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Research Article

PATHOLOGICAL INVESTIGATION OF GASTROINTESTINAL TRACT DISORDERS OF BOVINES

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ABSTRACT

The present study is an attempt to elucidate pathological investigation of gastrointestinal tract disorders of bovines. The investigations included detailed necropsy, isolation and identification of bacteria, *in-vitro* drug sensitivity and gross and histopathology studies on 30 bovine carcasses. Maximum isolations were from intestine followed by liver and heart blood. The bacterial organisms isolated were *Escherichia coli*, *Salmonella* Typhimurium*, Klebsiella pneumoniae*, *Pasteurella haemolytica, Proteus mirabilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*. *In-vitro* drug sensitivity revealed that most of these bacteria were sensitive to amikacin and resistant to tetracycline. Pathological studies revealed congestion and haemorrhages in various organs, cirrhosis, serofibrinous pneumonia, catarrhal or haemorrhagic enteritis, oedema and emphysema in lungs, congestion in kidneys, myocarditis and fibrinous pericarditis.

Key words: Bovine mortality, gastrointestinal tract disorders, pathology, *Escherichia coli, Salmonella* Typhimurium

India is predominantly an agricultural country with about 70% of its population dependent on income from agriculture. India is endowed with the largest livestock population in the world. The effective development of livestock industry depends upon the prevention and control of diseases among the animals. A number of viral, bacterial and parasitic diseases result in the mortality and decline in overall production of bovines. Among bacterial infections, salmonellosis and colibacillosis cause heavy mortality. To better define cause of the death and combat rising mortality, complete postmortem examination is must. For this purpose, a study was planned on pathological investigation of gastrointestinal tract disorders in bovines.

MATERIALS AND METHODS

A study was conducted on 30 buffaloes/ cows or calves brought to the Department for the postmortem examination. After examination of the exterior, the carcasses were opened to examine gross pathological alterations in various internal organs. At the time of post mortem, material for bacteriological studies was collected aseptically. Isolation of organisms was attempted from the heart blood, liver, lungs and intestinal contents (Cruickshank *et al.,* 1965). Bacterial growth was identified on the basis of colony characteristics, Gram's staining, haemolysis on blood agar and biochemical reactions. The organisms isolated were subjected to *in-vitro* drug sensitivity testing using various antimicrobials by the disc diffusion method (Bauer *et al.,* 1966).

Representative tissue samples from organs, which revealed lesions particularly on heart, liver, spleen, intestine, lung and kidneys were collected in 10 % buffered formalin for histopathological examination. The formalin fixed tissues were processed and embedded in paraffin wax. Paraffin sections of thickness 4-5 µ were stained with routine haematoxylin and eosin stain using Lilly Mayer's haematoxylin and 2 % water soluble eosin (Luna, 1968).

RESULTS AND DISCUSSION

Out of 30 bovine carcasses, different pathogens were isolated from intestine, liver, lung and heart blood of 23 cases. Bacterial organisms could not be isolated from seven cases. In three of the remaining seven cases, cysts suspected of *Echinococcus granulosus* were observed in liver, spleen and lungs.

Bacteriological studies: *Escherichia coli* (*E. coli*) was the most prominent organism isolated from different organs followed by *Salmonella* Typhimurium*, Staphylococcus aureus*, *Pasteurella haemolytica, Streptococcus pyogenes*, *Proteus mirabilis* and *Klebsiella pneumoniae.* Different bacteria isolated from different age group of animals affected are shown in Table 1.

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Maximum number of isolations was from the intestines followed by heart blood and liver (Table 2). Isolations from heart blood indicated bacteremia or septicaemia. In these cases, lesions were seen in number of organs indicating systemic infection. Various workers have also reported the isolation of these organisms from the carcasses of bovines (Verma *et al.,* 2001; Carlson *et al*., 2002).

In-vitro drug sensitivity pattern revealed that *E. coli* isolates were most sensitive to amikacin followed by neomycin, polymixin B and nitrofurantoin but resistant to co-trimoxazole, lincomycin, amoxyclav and ceftriaxone (Table 3). *S.* Typhimurium was found highly sensitive to amikacin, ceftriaxone and neomycin whereas, resistant to chloramphenicol, lincomycin and tetracycline. *K. pneumoniae* and *P. haemolytica* revealed maximum sensitivity to many antimicrobials like chloramphenicol, ceftriaxone, neomycin, nitrofurantoin, streptomycin etc. while, both were found resistant to ampicillin, amoxyclav, cloxacillin and tetracycline. *P. mirabilis* was found most sensitive to ampicillin and gentamicin followed by amikacin, chloramphenicol and streptomycin but found resistant to tetracycline. Both *S. pyogenes* and *S. aureus* showed most sensitivity to enrofloxacin, kanamycin and gentamicin. More or less similar results have been reported earlier (Singh, 2005; Lehreena, 2008). These antibiotic sensitivity tests are useful in effective drug administration to reduce treatment cost and to prevent the diseases in a better way.

