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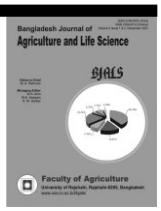

Prevalence of Enteric Bacterial Diseases of Chickens with Isolation and Characterization of Causal Agents and Pathology in Gazipur District of Bangladesh

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ABSTRACT

Enteric bacterial diseases are important cause of high economic losses in chicken industry due to increased mortality, decreased weight gain, increased medication costs and decreased feed conversion rates. Isolation and identification of enteric bacteria along with the pathology they produce is crucial to determine the cause of enteric diseases in chickens for formulating effective therapy and control measures. We investigated the prevalence of enteric bacterial diseases focusing on isolation and identification of enteric bacteria with gross and histopathologic changes in chickens. A total of 100 chicken (60 dead and 40 live) samples collected from Kazi Poultry Farm Laboratory of Gazipur Chowrasta Branch were analyzed by culture, biochemical tests, necropsy and histopathology. The prevalence of enteric bacterial diseases were 80% colibacillosis, 75% fowl typhoid, 50% pullorum disease, 60% fowl paratyphoid and 40% fowl cholera. *E. coli* fermented most of the basic sugars while *Salmonella* fermented only dextrose and mannitol and produced acid and gas. *Pasteurella multocida* fermented dextrose and sucrose with the production of only acid. Among the *Salmonella* organisms, only the *Salmonella paratyphi* displayed motility. Hemorrhagic intestine and congested lungs in colibacillosis, bronze color liver with necrotic foci in fowl typhoid and punctuate hemorrhage in the coronary groove fat in fowl cholera were predominant gross findings. Focal necrosis, congestion of vessels and heterophilic infiltrations were evident in different organs as the histopathologic changes. These findings help the poultry industry to maintain the gut health of chickens.

Introduction

Poultry industry is an emerging agribusiness sector starting practically during eighties in Bangladesh. Since early 1995 poultry farming is being considered as a well-established business in Bangladesh (Alam et al. 2020). Traditionally, poultry rearing is one of the most important sources of income for rural women especially for landless and marginal farmers in Bangladesh (Paul et al. 1990). It has been shown in a study by a non-government organization that more than 70% of

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the rural households in Bangladesh are involved in poultry rearing (BRAC, 2015). It has also been shown in another study that 20% of the population directly and 50% of the population indirectly earn their livelihood by poultry rearing (DLS, 2021). There are approximately 296.60 million chickens and 59.72 million ducks in Bangladesh (DLS, 2021). At present, poultry industry contributes 51% of total meat production of the country. Annually, the demand of egg is 104/head/year and availability is 104.23/head/year (DLS, 2021). The major constraints for poultry rearing are the infections with microorganisms and parasites, mismanagement, environmental stresses, and deficiency of essential vitamins and minerals. The microorganisms responsible for major enteric bacterial diseases are *Escherichia coli*, *Salmonella*, etc. (Haider et al. 2004). *Salmonella* and *E. coli* infections of poultry have been shown to be of critical importance in Bangladesh (Haider et al. 2008). Colibacillosis is a major problem and localized or systemic infection causes entirely or partly by avian pathogenic *Escherichia coli* (APEC) including colisepticemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), swollen-head syndrome, venereal colibacillosis, and coliform cellulitis (inflammatory process), peritonitis, salpingitis, orchitis, osteomyelitis/synovitis (turkey osteomyelitis complex), panophthalmitis, omphalitis/yolk sac infection and enteritis. Enteric health and nutrition are closely related. Poor enteric health can adversely affect feed digestion, gut motility and nutrient absorption by several means. Likewise, poor nutrition and feed quality can either increase the chicken's susceptibility to enteric disorders or diseases (Hafez et al. 1998). Nutritional factors that influence gut health include feed intake, palatability, feed ingredient quality, feed formulation and pellet quality. In addition, mistakes in feeding technique, the amount of fiber in the feed, the content and quality of the raw materials as well as sudden feed changes or restriction can result in changes in the intestinal flora and/or in the enzymatic activity which lead to digestive disorders. Most important enteric diseases are colibacillosis, fowl typhoid, pullorum disease, fowl cholera and necrotic enteritis. In Bangladesh, works on enteric bacterial diseases in chickens is scant. The enteric bacterial diseases are generally diagnosed on the basis of postmortem examination of the dead birds in Bangladesh. Very few attempts were taken to isolate the causal agents of enteric bacterial diseases in chickens in Gazipur district. Considering these views the research was planned to investigate the prevalence of enteric bacterial diseases of chickens along with isolation and characterization of causal agents and characterization of gross and histopathologic changes in tissues.

