

MULTIPLE FALSE DIVERTICULA AND INTESTINAL OBSTRUCTION DUE TO TOXOCARIOSIS IN BUFFALO CALF

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ABSTRACT

A month old male Murrah buffalo calf died after showing clinical signs of blood tinged mucoid diarrhoea, un-thriftiness and weakness was subjected to necropsy examination, revealed dilated intestine with 2 to 4 mm diameter size nodules on the serosal surface of duodenum and jejunum. Upon opening the intestine, it showed obstruction due to heavy worm-load and the worms were confirmed as *Toxocara vitulorum*. Histologically, intestine showed cross sections of adult worms, numerous eggs in the lumen, sloughing of epithelium, shortening and fusion of villi, and mononuclear cell infiltrations in mucosa and submucosa. The tunica muscularis of the intestine showed focal areas of coagulative necrosis with multiple pseudo-diverticula in the intestine.

Pseudo-diverticula contained mucosa, submucosa, necrotic debris containing inflammatory cells and eggs of *T. vitulorum* worm but did not reveal cross sections of the adult worms. The present case represents a rare case of toxocariosis in buffalo calf depicting multiple false diverticula and intestinal obstruction and hence reported.

Keywords: *Bubalus bubalis*, buffaloes, calf, false diverticula, intestinal obstruction, toxocariosis, *Toxocara vitulorum*

INTRODUCTION

Toxocara vitulorum (*T. vitulorum*) commonly occurs in the small intestine of buffalo and cow calves causing a disease called Toxocariosis (Soulsby, 1982; Starke-Buzetti, 2006) and responsible for high morbidity and mortality in young calves in South Asia (Gupta *et al.*, 1976; Patnaik and Pande, 1963). The life cycle of the parasite has been well elucidated in the literature (Roberts, 1990; Starke-Buzetti, 2006). Briefly, disease occurs when calve suckle milk or colostrum containing infective larvae from infected cow or buffaloes which leads to the development of adult worms in the small intestine of newborn calves in 4 to 5 weeks. Adult *T. vitulorum* worms are found mainly in the duodenum of 3 to 10 week old calves and shed 8000 to 1 lakh egg per gram in faeces per day (Roberts, 1989).

Clinically, the toxocariosis in calves is characterized by anorexia, straining while defecation, diarrhoea, loss of body condition, poor

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growth and digestive disturbances (Roberts, 1990; Roberts, 1993; Singh *et al.*, 2008). Mortality of 30 to 80% in 1 to 12 months old calves has been reported (Rast *et al.*, 2013). The severe infection can lead to intestinal and/ or biliary obstruction, intussusception, volvulus and intestinal perforation leading to peritonitis (Srivastava, 1963; Starke-Buzetti, 2006).

In literature, the diverticula of the intestine are divided into the congenital/ true and acquired/ false diverticula. The acquired diverticula are also called as pseudo-diverticula. The congenital diverticulum is said to be a hernia of mucosa, submucosa and muscularis; the pseudo-diverticulum, on the other hand, is a hernia of the mucosa and submucosa through the muscular wall of the intestine (Liu and Crawford, 2005).

False diverticula leading to multiple nodules on the serosal surface of the intestine with intestinal obstruction due to the heavy load of *T. vitulorum* (Toxocariosis) has not been reported so far in the world literature. Hence, present communication reports the unique case of bubaline toxocariosis associated with false diverticula and intestinal obstruction.

CASE HISTORY

One month Murrah Buffalo male calf was presented for necropsy in the second week of August month. A detailed history taken from the farm manager revealed that the calf had blood tinged diarrhoea four days back which later on turned to watery mucoid diarrhoea. The other clinical signs included unthriftiness and weakness for a week prior to death. The calf was treated with Metronidazole 20 mg/kg body weight by intravenous route and Dextrose 5% saline 10 ml/

kg body weight intravenously during illness. However, the calf died four days after the onset of treatment and the carcass was presented to the department for necropsy examination.

