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INCIDENCE AND HISTOPATHOLOGICAL STUDY OF COLIBACILLOSIS ON LIVER OF BUFFALO CALVES

Asopa Shesh[1]., Joshi, A[3]., Maria, A[2*]., Rani, S[1]. Dadhich, H[1]. Nazir, A. Bumla[4] and Sharma, S[3].
[1] *Department of Veterinary Pathology, RAJUVAS, Bikaner-334 001 (Raj.), India.*
[2] *Department of Veterinary Parasitology, ACVM-Jaipur India.302032.*
[3] *Department of Veterinary Physiology , RAJUVAS, Bikaner-334 001 (Raj.), India.*
[4] *Department of Livestock Product, ACVM-jaipur (Raj.), India.302032.*

**Corresponding author: mariaabbas122@gmail.com*

Colibacillosis occurs as an acute fatal septicaemic disease of young buffalo calves and is seen worldwide. The main determinant of the disease is decreased immune status of the young one along with improper management and stress factors, which also enhances the growth of opportunistic bacteria. The colisepticemic form of colibacillosis usually associated with an *E. coli* bacteraemia and results in the rapid death of the calf. The present paper highlighted the incidence and pathology of *E. coli* infection on liver of buffalo calves. The incidence of colibacillosis affecting liver was recorded as 11.52 per cent. Grossly, the affected liver showed focal areas of haemorrhages and presence of grayish, white, necrotic foci. Microscopically, liver sections showed varying degree of degenerative changes *viz.* fatty changes and coagulative necrosis, hyperplasia and proliferation of bile duct and dilated, blood filled hepatic sinusoids along with congested central vein. Some sections revealed hepatitis due to focal areas of infiltrating cells, predominantly neutrophils and few macrophages.

Key Words: Colibacillosis, Buffalo calves, Septicaemia, Liver.

The effective development of any livestock industry depends on the prevention and control of diseases among the animals. The rearing of buffalo calves is extremely delicate and so requires a lot of care, particularly for the prevention of diseases. Buffalo calves suffer from high mortality than cow calves (Tomar and Tripathi, 1991). Diarrhoea is a major problem in livestock production in Egypt and throughout the world (Farid *et al.*, 2001 and Ibrahim, 2007). Among bacterial pathogens, *E. coli* was the most prevalent agent observed in diarrhoeic buffalo calves and was often isolated alone. Buffalo calf scours, continued to pose a major health and threat for the buffalo-breeding (Nizza *et al.*, 2010). Diarrhoea due to *Escherichia coli* (also known as scours) is one of the most common diseases of young buffalo calves, despite vaccination programs, management measures and treatment with antibiotics and fluid therapy. Identification of infectious agents that cause scours is essential for implementation of effective prevention and treatment. (Radostits *et al.*, 2007).

E. coli produces enterotoxic and septicaemic colibacillosis in young buffalo calves. Calves that are deficient in immunoglobulins are most susceptible to colisepticaemic form (White and Andrews, 1986). The livestock owner sustains considerable loss, not only due to death of many young animals but also on the expensive treatment of the affected and loss in condition of the surviving animals. Mortality due to *E. coli* infection in buffalo calves was reported to be 25.49 per cent (Abd-Elrahman, 2011). The organism *E. coli* is ubiquitous and becomes pathogenic under certain conditions such as errors in feeding and management, inadequate housing, exposure to extremes of weather, insufficiency of colostrum, congenital weakness and inefficient production of antibodies. These conditions predisposes the animal to infection, enhances the growth of

opportunistic bacteria and therefore infection flares up. By invading the wall of small intestine, *E. coli* destroys the epithelium and causes bacteraemia and localizes in other organs including liver. The present report deals with the incidence and histopathological manifestations of colibacillosis on liver of buffalo calves.

MATERIALS AND METHODS

For present study, a total of 610 buffalo calves of below 6 weeks of age, irrespective of sex and breeds were examined from different slaughter houses of Rajasthan. Out of these, 68 buffalo calves suspected for colibacillosis were further processed for bacteriological and histopathological examination. Samples were collected in duplicate, one for bacteriological and another for histopathological examination.

