

## MICROBIOLOGICAL AND PATHOLOGICAL STUDIES ON HEPATIC AND LUNG DISORDERS IN BOVINE CALVES

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### ABSTRACT

Microbiological and pathological studies were undertaken on liver and lungs of bovine calves received for post mortem examination. The bacterial organisms isolated from liver and lungs were *E. coli* (15), *Klebsiella pneumoniae* (4), *Proteus mirabilis* (4), *Pseudomonas aeruginosa* (3), *Corynebacterium pyogenes* (3), *Staphylococcus aureus* (3), *Streptococcus pyogenes* (1) and *Citrobacter divergens* (1). Gross and histopathological studies revealed that main pathological conditions in liver were acute hepatitis, cirrhosis along with fatty changes congestion and haemorrhages. Gram-negative rod shaped bacilli were also demonstrated in hepatic lesions by Taylor's stain in which *E. coli* organism was isolated from liver. The characteristic pathological conditions observed in lungs were suppurative pneumonia, fibrinous pneumonia and acute interstitial pneumonia. Suppurative bronchopneumonia was associated with *Klebsiella pneumoniae*, while *Staphylococcus aureus* infection was characterized by acute interstitial pneumonia. *Streptococcus* infection in calves was associated with perivascular oedema in lungs. One case of cow calf revealed tuberculosis granuloma in which red coloured coccobacilli were demonstrated by Ziehl Neelsen stain.

**Key words:** Calf mortality, bacteriological studies, hepatic lesions, pulmonary lesions

Liver possesses varieties of functions that are central to the metabolic pathways i.e. metabolism and storage of carbohydrates, proteins, fats and vitamins, secretion of bile, synthesis of plasma proteins, detoxification of various toxic compounds and removal of waste substances from the blood (Sastri and Ramarao, 2001). Because of the highly specialized functions of the hepatic parenchymal cells and dual blood circulation, the liver gets first exposure to inimical agents i.e. infectious agents, parasites and toxins. Similarly, lungs are also exposed to various types of infectious or non infectious agents through respiratory passage. Diseased liver as well as lungs adversely affect the health and growth of animals particularly new born and young animals and may result in heavy economic losses (Purushothaman and Rajan, 1985). So to exploit dairy industry for maximum gains, a greater attention needs to be given to disease problems of calves. Keeping in view the above facts, microbiological and pathological studies were undertaken in bovine calves on liver and lungs.

### MATERIALS AND METHODS

The detailed post mortem examination was conducted on 52 carcasses of bovine calves received

in the Department of Veterinary Pathology, CCS Haryana Agricultural University, Hisar. The post mortem was conducted as soon as the carcasses were brought to the post mortem hall to avoid further autolytic and putrefactive changes. The representative pieces of liver and lungs from each carcass, in which the lesions were noticed, were collected in sterile petridish with the help of sterilized scissors and forceps for bacteriological examination. A portion of liver and lung was collected in 10 percent buffered formalin for histopathological examination.

**Isolation and identification of bacteria:** Isolation of bacteria from liver and lung tissue samples was conducted by inoculation of the samples on blood agar and MacConkey's lactose agar (MLA) plates as described by Cruickshank *et al.* (1965). The inoculated plates were incubated at 37°C for 24-48 h and examined for the presence of bacterial growth, if any. Blood agar plates were observed for the presence of haemolysis, if any. To obtain the organisms in pure culture, a single colony was picked up and reinoculated on fresh plates of blood agar and MLA. These plates were incubated again at 37°C for 24-48 h. After incubation, bacterial growth so obtained was observed for colony morphology and subjected to Gram's staining, catalase test and oxidase test for primary identification

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of bacteria. The pure cultures of bacteria were stored on blood agar slant/semisolid agar till further use.

For further characterization up to species level, the organisms were subjected to different biochemical tests like IMViC (Indole, Methyl Red, Voges Pro-Skaur and Citrate utilization test), nitrate reduction test, urease test and hydrogen sulphide production test on Triple sugar iron medium.

