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PATHOLOGICAL STUDY OF BACTERIAL DISEASES OF LIVER OF CATTLE

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ABSTRACT

The research was carried out to study the prevalence of liver infections of cattle in Gazipur district and to investigate the gross and microscopic lesions of the liver infections along with isolation and identification of non-specific bacteria causing infections of cattle. A total of thirty liver samples were collected from different slaughter houses of Gazipur district immediately after slaughter. Nutrient broth was used for taking the swab samples. Different microbiological protocols were used to isolate and identify the bacteria. Four bacterial species were isolated from the collected samples *e.g. Staphylococcus aureus*, *Staphylococcus epididermis, Escherichia coli* and *Klebsiella pneumoniae* subsp. *pneumonia*. The highest cultural prevalence was found in case of *Staphylococcus aureus* and *Escherichia coli* mixed infection (90%). Most frequent cases were abscess with hemorrhages. Multiple firm white nodular lesions with abscess, areas of congestion, necrotic patch were also found in this investigation. In *Staphylococcus aureus* infections, caseous necrosis surrounded with many neutrophils and fibrous connective tissue proliferation were seen. Leucocytic infiltration in presence of fatty change was found in case of *Escherichia coli* mixed with other anaerobes bacterial infections. The observed highest lesions were hemorrhages (83.34%) followed by abscess (76.67%), congestion (66.67%) and nodular lesions (60%). Therefore, the study might help to take preventive measures for the reduction of liver infections in cattle in the study area.

Keywords: Cattle, liver infections, bacteria, pathological lesions, Gazipur

Introduction

Livestock is an integral component of the complex farming system in Bangladesh. Livestock sub- sector offers employment and livelihood opportunities particularly for the rural poor, including the functionally landless, marginal and small-scale farmers, many of whom regard livestock as a main livelihood option. About 75 percent of people rely on livestock to some extent for their livelihood (Tareque and Chowdhury 2010). Livestock sub-sector provides full-time employment for 20% of the total population and part-time employment for another 50% (Begum *et al.* 2017). The contribution of livestock sector to the gross domestic product (GDP) is 1.73% in Bangladesh (Rahman *et al.* 2014).

Large ruminant especially cattle among other available livestock species are the most versatile component in relation to the existing integrated agricultural farming system in Bangladesh. The total cattle population of the country is about 24.5 million, which is about 1.79% of the world and 5.47% of Asian cattle population (FAO 2014). Number of cattle per livestock household is 3.5 and that of 0.94 for all household (BBS 2020). The national herd of Bangladesh constitutes of indigenous/local zebu cattle with some exotic and their crosses (not exceeding 10%). Among them non-descript Deshi, Pabna, Red Chittagong, Munshiganj Cattle, North Bengal Grey are indigenous types (Mason 1988) and also predominant. Holstein-Friesian, Jersy, Sahiwal, Hariana, Sindhi, Sahiwal-Friesian are some of the major exotic breeds. Some crossbreeds have been made through artificial insemination between indigenous and exotic breeds (Begum *et al.* 2017). Every year dairy cattle produces 80.06 lakh metric tons of milk where the demand for milk in our country is 150. 29 lakh metric tons. Another type of farming, Beef fattening which is practiced by the rural poor, especially landless, destitute and divorced women as a source of income (Ahmed *et al.* 2010). Local zebu cattle and their crosses with some exotic breeds are mainly raised for fattening.

The productivity is low mainly due to inadequate feed resources, low quality of feedstuffs, low genetic potentiality (as about 80% cattle of total population is indigenous with low productivity gene), reproductive inefficiencies including low fertility rates (45-47%) and high mortality rate, prevalence of various diseases. Incidence of diseases is a major constraint of cattle production in Bangladesh. Climate of our country along with the poor nutritional status of cattle, contribute to a high incidence of cattle diseases. Several major diseases are anthrax, hemorrhagic septicemia (HS), foot and mouth disease (FMD), black quarter (BQ), calf diarrhea, diseases caused by infestation with liver flukes, liver abscess and other infections in liver causes by bacteria (Ahmed *et al*. 2010). Among all the fatal diseases, liver diseases are very important because the functions of liver are related to the productivity of the cattle. Liver is the largest gland in the body and is considered to be the most important organ for mammalian metabolism, health condition and reproduction (Blood *et al*. 1989).