Materials and Methods

The study was conducted in the Pathology laboratory, Department of Pathobiology, Faculty of Veterinary Medicine & Animal Science (FVMAS), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from September 2017 to August 2018. For this investigation, a total of 100 samples were collected from the Kazi Poultry Farm Laboratory, Chowrasta Branch, Gazipur. Among the samples 60 were from dead layer and 40 from live chickens. Four hundred and sixty swab samples were cultured for each enteric disease from liver, spleen, lungs, heart, kidney, intestine and cloaca. Bacteriological swab samples were aseptically collected into nutrient (NB) and Tetrathionate broths. Samples for histopathologic examination were collected in 10% neutral buffered formalin.

Isolation and Identification of the Organisms

From the Nutrient Broth, subcultures were made in Nutrient agar and incubated for overnight. In the following day subcultures were performed in SS, EMB, BGA, TSI Agar, Blood agar and XLD agar from the isolated single colony and incubated for 48 hours at 37°C in incubator. The organisms were isolated and identified on the basis of their colony and staining characters and biochemical tests; the methods have been described elsewhere (Haider et al. 2004).

Motility Test

Inoculums were taken in Nutrient Broth from the pure isolated colony and were incubated overnight at 37°C in incubator. One drop of the fresh young broth culture was taken on a coverslip and "Hanging Drop" slide was placed onto the coverslip keeping the concave area of the slide right above the drop. The slides were observed under a microscope with a dim light under low and high-power objectives and at the periphery of the drop.

