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PATHOLOGICAL INVESTIGATION OF LUNG DISEASES OF CATTLE IN GAZIPUR DISTRICT

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ABSTRACT

The study was aimed to investigate the pathology of lung diseases of cattle in Gazipur district during November 2017 to August 2018. A total of 50 cattle lung samples were collected from slaughterhouse of Gazipur City Corporation. Histopathological samples were collected in 10% buffered formalin and bacterial samples were collected in nutrient broth. All the samples were transported to the laboratory at the Department of Pathobiology, Bangabandhu sheikh Mujibur Rahman Agriculture University, Gazipur. Different agars and stains were used for the isolation and identification of the bacteria. The overall prevalence of bacteria in abnormal and apparently healthy cattle lungs were 80.0%. The prevalence were *Pasteurella multocida* 66.7%, *Escherichia coli* 93.3%, *Staphylococcus aureus* 80.0% and *Klebseilla pneumonia* 33.3%, whereas the average of the cultural prevalence was 67.3%. On the blood agar, the presumptive *P. multocida* produced small colonies. The isolated of *E. coli* was shown greenish metallic sheen colonies on EMB agar. Colonies of *Staphylococcus aureus* were surrounded by zones of clear beta-hemolysis with golden appearance. On EMB agar *Klebseilla pneumonia* was produced pink color colonies without metallic sheen.

Keywords: Pathology, lung, disease, cattle, Gazipur

Introduction

Livestock agribusiness plays a significant role in our economy through contributing to poverty eradication by creating rural employment and to gear up the achievement of higher economic growth. Livestock is an integral component of agricultural economy of Bangladesh. Performing various functions as provisions of food, nutrition, income generation, savings, draft power, manure, fuel, transport and cultural function and earning foreign currency by exporting meat, hides and skin and value-added waste products, etc. About 36% of the total animal protein comes from the livestock and the rest 64% comes from poultry, fish and pluses (Hamid *et al.* 2017).

Bangladesh has huge number of livestock and poultry population with very high density but low productivity. The country has about 24.4 million cattle, 1.5 million buffaloes, 26.4 million goats, 3.6 million sheep, 296.6 million chicken and 59.7 million ducks (BBS 2020) Beef cattle are the important and potential sub-sector to economic development, where this sub-sector has a strategic value to fulfill the human need through progressively increase per capita income. Beef cattle are one of the potential commodities in national development. In Bangladesh beef cattle play an important role as sources of animal protein, income and fertilizer, employment opportunities, utilization of agricultural waste and as savings for farmers. The cattle population is spread throughout the country. In certain areas, beef cattle are profitable agribusiness for rural farmers which facilitated to develop meat processing companies and its bi-products such as hides, bone, horns and hooves are the raw materials for the development of other industries (Hossain 2016).

According to DLS 2016, Development of meat processing companies is largely determined by the availability and distribution of livestock. However, it is clear that many elements of the enabling environment for beef cattle agribusiness like poultry development is lacking in the Gazipur district. Beef cattle farming have a great scope, because this district is nearby the capital city Dhaka. Cattle farmer faces problem in lung diseases like other diseases in cattle. There is no research on lung diseases in cattle in

Gazipur district. For this reason, the study was undertaken to study the prevalence of lung diseases of cattle, and isolate and identify the bacteria from the collected lungs of slaughtered cattle in Gazipur district.

Materials and Methods

Sample collection and transportation: Fifty lungs samples from necropsy cases were collected from slaughter house of Gazipur district, Bangladesh from November' 17 to September' 18. Among 50 lung samples, 30 were from abnormal lung and 20 were from apparently healthy lung. The samples for bacteriological study were collected in nutrient broth. Theses samples were brought to the department of Pathobiology, BSMRAU, Gazipur in ice box with ice. The tissue samples were collected for histopathological examination in plastic container containing 10 percent buffered formalin.

Lung swabs sample collection: Lung swabs were collected by using cotton tipped swabs from lung. After collection the swabs were inserted into sterilized screw capped test tubes containing Nutrient broth.