A very few researches related to bacterial diseases of liver of cattle are done in this area. Therefore, the present work was carried out to study the prevalence of liver diseases of cattle in Gazipur district and to investigate the gross and microscopic lesions of liver diseases of cattle as well as to isolate and identify the bacteria causing infections in the liver of cattle.

Materials and Methods

A total of thirty samples from cattle liver were collected for the current study. Liver samples were collected from different slaughter houses of Gazipur district, Bangladesh. The samples were pathologically investigated that included isolation and identification of non-specific bacteria along with gross and histopathological study. Swabs were taken from different parts of the liver for isolation and identification of bacteria. This pathological investigation was conducted from the time period of September' 2018 to September'2019 and was performed in the Microbiology section of Central Laboratory in the Faculty of Veterinary Medicine and Animal Science and Pathology laboratory under the Department of Pathobiology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Transportation of samples: The samples were collected immediately after slaughter and brought to the laboratory of the department of Pathobiology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur in ice box with ice.

Collection of bacteriological samples: Cotton tipped swabs were used after sterilization by autoclaving for the collection of swabs. Swab samples were taken aseptically from different sides of liver and kept in screw capped test tubes containing 10 ml of nutrient broth for bacteriological study.

Preservation of samples for histopathology: 10% formalin was used for the preservation of samples after collection in plastic containers for histopathological study.

Media used for bacterial culture: Nutrient agar, Eosin Methylene Blue (EMB) agar, MacConkey agar, Mannitol salt agar, and *Salmonella Shigella (*SS) Agar were used for bacterial culture.

Morphological characterization: The presumptive colonies of different bacteria in various media were characterized microscopically using Gram's stain.

Carbohydrate fermentation test and biochemical test: Five basic sugars such as glucose, sucrose, lactose, mannitol and maltose were used for sugar fermentation tests (Haider *et al*., 2008). Catalase test was performed followed by standard procedure (Haider *et al*., 2008).

Processing of Tissues: Tissue samples collected from different parts of liver were collected and fixed in 10% neutral buffered formalin and further processed for histopathological examination (Haider *et al*., 2008).

Photomicrography: Photomicrography was taken using photomicrographic camera (ZEISS AxioCam ERc5s) facilities facilitated by Department of Gynecology, Obstetrics and Reproductive Health of BSMRAU.

Results and Discussion

Isolated bacteria from liver samples with their prevalence percentage are shown in Table 1. The highest cultural prevalence rate was found in *Staphylococcus aureus* and *Escherichia coli* mixed infection and the lowest was found in *Klebsiella pneumoniae* subsp. *pneumonia* infection.

The isolated *Klebsiella pneumoniae* subsp. *pneumonia* organisms were catalase positive.

Colony characteristics of bacteria on different agar media were observed. Bacteria were confirmed through specific colony characteristics on different agar media. For further confirmation some biochemical tests were also performed. Four species of bacteria were isolated and identified named as *Staphylococcus aureus*, *Staphylococcus epididermis, Escherichia coli* and *Kleibsiella pneumoniae* subsp. *pneumoniae.* Mixed bacterial infections were also observed such as *Staphylococcus aureus* mixed with *Escherichia coli*, *Escherichia coli* mixed with *Staphylococcus epididermis*, *Staphylococcus aureus mixed with Klebsiella pneumoniae* subsp. *Pneumonia. Staphylococcus aureus, Escherichia coli and Staphylococcus epididermis* were also found in mixed condition.

The cultural prevalence of *Staphylococcus aureus* mixed with *Escherichia coli*; *Escherichia coli;* mixed of *Staphylococcus aureus, Escherichia coli and Staphylococcus epididermis; Staphylococcus aureus mixed with Klebsiella pneumoniae* subsp. *Pneumonia*; *Staphylococcus epididermis* mixed with *Escherichia coli*; *Kleibsiella pneumonia* subsp. *pneumonia* were 90%, 83.33%, 73.33%, 66.67%, 80%, and 60%, respectively (Figure 1). The prevalence rate was highest in case of *Escherichia coli* infection mixed with *Staphylococcus aureus* and it was 90%.

