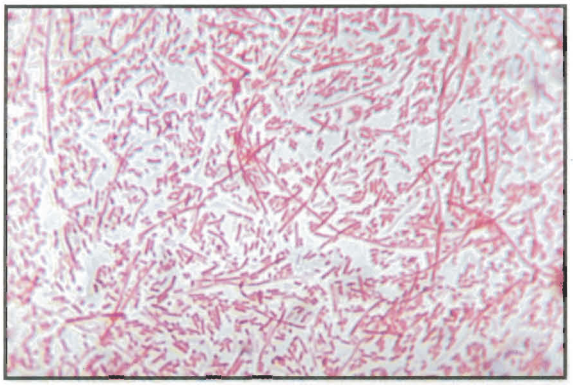

Fig. 1. Isolated *Salmonellae* showing rod shape, short to long chain forming bacteria (Modified Gram's stain, X 830).

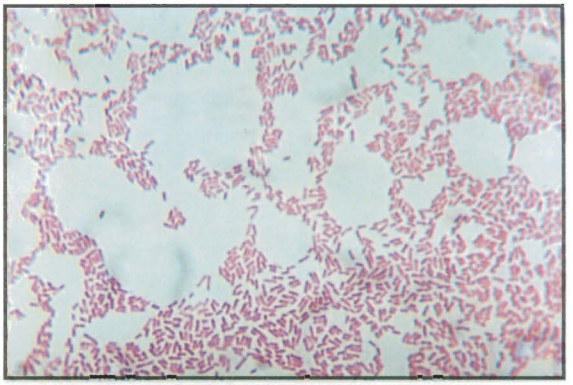

Fig. 2. Isolated *E. coli* showing short rod, varying from coccoid to bipolar shape (Modified Gram's stain, X 830).

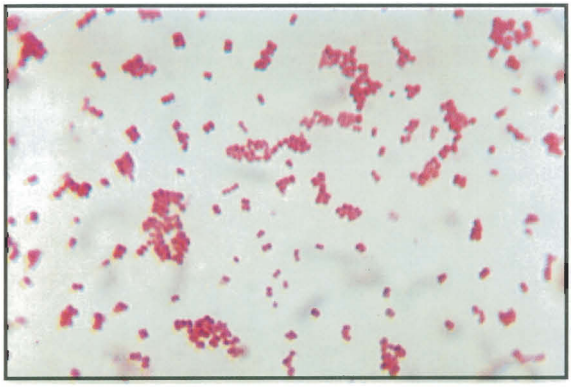

Fig. 3. Isolated *Staphylococcus* showing cocci bacteria and arranged in grape like clusters (Modified Gram's stain, X 830).

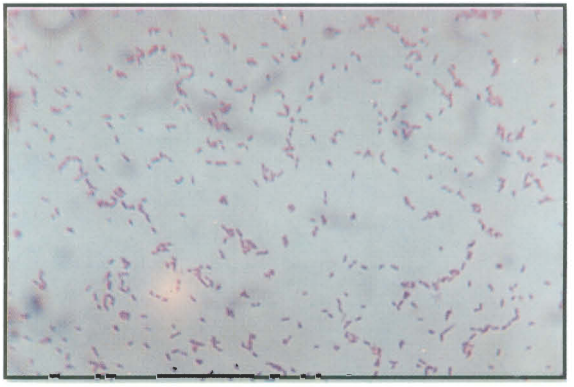

Fig. 4. Isolated *Sreptococcus* organisms arranged in pairs and short to long chains (Modified Gram's stain, X 830).

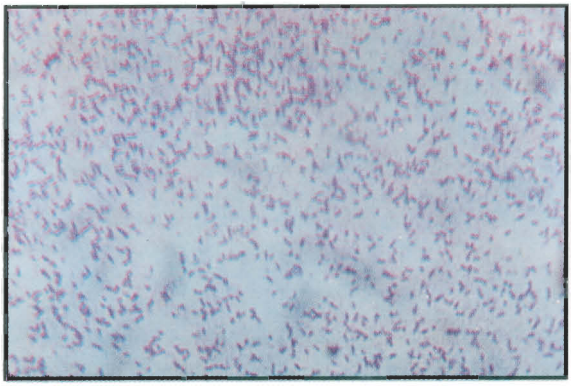

Fig. 5. Isolated *Pasteurella multocida* showing small coccoid rod to coccobacilli with a bipolar appearance (Leishman's stain, X 830).