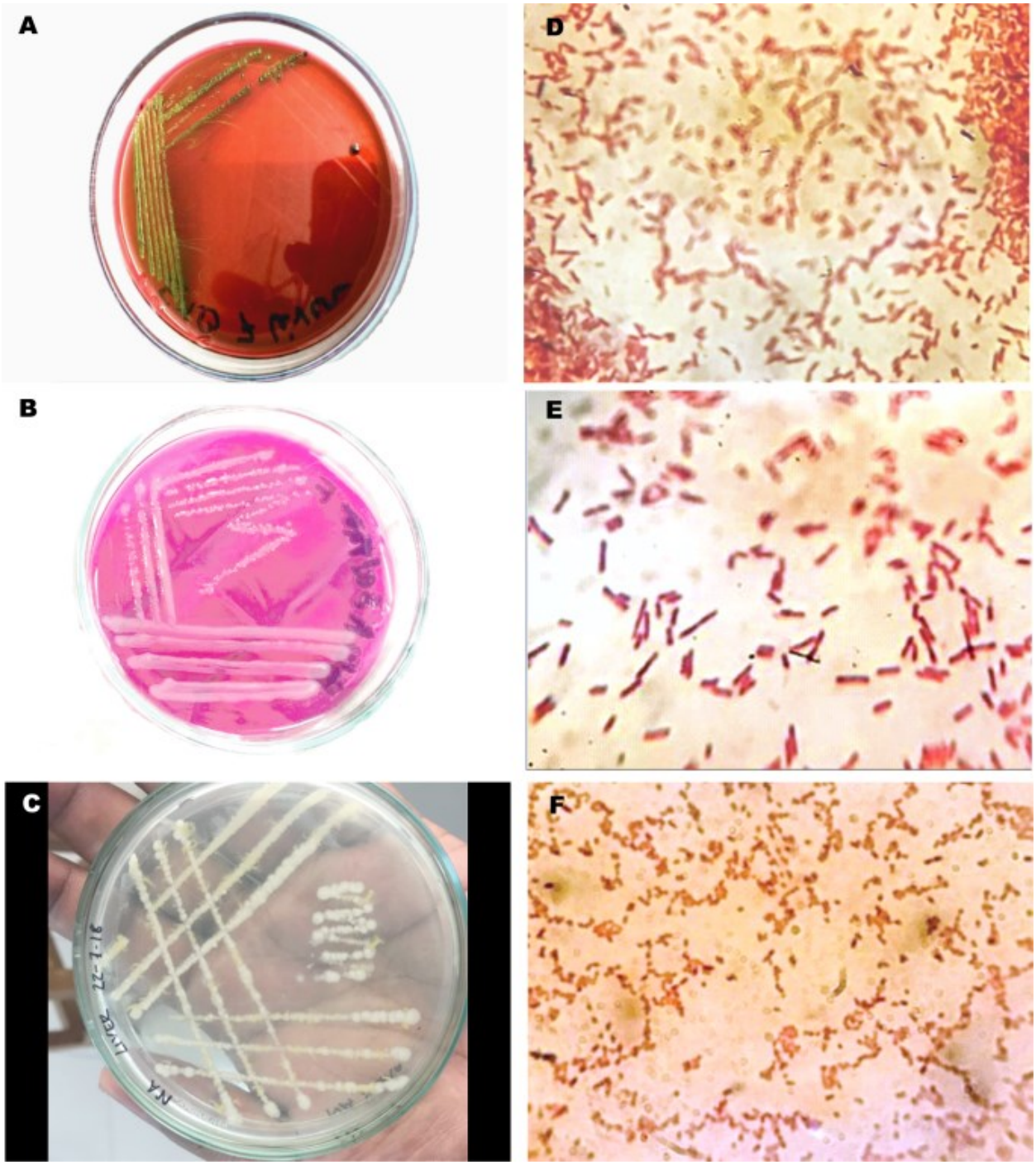

Fig. 1. Colony characters and morphologic features of cultured bacteria isolated from chickens. *E. coli* produced green metallic sheen colonies on eosin methylene blue agar (A) and were Gram negative rod shaped (D). Salmonella produced small, opaque, white colonies on brilliant green agar (B) and were small rods arranged in single or pair (E). *Pasteurella multocida* displayed round, flat colonies of sticky, mucoid consistency on nutrient agar (C) and were Gram negative small rod or coccobacilli arranged in single, pair or occasionally in short chains (F).

Staining of bacteria

The bacteria were stained with Gram's stain to observe their morphological characteristics by taking inoculum from pure cultures (Haider et al. 2004).

Biochemical Tests

A number of biochemical tests were performed for confirmation of the isolates (Tanu et al. 2011). Sugars such as glucose, lactose, mannitol, arabinose, xylose, dulcitol, inositol and sucrose were subjected to fermentation by the bacterial cell to determine fermentation status with their products (acid or acid and gas) as described by Freeman, 1995.

Gross and histopathology

Gross changes in various organs associated with bacterial diseases were observed carefully and were recorded in a semi-structured data collection form. The procedures have been described previously (Blackall and Hofacre, 2020). For histopathology, representative tissues from liver, intestine, and lungs were collected in 10% neutral buffered formalin. After overnight fixation, the tissues were trimmed and re-fixed in fresh 10% neutral buffered formalin for 12 hours on a shaker and were processed following standard procedure (Haider et al. 2008). Briefly, the tissues were dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin. The paraffin embedded tissue sections, cut at 5 µm in thickness, were stained with hematoxylin and eosin, and observed under a light microscope.

Photomicrography

Photomicrographs of representative tissue sections were grabbed using photomicrographic camera system (ZEISS AxioCam ERc5s, Carl Zeiss, Germany) at the Department of Gynecology, Obstetrics & Reproductive Health, FVMAS, BSMRAU, Gazipur.

