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**Research Article**

# PATHOLOGICAL INVESTIGATION ON BUFFALO CALVES SUFFERING FROM GASTROINTESTINAL TRACT DISORDERS

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**ABSTRACT**

The present investigation was undertaken to study the pathology of gastrointestinal tract disorders in buffalo calves for which 49 calves up to one year of age were thoroughly examined for bacteriological isolations, gross and histopathological lesions. Of these, 43 calves showed various pathological conditions of gastrointestinal tract categorized as gastroenteritis associated with *Escherichia coli* (19 cases), enteritis associated with *E. coli, Proteus spp., Klebseilla spp., Salmonella* Typhimurium *4,5:i:1* (13 cases), enteritis due to ascariasis (4 cases), enteritis due to coccidiosis (3 cases), hepatitis associated with *Escherichia coli* (three cases) and gastritis associated with *E. coli* (one case). Gross lesions in visceral organs along with gastrointestinal tract revealed vascular changes, consolidation, necrotic changes and abscess formation. Histopathologically, lesions in intestine and abomasum were desquamation of epithelium, ulcer formation due to complete denudation of epithelium, lymphoid depletion in Peyer's patches, oedema and infiltration of mononuclear cells. Degenerative changes in liver alongwith micro-granuloma formation and bile duct hyperplasia were also seen. Mesenteric lymph nodes were reactive and exhibited depletion of lymphoid tissue and reticular cell proliferation. Spleen also showed reticular cell hyperplasia and depletion of lymphoid cells in white pulp. Lungs exhibited serofibrinous pneumonia, micro-granuloma, thickened pleura due to serofibrinous exudate and leucocytes, desquamation of bronchial epithelium, emphysema and atelectasis of alveoli. Heart showed hyaline degeneration, sacrocysts and focal necrosis of muscle fiber. Kidneys showed congestion and mild degenerative changes.

**Key words:** Pathology, buffalo calves, gastrointestinal tract disorders, enteritis, hepatitis

Disorders of gastro-intestinal tract are very important causes of morbidity and mortality in young buffalo calves and cause a great economic loss to buffalo owners (Roy *et al.*, 1997; Rathore, 1998; Saxena *et al.*, 2002). Gastro-enteritis, gastritis, enteritis, septicaemia, peritonitis and naval abscess are the common causes of death in young animals. Colibacillosis and colisepticaemia have been found to be most devastating, causing heavy mortality in neonatal calves. For the prevention and control of mortality in buffalo calves, it is desirable to know the etiology and clinico-pathological aspects of the disease conditions causing mortality in buffalo calves. The present work is aimed at etiopathological studies on gastro-intestinal tract disorders in buffalo calves.

## MATERIALS AND METHODS

A total of 49 buffalo calves received for necropsy in the Department were thoroughly examined for postmortem lesions. Of these, 43 cases showed lesions in gastro-intestinal tract. During the course of post mortem examination, representative tissues such as heart, lung, fore stomach, intestine, liver, spleen, mesenteric lymph nodes and kidneys were collected on ice and 10% formal saline for microbiological and histopathological studies, respectively. Identification of all isolates was done following the procedure of Quinn *et al.* (1994). The cultures suspected for *Salmonella* and *Escherichia coli* were sent to the Central Research Institute, Kasauli (H.P.) for further confirmation and typing. The formalin fixed tissues were processed and embedded in paraffin wax. Paraffin sections were cut at the thickness of 4-5 µ and stained with routine haematoxylin and eosin stain using Lilly Mayer's haematoxylin and 2% water soluble eosin (Luna, 1968).