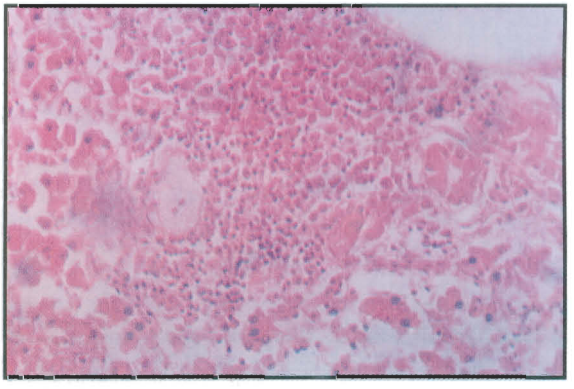

Fig. 6. Section of liver showing congestion, haemorrhages, focal degeneration, focal necrosis with infiltration of mononuclear and round cells and congestion of the central veins in salmonellosis (H & E, X 333).

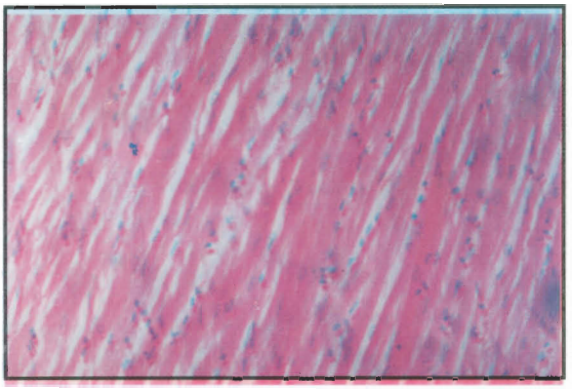

Fig. 7. Section of heart showing scatteredly distributed degenerated muscle fibers with few infiltrations of heterophils and mononuclear cells with round nucleus in salmonellosis (H & E, X 333).

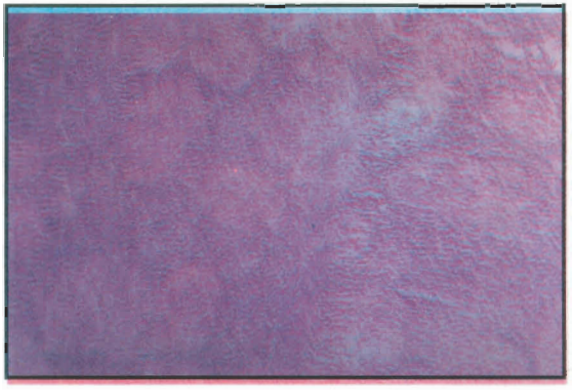

Fig. 8. Section of spleen showing focal degeneration and necrosis of lymphocytes in salmonellosis (H & E, X 333).

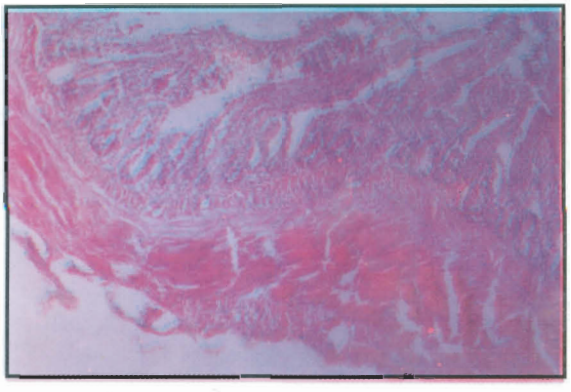

Fig. 9. Section of intestine showing congestion, haemorrhage and infiltration of inflammatory cells in salmonellosis (H & E, X 333).

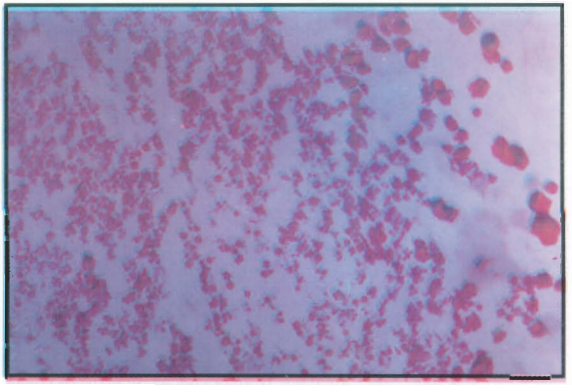

Fig. 10. Section of ovary showing deformed ova with mild to moderate haemorrhage in salmonellosis (H & E, X 333).