Pathological Studies

***E. coli* Infection:** *E. coli* infection alone was observed in 12 cases, while it was associated with *K. pneumoniae* (one case) and *P. mirabilis* (one case). Gross lesions observed were congestion, petechial haemorrhages, hepatomegaly and thin fibrinous covering on the liver. In most cases, lung lobes were congested and consolidated along with severe haemorrhagic enteritis. Petechial haemorrhages on the epicardium and endocardium were also observed. Tracheal lumen was found filled with foam and intestinal serosa was congested in concurrent infection of *E. coli* and *K. pneumoniae.* On the other hand, adhesions of liver to the visceral peritoneum, consolidation in lungs, congested and haemorrhagic mucosa of intestine and distended gall bladder were observed in concurrent infection of *E. coli* and *P. mirabilis*.

Microscopically, in most cases liver revealed fibrinous perihepatitis, pericellular cirrhosis, fatty changes, while in lungs, there was serous or serofibrinous exudate in the lumen of alveoli consisting of leucocytic infiltration mainly neutrophils and lymphocytes along with thickening of interlobular septa due to fibrinous exudate. In intestine there was desquamation of mucosal epithelium in some cases and mononuclear cell infiltration in mucosa replacing villi giving sheet like appearance along with goblet cell hyperplasia. Myocarditis, fibrinous pericarditis and depletion of lymphocytes in white pulp of spleen were also observed. Similar hepatic and/or pulmonary, intestinal, cardiac lesions due to collibacillosis have also been described by Jubb *et al.* (1985, 1993) and Khan and Khan (1997) in bovines. Some serotypes of *E. coli* colonise the intestine and elaborate the toxin, thus are enterotoxic in nature. The strains which do not elaborate toxin, adhere closely to the cells and produces intense inflammatory reaction in various organs like liver, intestine, lungs etc. (Greenwood *et al*., 2002).

***Proteus* Infection:** Grossly liver was enlarged and pale with firm consistency as also reported by Lehreena *et al.* (2010). Focal areas of congestion and consolidation were

Table 1
Isolation of bacteria in relation to age group of animal affected

Bacteria isolates	Age groups					
	<1 month	1-6 months	6-12 months	1-3 years	3-6 years	≥ 6 years
E. coli	3	1	1	1	2	4
S. Typhimurium	-	-	2	2	-	-
Klebsiella pneumoniae	1	-	-	-	-	-
Pasturella haemolytica	1	-	1	-	-	-
Proteus mirabilis	-	-	-	-	-	-
Staphylococcus aureus	-	-	1	-	-	-
Streptococcus pyogenes	-	-	-	1	-	-
E. coli + *K. pneumoniae*	-	-	-	-	-	1
E. coli + *P. mirabilis*	-	-	1	-	-	-

Table 2
Bacteria isolated from various organs and heart blood of the bovine carcasses

Bacteria	Intestine	Liver	Heart blood	Lung	Total (%)
E. coli	14	9	11	2	36 (56)
S. Typhimurium	3	2	1	2	8 (13)
S. aureus	0	1	4	2	7 (11)
P. haemolytica	1	1	1	1	4 (6)
S. pyogenes	1	1	1	1	4 (6)
P. mirabilis	2	1	0	0	3 (4)
K. pneumoniae	1	0	0	1	2 (3)
Total (%)	22 (34.38)	15 (23.40)	18 (28.12)	9 (14.06)	64

observed in cardiac and apical lobes of lungs. Microscopically, central vein and sinusoids in liver were found severely congested along with infiltration of neutrophils and lymphocytes. Lungs revealed changes of red hepatization along with infiltration of neutrophils and lymphocytes in the alveoli similar to findings of Lehreena *et al.* (2010). The production of hemolysin, urease enzyme, fimbriae and flagellum-mediated motility has been postulated as virulence factors to cause fever, bacteremia and death (Bahrani *et al.*, 1991).

***Klebsiella* Infection:** Grossly there was hepatomegaly and irregular foci of congestion and consolidation in different lobes of the lungs. Microscopically liver revealed hepatitis, bile duct hyperplasia and fatty changes. Intestine revealed enteritis characterized by congestion, desquamation of villi and infiltration of lymphocytes in lamina propria (Fig. 1). Interstitial pneumonia and tracheitis characterized by leucocytic infiltration in mucosa were seen. There was congestion and depletion of lymphocytes in white pulp of spleen along with reticuloendothelial cell proliferation. The pneumonic lesions in lungs, and hepatitis and fatty changes in liver were similar to the findings of Singh *et al.* (2006) and Lehreena *et al.* (2010). Factors that are implicated in the virulence include the capsule, lipopolysaccharide, iron scavenging systems, fimbrial and non-fimbrial adhesins (Williams and Tomas, 1990; Brisse *et al.,* 2006).