Pathological examination

The detail necropsy was conducted and gross lesions were recorded. The tissue samples from the different portion of duodenum, jejunum, and ileum collected and fixed in 10% formal saline for 24 h were processed and embedded in paraffin blocks. Sections of 5 µm were taken on slides and stained with haematoxylin and eosin (Kim, *et al.*, 2019).

Parasitological examination

The intestinal contents were examined by direct smear examination and floatation method with a saturated salt solution. The adult worms and eggs were identified as per the keys described (Soulsby, 1982). Microscopic examination of worms found in intestine confirmed as ascarid infection.

RESULTS AND DISCUSSION

Necropsy examination revealed emaciated and dehydrated carcass. The tail was soiled with faeces. The abdominal and thoracic organs except the intestinal tract were grossly normal. Intestinal serosa showed numerous raised nodules of 2 to 4 mm in diameter size (Figure 1).

The nodules were present throughout the intestine but were more numerous and diffuse towards duodenum and jejunum. Intestine upon opening revealed obstruction due to heavy roundworm load (Figure 2).

The worms were more numerous towards

duodenum and anterior part of jejunum. The mucosal contents were brownish, mixed with catarrhal exudates and underneath showed hyperemia. Histologically, the cross section of intestine showed severe sloughing of epithelium, shortening and fusion of villi with mononuclear cellular infiltration in mucosa and submucosa and cellular debris along with eggs of *T. vitulorum* in the lumen. Higher magnification of the section revealed mononuclear cell *viz.* lymphocytes, eosinophils, sparse population of macrophages and recruitment of fibroblast at submucosa. The section of intestine also showed cross-section of adult worms in the lumen, cellular debris and accumulation of inflammatory cells and eggs of *T. vitulorum* (Figure 3).

The load of the parasite in the lumen was so huge that the mucosa at many places was compressed. The tunica muscularis of the intestine showed focal areas of coagulative necrosis with focal loss or disappearance of muscularis (Figure 4).

The section of intestinal nodule showed a herniated loop containing mucosa and submucosa without any aperture or opening to muscularis in the initial section (Figure 5).

The serial cross-section of intestinal nodules revealed hernia of mucosa and submucosa through weaken tunica muscularis (muscularis wall). Tunica adventitia (serosa) formed a capsule of the herniated loop (Figure 6).

Upon observation of the initial and serial sections of intestine, it was revealed that the areas of coagulative necrosis in muscularis created numerous spaces/ aperture through which mucosa and submucosa herniated and stretched through the tear in tunica muscularis possibly due to pressure from heavy worm load and depicted as a circular elevation/nodule on the serosal surface of intestine.

The microscopic changes in the herniated loop showed a lumen of the intestine with mucosa and submucosa (Figure 7).

The microscopic changes in the herniated loop were similar to those seen in mucosa and submucosa of the intestine, *i.e.* necrosis with sloughing of epithelium, accumulation of cellular debris and 4 to 5 eggs with albuminous pitting. However, the herniated loop lacked adult parasite. The aperture created due to the weakening of the wall of muscularis of intestine could have been very small due to which only eggs were escaped into the herniated portion.

The eggs in the intestinal and herniated part of intestine were sub-globular to oval in shape and brownish in colour with pitted albuminous layers. The size of the egg in the intestinal section and intestinal content ranged from 60 to 95 μm . In the H and E stained section of intestine, the wall of the egg was thick, eosinophilic with albuminous pitting and contained embryonic mass in the centre.