In this study, several mixed bacterial infections such as *Staphylococcus aureus* mixed with *Escherichia coli*; *Escherichia coli* mixed with *Staphylococcus epididermis*; *Staphylococcus aureus mixed with Klebsiella pneumoniae* subsp. *Pneumonia*; mixed of *Staphylococcus aureus, Escherichia coli and Staphylococcus epididermis* were isolated which concur with the previous findings (Al-Nakeeb and Al-Fetly 2016, Borai *et al*. 2013, Sohair and Eman 2009).

The isolated *Staphylococcus aureus* produced gray white colonies on nutrient agar media and yellow colonies on Mannitol salt agar media (Figure 2A). The isolated *Staphylococcus epididermis* organisms produced white colonies on nutrient agar and pink colonies on Mannitol salt agar (Figure 2B). *Escherichia coli* produced circular with a convex elevation and a smooth surface on nutrient agar; green metallic sheen on EMB agar (Figure 2C); pink colonies on SS agar and pink colonies on MacConkey agar (Figure 2D). *Kleibsiella pneumonia* subsp. *pneumoniae* produced pink colonies on MacConkey agar (Figure 2E) but there was no growth on Mannitol salt agar.

Staphylococcus aureus was isolated from liver sample which concur with the previous reports (Al- Nakeeb and Al-Fetly 2016, Borai *et al*. 2013), Sohair and Eman (2009). It has also been reported that *Staphylococcus aureus* produced gray white colonies on Nutrient agar and yellowish colonies on Mannitol Salt agar (Habib *et al.* 2015).

Staphylococcus aureus were cocci shaped bacteria with clusters in arrangements and Gram's positive (Figure 3A). The morphology of isolated *Staphylococcus aureus* was cocci shaped with clusters in arrangements and Gram's positive which corresponds to the findings of Vos *et al*. (2009). *Staphylococcus epididermis* were cocci shaped organisms, pairs in arrangements and Gram's positive in reaction (Figure 3B). *Escherichia coli* were bacilli

Table 1. Number of bacteria isolated from liver sample of cattle

Bacteria	Total Sample	Positive
Staphylococcus aureus + *Escherichia coli* (Mixed)	30	27
Escherichia coli		25
Staphylococcus aureus+ *Escherichia coli* + *Staphylococcus epididermis* (Mixed)		22
Staphylococcus aureus + *Klebsiella pneumoniae* subsp. *pneumonia*		20
Staphylococcus epididermis + *Escherichia coli* (Mixed)		24
Klebsiella pneumoniae subsp. *pneumonia*		18