Results

Prevalence of the enteric bacterial diseases

The prevalence of enteric bacterial diseases were 80.00% colibacillosis, 6.96% fowl typhoid, 13.91% pullorum disease, 19.57% fowl paratyphoid and 8.7% fowl cholera (Table 1). Among the diseases, prevalence of colibacillosis was the highest followed by salmonellosis and fowl cholera in the present study. The organ-wise prevalence of colibacillosis were 11.30%, 10.22%, 11.08%, 9.57%, 7.83%, 12.17% and 17.83%; salmonellosis were 7.39%, 3.48%, 3.91%, 6.02%, 3.04%, 5.65% and 10.87%, and fowl cholera were 1.74%, 0.65%, 0.87%, 1.52%, 0.65%, 1.96% and 1.96% in liver, spleen, lungs, heart, kidney, intestine and cloaca, respectively (Fig. 1). The disease producing bacteria were more abundant in cloaca and least in the kidney.

Colony characteristics of different bacteria

Isolated *E. coli* produced white round colonies on nutrient agar, greenish colonies with metallic sheen on Eosin methylene blue (EMB) agar (Fig. 2A), bright pink colonies on MacConkey agar, pink colonies on salmonella shigella (SS) agar and yellowish green colonies surrounded by an intense yellow green zone on Brilliant green agar. Isolated *Salmonella spp.* formed round, white dew drop-like colonies on nutrient agar. The organisms also formed round, raised, transparent single colonies with black centers on Salmonella Shigella (SS) agar. On xylose lysine deoxycholate (XLD) agar, *Salmonella* produced black colonies. Red to pink white colonies surrounded by brilliant red zones formed on Brilliant Green (BG) agar (Fig. 2B). Pink colonies were produced by *Salmonella* on Eosin methylene blue (EMB) agar. After overnight incubation *Pasteurella multocida* produced diffused turbidity in nutrient broth; round, flat colonies of sticky, mucoid consistency on nutrient agar (NA) (Fig. 2C), slight growth with colorless colonies on Eosin methylene blue (EMB) agar media, round, grayish and mucoid colony with musty odor on Blood agar media and no growth on MacConkey agar.

Staining characteristics

E. coli were Gram negative, pink color, short rod to coccoid shape with Gram's Stain (Fig. 2D). Isolated *Salmonella* were Gram negative, rod shaped, pink color bacilli with Gram's stain (Fig.2E). Microscopic examination of *Pasteurella multocida* with Gram's stained smears from NA and BA revealed Gram- negative small rod or coccobacilli and arranged in single, paired or occasionally in short chain (Fig. 2F).

