

Pathomorphological changes associated with Paramphistome infection in rumen of buffalo

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Abstract

Paramphistomosis, a widespread disease of ruminants, is considered a copacetic obstacle in animal health and productive performance. Considering the economic importance of this disease, rumen infected with amphistomes was collected from a local butcher in and around Srinagar city. Samples were fixed in 10% formalin for studying the histomorphological alterations. Sections of the parasite were observed attached to mucosa in between the ruminal papillae. There was disintegration and disorientation of ruminal papillae along with cellular infiltration. Further, the ruminal mucosa showed disintegration along with mononuclear cell infiltration and necrosis with thickening of the muscularis mucosa. In conclusion, amphistomosis causes histomorphological alteration in the rumen that could alter the function of the organ.

Keywords: Buffalo, Histopathology, Paramphistomosis, Parasite, Rumen

Highlight

- Rumen of buffaloes infected with amphistomosis was studied for histopathology.
- Disintegration and disorientation of ruminal papillae along with cellular infiltration was found.
- Mucosa of rumen showed disintegration along with mononuclear cell infiltration and necrosis with thickening of muscularis mucosa.

The livestock population in India is susceptible to many diseases, among which helminth parasitism in general and platyhelminths in particular have been a major health concern, severely limiting animal productivity. The helminth parasites cause productive losses in terms of mortality, morbidity, condemnation of organs at the time of slaughter and cost incurred on their treatment and control (Shahardar, 2013). Different types of platyhelminth parasites recorded from livestock throughout the globe include *Fasciola hepatica*, *F. gigantica*, *Dicrocoelium dendriticum*, *Paramphistomum* spp., *Moniezia expansa*, *M. Benedeni* and *Avitellina centripunctata*, *Stilesia hepatica*, *S. Globipunctata* (Al-Khafaji and Rhaymah, 1993). Amphistomosis caused by Paramphistomatidae is a widespread disease of buffaloes, cattle, sheep and goats. Acute amphistomosis has been reported in young animals, while older animals are capable of withstanding massive infections (Soulsby, 1982). Fluke infections due to *Paramphistomum* spp. are widespread in Asia, with a high prevalence in South and Southeast Asia (Debbra *et al.*, 2018). Rumen fluke species in cattle and buffaloes in Asian countries belong to two families: Paramphistomidae and Gastrothylacidae, and among which *Paramphistomum cervi* and *Paramphistomum epiclitum* are the most

common species and the most significant cause of diseases in cattle and buffaloes (Rafiq *et al.*, 2020). The prevalence of amphistome infection in cattle of Kashmir ranges from 7.10-7.34% (Aiman *et al.*, 2017; Shah *et al.*, 2019). The clinical signs are noticed only in heavily infected, and advanced cases show anorexia, unthriftiness, general depression, retarded growth, emaciation/loss of weight, poor wool growth, falling of wool, diarrhoea, anaemia, oedema and mortality (Agrawal *et al.*, 2004). The frequency and significance of lesions caused by the adult rumen fluke are unclear (Toledo *et al.*, 2006), and it is suggested that the adult stage of rumen fluke is harmless, but experimental studies have shown that it is associated with ruminal papillae atrophy and ulceration at the site of fluke attachment in case of heavy infections (Fuentes *et al.*, 2015; Mohanta *et al.*, 2017).

The pathobiological effects of different parasites vary depending on their habit, parasitic load and the health status of the host. The pathological lesions due to helminth parasites include marked oedema of mucosae and submucosae of forestomach, small intestine, catarrhal changes, haemorrhages, degeneration, fibrosis, hyperplasia of glandular tissue, proliferation of connective tissue, biliary cirrhosis and granulomatous

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inflammation (Soulsby, 1982; Sreedevi *et al.*, 2005). Several authors have reported the prevalence of amphistomes in livestock of India, but the histological changes induced by these parasites, particularly in buffaloes, are scarce (Yadav *et al.*, 2019; Arshad *et al.*, 2022) and therefore, the present work was undertaken to study the histological changes in the amphistome infected rumen of buffalo.