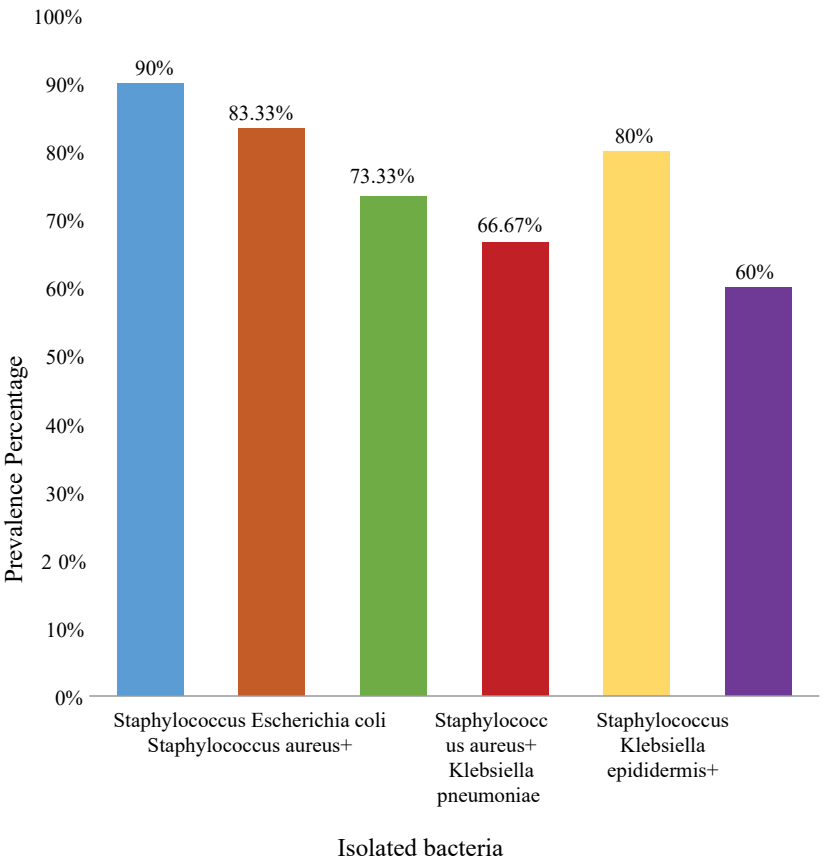

Figure 1. Cultural prevalence (%) of bacteria in liver infections of cattle in Gazipur District

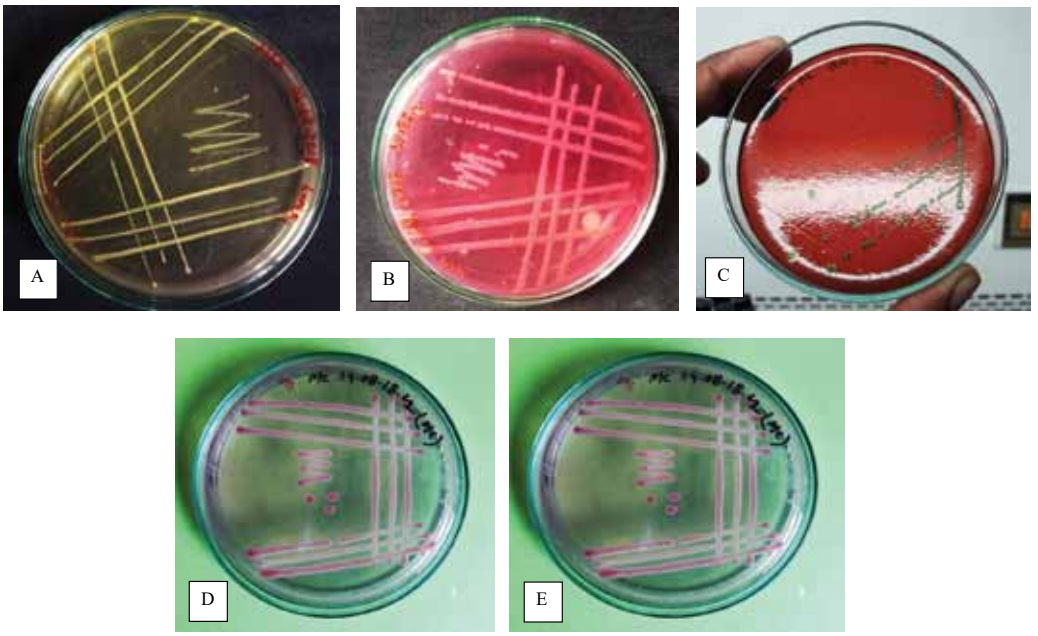

Figure 2. Colony characteristics of isolated bacteria. A) Colonies of *Staphylococcus aureus* on Mannitol salt agar; B) Colonies of *Staphylococcus epididermis* on Mannitol salt agar; C) Colonies of isolated *Escherichia coli* on EMB agar; D) Colonies of isolated *Escherichia coli* on SS agar; E) Colonies of isolated *Klebsiella pneumoniae* subsp. *pneumonia* on MacConkey agar.

shaped organisms; singly and pairs in arrangements and Gram's negative in reaction with Gram's staining (Figure 3C). *Klebsiella pneumoniae* subsp. *pneumonia* was rod shaped bacteria which appeared singly, in short chains and Gram's negative (Figure 3D).

Escherichia coli was also isolated from liver sample which concur with the previous findings (Al-Nakeeb and Al-Fetly 2016), (Sohair and Eman 2009). *Escherichia coli* produced green metallic sheen on EMB agar and bright pink colonies on MacConkey agar which is similar to the another report (Marjan *et al*. 2012). The morphology of isolated bacteria was rod shaped and Gram's negative which was also reported (Zinnah *et al*. 2007).