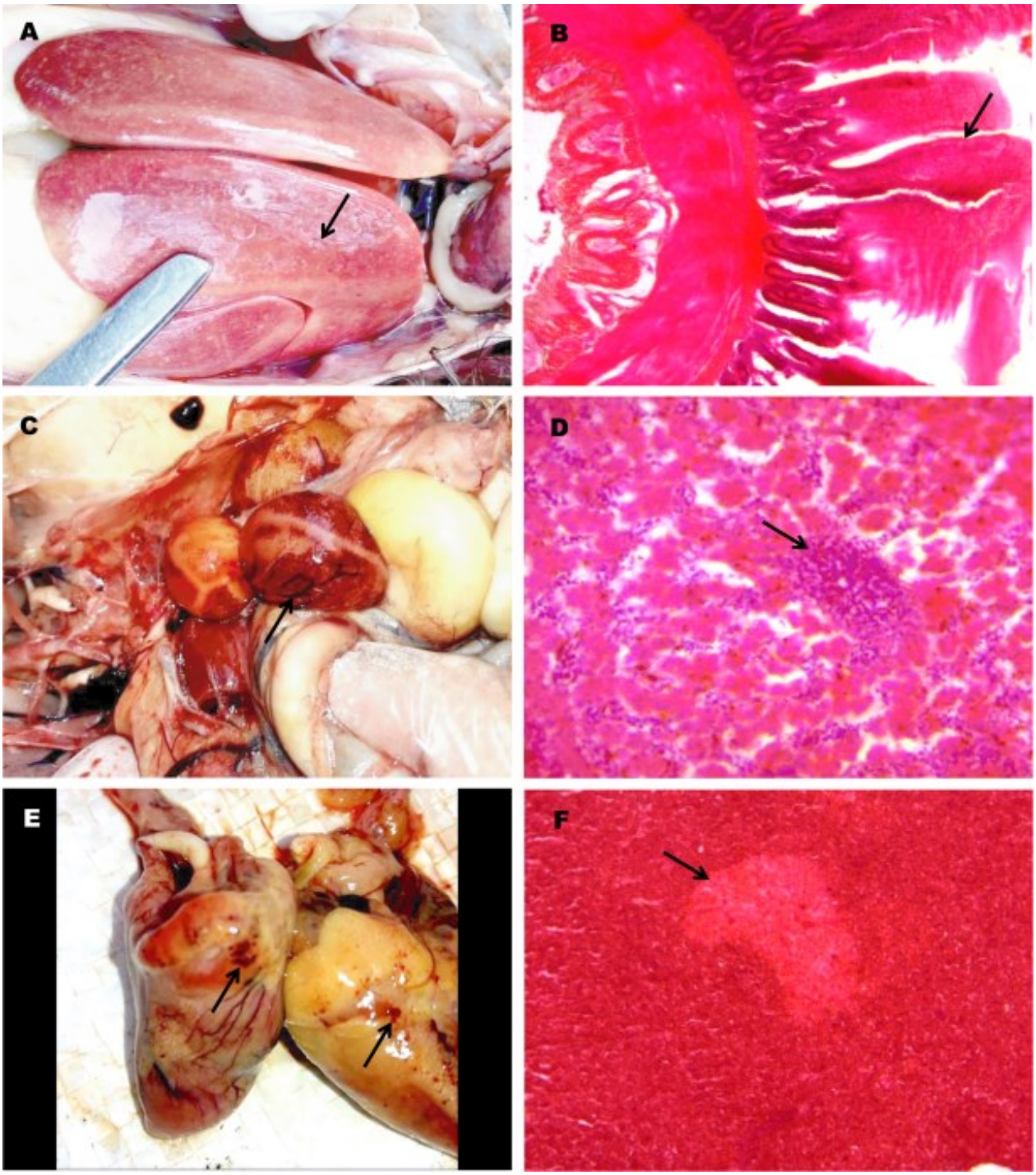

Fig. 2. Gross and histopathologic lesions in colibacillosis, salmonellosis and pasteurellosis. Grossly, the liver was hemorrhagic and displayed numerous necrotic foci (arrow) on the surface in colibacillosis (A). The section of intestine showed severe destruction of intestinal villi (arrow) and infiltration of heterophils and lymphocytes in the mucosa, submucosa and lamina propia (B). In Salmonellosis, the ova were irregular, deformed and hemorrhagic (C, arrow). The section of liver showed multifocal hepatocytes necrosis and huge infiltration of heterophils (D; arrow). Petechial hemorrhages in the fat of coronary groove of heart in pasteurellosis (E) and section of *Pasteurella multocida* infected liver showed focal area of hepatocyte necrosis and heterophilic infiltration (F).

Motility test

E. coli displayed motility when examined under microscope with hanging drop slide preparation. Isolated Paratyphoid Salmonellae, on the other hand, were motile while *Salmonella* Pullorum and *Salmonella* Gallinarum were non-motile when examined under light microscope with hanging drop slide preparation. The isolates of *Pasteurella multocida* were also non-motile.