**Pathological studies:** During postmortem examination, the gross lesions were recorded in liver and lungs of bovine calves. The formalin fixed tissues were processed for paraffin embedding technique. The tissues were properly trimmed, washed in running tap water, dehydrated in graded ethanol, cleared in benzene and embedded in paraffin wax (melting point 60-62°C). The sections were cut at the thickness of 3-4 mm and stained with Haematoxylin and Eosin (Luna, 1968).

**Demonstration of bacterial organism in tissue sections:** The tissue sections from suspected cases of bacterial infection were stained by Taylor's stain for the demonstration of Gram positive and Gram negative bacteria and Ziehl-Neelsen for acid fast organisms (Luna, 1968).

## RESULTS AND DISCUSSION

**Isolation and identification of bacteria:** Total number of isolates obtained from liver and lungs were *E. coli* (15), *Klebsiella pneumoniae* (4), *Proteus mirabilis* (4), *Pseudomonas aeruginosa* (3), *Corynebacterium pyogenes* (3), *Staphylococcus aureus* (3), *Streptococcus pyogenes* (1) and *Citrobacter divergens* (1). Various workers (Joon and Kaura, 1993; Manteca *et al.*, 2001; Verma *et al.*, 2001) have also reported isolation of most of these organisms from the carcasses of bovine calves.

### Pathological studies

#### Hepatic disorders

Pathological lesions in liver were seen in 35 cases. Main pathological conditions were acute hepatitis, fibrosis/cirrhosis, fatty changes, bile duct hyperplasia, congestion, haemorrhages and telangectasis.

**Gross lesions:** Liver in which *E. coli* organisms were isolated revealed large greyish white areas of necrotic foci (Fig. 1) along with distension of gall bladder. There was pale discoloration or haemorrhages

in liver in *P. mirabilis* infection. In *K. pneumoniae* infection, haemorrhages were noticed in liver along with yellowish discoloration. In *S. aureus* infection, liver revealed congestion, haemorrhages and presence of greyish white areas. In *S. pyogenes* infection, liver revealed congestion and haemorrhages. In *C. pyogenes* infection, there was yellowish discoloration of liver. In other cases showing lesions of hepatitis where no etiological agent of pathological significance could be isolated, liver appeared slightly enlarged and discoloured/mottled having haemorrhages, congestion and necrotic foci.

Cirrhosis was seen in two cases; one was associated with *E. coli* infection and in another, no bacterial organism could be isolated. The liver was hard and firm in consistency, pale in colour and having uneven surface. The gall bladder was also distended. **Microscopic lesions:** Liver infected with *E. coli* in buffalo calves revealed hepatitis characterized by haemorrhages, congestion, necrosis of hepatocytes and leucocytic infiltration mainly neutrophils and lymphocytes. Besides, there was fibrinous hepatitis along with fatty changes in some cases. Gram negative rod shaped bacilli were seen in the section of different cases of hepatitis by Taylor's stain indicating *E. coli*. Hepatic lesions of similar lesions have also been described by Jubb *et al.* (1985) in calves due to colibacillosis.

In *P. mirabilis* infection, liver revealed haemorrhages and congestion in central veins and sinusoids along with telangectasis (Fig. 2). Hepatitis characterized by infiltration of neutrophils and lymphocytes along with fatty changes was also observed in a buffalo calf. In *K. pneumoniae* infection, liver revealed mild hepatitis characterized by infiltration of lymphocytes, bile duct hyperplasia and fatty changes. In one case, mild fibrosis at focal areas in parenchyma was also evident. In *S. aureus* infection in cow calves, liver revealed congestion and in buffalo calf, there was hepatitis characterized by mononuclear cells infiltration mainly macrophages and lymphocytes along with mild fibrous tissue proliferation and telangectasis. In *S. pyogenes* infection liver revealed haemorrhages and congestion. In *C. pyogenes* infection in one case, there was congestion and hepatitis characterized by lymphocytic infiltration, mild fibrous tissue proliferation and accumulation of oedematous fluid in portal area

while in other case there was congestion along with mild fatty changes. In other cases, showing lesions of hepatitis where no etiological agent of pathological significance could be isolated, characteristic changes of hepatitis were infiltration of lymphocytes in most of cases along with necrosed hepatocytes, haemorrhages and congestion. Besides, in a few cases, there was bile duct hyperplasia. In one buffalo calf, liver showed perihepatitis characterized by the presence of lymphocytes and fibrin mass and the portal triad areas contained lymphocytes and oedematous fluid. More or less similar lesions of hepatitis have been reported in various bacterial infections by other workers (Verma *et al.*, 1980; Singh *et al.*, 2006; Seema *et al.*, 2007).