Staphylococcus epididermis was another single bacteria isolated from liver sample which partially concur with the previous findings (Al-Nakeeb and Al-Fetly 2016, Sohair and Eman 2009). *Staphylococcus epididermis* produced white colonies on Nutrient agar, pink colonies on Mannitol Salt agar. Their morphology were cocci shaped, pairs in arrangements and Gram's positive. *Klebsiella pneumoniae* subsp. *pneumonia* was the last single bacteria that isolated from liver sample which resembles to the previous report (Sohair and Eman 2009). *Klebsiella pneumoniae* subsp. *pneumonia* produced pink colonies on Macconkey agar but there was no growth on Mannitol Salt agar**.** The morphology of the bacteria was rod shaped, arranged in short chains and Gram's negative.

Staphylococcus aureus were catalase positive and fermented mannitol, maltose, and sucrose but could not ferment arabinose (Figure 4). This result was similar with the previous reports (Lund *et al*. 2000), Roginski *et al*. 2003, Vos *et al.* 2011). *Staphylococcus epididermis* were catalase positive and fermented maltose and sucrose but could not ferment mannitol and arabinose which corresponds with the previous finding (Al-Sariy 2014). *Escherichia coli* fermented five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol which was indicated by acid and gas production and this result was similar to another report (Cheesbrough 1985). *Kleibsiella pneumonia* subsp. *pneumoniae* fermented D-mannitol, maltose, sucrose, D-glucose, lactose and L-arabinose (Sohair and Eman 2009).

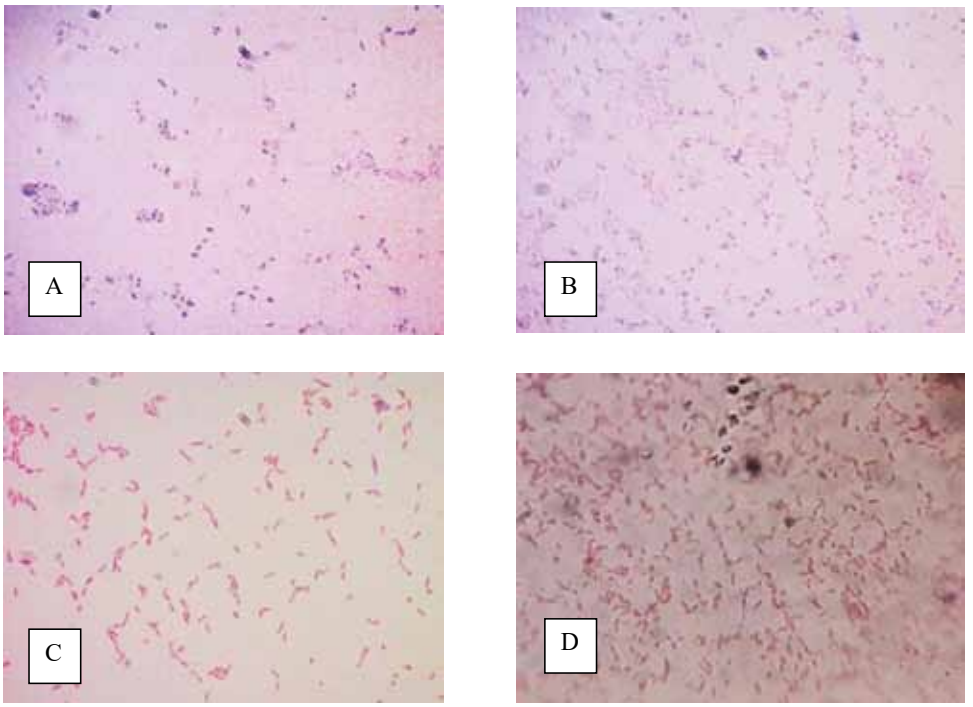

Figure 3. Morphological characteristics of isolated organisms. A) Isolated *Staphylococcus aureus* showed positively stained cocci shaped bacteria (Gram's stain, X1000); B) Isolated *Staphylococcus epididermis* showed positively stained cocci shaped bacteria (Gram's stain, X1000); C) Isolated *Escherichia coli* showed negatively stained bacilli shaped bacteria (Gram's stain, X1000); D) *Klebsiella pneumoniae* subsp. *pneumonia* showed rod shaped negatively stained bacteria arranged in short chains (Gram's stain, X1000).