Isolation and identification of *E. coli* organism from liver of buffalo calves:

For bacteriological examination, sterile cotton swabs were used for aseptic collection of the samples from the sites showing visible abnormality at necropsy for the bacterial isolation and streaked on Mac-Conkey agar petriplates within two hours of sampling. The inoculated plates were incubated at 37^0 C for 24 hours. After this, single pink colony from the Mac-Conkey lactose agar showing lactose fermentation was fished out and streaked on Eosine Methylene Blue (EMB) agar petriplates. The inoculated plates were then incubated at 37^0 C for 24 hours. From EMB agar the colonies showing metallic luster, were further subjected to primary and secondary identification tests as per the method described by Cowan and Steel (1965) and Carter (1975).

Histopathological Study:

After confirmation of bacteriological examination, liver samples were processed for histopathological examination. Samples were examined grossly for alteration in morphology in terms of shape, size, colour, consistency, odour, location and type of the lesions. As far as possible, the colour of liver was noted immediately after collection and prior to fixation. The samples were promptly preserved in 10 per cent formal saline and processed mechanically for paraffin embedding by Acetone and Benzene technique (Lillie, 1965). The sections of 4-6 micron were cut and stained with Haematoxylin and Eosin.

RESULTS AND DISCUSSION

In the present study, total 217 *E. coli* isolates were obtained from different organs, out of which, 25 were isolated from liver. Therefore, the incidence of colibacillosis affecting liver was recorded as 11.52 per cent. A higher incidence was recorded by Verma and Kalra (1975) as 33.33 per cent and Seema (2007) as 19.11 per cent.

Bacteriological Study:

The isolation and identification of bacteria (*Escherichia coli*) was carried out from 25 samples of liver. The 25 tissue samples inoculated on Mac-Conkey agar plates revealed the presence of lactose fermenting pink colonies (Fig. 1).

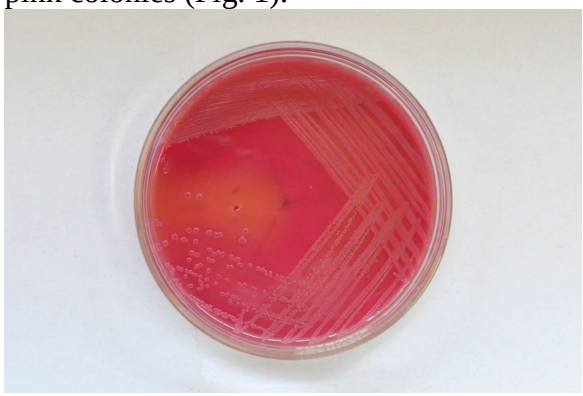

Fig.1 Photograph of MacConkey lactose agar petriplate showing pink colonies of bacteria.

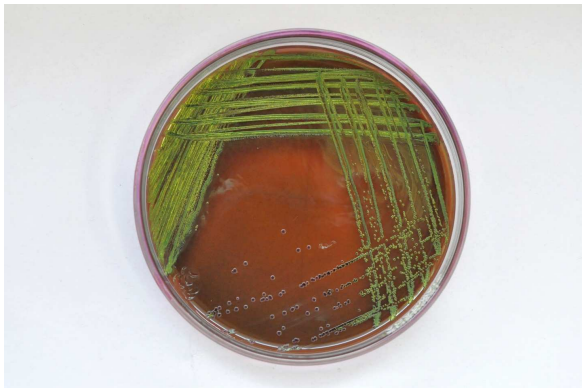

Fig.2 Photograph of *Escherichia coli* colonies showing metallic sheen on EMB petriplates.