The adult worms were stout and whitish to grey in colour. The average size of the male parasite was 25 cm by 5 mm and the females of 30 cm by 6 mm. The cuticle was thin and translucent as the internal structure of the worm could be seen through the outer wall. Microscopic examination of intestinal scraping revealed round/ sub globular eggs with albuminous pitting. Based on the morphology of adult parasite and egg, the location of the parasite in the intestine and gross as well as histopathology examination, the case was confirmed as *T. vitulorum* (Toxocariosis) infection. In the present investigation, the toxocariosis was reported in one month calf and infection could have acquired through colostrum/ milk as the larvae of *T. vitulorum* are present in greatest number in colostrum and milk after calving and



Figure1. Intestine showing multiple raised nodules on the serosal surface with impaction due to worm.



Figure 2. Intestine showing heavy worm load leading to obstruction.

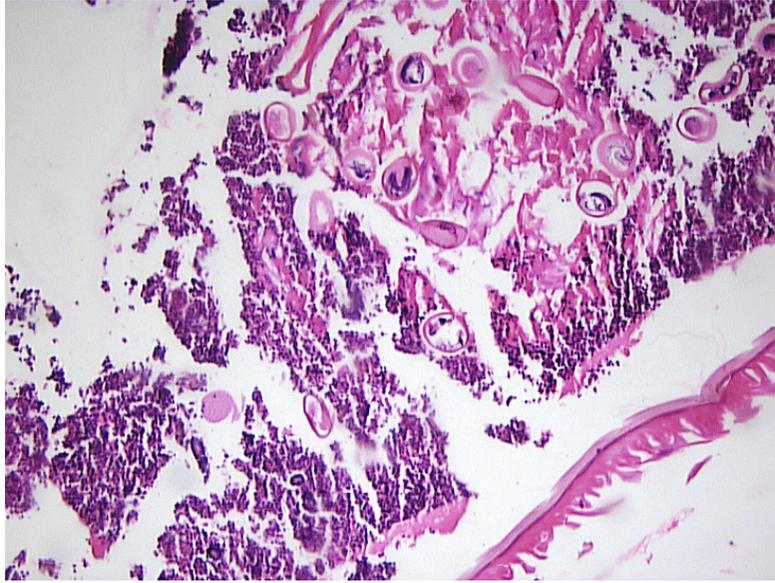


Figure 3. Intestinal lumen containing cellular debris, inflammatory cells and eggs of the *T. vitulorum* (H & E, 100X).

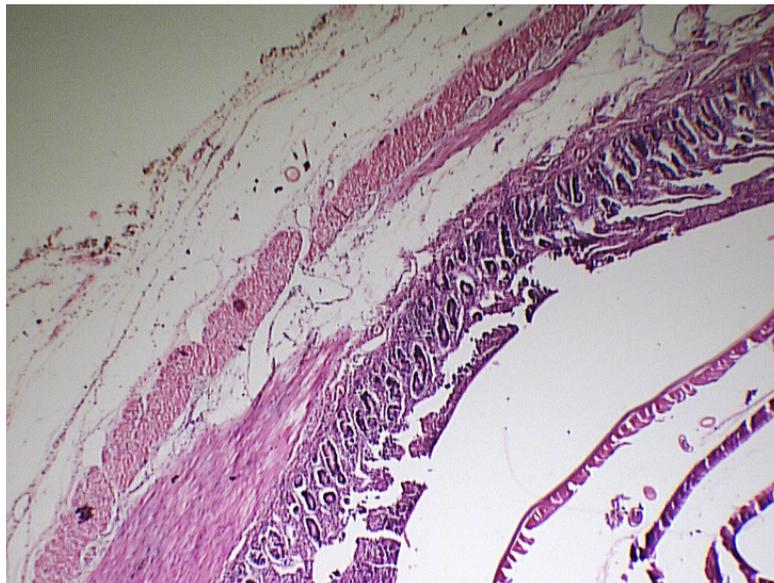


Figure 4. Section of intestine showing focal areas of necrosis and loss of tunica muscularis of the intestine (H & E, 100X).