Gross lesions: In this investigation, a total of thirty liver samples were collected from different locations of Gazipur district in Table 2. All the liver samples were grossly and histopathologically examined and lesions were recorded. The detected predominant lesion in the liver was multiple abscesses on the surface. Creamy viscid pus was found in several samples. Nodules formations with hemorrhages were also found in few samples. Dark spot, congestion, necrotic patch were found in liver samples. In liver samples, multiple firm white nodular lesions with abscess and hemorrhages were found (Figure 5A). In some cases, different stages of multiple abscessation were found with dark spots on liver surface (Figure 5B). Cut surface showed whitish pus or cheesy material (Figure 5C).

Several gross lesions were found in liver samples. The most frequently observed gross lesions were abscess and hemorrhages. Multiple firm white nodular lesions with abscess were found which corresponds with the previous finding. Areas of congestion, necrotic patch and hemorrhages were also found in liver surface (Figure 5D). It has also been reported that whitish pus or cheesy material were seen from the cut surface of liver (Borai *et al*. 2013).

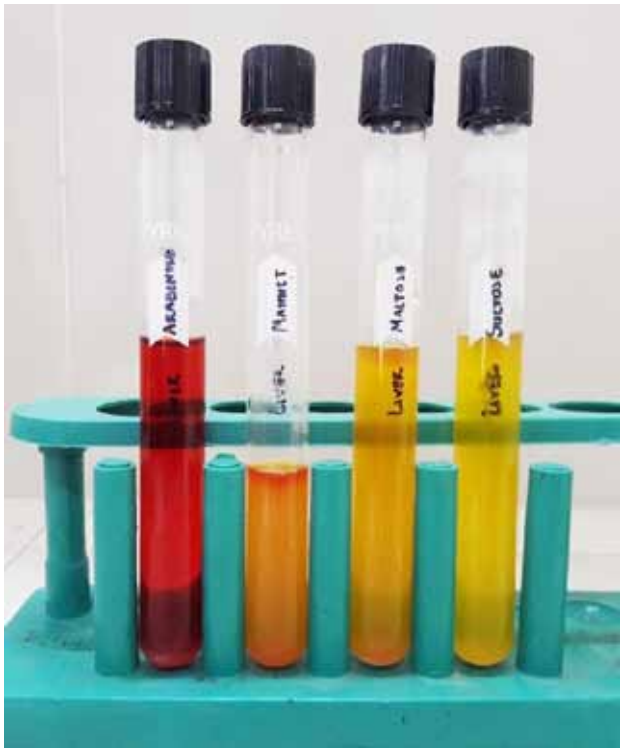

Figure 4. Sugar fermentation test for *Staphylococcus aureus.*

Table 2. Prevalence percentage of overall lesions in the liver of cattle

Lesions observed	Total samples	Positive	Prevalence (%)
Abscess	30	23	76.67
Nodular lesions		18	60
Haemorrhages		25	83.33
Pus and Congestion		20	66.67

Histopathologic lesions: In tissue section of liver cirrhosis, presences of nodules and fibrous septa with effacement of the lobular architecture were found (Figure 6A). Areas of congestion, necrotic patch and hemorrhages were found in liver surface. Fatty liver changes with infiltration of lymphocytes, fat lobules and hemorrhages were found (Figure 6B). In case of lumen of an abscess, degeneration with lymphocytic infiltration was observed in the liver section (Figure 6C). Infiltrations of lymphocytes together with fatty change were found in liver tissue (Figure 6D). Granulomatous lesions of the liver surrounded by inflammatory cells and multinucleated giant cells infiltration were seen (Figure 6E). Hepatic congestion with infiltration of inflammatory cells was also found in portal blood vessels (Figure 6F). Hepatic fibrosis with inflammatory cells (Figure 6G) and Lymphocytic infiltration in the liver tissue section were also found (Figure 6H).