The bacteria subcultured on EMB agar plates resulted into colonies exhibiting

metallic sheen, a characteristic feature of *Escherichia coli* (Fig. 2). These organisms when subjected to primary identification tests revealed following properties:

Gram Reaction	G(negative)
Morphology	Bacilli
Catalase	+
Oxidase	-
O/F Test	Fermentative
Growth in TSI slant	A/A Gas
Indole	Positive
M.R.	Positive
V.P.	Negative
Citrate	Negative

Histopathological Study:

Grossly, there were presence of grayish, white, necrotic foci and haemorrhages on the affected liver surface. Similar observations have been made by Singh *et al.* (1996) and Seema (2007). Microscopically, hepatocytes revealed varying degree of degenerative changes *viz.* fatty changes and coagulative necrosis (Fig. 3).

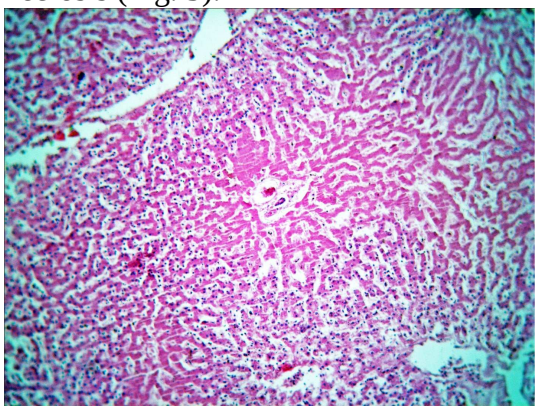

Fig. 3 Microphotograph of liver showing coagulative necrosis in hepatocytes around the central vein. H&E, 100 X

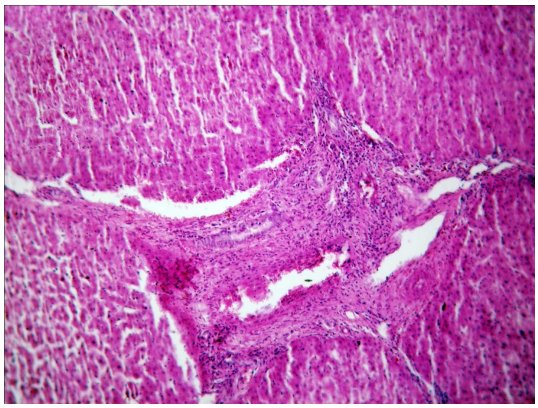

Fig.4 Microphotograph of liver showing marked infiltration of neutrophils and few macrophages in triad area. H&E, 100 X

Some sections revealed infiltration of neutrophils and macrophages in triad area (Fig. 4). Hepatic sinusoids were dilated and filled with blood along with congested central vein. Hepatitis due to focal areas of infiltrating cells, mainly neutrophils and few macrophages and lymphocytes and hyperplasia and proliferation of bile duct (Fig. 5) were also observed. Almost similar findings were observed by Singh and Singh (1983), Singh *et al.* (1996), Sastry (2001) and Jubb *et al.* (2007).

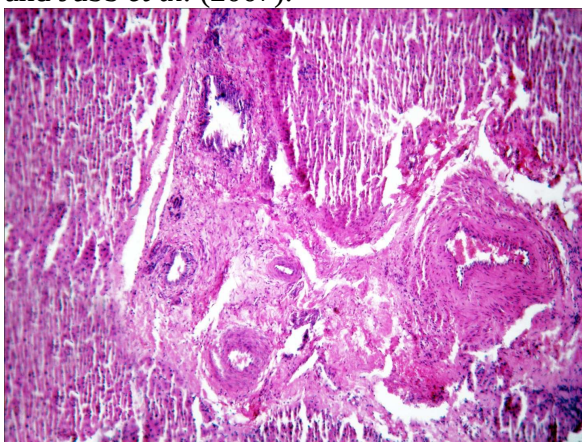

Fig. 5 Microphotograph of liver showing hyperplasia and proliferation of bile duct. H&E, 100 X

Above findings may be in accordance to the fact that liver functions are depressed due to blocking of bile secretion and pressure on hepatocytes by bacterial emboli in *E. coli* infection (Golovko *et al.*, 1984). Dilatation and congestion of hepatic sinusoids, hepatitis and petechial haemorrhages were occurred due to toxaemic effect of *E. coli* infection.

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