## RESULTS AND DISCUSSION

Out of 49 calves, 43 calves showed pathology in gastro-intestinal tract. Main pathological conditions found were gastroenteritis in 19 cases (*E. coli*), enteritis associated with *E. coli, Proteus spp., Klebseilla spp., Salmonella* Typhimurium *4, 5:i:1* (13 cases), ascariasis (four cases), coccidiosis (three cases), hepatitis associated with *E. coli* (three cases) and gastritis associated with *E. coli* (one case). These findings are supported by Roy *et al*. (1997). A relatively high rate of *E. coli* infection in buffalo calves (36 cases) may be due to errors in colostrum

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feeding, inefficient production of antibodies and stress which enhance the growth of opportunistic bacteria and therefore, flare up the infection. The macroscopic and the microscopic findings are discussed below:

**Gastritis:** Lesions suggestive of gastritis were observed in one case from which *E. coli* serotype 02 was isolated from abomasal and intestinal contents. Grossly, abomasal mucosa revealed the presence of ulcers of 5-6 mm in diameter with black margins (Fig. 1). The mucosa was highly congested with blood tinged contents. Microscopically, abomasal mucosa revealed extensive areas of coagulative necrosis. Some part of mucosa was lost indicating ulcer formation. Lamina propria under necrosed tissue was exposed and revealed congestion. The adjoining area in the muscular tissue showed the infiltration of lymphocytes.

**Enteritis and Gastroenteritis:** Lesions suggestive of enteritis and gastroenteritis were observed in 32 buffalo calves (74.4%). Main infectious agent isolated were *E. coli*, *S.* Typhimurium, *Proteus* spp. and *Klebsiella* spp. The details are:

**Enteritis due to *E. coli* Infection:** *E. coli* alone was isolated from heart blood of 19 carcasses of buffalo calves (41.7%). In 13 cases there was mixed infection of *E. coli* with *S.* Typhimurium, *Proteus* spp. and *Klebsiella* spp. *E. coli* was the most common bacteria isolated from fecal samples of diarrheic calves in previous studies (China *et al.,* 1998; Harbby, 2002). Enteritis in newborn calves is known cause high morbidity and mortality rates, leading to significant economical losses in Egypt (Ashraf, 2007). *E. coli* serotype O25 was the most prevalent in this study followed by O9, O44, O101, O128 and others. Gross examination revealed diffuse congestion of intestinal mucosa leading to thickening. The intestinal contents were loose and mixed with mucus. The mesenteric lymph nodes appeared enlarged and congested (Fig. 2). Severe haemorrhages were present on splenic surface, kidney cortex, epicardium and endocardium. There were areas of congestion and consolidation in the lungs. The bronchiolar epithelium was desquamated and lumen contained an excessive amount of thick mucus and desquamated epithelial cells. The liver appeared congested with petechial haemorrhages along with thin fibrinous covering. In addition, congestion of peritoneum and an increased amount of fluid in the pericardial and peritoneal cavity were observed in one case.

Microscopically, intestine revealed congested blood vessels in lamina propria and submucosa along with extensive stunting of villi. There were an increased number of reticular cells in the lamina propria of the villi in the distal ileum. Coagulative necrosis of intestinal epithelium and goblet cell hyperplasia were observed (Fig. 3). The crypts of the mucosal glands were atrophied and

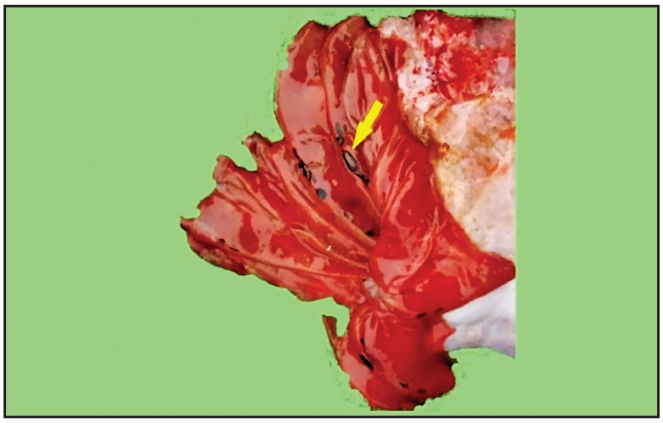

Fig 1. Ulcer and reddened mucosa in abomasal mucosa of a buffalo calf.