Isolation identification of the organisms: Test tubes containing samples were incubated for 24 hours at 37^0C from the nutrient broth, subculture was also made on Nutrient agar, Eosin methylene blue (EMB) Mannitol salt agar, Blood agar, MacConkey agar and incubated at 37°C for overnight. The identification of the organisms was performed by the test as described by Haider *et al.* 2008;

On the basis of colony and staining characters, and biochemical test of organisms were isolated and identified.

Motile test: Young broth culture of the organisms, incubated at or below the optimum temperature (e/ g. 37°C and 22°C), was examined in 'hanging drop' preparation, using a high-power oil immersion objective with reduced illumination.

Staining method: The bacteria were staining using modified Gram' staining (Haider *et al.* 2008).

Carbohydrate fermentation test: This carbohydrate fermentation test was performed to determine to fermentation of sugar (glucose, maltose, lactose, sucrose and mannitol) by bacterial cells.

Maintenance of stock culture: During the experiment it was necessary to preserve the isolated selective organisms for longer periods. For this purpose, the organisms were inoculated at 37°C for 24 hours. After the growth of the organisms the tubes were sealed with paraffin wax and kept in the refrigerator at 4°C for further studies.

Gross pathology: The postmortem examination in all the cases was performed for the pneumonic cattle lung at necropsy, gross tissue changes were observed and recorded carefully and representative tissue sample containing lesions were fixed in 10% neutral buffered formalin for histopathological studies (Booker et. al. 2008).

Histopathology: The formalin fixed tissues were trimmed, processed, sectioned and stained as per standers procedure. Specific lesions containing sample from each group were used in histopathological study (Luna 1968).

Photomicrograph: Photomicrography was taken at the Department of Gynecology, Obstetrics and Reproductive Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Data interpretation: At the end of the study period all finding were compiled, examined and presented for comprehensive interpretation.

Results and Discussion

Bacteriological and histopathological cattle lung sample were collected from slaughterhouse the abnormal apparent were 30 out of 50. The cattle were in different age group and sex. These necropsy cases of sample were collected for isolation and identification of bacteria and histopathology study of identified cases. This research was performed in Microbiological section of central laboratory of faculty of Veterinary Medicine and Animal Science and pathological laboratory, Department of Pathobiology, Bandabandhu Sheikh Mujibur Rahman Agriculture University, Gazipur during the study period of November' 17 to September 18.

Prevalence of isolated and identified of all bacterial: The prevalence of bacteria was 80% in apparently healthy and abnormal cattle lung of total 50 samples (Table 1). The prevalence was *P. multocida* 66.7% *E. coli* 93.3% *Staphlococcus aureus* 80.0% and *Klebseilla pneumonia* 33.3% whereas the average culture prevalence rate was 67.3% (Table 2).

Morphology: The organisms were found gram-negative, pink color, bipolar bacillary of *Pasteurella multocida* with Leishman's stain (Figure 1). Gram negative, pink color, rod to coccoid shape *E. coli* was found with gram's stain.

Table 1. Prevalence of bacteria from lung swabs of apparently healthy and abnormal lung collected from slaughterhouse

Parameters	Values
No. of sample examined	50
Total number of +ve isolates	40
Overall prevalence rate (%)	80

Table 2. Cultural prevalence of bacteria in different lung samples examined

Lung swabs	Total swabs	Positive	Cultural prevalence (%)
P. multocida	30	20	66.7
E. coli	30	28	93.3
Staphlococcus aureus	30	16	80.0
Klebsiella pneumonia	20	10	33.3
Total	110	74	67.3

Table 3. Results of different sugars test of isolated bacteria from lung swabs of cattle

	P. multocida		*E. coli*		*Staphylococcus spp.*		*K. pneumonia*	
Sugars	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid
Dextrose	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Maltose	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Sucrose	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Mannitol	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

