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LIST OF ABBREVIATION

Abbreviation and Symbol	Elaboration
%	Percent
et al.	And his associate
CVASU	Chittagong Veterinary and Animal Sciences University
E.coli	Escherichia coli
MC	MacConkey agar
EMB	Eosin Methylene Blue agar

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The Author

ABSTRACT

Avian colibacillosis is considered to be a major bacterial disease in the poultry industry worldwide. The prevalence of colibacillosis in broiler chickens was studied from January to February 2017 that was presented at Department of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University. 50 swabs from liver, lungs, cloaca of 30 dead birds were collected in sterile nutrient broth as well as 30 liver samples for histopathology. Bacteria were isolated through cultural properties in MacConkey and Eosin Methylene Blue agar. Tissue samples were studied under light microscope after routine Hematoxylline and Eosin staining. Cloudy thickened air sacs, pericarditis, congestion in the liver, lung and spleen were observed in dead birds. On histopathological examination heterophils, lymphocytes, macrophages and fatty liver change found in liver. Based on cultural properties 31 (62%) isolates were found positive. All isolates of Boalkhali were found positive in bacteriological culture technique and no isolates from Betbunia was found positive.

Keywords: Colibacillosis, commercial broiler, histopathology, *E. coli*

CHAPTER-I: INTRODUCTION

In the world more than a billion people currently live in extreme poverty, these people are powerless, isolated, vulnerable and malnourished (Mack et al., 2004). For this section of people poultry production can be one of the effective tools of combat food security and poverty many parts of the world (Hensen, 2004, Mack et al., 2004). The development and growth of poutry industry over the last 50 years has been one of the remarkable achievements in the history of agriculture. More than 130 hatcheries produce 3.4 million day-old chicks per week and 30,000 commercial broiler and layer farms supply 0.26 million tons of poultry meat and 5210 million eggs per year (Rahman, 2003).With a view to meet the protein gap with shortest possible time, a number of poultry farms have been established on commercial basis in and around the cities and towns and are operated under intensive management. Poultry not only provides protein but also provides full time employment to about 20% of the population and about 50% peoples are associated with this sub sector as part timers. Moreover, poultry farming is a quick returnable enterprise but one of the major constrains in the development of poultry industries is the outbreak of the diseases. Among the different poultry diseases avian Colibacillosis has been found to be a major infectious disease of all ages of birds which causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting in death and in its subacute form by pericarditis, air sacculitis and peri-hepatitis (Calnek *et al*., 1997). Yolk sac infection (YSI) is a major cause of mortality of broilers during the first week of post-hatching (Bains, 1979). Broilers suffering from colibacillosis are depressed; show respiratory distress and growth retardation. Mortality usually remains below 5%, but morbidity often reaches more than 50% (Wray *et al*., 1996; Vandekerckhove *et al*., 2004). Day Old Chicks may become infected via the yolk sac, but in older chicks the infection is considered to be mainly airborne. Young broiler

chickens up to three weeks of age are highly susceptible to the disease. But colibacillosis commonly occur in three to twelve weeks of age and characterized by intestinal disease. However, various risk factors may increase the susceptibility of broilers to colibacillosis. In addition, the *E. coli* concentration in the air of the broiler house is an important factor. It causes serious loss specially if there is a bad management or stress in broilers such as complicating infections like chronic respiratory disease (CRD) or Mycoplasma (Talha et al., 2003; Rahman, 2003b; Hossain et al., 2004). Heavy loss occurs in broilers due to morbidity, mortality, reduced production and poor chick quality (Islam et al., 2003; Rahman, 2003b; Rahman et al., 2004; Hossain et al., 2004). Investigation on colibacillosis is still scanty in Bangladesh. The disease pattern is variable. This study was undertaken to know the status of colibacillosis in commercial poultry farms.