Biochemical tests

E. coli fermented dextrose, lactose, maltose, sucrose, dulcitol, arabinose and mannitol and produced both acid and gas but did not ferment inositol (Table 2). *Salmonella spp*., on the other hand, fermented dextrose, mannitol and dulcitol with the production of acid and gas but did not ferment lactose, maltose, sucrose, arabinose and inositol. It was worthy to note that the *Pasteurella multocida* fermented dextrose and sucrose with the production of only acid and did not ferment lactose, maltose, dulcitol, arabinose, innositol and mannitol. The *Salmonella gallinarum*, *Salmonella pullorum* and *Paratyphoid salmonellae* were differentiated by biochemical and motility tests. The *Salmonella gallinarum* fermented dextrose, mannitor and dulcitol and was non-motile (Table 4). The *Salmonella pullorum* fermented dextrose and mannitol and were also non-motile. Similar to *Salmonella gallinarum*, the *Paratyphoid salmonella* fermented dextrose, mannitol and dulcitol. However, *Paratyphoid salmonella* was motile whereas *Salmonella gallinarum* was non-motile.

Gross and microscopic lesions of enteric bacterial disease

In colibacillosis, hemorrhages and necrotic foci in the liver (Fig. 2A), pericarditis in heart, fluid accumulation and hemorrhages in intestine and congestion in lungs and kidney were seen. Microscopically, destruction of intestinal villi, necrosis and infiltration of heterophils and lymphocytes in the mucosa, submucosa and lamina propria were evident (Fig. 2B). Liver showed focal coagulation necrosis of hepatocytes and heterophil infiltration. Heterophil infiltration along with congestion in kidney and spleen, pericarditis in heart, congestion and serofibrinous exudation in lungs were found. Postmortem lesions in Salmonellosis included pericarditis with hemorrhages, swollen and dark coppery bronze liver often with necrosis, and deformed and hemorrhagic ova (Fig. 2C). Heterophilic infiltrations and congestions in intestine, kidney, lungs, heart muscle, spleen and liver were also seen (Fig. 2D). Swelling of liver, whitish necrotic foci, relatively smaller in size compared to salmonellosis, petechial hemorrhages at the base of heart and abdominal fat, and hemorrhage and focal necrosis in the spleen of chickens were often found in pasteurellosis (Fig. 2E). Section of *Pasteurella multocida* infected liver showed focal area of hepatocyte necrosis and heterophilic infiltration (Fig. 2F), lungs showed serous exudates in the air vesicle and respiratory bronchiole, congestion and heterophilic infiltration in the intestine.

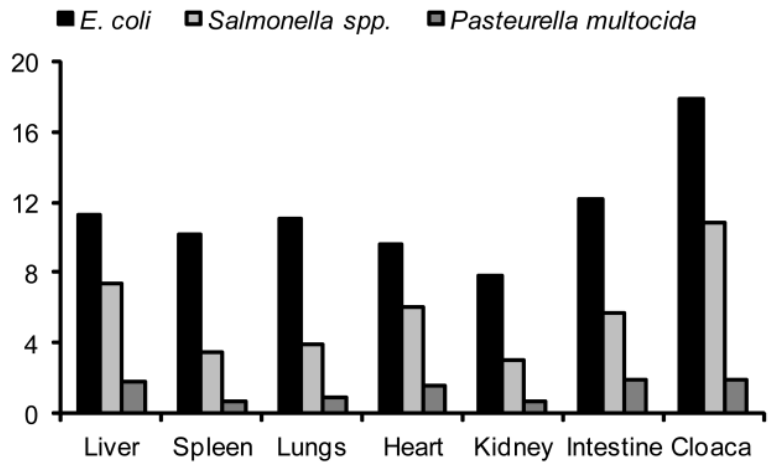

Fig. 3. Prevalence of Escherichia, Salmonella and Pasteurella organisms in different organs of chickens. *E. coli* was the highest in prevalence followed by *Salmonella spp*. and *Pasteurella multocida*. Among the liver, spleen, heart, lungs, kidney, intestine and cloaca, the organisms were most prevalent in cloaca.