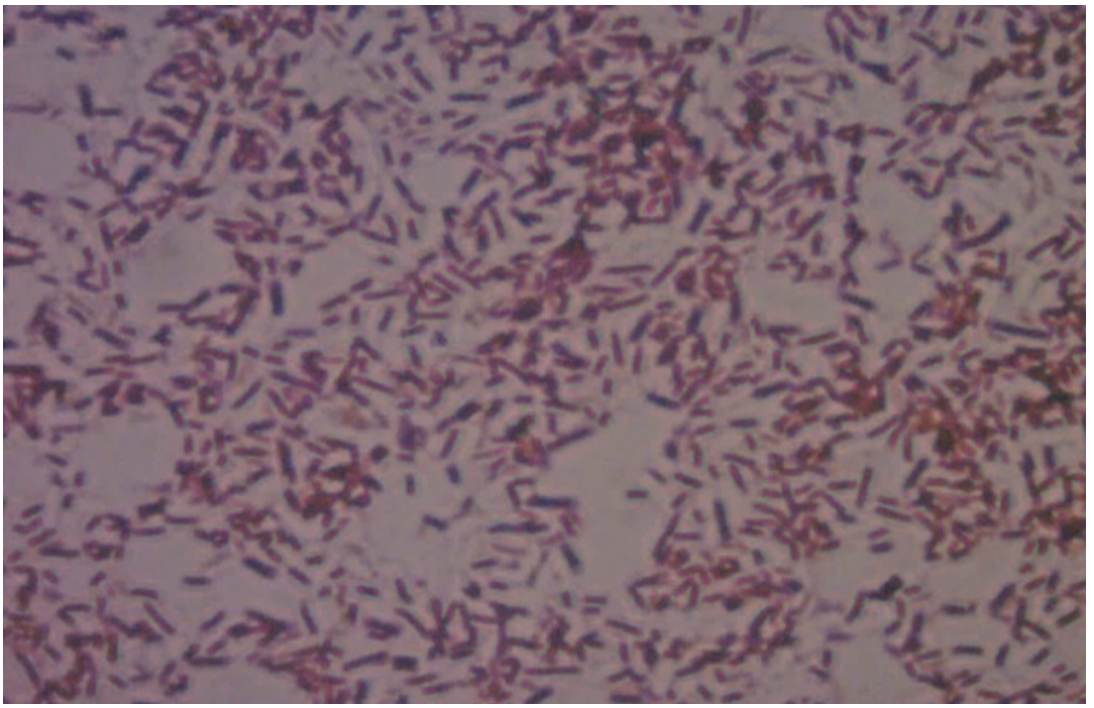


Figure 1. Isolation *P. multocida* was shown negative bipolar bacillus shape (Leishman's stain X100)

Gram positive, purple color, coccoid shape *Staphylococcus spp.* was characterized with gram's stain. Gram negative, pink color rode shape *Klebsiella pneumonia* was seen with gram stain.

Colony character: The growth of *P. multocida* in nutrient broth was characterized by diffused turbidity of the medium and in few occasions, pellicle was formed. The presumptive *P. multocida* produce small colonies on nutrient agar media. The other characteristics of these colonies included whitish, districted, opaque, circular and translucent in appearance. The presumptive *P. multocida* produced small colonies on blood media. The other characteristics of these colonies included whitish, discrete, opaque, and circular and translucent in appearance.no hemolytic was noticed on the blood agar media. Culture on MacConkey agar plates was not yielded colonies. Culture of *P. multocida* on EMB agar yielded small, circular, smooth, convex, translucent, glistening colonies which tended to coagulase. Metallic sheen was absent.

The growth of *Escherichia coli* on nutrient broth (NB) colonies are large thick, greyish white, moist, smooth, opaque or translucent discs. Colonies are large, thick, greyish white, moist, smooth, opaque or translucent discs on Nutrient agar (NA). Cultured of *E. coli* on MacConkey agar agar colonies are bright pink due to lactose fermentation and alpha hemolysis. The isolated of *E. coli* on EMB agar characterized Greenish metallic sheen colonies.

Staphylococcus shows on the nutrient broth the cultured characterized no turbidity, opaque, translucent. Colonies of *Staphylococcus aureus* are frequently surrounded by zones of clear beta-hemolysis the golden appearance.

The colonies that were confirmed by staining reaction and catalase test were streaked on mannitol salt agar plate (MSA), incubated at 37°C, and examined after 24-48 h for growth and fermentation. The presence of growth and change of pH in the media (red to yellow color) regarded as presumptive identification of *Staphylococcus aureus*.