Chittagong is also a commercial poultry rearing zone of Bangladesh. A significant number of people depend on poultry business. This is important to know the prevalence of colibacillosis in commercial poultry. This study was conducted with the aim to

1. Know the prevalence of E. coli in commercial poultry in Chittagong
2. Know the pathological changes in different organs due to colibacillosis

CHAPTER-II: MATERIALS AND METHODS

Study area and duration

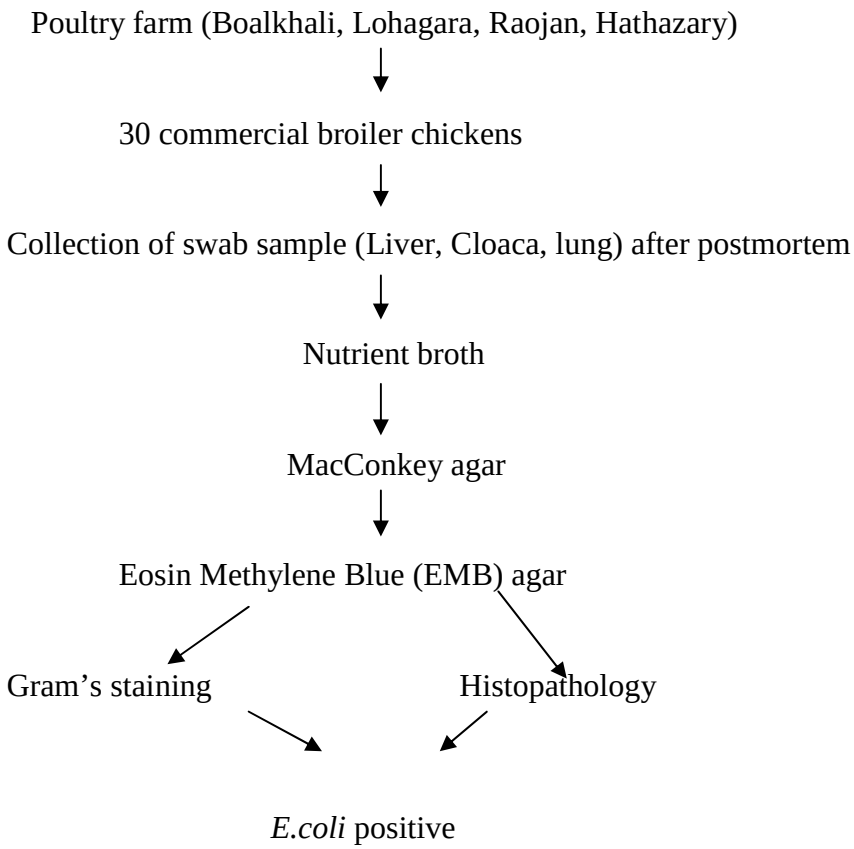
The study was conducted on commercial broiler chicken of various farm of Chittagong, Bangladesh during the period of January to February 2017.

Study population

A total number of 30 dead chickens were brought from various farms of Chittagong to Department of Pathology and Parasitology, CVASU. Sterile swabs were used to take swabs from liver, lungs and cloaca from all 30 chickens.

Study Design

This study was done by using the following study design:



Collection of clinical history about clinical findings

The clinical history was collected from farm owner by cross question method. These questions were asked easy and the scientific and technical terms were avoided.

Postmortem examination and sample collection

Postmortem examinations of the dead chicken were done by maintaining standard procedures. Sample was collected from liver and yolk sac and by using sterile cotton swab and inoculated into test tube containing Nutrient broth and liver sample kept in Bouin's solution for histopathology.

Media used for bacterial isolation

MacConkey (MC) agar and Eosin Methylene Blue (EMB) agar were used as solid media. Nutrient broth was used as enrichment of all bacteria. Gram's staining reagents was used for identification of *E. coli.*

Table-1: Methods of culture was done

Sl	**Agar**	**Incubation time and temperature**	**Observations**
1.	MacConkey agar	Incubated at 37^0 temperature for 24 hours.	Dark pink colored raised colony
2.	Eosin Methylene Blue (EMB) agar	Incubated at 37^0 temperature for 24 hours	Characteristic metallic sheen

Data analysis

Data was stored in MS excel (Microsoft Word 2007) and descriptive analysis was done in this study.