Rumen of ten bovines infected with *Amphistome* spp. was collected from a local butcher in and around Srinagar city. Some of the attached amphistomes were collected for morphological identification, and the rest, along with the tissue, were fixed in 10% formalin to record the histomorphological alterations. Paraffin embedding technique was followed for micro-sectioning of tissues, which were then cut into small portions of 2-3 mm thickness, washed in water for several hours, followed by dehydration in ascending grades of alcohol. Clearing of tissues was performed in benzene and embedding in paraffin. Casting of blocks was carried out in L-molds (two L- L-shaped pieces), which facilitated the manipulation of size as per the requirement (Culling, 1974).

A rotary-type microtome was used for cutting paraffin sections of 5 mm thickness. Continuous ribbons (6-7 inches long) of the material were cut and laid on the surface of a constant temperature water bath (around 55°C). The sections were separated with a heated scalpel after they spread completely. The cut sections were mounted on clean glass slides using Mayer's egg albumin as section adhesive. The mounted slides were marked for identification and dried in a paraffin oven at 60°C for one hour. The tissue sections were stained by Harris haematoxylin and eosin staining method. The paraffin sections were deparaffinized with xylene before hydration through graded alcohol to distilled water. Staining was done as per the standard procedure, followed by dehydration in ascending grades of alcohol. Clearing was performed in xylene and mounting in DPX. A drop of DPX mount was placed on a cover slip, and the section on the slide was pressed on it after taking out from xylene. The slide was inverted, and the cover slip was pressed with a rod to remove air bubbles if any were trapped.

Paramphistomosis has been neglected owing to less severe pathogenic effects due to adult stages which elapse their maturity in the rumen and reticulum without any prominent changes (Tehrani *et al.*, 2015). The pathology of amphistome infection has been systematically studied in goats and sheep (Singh *et al.*, 1984; Rolfe *et al.*, 1994) but not in the case of buffaloes (Yadav *et al.*, 2019; Arshad *et al.*, 2022).

No major pathological lesion was found in the fore stomach grossly, but rumen papillae were atrophied,

and the affected area looked whitish in colour, although paramphistomes were found attached to the wall of the rumen and reticulum. Soulsby (1982) reported that adult amphistomes do not cause any serious damage to the host, and in heavy infections, they may cause loss of ruminal papilla. Yadav *et al.* (2019) also reported similar findings in their study. On histological examination of the rumen infected with paramphistome species, sections of the parasite were observed attached to the mucosa in between the ruminal papillae. Histopathological examination of the micro sections revealed disintegration and disorientation of ruminal papillae along with cellular infiltration (Fig. 1). Ruminal mucosa showed disintegration along with mononuclear cell infiltration (Fig. 2) and necrosis with thickening of muscularis mucosa. Rolfe *et al.* (1994) observed severe damage to the mucosa of rumen with eosinophil infiltration. Mukherjee and Deorani (1962) demonstrated the massive infection of a sheep with amphistomes and the histopathology of parasitized rumen. Singh *et al.* (1984) observed histopathology of the duodenum and rumen during experimental infection with *P. cervi*. Shahnawaz *et al.* (2012) and Tehrani *et al.* (2015) reported mononuclear cell infiltration in the submucosa of the ruminal papillae, necrosis and degenerative changes as reported in our study.