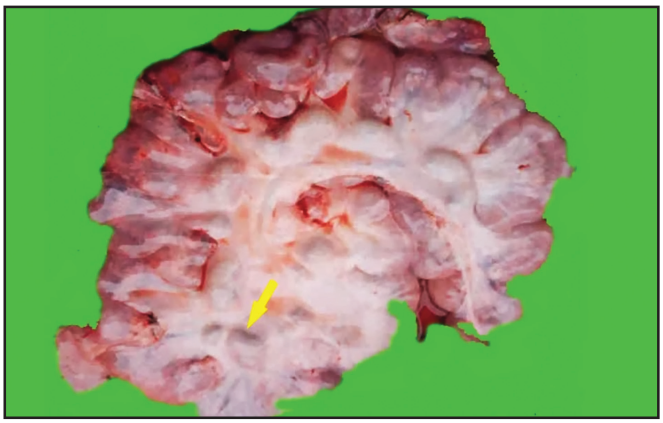

Fig 2. Enlargement of mesenteric lymphnodes in a buffalo calf.

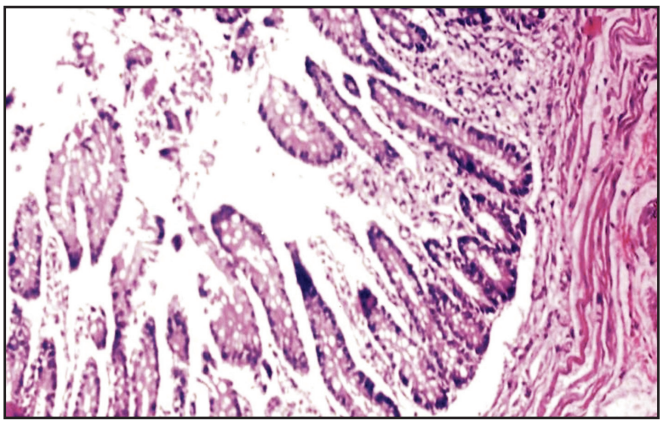

Fig 3. Desquamation of mucosal epithelium with goblet cell hyperplasia with leucocytic infiltration in intestine. (H.&E. X33)

surrounded by leucocytes particularly neutrophils and macrophages. There was depletion of lymphocytes in mesenteric lymph nodes (Fig. 4) and spleen. Haemorrhages were present in the parenchyma of liver and the bile duct epithelium was hyperplastic in some cases. Perivascular lymphoid infiltration and hyperplasia of Kupffer cells was evident. Congestion and oedema were observed in myocardium along with thickened pericardium due to serofibrinous exudate. At places, haemorrhages between myocardial muscle fibers were evident. There was congestion and haemorrhages in kidney parenchyma along with perivascular fibroblast proliferation and infiltration in interstitial tissues particularly by lymphocytes. There was congestion of blood vessels along with fibrinous exudate in the alveoli and interstitial tissues. Mild leucocytic infiltration particularly by lymphocytes in interalveolar septae leading to their thickening.

**Enteritis due to *Salmonella* Infection:** *Salmonella* sp. (*S.* Typhimurium *4, 5:i:1, 2*) was isolated from three buffalo calves. Macroscopic examination revealed congested intestinal mucosa of duodenum and jejunum and intestinal contents were loose in consistency. Oesophageal mucosa appeared mildly congested. Spleen appeared congested and petechial haemorrhages were present on its surface. Focal congestion and consolidation of lung was present affecting apical and cardiac lobes. The bronchial mucosa appeared congested.

Microscopic examination showed congestion in mucosa, submucosa and serosa of intestines, desquamation and stunting of villi along with their fusion. At places, mucosal glands were necrosed and were surrounded by lymphocytes and macrophages (Fig. 5). Marked goblet cell hyperplasia was seen in mucosa and submucosa along with leucocytic infiltration particularly by lymphocytes and few neutrophils. There were erosion and ulceration of mucosal epithelium of oesophagus along with mild lymphocytic infiltration in submucosa. The central vein and hepatic sinusoids were congested along with degenerative changes in hepatocytes. The bile duct epithelium appeared hyperplastic. Liver also revealed lesion resembling microgranuloma surrounded by hyperplastic Kupffer cells and giant cells (Fig. 6). In the portal areas, perivascular mononuclear cell infiltration was observed.In the cortex of mesenteric lymphnodes, there was an evidence of increased number of lymphoid aggregates.The capillaries in lung parenchyma appeared congested and alveolar lumen was filled with serofibrinous exudate. In most of the alveoli, the lining epithelium appeared hypertrophied assuming the structure of cuboidal epithelium. Areas of emphysema with some broken alveoli were also seen. The bronchial epithelium revealed marked hyperplastic alteration and desquamation. The splenic parenchyma appeared moderately congested along with hyperplasia of reticuloendothelial cells.The glomeruli as well as capillaries in the renal parenchyma were dilated and full of RBC's.