CHAPTER- III: RESULTS

Results of cultural and biochemical examination

Among 50 swab samples collected from liver, lung and cloaca of 30 broiler chicken. 70% samples are positive in MacConkey and 62% samples are positive in EMB agar which is also positive in Gram's staining. In the microscopic examination of Gram's staining, all the positive samples are found as Gram-negative, pink colored, rod shaped bacteria which are arranged in single or in pairs.

Table-2: The examination result at a glance done for isolation of *E. coli*

SL	Name of the media /test	Total no. of sample	No. of positive sample	Percentage (%)
1.	MacConkey agar	50	35	70
2.	Eosin Methylene Blue (EMB) agar	50	31	62
3.	Gram 's staining	50	31	62

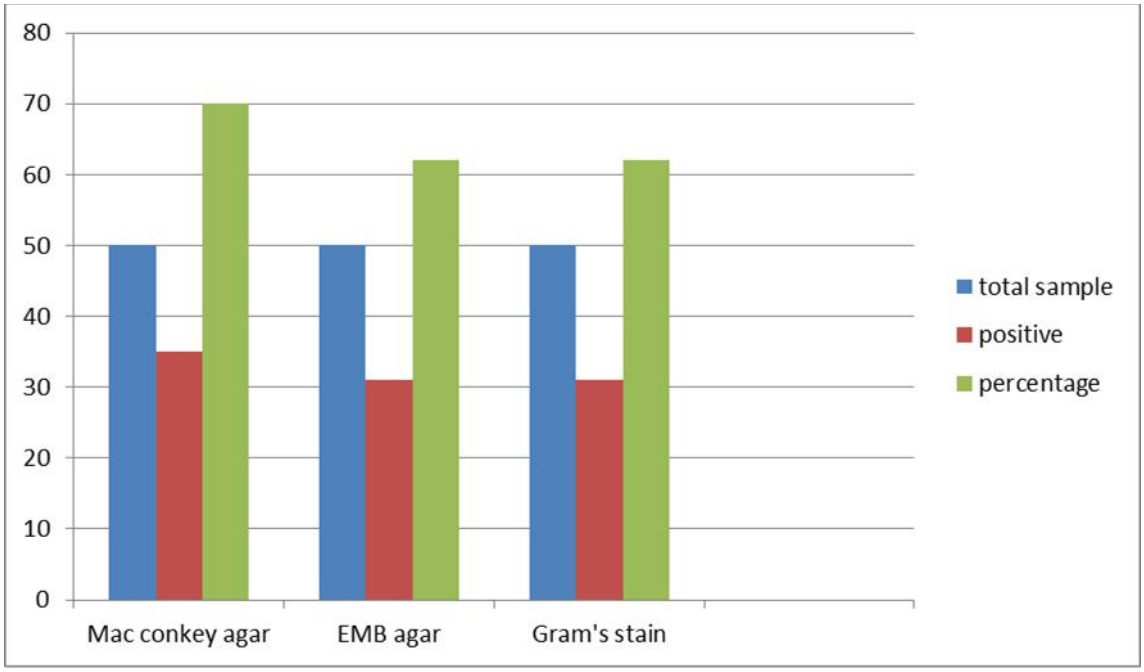


Figure-1: Prevalence of *E. coli* in different media

Table-3: Prevalance of E.coli in different sampling sites

Area	No of sample	EMB Agar (+ve)	Prevalance (%)
Raojan	10	10	100
Darmapur	6	3	50
Vatiari	9	4	44.44
Anowara	5	2	40
Hatazary	7	4	57.14
Betbunia	2	0	0
Lohagara	3	1	33.33
Boalkhali	5	5	100
Abul Khayer steel mill	3	2	66.67

Table-3 shows the prevalence of *E. coli* in different sampling sites. Out 50 samples the highest prevalence of was found in Boalkhali (100%), Raojan (100%) and lowest prevalence in Betbunia (0%)