There were some cases in which characteristic lesions of hepatitis particularly infiltration of lymphocytes along with necrosed hepatocytes, haemorrhages and congestion were seen but no bacteria could be isolated from these cases. It might be because of the stage of infection. In some cases, histopathological picture of hepatitis was suggestive of viral infection. It appears that in most cases, hepatitis was one of the lesions in systemic infection as the lesions in other organs were also noticed.

Cirrhosis was also noticed in two cases which might be due to bacterial infection or toxic effect of chemical or toxin. Haemorrhages and congestion in liver were mostly associated with septicaemia. These findings were similar to those reported by Bourque *et al.* (2001) who described hepatic fibrosis in calves. Histopathologically, cirrhosis was characterized by the fibrous connective tissue proliferation within and around lobules along with infiltration of lymphocytes and pseudolobulation (Fig. 3).

### **Lung disorders**

Pathological lesions in lungs were seen in 31 cases. These lesions were suggestive of pneumonia. In addition, compensatory emphysema and one case of tuberculosis were also noticed.

**Pneumonia:** Pneumonia was evident in all the cases of bovine calves in which *E. coli* was isolated. Grossly, infection with *K. pneumoniae*, *P. aeruginosa*, *C. divergens*, *C. pyogenes* and *S. aureus* showed the presence of consolidation of lungs along with congestion and haemorrhages. In cases showing

lesions of pneumonia in which no etiological agent of pathological significance could be isolated showed consolidation along with the presence of greyish white colour raised nodules filled with pus, haemorrhages and congestion.

Microscopically, pneumonia was evident in all calves in which *E. coli* was isolated. In majority of cases it was characterized by serous or serofibrinous mass in the lumen of alveoli along with congestion and haemorrhages in capillaries and leucocytic infiltration mainly neutrophils and lymphocytes. In some cases, there was thickening of interlobular septa and fibrinous exudate in bronchi/bronchioles.

Pneumonia was observed in all the four cases in which *P. mirabilis* was isolated. In two cases, it was of fibrinous type characterized by the presence of fibrin, congestion and infiltration of neutrophils and lymphocytes in the alveoli. In one case, alveoli were filled with lysed and intact neutrophils indicating suppurative pneumonia (Fig. 4). Pneumonia was also seen in all the three calves that were infected with *K. pneumoniae*. In one case, there was suppurative bronchopneumonia characterized by the presence of large number of neutrophils in the alveoli as well as bronchi along with hyperplasia of bronchial mucosa, haemorrhages and congestion. The gram-negative red rod shaped bacteria were demonstrated in the exudate.

*S. aureus* infected cases revealed congestion that was noticed in a case of buffalo calf whereas in cow calves, there was either congestion or acute interstitial pneumonia characterized by the presence of thickening of alveolar septa due to congestion in capillaries and the presence of lymphocytes and neutrophils in the interstitial tissue. Infection of *C. pyogenes* revealed the presence of congestion and hepatitis characterized by lymphocytic infiltration, mild fibrous tissue proliferation and accumulation of oedematous fluid in portal area in one case while in other case there was congestion along with mild fatty changes.