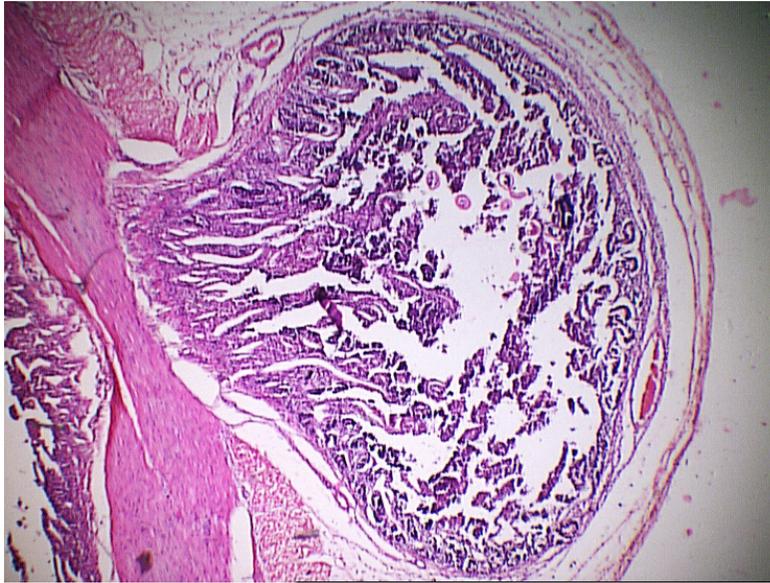


Figure 5. The section of intestinal nodule showing herniated loop containing mucosa and sub mucosa without any aperture or opening to muscularis in initial section (H & E, 100X).

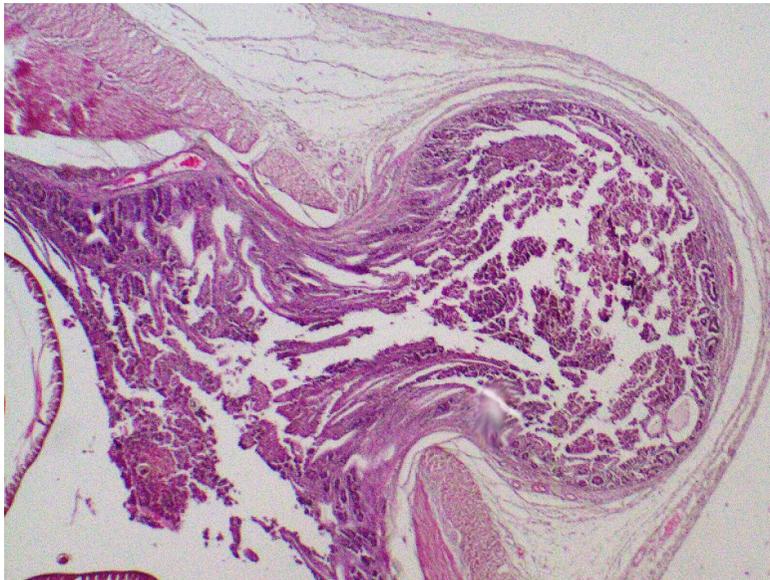


Figure 6. Serial section of intestine showing herniation and stretching of submucosa through opening in tunica muscularis (H & E, 20X).