***Staphylococcus* Infection:** Grossly, there was congestion, haemorrhage and hepatomegaly in liver as reported by Lehreena *et al.* (2010). Congestion and consolidation of cranioventral lobes of the lungs, epicardial and endocardial haemorrahges, fibrinous covering over pericardium along with oedematous fluid was also observed.

Microscopically, there was hepatitis characterized by necrotic hepatocytes along with infiltration of

Table 3
***In-vitro* drug sensitivity (% sensitivity) of various bacteria isolated from different organs to different antimicrobials**

Drug/concentration (mcg)	Percent sensitivity of indicated bacteria to different antimicrobials						
	E. coli (n=36)	*S.* typhi-murium*(n=8)*	*K. pneu-moniae (n=2)*	*P. haemolytica (n=4)*	*P. mirabilis (n=3)*	*S. aureus (n=7)*	*S. pyogenes (n=4)*
Amikacin (30)	94.12	100.00	50.00	100.00	66.67	00.00	100.00
Ampicillin (10)	35.29	20.00	00.00	00.00	100.00	85.71	100.00
Amoxyclav (10)	11.76	20.00	00.00	00.00	33.33	14.28	25.00
Cloxacillin (10)	23.52	20.00	00.00	00.00	33.33	28.56	50.00
Chloramphenicol (30)	26.47	00.00	100.00	00.00	66.67	71.42	75.00
Ceftriaxone (30)	11.76	60.00	100.00	100.00	66.67	100.00	50.0
Ciprofloxacin (10)	35.29	40.00	50.00	100.00	33.33	28.56	25.00
Cefotaxime (30)	23.52	00.00	00.00	100.00	66.67	100.00	100.00
Cephaloridine (30)	41.17	20.00	100.00	100.00	33.33	42.86	50.00
Colistin (25)	17.64	00.00	100.00	100.00	66.67	57.14	75.00
Cotrimoxazole (25)	00.00	40.00	100.00	100.00	33.33	42.86	50.00
Enrofloxacin (10)	35.29	40.00	100.00	100.00	66.67	100.00	100.00
Gentamicin (10)	29.41	60.00	100.00	100.00	100.00	100.00	100.00
Kanamycin (30)	23.52	40.00	50.00	100.00	66.67	100.00	100.00
Lincomycin (15)	5.88	00.00	00.00	100.00	33.33	57.14	50.00
Neomycin (30)	88.23	100.00	100.00	100.00	33.33	100.00	100.00
Nitrofurantoin (200)	76.47	20.00	100.00	100.00	66.67	71.42	100.00
Ofloxacin (5)	35.29	40.00	100.00	100.00	66.67	71.42	75.00
Polymyxin B (25 units/ disc)	88.23	60.00	100.00	100.00	33.33	42.86	25.00
Streptomycin (25)	17.65	40.00	100.00	100.00	66.67	00.00	00.00
Tetracycline (30)	35.29	00.00	00.00	00.00	00.00	100.00	00.00

heterophils and macrophages. Interstitial pneumonia was observed in lungs characterized by thickening of inter alveolar septa due to the presence of erythrocytes, lymphocytes and neutrophils in the interstitial tissue as reported by Lehreena *et al.* (2010). In intestine there was necrosis of mucosal glands, denuded mucosa due to ulcer formation in most of the cases along with goblet cell hyperplasia and depletion of lymphocytes in Peyer's patches in some cases. In heart there was necrosis and fragmentation of cardiac muscle fibers with infiltration of leucocytes, while in kidneys only coagulative necrosis of renal tubules was observed along with mild leucocytic infiltration. The invasion of host tissues by staphylococci involves the production of a large number of extracellular proteins, exotoxins and endotoxins which are responsible for various lesions in visceral organs (Bhakdi and Tranum-Jensen, 1991).

***Streptococcus* Infection:** Gross lesions observed were congestion in lung, pericarditis and red and swollen intestinal mucosa. Microscopically, liver revealed congestion and haemorrhages similar to findings of Lehreena *et al.* (2010). Intestine showed haemorrhagic enteritis along with desquamation of villi. Heart revealed pericarditis characterized by infiltration of lymphocytes and neutrophils in pericardium. The virulence factors of the organism are adherence, evasion of host immunity and tissue damage. Most strains of *S. pyogenes* produce one or more toxins that are called pyrogenic exotoxins which induce fever and septicaemia (Kilian, 1998).