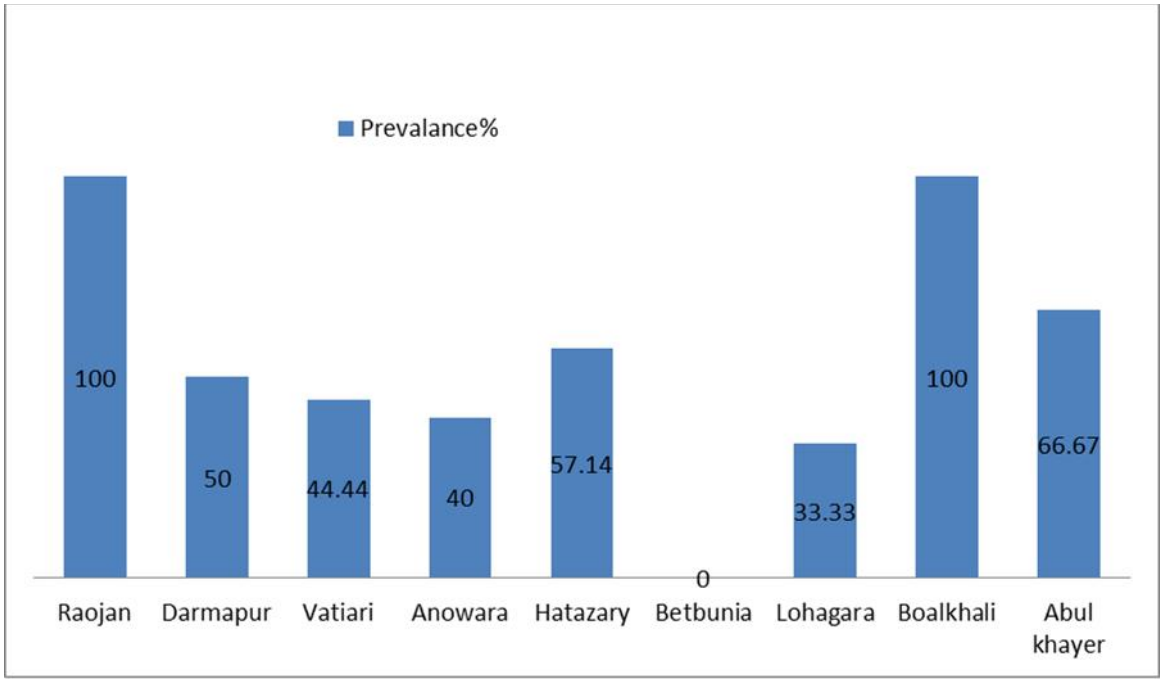


Figure-2: Prevalance of *E. coli* in different sampling sites

Colony characters:

Pink colonies on MacConkey agar and greenish colonies with metallic sheen on EMB Pink colonies on MacConkey agar and greenish colonies with metallic sheen on EMB agar (Figure-3) after overnight incubation were confirmed as *E. coli* these colony characteristics correspond with the finding others.

Staining characters:

Microscopy revealed Gram-negative, pink ,short rod-shaped organism arranged singly or pairs (Figure-4).

Pathological study

Postmortem findings revealed cloudy and thickened air sacs (air sacculitis), congested liver and consolidate lung in some chickens. The pericardium was thickened and the spleen enlarged with congestion. All these lesions indicated the septicaemic form of colibacillosis. E. coli can cause several disease conditions but in the present study not all conditions induced by E.coli were recorded.

Microscopic lesions:

The liver showed coagulation type of focal necrosis, infiltration of heterophils, lymphocytes and macrophages mainly in portal area and fatty change (Figure-5).