Cultured of *Klebseilla pneumonia* on MacConkey agar plate was found short, plump, straight rods, round dark circle and the colonies were opaque, mucus and pink in color. On EMB agar *Klebseilla pneumonia* was produced pink color no metallic sheen.

Leishman's staining technique: Rod shaped bacteria with bipolar appearance were observed after Leishman's staining of fresh culture of the organisms which was indicative of *P. multocida.* The rod-shaped gram negative organisms arranged singly, paired or in short chain indicated *E. coli and Staphylococci* were gram positive and arranged in cluster.

Motility: Organisms were found motile when examined under microscope with hanging drop slide preparation.

Sugar fermentation tests: In sugar fermentation tests, *P. multocida* were fermented 4 sugars out of 5 sugars (glucose, maltose, sucrose, lactose and mannito) except the lactose in which no acid and gas production. Isolated *E. coli* were fermented glucose, lactose, maltose and mannitol Figure 2). *Klebseilla pneumonia* and *Staphylococcus aureus* were fermented 5 sugars out of 5 sugars.

Gross lesions: The gross of lung focal necrosis and hemorrhage, lung congested with petechialtion and dark-red discoloration, congestion and consolidation, and hepatization red and gray of the lung, bronchopneumonia, branchioalveola pneumonia, Intra-alveolar fibrin clumps and hemorrhagic pneumonia, thickened interalveolar septa due to congestion (Figure 3).

Microscopic lesions: Microscopic lesions of the lung showed pneumonia, bronchopneumonia, bronchioalveolar pneumonia, Intra-alveolar fibrin clumps and infiltration by macrophages and neutrophils neutrophils, pneumonia and hemorrhagic pneumonia, thickened interalveolar septa due to congestion and infiltration by neutrophils, fibrin and few neutrophils with nodule formation (Figure 4).

Lung diseases caused by bacteria is one of the most causes of mortality, morbidity and economical loss in cattle in Bangladesh. The total prevalence rate of bacteria 80% (30 out of 50) and were the average cultural prevalence rate of bacteria were 67.3% (74 out of 110). The prevalence value was found that was similar to the findings of Haider *et al.* 2007. The prevalence rate is slightly higher than the other authors (Akbor *et al.* 2007b). Akbor *et al.* 2007b was conducted their study in aged male buffalo in the Barisal district. But in present study, the author was done the study in different breed, age group and sex cattle in Gazipur district. Both studies were conducted over the year. So, there is no season variation.

The colony character of *Pasteurella spp* on nutrient broth the growth of *P*. multocida in NB was characterized by