Cases in which no etiological agent of pathological significance could be isolated revealed various types of pneumonia including suppurative, fibrinous, serofibrinous, interstitial and bronchopneumonia. The suppurative pneumonia was characterized by the presence of lysed and intact neutrophils and

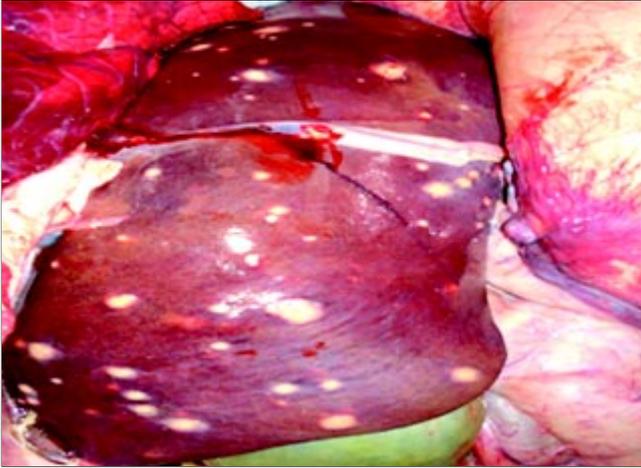


Fig 1. Liver showing large number of greyish white raised areas in an *E. coli* infected buffalo calf. Portion of consolidated lung on left side can be seen in picture.

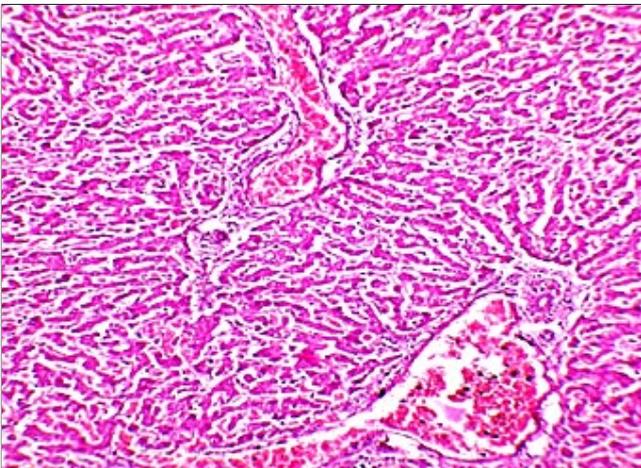


Fig 2. Section of liver showing haemorrhages and congestion in veins and sinusoids along with telengectasis in a *Proteus* infected buffalo calf. (H. & E. x 200)

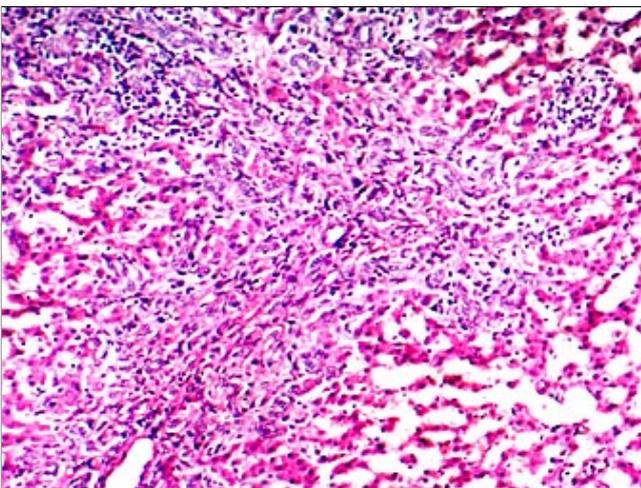


Fig 3. Liver section showing cirrhosis characterized by infiltration of lymphocytes, fibrous connective tissue proliferation, bile duct hyperplasia and pseudolobulation in a buffalo calf. (H. & E. x 400)

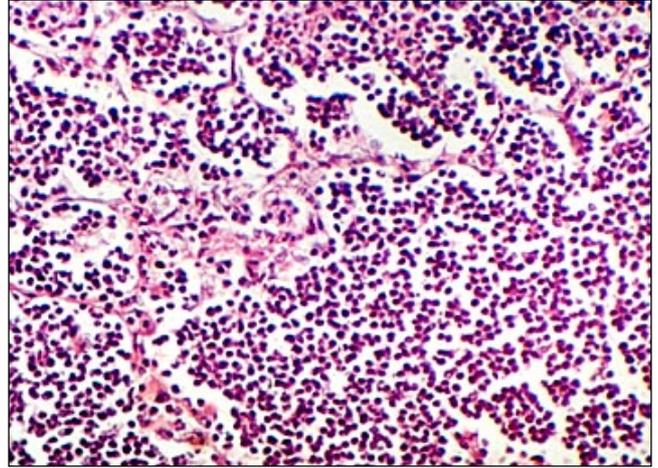


Fig 4. Section of lung showing suppurative pneumonia characterized by the presence of lysed and intact neutrophils in alveoli in a *Proteus* infected buffalo calf. (H. & E. x 400)