**Hepatitis:** The lesions suggestive of hepatitis were observed in three buffalo calves from which *E. coli* was isolated. Macroscopic examination revealed pale and enlarged liver. A fibrinous covering and small necrotic foci were present on the surface of liver. Irregular patches of congestion and consolidation were observed in all lobes of both the lungs. The mucosa of the small intestine appeared congested and the intestinal contents were of loose consistency. Numerous petechiae were observed on the cortical surface of kidneys. The spleen appeared congested. Mesenteric lymph nodes were enlarged and congested.

Microscopic examination of liver revealed that central vein and hepatic sinusoids were congested and dilated along with fatty changes in hepatocytes. In one case, there were foci of coagulative necrosis with pyknotic and hyperchromatic nuclei. The bile duct epithelium was hyperplastic and in some places, it was desquamated. At places, tendency to form lymphoid follicles was observed. Lungs revealed acute congestion and presence of serous exudate in alveoli. There was infiltration of mononuclear cells in the inter-alveolar septa leading to thickening of walls of alveoli. Intestine revealed mild congestion. The glomeruli and other capillaries were congested. There were haemorrhages in interstitial connective tissue both in cortex and medulla.

**Ascariasis:** *Ascaris* infestation was observed in four buffalo calves. Grossly, intestine revealed adult *Neoascaris vitulorum* worms present in its lumen. The intestinal mucosa was congested with contents mixed with traces of bloods. Mesenteric lymph nodes appeared oedematous and congested leading to its enlargement.The liver appeared somewhat swollen and pale in colour. On its surface, petechial haemorrhages were observed. The ecchymotic haemorrhages were present on the cortical surface of both the kidneys.

Microscopic examination revealed extensive desquamation of mucosal epithelium resulting into

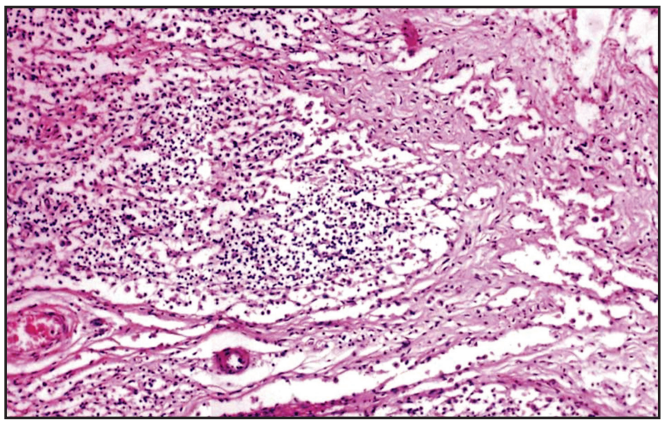

Fig 4. Depletion of lymphocytes and fibroblasts proliferation in medullary portion of mesenteric lymphnodes. (H.&E. X33)

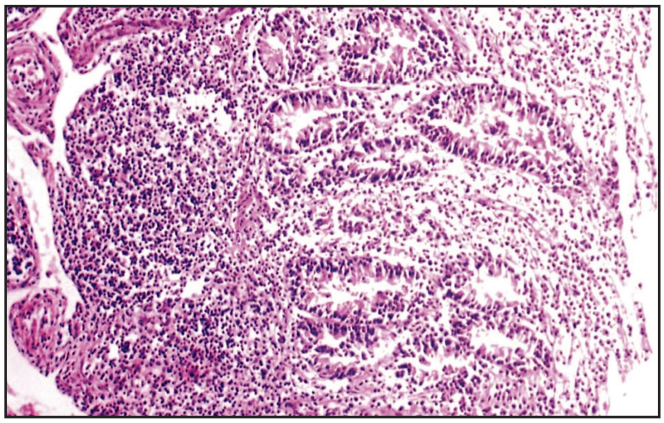

Fig 5. Severe infiltration, necrosis of secretary glands and their replacement by lymphocytes and macrophages in intestine. (H.&E. X33)