In histopathological investigation, leucocytic infiltration with the presence of fatty change of hepatocytes were found which results due to *Escherichia coli* mixed with other anaerobes bacterial infections. This histopathological study of liver was supported by previous report (Borai *et al*. 2013). Caseous necrosis surrounded with many neutrophils and fibrous connective tissue proliferation was seen and it happened due to the presence of *Staphylococcus aureus* in liver samples. Presence of cirrhosis, nodules, fibrous septa and mononuclear leucocytic infiltration in liver tissue were found that corresponds to previous report (Borai *et al*. 2013). Infiltration of lymphocytes, degenerating hepatocytes were seen in case of liver abscess. Infiltration of neutrophils surrounded by fibrous connective tissue capsule, congestion with infiltration of inflammatory cells in liver was observed in this histopathological study due to the presence of *Corynebacterium pyogenes* infection. *Staphylococcus aureus, Corynebacterium pyogenes,*

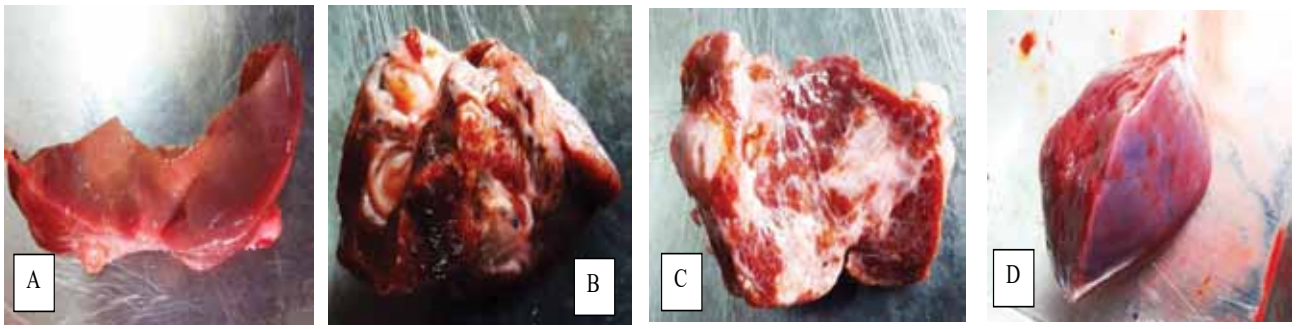

Figure 5. Gross lesions in liver. A) Cattle liver with abscess, hemorrhages and multiple nodules; B) Cattle liver with multiple abscess and dark spots; C) Presence of whitish pus or cheesy material on cut surface of liver; D) Congestion, necrotic patch and hemorrhages on liver.