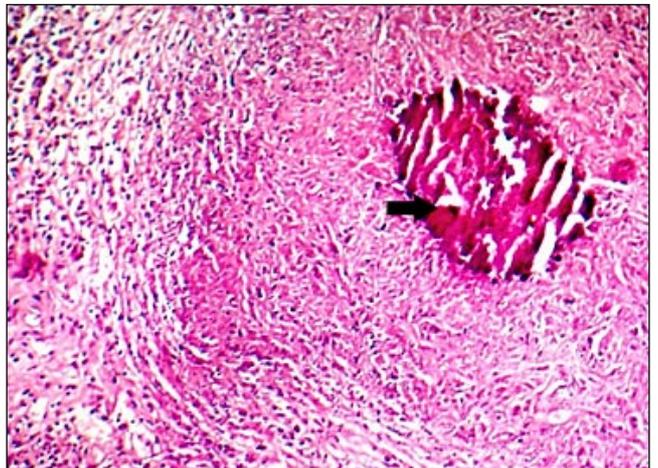


Fig 5. Lung section showing granuloma characterized by caseative necrosed mass along with calcification at the centre (arrow) surrounded by zone of epithelioid cells, macrophages and lymphocytes in a cow calf. (H. & E. x 200)

macrophages along with the giant cells. Serofibrinous pneumonia was characterized by the presence of serous fluid containing neutrophils, lymphocytes and fibrin mass along with thickening of interlobular septa. Interstitial pneumonia was characterized by the thickening of alveolar wall due to presence of the lymphocytes and congestion in capillaries. The compensatory emphysema was also evident in some areas. Taylor's stain of sections from lungs affected with interstitial pneumonia revealed presence of red rod shaped bacilli in the exudate and in the vicinity of alveolar epithelium.

Regarding pulmonary lesions, serofibrinous pneumonia has also been reported by other workers

(Arora and Kalra, 1973; Jubb *et al.*, 1993). *K. pneumoniae* has been found to be associated with septicaemia and reported to cause suppurative bronchopneumonia and hepatitis in calves (Verma *et al.*, 1980; Jubb *et al.*, 1993). The lesions in which no etiological agent was isolated might be due to certain respiratory viral infections.

**Tuberculosis:** One case of a cow calf of age six months was suffering from tuberculosis as evidenced by histopathological lesions in lungs and demonstration of acid fast organism by special staining method. This calf also had concurrent infection of *E. coli*. Grossly, lungs revealed greyish white nodules consisting of cheesy and calcified mass and remaining parts of the lungs were showing hepatization. Microscopically, lungs revealed the presence of granuloma characterized by areas of caseative necrosis along with calcification at the center surrounded by zone of epithelioid cells, macrophages and lymphocytes (Fig. 5). The calcification was confirmed by Von Kossa's stain where calcified areas appeared black in colour. Tuberculosis organisms could be demonstrated in sections by Ziehl Neelsen stain which appeared as red colour coccobacilli undistinguishable from tuberculous bacilli. Pulmonary tuberculosis in calves of more than a few weeks of age has been described by Jubb *et al.* (1985). It might be due to congenital infection or due to drinking of milk infected by TB organisms and crowded conditions may play a major role particularly in pulmonary infection.

On the basis of the results of this study, it may be concluded that most of the lesions in liver and lungs of buffalo calves and cow calves were circulatory (haemorrhages) and inflammatory in nature and *E. coli* infection emerged as the major infection involved in the calf mortality.

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