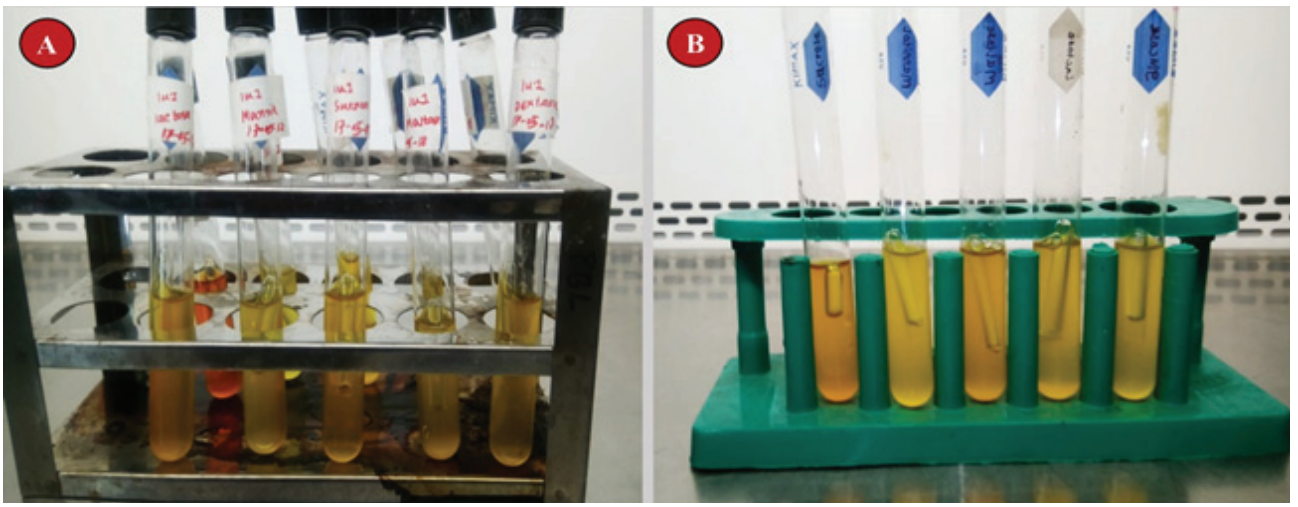


Figure 2. A) Isolated *Staphylococcus* fermented glucose, maltose, sucrose, lactose and mannitol; B) Isolated *E. coli* fermented glucose, lactose, maltose and mannitol.