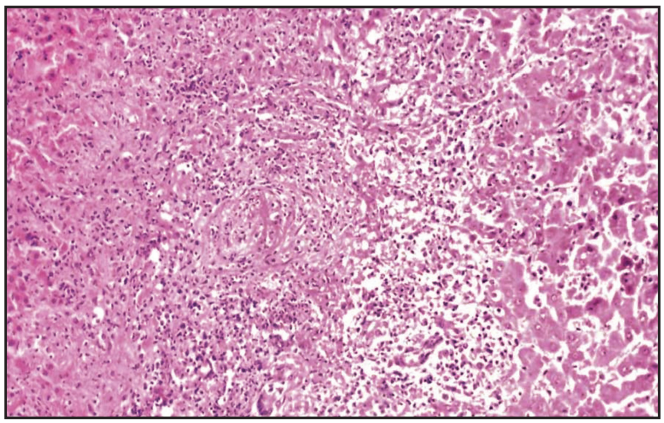

Fig 6. Microgranuloma surrounded by hyperplastic Kupffer cells and giant cells in liver parenchyma. (H.&E. X33)

disorganized appearance of villus structures in intestines. The blood vessels in the mucosa and submucosa were congested. The lamina propria revealed small areas of haemorrhages along with the infiltration of neutrophils and few eosinophils. The mesenteric lymph nodes revealed mild lymphoid depletion with congested capillaries. The bile duct was very much dilated and its wall was much thickened. The lining epithelium was desquamated extensively. Around the bile duct, there was proliferation of fibrous connective tissue and mononuclear cell infiltration along with a few eosinophils. The hepatic structure was partially disorganized and the hepatic cells revealed focal necrosis. Due to migrating ascarid larvae, lesions are also seen in the liver and lungs including scarring of liver with diffuse fibrosis and interstitial pneumonia. In ascariasis, degenerative and extensive vascular changes were invariably present in the kidneys. This may possibly be due to the toxins liberated by the parasites (*Ascaris*). The production of toxins by *Ascaris* spp. has already been reported by Srivastava (1963) and Gupta *et al*. (1978).

**Coccidiosis:** Coccidiosis was observed in two cases. Macroscopic examination of the small intestine revealed focal congestion. The intestinal contents were loose, semi liquid in consistency and mixed with blood. The liver appeared somewhat enlarged and pale. The mesenteric lymph nodes were congested and enlarged. Other organs *viz.* kidneys, spleen, heart and lungs did not reveal gross pathological lesion.

On microscopic examination of the intestinal section, different development stages of oocysts in lining epithelium could be seen. The intestinal villus structure was disorganised alongwith extensive desquamation of the mucosal epithelium. The blood vessels in the mucosa and submucosa were acutely congested and petechial haemorrhages were noticed in lamina propria. There was infiltration of macrophages, eosinophils and a few neutrophils and lymphocytes at places in the mucosa. In general, the tissue changes were similar to those described by Jubb *et al*. (1993) and Charan and Pawalya (1997).

In this study, gross lesions were observed in intestinal tract, liver, lymph nodes, spleen, kidney, heart and lungs. Similar findings have also been reported by Libby *et al*. (1997), Khan and Khan (1997) and Carlson *et al*. (2002). More or less similar histopathological changes as observed in this study in intestine, abomasum and Peyer's patches have been reported by Singh *et al*. (1996) and Libby *et al*. (1997). The mesenteric lymph nodes showed depletion of lymphocytes and reticular cells proliferation in medulla replacing lymphoid tissue. Acute congestion of capillaries was also seen. The observations of Maity *et al*. (2000) support these findings.

Changes observed in liver and spleen in this study are in conformity with Jubb *et al.* (1993), Libby *et al*. (1997) and Alam *et al*. (2001). Heart exhibited congestion of capillaries and thickening of pericardium due to serofibrinous exudate. Singh *et al*. (1996) and Khan and Khan (1997) have also reported the similar changes in heart. Pathological changes in lungs were similar to these reported by Jubb *et al.* (1993), Singh *et al*. (1996) and Libby *et al*. (1997). Trachea and kidneys showed vascular and degenerative changes. Observations of Khan and Khan (1997) support the present findings.