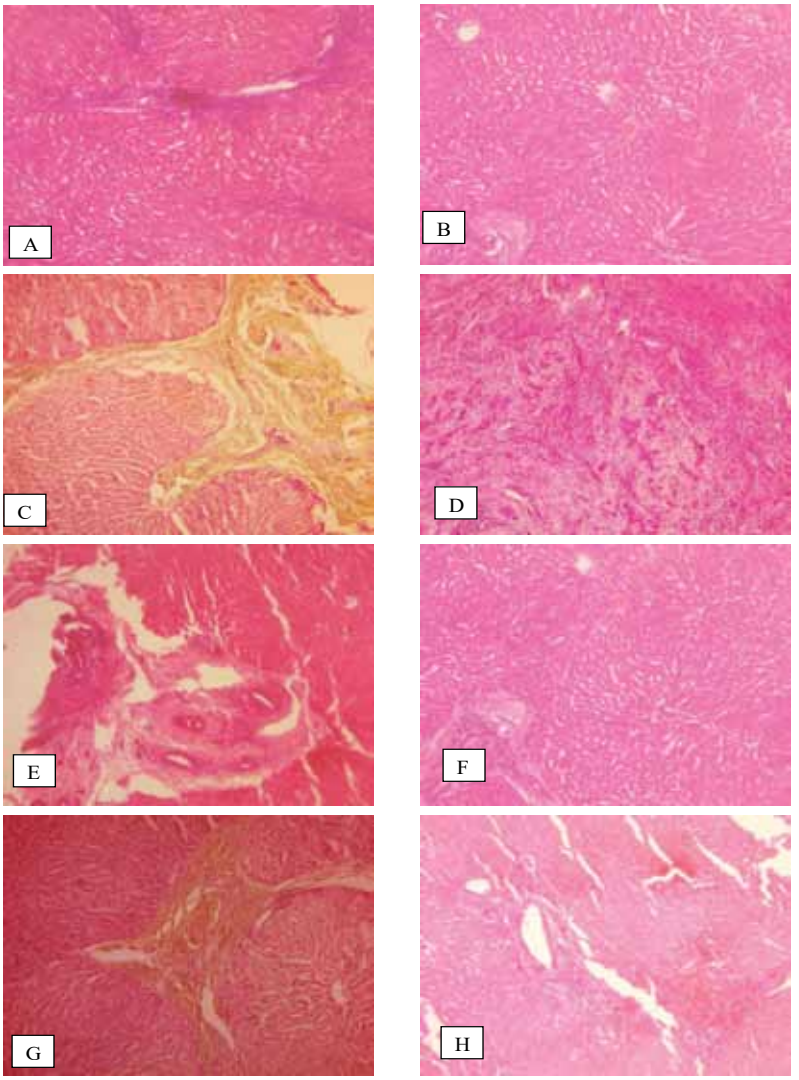

Figure 6. Histopathological Changes of liver infections in cattle. A) Cirrhosis in the Liver tissue sections; B) Fatty changes in the liver; C) Tissue degeneration with lymphocytic infiltration; D) Fatty changes in the liver with infiltration of Lymphocytes; E). Granulomatous lesions of the liver surrounded by inflammatory cells and multinucleated giant cells infiltration (H & E, X100). F). Hepatic congestion with infiltration of inflammatory cells (H & E, X100); G). Hepatic fibrosis with lymphocyte infiltration (H & E, X100); H) Lymphocytic infiltration in the liver tissue section (H & E, X100).

Escherichia coli are the main bacteria which causes of liver abscess. This finding are almost similar with previous report (Sohair and Eman 2009) whereas another findings (Al-Nakeeb and Al-Fetly 2016, Borai *et al*. 2013) reported that not only the single bacteria but also the mixed organisms like *Escherichia coli* mixed with *Staphylococcus aureus; Escherichia coli* mixed with *Staphylococcus epididermis*, *Staphylococcus aureus* mixed *with Klebsiella pneumoniae* subsp. *Pneumonia. Staphylococcus aureus, Escherichia coli and Staphylococcus epididermis* causes liver abscess in cattle.

Conclusions

Highest cultural prevalence was observed in case of *Escherichia coli* and *Staphylococcus aureus* mixed infection (90%) among the total examined samples. The prevalence rate were 83.33%, 73.33%, 66.67%, 80%, 60% in case of *Escherichia coli,* mixed of *Staphylococcus aureus, Escherichia coli and Staphylococcus epididermi, Staphylococcus aureus mixed with Klebsiella pneumoniae* subsp. *Pneumonia*, *Staphylococcus epididermis* mixed with *Escherichia coli*, *Kleibsiella pneumonia* subsp. pneumonia infection, respectively. The most frequently observed gross lesions were abscess and hemorrhages. Multiple firm white nodular lesions with abscess, areas of congestion, necrotic patch were also found. Whitish pus or cheesy material was seen from the cut surface of liver. In case of *Staphylococcus aureus* infections, caseous necrosis surrounded with many neutrophils and fibrous connective tissue proliferation were seen. Leucocytic infiltration with the presence of fatty change of hepatocytes was found in case of *E. coli* mixed with other anaerobes bacterial infections. Presence of cirrhosis, nodules, fibrous septa and mononuclear leucocytic infiltration in liver tissue were also found. Infiltrations of neutrophils surrounded by fibrous connective tissue capsule, congestion with infiltration of inflammatory cells in liver were observed in liver abscess which caused due to bacterial infections. The observed highest lesions were haemorrhages (83.34%) followed by abscess (76.67%), pus with congestion (66.67%) and nodular lesions (60%).

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