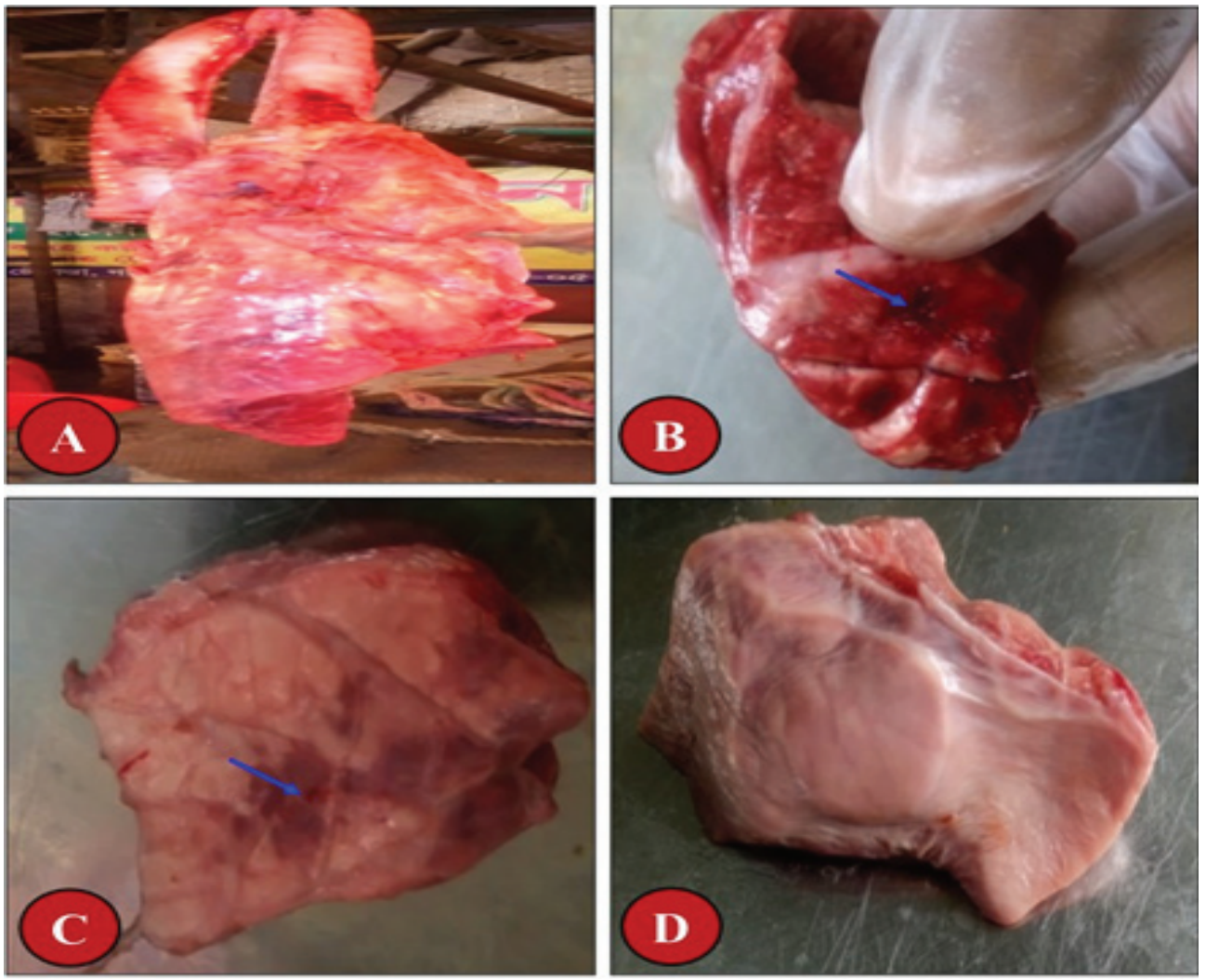


Figure 3. Photograph shows gross lesions in lung samples of cattle. **A)** Congestion and empyema, **B)** Focal necrosis and hemorrhage, **C)** Petechial hemorrhage and dark-red discoloration, and **D)** Congestion and consolidation.