From the above study it can be concluded that affections of digestive system such as enteritis (due to colibacillosis, salmonellosis, ascariasis and coccidiosis), gastroenteritis, hepatitis and gastritis caused maximum mortality among buffalo calves of less than one month of age. So they require adequate care and management such as feeding of colostrum, better health care and proper housing to avoid seasonal stresses. To minimize the prevalence of ascariasis and coccidiosis, deworming at proper age should be done.

## REFERENCES


Alam, J., Thakur, S.K., Sinha, B.K. and Prasad, L.N. (2001). Pathology of spontaneous liver affections in buffaloes. *Indian J. Vet. Pathol.* **25**: 85-86.

Ashraf, N.M.R. (2007). Enzootic gram negative bacteria associated with diarrhea in neonates in Egypt. Ph.D thesis, Dept. of Microbiol., Fac. Vet.Med., Alex. Univ., Egypt.

Carlson, S.A., Stoffregan, W.C. and Bolin, S.R. (2002). Abomasitis associated with multiple antibiotic resistant *Salmonella enterica* serotype Typhimurium phagetype DT 104. *Vet. Microbiol.* **85**: 233-240.

Charan, K. and Pawalya, R.V.S. (1997). Coccidiosis in buffalo calves. *Indian Vet. Med. J.* **21**: 154-156.

China, B., Pirson, V. and Mainil, J. (1998). Prevalence and molecular of attaching effacing *E. coli* among calf populations in Belgium. *Vet. Microbiol*. **63**: 249-259.

Gupta, P.P., Singh, B., Mandal, P.C., Gill, B.S. and Grewal, G.S. (1978). A postmortem study of mortality pattern in adult buffaloes in Punjab, India. *Indian J. Anim. Sci*. **48**: 669-673.

Harbby, H.A. (2002). Bacterial causes of diarrhea insmall animals (Kids, lambs and calves) in Sultanate of Oman. J. Egypt *Vet. Med. Assoc.* **62**: 227-235.

Jubb, K.V., Kennedy, P.C. and Palmer, N. (1993). Pathology of Domestic Animals. (4th edn.), Vol. 2. Academic Press Inc., San Diego, California.

Khan, A. and Khan, M.Z. (1997). Gross and histopathological findings in buffalo and bovine diarrhoeic neonates. *Veterinarski Archiv.* **67**: 203-213.

Libby, S.J., Adams, L.G., Ficht, T.A., Allen, C., Howard, A., Whitford, A.A., Buchmeier, N.A., Bossie, S., Guiney, G.D. (1997). *SPV* genes on the *Salmonella dublin* virulence plasmid are required for severe enteritis and systemic infection in natural host. *Infec. Immun.* **65**: 1786-1792.

Luna, L.G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. (3rd edn.), McGraw Hill Book Company, New York.

Maity, S.B., Deb, P., Das, R. and Som, T.L. (2000). Pathology of lymph nodes in cattle. *Indian J. Vet. Pathol.* **24**: 32-34.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (1994). Clinical Veterinary Microbiology. Wolfe Publishing Co., London, U.K.

Rathore, B.S. (1998). An epidemiological study on buffalo morbidity and mortality based on four year observations on 18, 630 buffaloes maintained at 28 livestock farms in India. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **19**: 43-49.

Roy, P.K., Ghosh, A., Pal, R.K. and Basu, S.B. (1997). Mortality pattern in Jersey x Tharparkar crossbred female calves. *Indian Vet. J.* **74**: 673-676.

Saxena, B.C., Arya, S.R.S. and Bindal, V. (2002). Disease prevalence and productivity losses in bovines-a study. *Cheiron* **31**: 74-77.

Singh, D.V., Sodh, S.P.S., Brar, A.P.S. and Grewal, J.J. (1996). Certain pathological profiles of endotoxic shock in buffalo calves. *Buff. J.* **12**: 283-288.

Srivastava, S.C. (1963). *Neoascaris vitulorum* (Goeze, 1782, Travasses, 1907) in intestinal perforation with its localization in liver of buffalo calves. *Indian Vet. J.* **40**: 758-762.