Figure- 3: E. coli colonies showing greenish colour with metallic sheen on EMB Agar

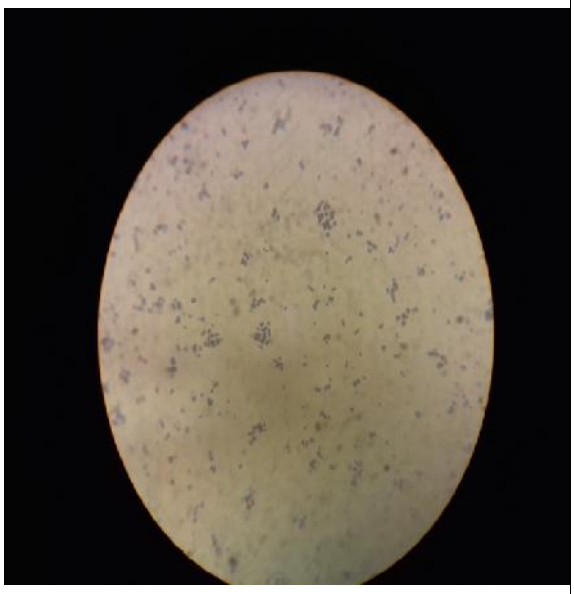


Figure- 4: E. coli in Gram's stain showing Gram negative, pink, short rod-shaped organisms

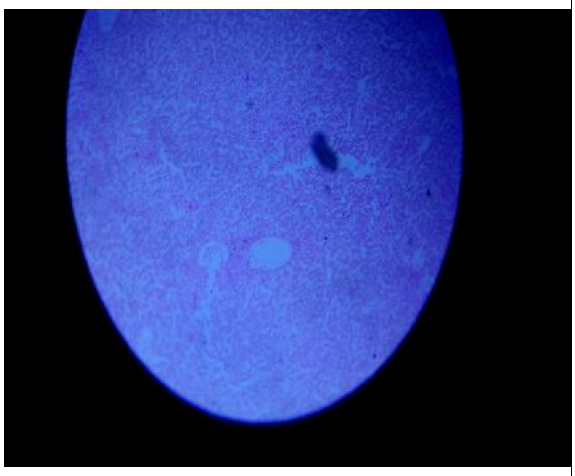
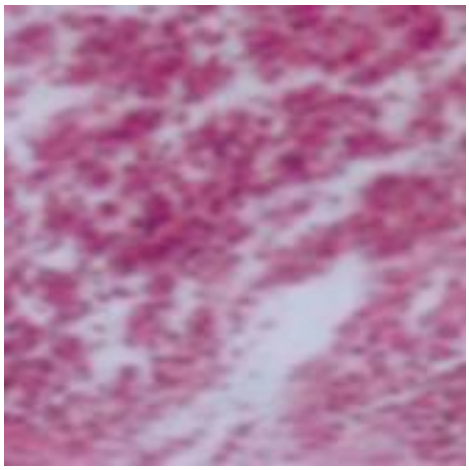
	

Figure- 5: Section of liver with *E. coli* infection showing fatty change	Figure-6: Section of liver with *E.coli* infection showing Mononuclear cell infiltration

CHAPTER-IV: DISCUSSION

Avian Colibacillosis caused by *E. coli* is a major health problem in poultry industry. The study was conducted with the aim of isolation and identification of *Escherichia coli* present in liver and yolk sac of dead chicken. In this study, chicken clinically infected with colibacillosis manifested characteristics clinical sign such as watery diarrhea, anorexia, weakness and loss of body weight. A similar type of clinical sign of colibacillosis was also recorded (Kim et al., 1996). Here this study observed that 62 *E. coli* was present in broiler Chicken. The results was partially agreed with the findings of Maiorka *et al.* (2006) where he reported that at hatching, chick was still microorganism-free but after hatching a significant number of microorganisms invaded and colonized in different organ. In this study, the average finding of *E. coli* was 62% from commercial broiler which is similar to the earlier reports of Suha *et al.* (2008) who reported 43.50%. Rahman *et al.* (2004) reported 67.73% and Ahmed *et al.*, (2009) found 52.26% colibacillosis in commercial broiler which is higher than this study. The result is also similar with Bhattacharjee *et al.*, (1996) reported 40.82% of *E. coli* in chicken from Bangladesh. Morphology, staining and cultural characteristics of the bacteria in different cultural media as recorded in the study were almost similar as reported by Choudhury *et al.* (1985). They reported that staining and morphology of isolated *E. coli* exhibited Gram negative, small rod, arranged in single or pairs, non-spore former.