***Salmonella* Infection:** Grossly, there was mottling of liver along with necrotic foci, consolidation of lungs, petechial haemorrhages on the epicardium and fibrinous pericarditis. Severe congestion of the intestinal serosa and haemorrhagic enteritis along with congested and engorged mesenteric veins were also observed.

Microscopically, liver showed changes of cirrhosis with diffuse fatty changes, fibrosis and infiltration of neutrophils, lymphocytes and macrophages leading to pseudolobulation (Fig. 2). Interstitial pneumonia, haemorrhagic enteritis, loss of villi and fibrinous pericarditis were also observed. These lesions in visceral organs observed were similar to the observations of Singh (2005). This bacterium invades the intestinal epithelium in the terminal ileum, resulting in exfoliation of epithelial cells and stunting of villi (Frost *et al.,* 1997).

***Pasteurella* Infection:** Grossly there was haemorrhagic tracheitis with serosanguinous fluid in lumen of trachea, pneumonia with red hepatization and consolidation particularly in apical and cardiac lobes of the lungs. Haemorrhages on epicardium and intestines were also observed.

Microscopic lesions observed were haemorrhagic tracheitis characterized by desquamation of mucosal epithelium with leucocytic infiltration mainly neutrophils and lymphocytes in mucosa. Serous pneumonia characterized by presence of serous exudate containing inflammatory cells predominantly neutrophils and a few lymphocytes were observed in lung parenchyma. Lesions seen in trachea and lungs were in accordance with the findings of Jubb *et al.* (1993). Haemorrhagic enteritis and myocarditis were also seen. According to Quinn *et al.* (1994), the endotoxins produced by *P. haemolytica* (now known as *Mannheimia haemolytica*) might be responsible for septicaemia and various lesions in the visceral organs.

Two cases of buffalo and one case of cow revealed cysts in various organs like liver, lung and spleen which revealed lesions suspected of *Echinococcus granulosus* infection. Microscopically, in liver there were fibrosis characterized by fibrous connective tissue proliferation and infiltration of mononuclear cells and eosinophils around the cysts wall. Azlaf and Dakkak (2006) also observed the similar changes in liver and lungs of bovines affected with hydatid cysts.

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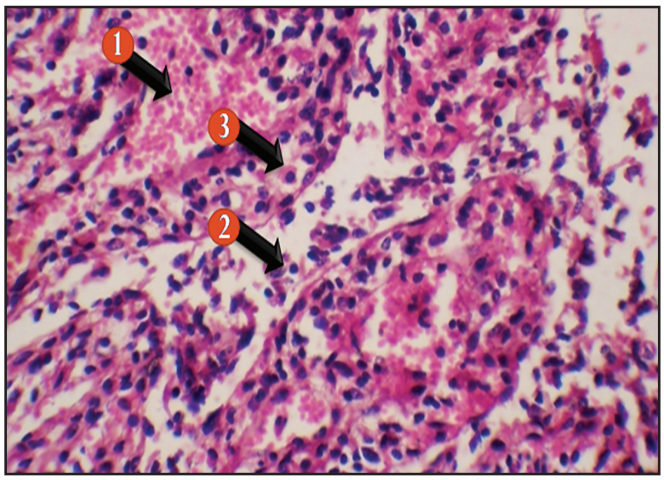

Fig 1. Mild enteritis characterized by congestion (1) and infiltration of lymphocytes (2) and macrophages (3) in between villi and lamina propria (*Klebsiella pneumoniae* was isolated)

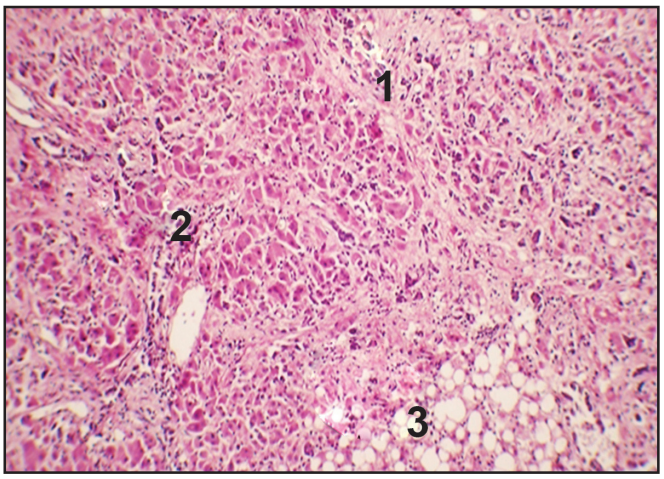

Fig 2. Cirrhosis in liver characterized by fibrous connective tissue around group of hepatocytes (1) along with infiltration of inflammatory cells (2) and fatty changes (3) in right lower portion (*Salmonella* Typhimurium was isolated)

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