Table 1. Overall prevalence of enteric bacterial diseases (N=460)

Sl. No.	Name of enteric bacterial diseases	Total swabs examined	Total positive cases	Cultural prevalence (%)
1	Colibacillosis	460	368	80.00
2	Fowl typhoid	460	32	6.96
3	Pullorum disease	460	64	13.91
4	Fowl paratyphoid	460	90	19.57
5	Fowl cholera	460	40	8.70

Table 2. Sugar fermentation tests of different isolated organisms

SL.NO.	Sugar	*E. coli*	*Salmonella*	*Pasteurella multocida*
1	Dextrose	AG	AG	A
2	Lactose	AG	–	–
3	Maltose	AG	–	–
4	Sucrose	AG	–	A
5	Dulcitol	AG	+/-	–
AG	Arabinose		–	–
–	Inositol		–	–
8	Mannitol	AG	AG	–

A=Acid, G=Gas, AG=Acid Gas

Table 3. Results of different sugar fermentation and motility tests of isolated *Salmonella*

Sugar fermentation test						
Dextrose	**Sucrose**	**Lactose**	**Mannitol**	**Dulcitol**	**Motility**	**Name of the bacteria**
AG	–	–	AG	AG	–	*Salmonella* gallinarum
AG	–	–	AG	–	–	*Salmonella* pullorum
AG	–	–	AG	AG	+	Paratyphoid salmonellae

AG=Acid Gas

Discussion

Overall prevalence of enteric bacterial diseases

Identification and isolation of enteric bacteria were done by collecting swab samples from different organs in chickens. From the total 100 samples, prevalence rate of colibacillosis, fowl typhoid, pullorum disease, fowl paratyphoid, fowl cholera found were 80.00%, 6.96%, 13.91%, 19.57 and 8.70%, respectively. The prevalence rate of colibacillosis detected in this study is similar to the findings of Haider et al. (2004) and slightly higher than the rate reported by Hasan et al. (2016) and Ahmed et al. (2004). The variation in prevalence rate might be due to the differences in age and breeds of the chickens as well as their management, vaccine and nutrition. The prevalence of fowl typhoid was 75% and pullorum disease was 50% in this study which are slightly higher than the prevalence reported by other authors (Ahmed et al. 2008; Haider et al. 2008; Hassan et al. 2010). The prevalence of fowl paratyphoid was 60% and fowl cholera was 40% which are also slightly higher than the prevalence reported by Cheesbrough (2006), Hassan et al. (2010), Hossain et al. (2013), Panna et al. (2015).

Colony characteristics

The *E. coli* showed pink colonies on MacConkey agar and greenish colonies with metallic sheen on EMB agar, yellow to greenish-yellow colonies surrounded by an intense yellow-green zone on BGA which are similar with the finding of Haider et al. (2004), Hassan et al. (2010) and Tanu et al. (2011). The colony character of *Salmonella* spp. on different media were characterized by turbidity in nutrient broth; circular, smooth, opaque and translucent colonies on NA, colorless colonies with black centers on SS agar, pale pink color colonies against a pinkish background on BGA; pale, smooth, transparent and raised colonies on MacConkey agar; large, colorless colonies on EMB agar media, the same colony characteristics are also reported by Cheesbrough, 2006 and Hassan et al. 2010. The colony character of turbidity in nutrient broth; round, flat colonies of sticky, mucoid consistency on NA; round, grayish and mucoid colony with musty odour on BA media, slight growth with

colorless colonies on EMB agar media and no growth found on MacConkey agar by *Pasteurella multocida* are similar to colony characters of the bacteria documented by Cheesbrough, (2006) and Hassan et al. (2010).

Staining characteristics

The *E. coli* showed Gram negative, pink color short rod to coccoid shape with Gram's stain (Cheesbrough, 2006; Haider et al. 2004; Kabir 2010). *Salmonella*, on the other hand, showed the morphology of small rod shape, Gram negative, single or paired in arrangement similar to the features reported by other authors (Hossain et al. 2004). The staining character of *Pasteurella* was small rod or coccobacilli and arranged in single, paired or occasionally in short chain which correspond with the staining characters of the organisms documented by other authors (Hassan et al. 2010).