CHAPTER-V: CONCLUSION

Avian colibacillosis caused by *E. coli* is a major health problem in a poultry industry. Although unrespective sample size was investigated in this study but based on the findings it can be assumed that a higher population of chickens in our country is suffering from colibacillosis. The poor sanitation and handling of sewage and poor feeding, poor housing overall poor hygienic management and vaccination failure could be a source contamination. Colibacillosis can be treated if appropriate antibiotics are chosen to treat the disease.

CHAPTER-VI: REFERENCES

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APPENDIX

Formula of various bacteriological media

A. MacConkey Agar

Peptone……20.0g

Lactose……10.0g

Bile salts……....5.0g

Sodium chloride……5.0g

Neutral red……..0.075g

Agar…….....12.0g

Distilled water…….....1000ml

B. Eosin Methyl Blue (EMB) Agar

Peptone…….....10.0g

Sucrose…….....5.0g

Lactose……..……5.0g

Dipotasssium phosphate……..…….....2.0g

Agar……13.5g

Eosin…….0.4g

Methylene blue…….0.065g

Distilled water……1000ml

LIMITATIONS

→ Sample size of this investigation was not representative to the population due to short period of the study.

→ Timing was not sufficient for such type of study.

→ Follow up was not completed for each and every case.

→ Lack of my subject related journals to help the work.

Diagnostic information's

Case no: Date……..

Basic information’s:

1. Name of the farm:

2. Name of the owner:

3. Address of the farm:

4. Type of farm: (layer/Broiler).

5. Housing system: (intensive/semi intensive)

6. Floor type:(Litter/Concentrate/Slat/Mud/Others)

7. a) Vaccination: 1=Properly, 2=Improperly b) Vaccination: (Good/Moderate/Not performed).

8. a) Biosecurity: Visitor access: 1=Not restricted, 2=Restricted. Foot bath: 1=Present, 2= Absent Wild birds/animal access: 1=Yes, 2=No. Chick care management: 1= Properly 2=Not properly.

9. Age of bird:……

10. Flock size:……

11. Morbidity……

12. Mortality……..

Clinical findings:

A. General systemic status

1……2……..3……

B. Respiratory system:

1……2……3……

C. Digestive system:

1……..2…….3……..

D. External organ:

1……2……..3…….

Information about clinical findings:

A. General systemic status:

Clinical findings	Nature/additional information
Dullness/Depression	Colisepticemic form
Dehydration	Due to enteritis
Ruffled feathers	Colisepticemic form
Weakness	Coligranuloma form

B. Respiratory system:

Clinical findings	Nature/additional information
Gasping/open beck breathing	In case of pericarditis due to colibacillosis and gasping followed by death.
Respiratory distress	In colisepticemia form due to bronchopneumonia

C. Digestive system:

Clinical findings	Nature/additional information
Diarrhoea	Due to enteritis and yellow colour faeces in young
Anorexia	In later stage of swollen head syndrom
Reduce feeding	Colisepticemic form

D. External organ:

Clinical findings	Nature/additional form
Pasty vent	In Colisepticemia form, due to steaky dropping
Swollen and inflamed naval region	In case of yolk sac infection, due to oedema in naval region.
Swollen joint	In case of infective arthritis due to septicaemia and synovitis. Here mainly hock joint is mainly affected.
Omphalitis	In case of yolk sac infection.

BIOGRAPHY

Setara Akter is an intern student of Doctor of Veterinary Medicine (DVM) degree at Chittagong Veterinary and Animal Sciences University (CVASU). She is daughter of Md. Abul Kalam Ajad and Hazera Begum. She passed the SSC examination in 2009 and HSC examination in 2011. She successfully completed her DVM 4th year theory courses and clinical training in Madras Veterinary College and Veterinary College and Research Institute (VCRI), Namakkal, India. She has great interest in veterinary pathology for higher study.