The present study provides insights into the pathology associated with paramphistome infections in the rumen of buffalo, but a detailed study needs to be done in order to establish the true prevalence of the parasite in buffaloes and also understand the immunological and molecular mechanism lying behind the cause of pathogenicity so that appropriate control measures could be adopted to prevent further damage to livestock industry.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: ZAW, IMA, KHB, RAS: Carried out the study; SA: Helped in the preparation of the final draft; SAR, MNH: Helped in sampling; SAS, MS: Carried out the histopathological study.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

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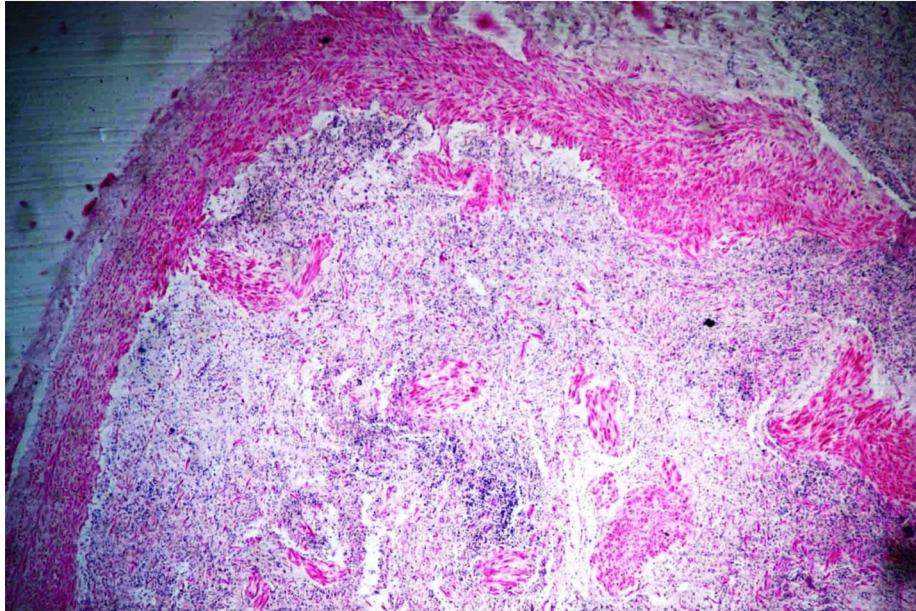


Fig. 1. Photomicrograph of a section of rumen showing disintegration and disorientation of rumen papillae, H&E stain

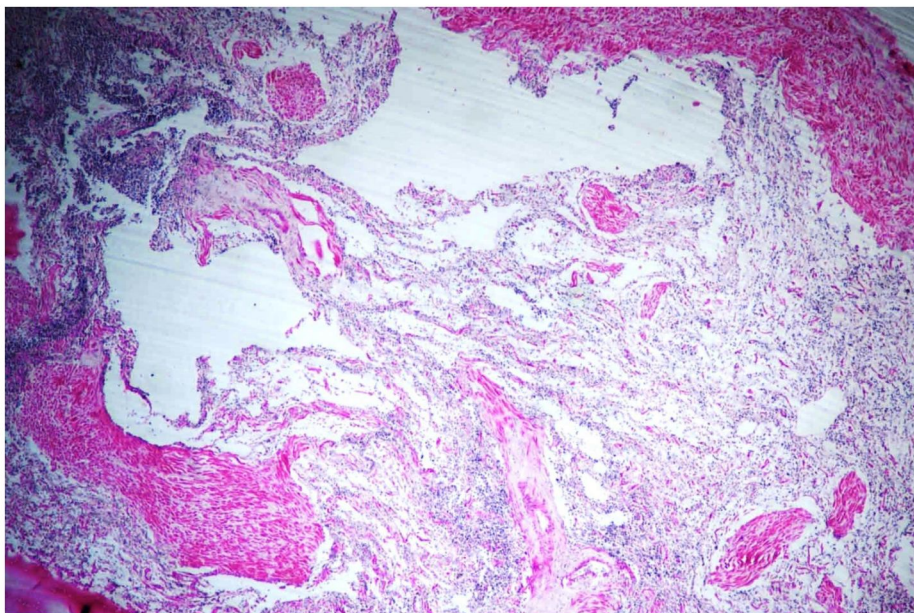


Fig. 2. Photomicrograph of a section of rumen showing disintegration of rumen mucosa and mononuclear cell infiltration, H&E stain

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