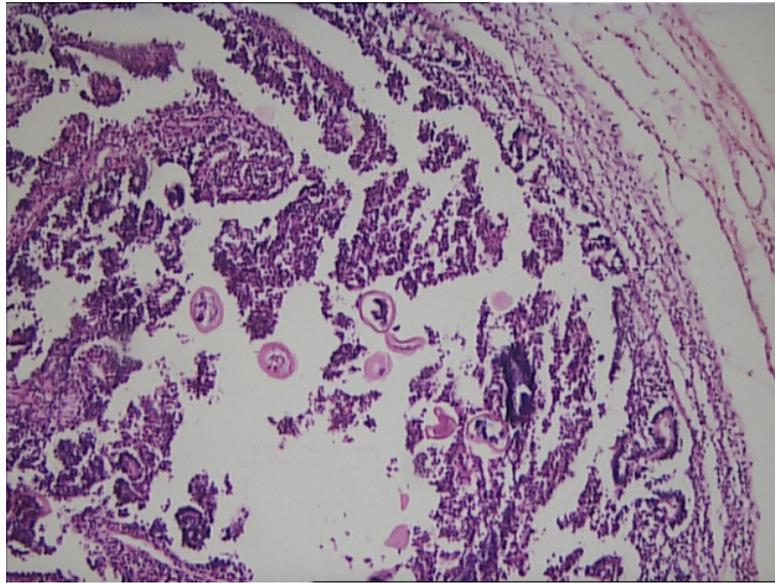


Figure 7. Section of herniated portion of intestine showing sloughing of epithelium and accumulation of inflammatory cells into lumen and eggs of the *T. vitulorum*. (H & E, 400X).

larvae become an adult in 4 to 5 weeks (Soulsby, 1982). Thus, adult worms are commonly found in 1 to 3 months calves (Ferreira and Starke-Buzetti, 2005). Prevalence of *T. vitulorum* is more in buffalo than that of cattle as supported in earlier studies (Starke-Buzetti, 2006).

Morphology of adult parasite and egg, the location of the parasite in the intestine helped to arrive at a diagnosis of toxocariosis. However, the presence of numerous elevated nodules on the serosa of intestine lead to suspicions of other parasitic infestation viz. oesophagostomiosis. The serial histological sections taken from the intestine revealed that the nodules were actually the herniation of mucosa and submucosa through a minute pore created through weaken tunica muscularis referred as pseudo-diverticula. In the present case, pseudo-diverticula were multiple and observed throughout the small intestine but were more numerous in duodenum and jejunum.

The pseudo-diverticula have been well described in humans and affects approximately 50% of individuals over 60 years of age in Western countries (Liu and Crawford, 2005). In humans, multiple pseudo-diverticula are commonly observed (Liu and Crawford, 2005) with the involvement of small bowel (Lin *et al.*, 2005). The age (Liu and Crawford, 2005) and low fibre diet have been suggested as a cause of diverticula (Fischer *et al.*, 1985). In animals, diverticular diseases are rare and have been reported only in dog, cat, horses (Maxie, 2016) and sheep (Penades *et al.*, 2010). Progressive weakening of intestinal smooth muscle and increased intra intestinal pressure has been said to be a reason for pseudo-diverticula (Rizwan *et al.*, 2011).

In the present case, intestinal muscularis showed severe coagulative necrosis and thereby weakening of intestinal wall and focal loss or

disappearance of tunica muscularis. The *T. vitulorum* worm load in the lumen of intestine was so huge that could have resulted in necrosis and lysis of tunica muscularis. The intra-intestinal pressure due to heavy worm load and loss of the portion of tunica muscularis could have resulted in herniation of mucosa and submucosa of intestine through weaken muscularis causing pseudo-diverticula. Pathology of *T. vitulorum* in buffalo calf associated with different clinical manifestation viz. diarrhoea, poor growth, digestive disturbances, intestinal and biliary obstruction, intussusception, volvulus, intestinal perforation with peritonitis has been reported (Roberts, 1990; Singh, *et al.*, 2008; Srivastava, 1963). However, multiple pseudo-diverticula a causing nodular lesion on the serosal surface of the intestine has not been so far reported in the world literature.

This kind of lesion associated with *T. vitulorum* is being reported for the first time. It could be mistaken for oesophagostomiosis where similar nodules over the serosal surface of duodenum and jejunum are observed. However, the lesions of oesophagostomiosis (larvated nodules with a small opening in the mouth) differ significantly from *T. vitulorum* lesions. Hence, the present case represents a unique and probably a rare case of Toxocariosis in buffalo calf.

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