Motility test

Motility is considered an important feature to differentiate enteric bacterial species. Isolated *E. coli* and *Paratyphoid salmonella* displayed motility on hanging drop preparation while *Salmonella* Pullorum and *Salmonella* Gallinarum were non-motile. The isolates of *Pasteurella multocida* were also non-motile. The motility of the enteric bacteria in this study are supported by Haider el al. 2004 and Tanu et al. 2011.

Biochemical tests

Biochemical tests are important tools for the identification and characterization of bacteria species based on differences in the biochemical activities of the bacteria. Bacteria ferments sugars with the production of both acid and gas or either acid or gas. In sugar fermentation tests, *E. coli* fermented 7 out of the 8 basic sugars used in this study except inositol in which neither acid and nor gas production was observed (Cheesbrough, 2006; Haider el al. 2004). The *Salmonella* spp. fermented dextrose, sucrose and mannitol but did not ferment maltose and lactose. These fermentations of sugars producing acid without gas formation are similar to biochemical activities on sugars by Salmonella as observed by Christensen et al. 2014). The fermentation of dextrose and sucrose by *Pasteurella multocida* with the production of only acid is supported by the findings of Freeman, (1995) and Haider el al. (2004).

Gross and Histopathological lesions

Gross and microscopic lesions produced during the course of development of disease in different organs are important hall marks of disease diagnosis. Each of the enteric bacteria produces unique lesions in different organs. *E. coli* produces hemorrhages and necrotic foci in the liver, pericarditis in heart, fluid accumulation and hemorrhages in intestine, congestion in lungs and kidney of chickens (Lolan et al. 2020; Kabir, 2010; Tanu et al. 2011). Necrosis and infiltration of heterophils and lymphocytes in the intestinal mucosa, focal coagulation necrosis of hepatocytes with heterophilic infiltration in liver, infiltration of heterophils in kidney, serofibrinous exudation in lungs, pericarditis and heterophilic infiltration in heart were commonly encountered microscopic features in Collibacillosis (Khan et al. 1998; Lolan et al. 2020; Tanu et al. 2011). Gross lesions in salmonellosis included hemorrhage and congestion in intestine, liver, spleen and ovaries and focal necrosis in liver and spleen. Ovaries showed ova of different shape. There was button like ulcerations on cecal tonsils. Microscopic lesions found in intestine in all affected samples included hemorrhage and heterophilic infiltrations (Gast and Robert, 2020; Haider el al. 2008; Robinson et al. 2000). Macroscopically, smaller necrotic foci submerging from the liver surface and punctuate hemorrhage on the fat of coronary groove help differentiating fowl cholera from salmonellosis. Heterophil infiltration and congestion were predominant microscopic features found in fowl cholera as evident in collibacillosis and salmonellosis (Blackall and Hofacre, 2020; Gast and Robert, 2020; Haider et al. 2008).

Conclusions

Poultry industry is a rising and potential sector of Bangladesh. Enteric bacterial diseases are the major threat for the gut health and production. Prevention of the diseases is essential for sustainable poultry production and ensuring safe poultry meat and eggs. Five different bacterial diseases namely colibacillosis (80%), fowl typhoid (6.96%), pullorum disease (13.91%), fowl paratyphoid (19.57%) and fowl cholera (8.70%) were identified in the present investigation. *E. coli, Salmonella* Pullorum, *Salmonella* Gallinarum, non-motile salmonellae (Paratyphoid group), and *Pasteurella multocida* were isolated and identified from different chicken samples by culture, staining

and biochemical tests along with the pathology they produced. Highest cultural prevalence was found from cloacal swabs collected from dead and live chickens. Predominant lesions encountered were hemorrhages in intestine and focal necrosis in liver. The findings of the current study will help the researcher for further investigations and suggesting poultry farmers to take necessary control measures for combating these diseases. The identification and characterization of the pathogen will play a vital role regarding this aspect.

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Authors' Contribution: Conceptualization, SS; Formal analysis, SS; Methodology, MAZ and ARU; Investigation, MGH, Writing-original draft preparation, MTI and. MGH; Writing-review and editing, ANMAR; Supervision, MGH. All authors have read and agreed to the published version of the manuscript.

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