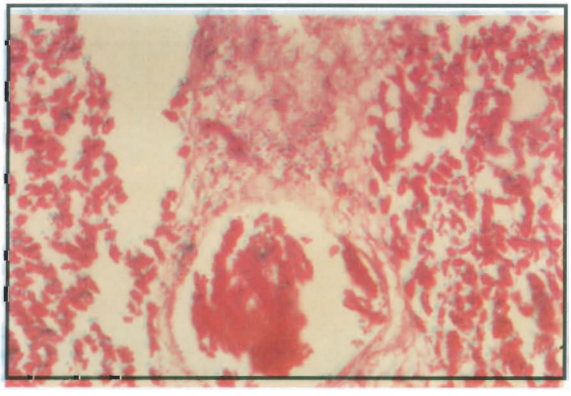

Fig. 11. Section of lung showing infiltration of heterophils, fibrinous exudation and pink colored fluid around the blood vessels in colibacillosis (H & E, X 333)

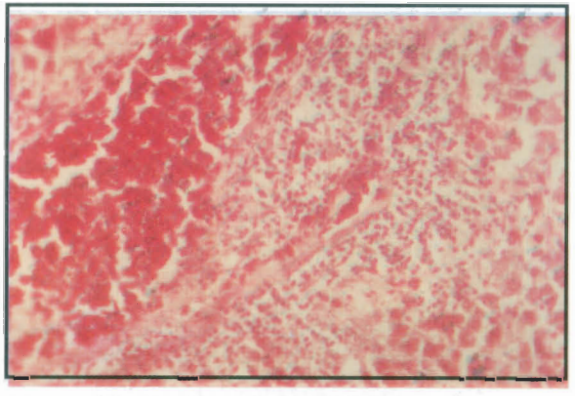

Fig. 12. Section of liver showing infiltration of heterophils, lymphocytes and macrophages mainly in portal areas in colibacillosis (H & E, X 333).

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, mannitol and sucrose. 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 (Carter, 1979; Brooks *et al.*, 2002). This study recorded an overall 51.66% occurrence of *Staphylococcus* organisms in the intestinal swabs of chickens which is comparatively lower than Nagase *et al.* (2002) 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.

Streptococcus

The organisms formed small white, hard dew 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). *Streptococcus* spp. fermented lactose and sucrose with acid production. Mannitol was not fermented. The *Streptococcus* spp. showed negative to catalase and coagulase tests. The cultural characters, staining and biochemical tests confirmed the organism as *Streptococcus*. The cultural characters, morphological and biochemical properties were similar to the findings of Mahanta (1966) and Cheesbrough (2000). The lowest prevalence of the organisms 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 coccoid rod in shape, gram- negative coccobacilli with a bipolar appearance with Leishman's stain (Fig. 5). The bacteria were positive to oxidase 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 Sarker (1976). In this study the cultural, morphological and biochemical characters corresponded the findings Merchant and Packer (1967), Chauhan and Roy (1996). The present study showed the low percentage of fowl cholera organism in Sonali chickens and this may be due to proper vaccination with fowl cholera vaccine.

Pathology

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

Salmonellosis

This investigation recorded a total of 11 (36.66%) cases of salmonellosis out of 30 sick / dead chickens. The affected birds exhibited somnolence, weakness, poor growth and inappetance. Chalk white excreta sometimes stained with greenish brown adhered with the vent. Labored breathing and gasping were observed. The adult affected birds showed depression, anorexia, diarrhea 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 pneumonic. There was catarrhal inflammation in the intestine. The ova were deformed, discolored and cystic.

Microscopically the section of the liver showed congestion, hemorrhages, focal degeneration, focal necrosis with infiltration of mononuclear and round cells and congestion of the central veins (Fig. 6). The pulmonary lesions consisted of diffuse congestion and hemorrhage associated with sero-fibrinous exudation. Section of the heart showed scatteredly distributed degeneration of muscle fibers with few infiltrations 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, haemorrhages and infiltration of inflammatory cells. In many instances sloughing of mucosal epithelia was recorded (Fig. 9). The ovary showed deformed shaped ova with mild to moderate hemorrhages (Fig. 10).

Colibacillosis

A total of 29 (96.66%) *E. coli* was isolated from 30 sick / dead birds, of which only 8 (26.66%) cases were found to be associated with marked pathological lesions and disease (Table 1). The birds were found lethargic, dehydrated and depressed with poor growth performance. Gross lesions included thickening of the air sacs, caseous exudation on the respiratory 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 colored fluid around the blood vessels (Fig.11). There was 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 (96.66%) in the present study, were relatively lower than the reports of other authors (Wilkins *et al.*, 2002; Saleque *et al.*, 2003). Salmonellosis and colibacillosis were confirmed by isolating the organisms from infected birds, necropsy findings and histopathologic lesions. All the parameters used for the diagnosis of salmonellosis and colibacillosis corresponded with the findings of many authors (Chishti *et al.*, 1985; North and Bell, 1990; Chauhan and Roy, 1996; Jordan and Pattison, 1996; Ley and Yoder, 1997; Talha *et al.*, 2001; Pikpinyo *et al.*, 2002; Shome *et al.*, 2002; Wilkins *et al.*, 2002 and Saleque *et al.*, 2003).

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

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