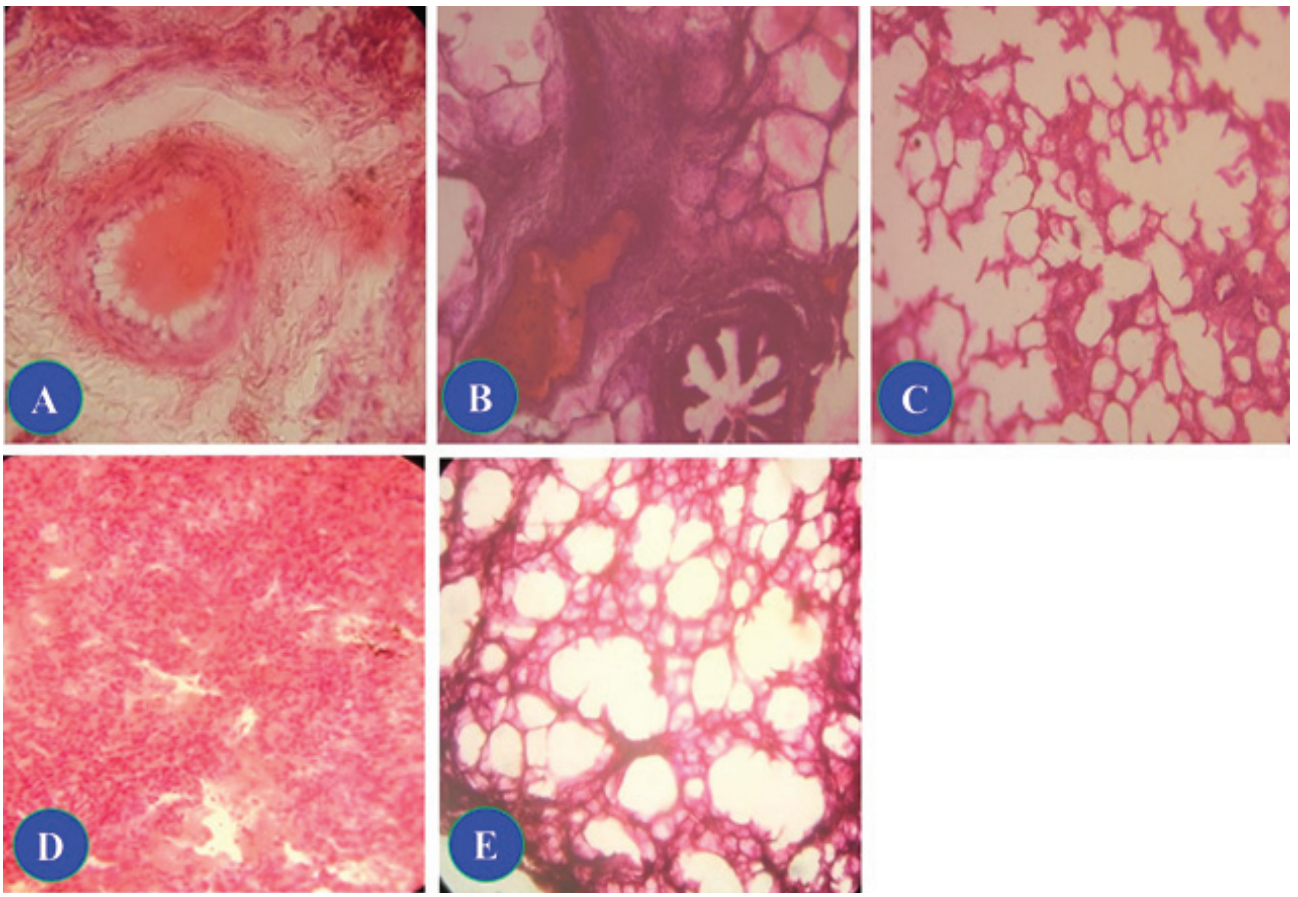


Figure 4. Histopathological changes in different lung samples of cattle. **A)** Bronchopneumonia (H &E; 40X), **B)** Infiltration of neutrophils and hemorrhage (H &E; 100X), **C)** Branchio-alveola pneumonia (H &E; 40X), **D)** Intra-alveolar fibrin clumps and infiltration of macrophages and neutrophils (H &E; 40X), and **E)** Thickened interalveolar septa with congestion and infiltration of neutrophils (H &E; 40X).

diffused turbidity of the medium and in few occasions pellicle was formed. On nutrient agar the presumptive *P. multocida* produce small colonies on NA media. The other characteristics of these colonies included whitish, districte, opaque, circular and translucent in appearance. The colony and staining character of *P. multocida* showed gram negative, pink color, coccobacilli in shape and hanging drop technique all the isolated revealed non-motile similar finding with other authors (Akbor *et al.* 2007b). The colony and cultural characteristics are remained same in always. It does not vary in the place and season.

E. coli on nutrient broth the growth of *E.coli* On NA colonies are large thick, greyish white, moist, smooth, opaque or translucent discs. The staining character of *E. coli* showed Gram negative, pink color, rod shape and hanging drop technique all the isolated revealed motile similar finding with other authors (Zinnah *et al.* 2007, Tonu *et al.* 2011, Matin *et al.* 2017).

The colony and staining character of staphylococcus showed gram positive, purple color cocci shape and hanging drop technique all the isolated revealed non-motile similar fining with other authors (Greenwood *et al.* 2002).

The colony and staining character of *K. pneumonia* showed gram negative, pink color, rod shape and hanging drop technique all the isolated revealed non-motiles similar finding with other authors (Greenwood *et al.* 2002).

In sugar fermentation tests, *P. multocida* were fermented were 4 sugars out of 5 sugars except the lactose in which no acid and gas production. The similar findings were detected by other authors (Cowan 1974, Akbor *et al.* 2007b). In sugar fermentation tests, *E. coli, Klebseilla* and *Staphylococcus* were fermented 5 sugar out of 5 sugars were fermented (Tonu et al. 2011, Islam et al. 2016, Matin *et al.* 2017, Zinnah *et al.* 2017).

In this study, the gross and histopathological lesion of lung focal necrosis and hemorrhage, lung congested with petechialtion and dark-red discoloration, congestion and consolidation, and hepatization red and gray of the lung, bronchopneumonia, branchioalveola pneumonia, Intra-alveolar fibrin clumps and infiltration by macrophages and neutrophil, neutrophils and hemorrhagic pneumonia, thickened interalveolar septa due to congestion and infiltration by neutrophils and fibrin and few neutrophils (Saharee 1992). The microscopic lesions were formed nodule. This finding of the present study was corresponded with the findings of Akbor *et al.* 2007a. In this study, qualitative data were recorded for the gross and histopathological lesions.

Conclusion

The total bacterial prevalence was 80% that were collected from abnormal and apparently healthy lung of cattle. In this study *P. multocida*, *E. coli*, *Staphlococcus aureus* and *Klebseilla pneumonia* bacteria were isolated and identified. The important gross lesions were focal necrosis, red and gray hepatization and consolidation of the lung. Bronchopneumonia and branchioalveola pneumonia with nodule formation were very common microscopic lesions in the affected lungs of cattle. The findings of this study will help the veterinarian for diagnosis of causal agents and antibiogram for the proper treatment.

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