



## Patho-morphological Study of *Gigantocotyle* spp. Infection in Water Buffaloes (*Bubalus bubalis*)

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### ABSTRACT

A total of 12 adult buffaloes were presented for the necropsy over the period of 6 months at the Department of Veterinary Pathology, CGKV, Anjora, Durg, Chhattisgarh. Prevalence of *Gigantocotyle* spp. parasite infection was found to be 16.67% (2/12). Significant number of parasites was found in the bile duct and ductules of the infected liver along with the necrotic debris caused by parasite. Other gross lesions observed were included hepatitis, haemorrhages and fibrosis of the liver. Black color hard nodules at the site of mucosal attachment of parasites were noticed. Microscopic changes observed were included bile duct hyperplasia, haemorrhages, moderate fibrosis and granulomatous nodule characterized by infiltration of inflammatory cells.

**Keywords:** *Gigantocotyle* spp. parasite, Gross Pathology, Liver, Microscopic Pathology, Water buffalo

The water buffalo (*Bubalus bubalis*) or domestic water buffalo is a large bovid originating in the Indian subcontinent, South Asia and China. Many peoples of these areas are relying on buffaloes as compare to other animals. They make a critical contribution to farmers by providing milk, meat, skin, manure and traction (Cockril, 1977 and Haque *et al.*, 2011). However, buffalo has the genetic adaptability to survive in adverse climatic conditions of tropical areas, but negligence towards proper nutrition and management making them vulnerable for the various diseases.

The gastrointestinal tract (GIT) of animals harbours a variety of parasites chiefly helminthes, which cause clinical and sub clinical parasitism. These parasites adversely affect the well being of animals and cause massive economic losses to the livestock industry (Anuracpreeda *et al.*, 2008). *Gigantocotyle* spp. parasite is a very common trematode found in the liver of water buffalo and has a wide geographical distribution in India and neighboring countries. The fresh water snail *Gyrulus convexiculus*

serves as the intermediate host (Naeem *et al.*, 2014). The parasite localized in the main bile ducts and intrahepatic ductules damages the organ and causes reduction in meat, milk and growth rate of the animal. Parasitism may cause even death of the host (Haque *et al.*, 2011).

The prevalence of infection is very high in Indian subcontinent; about 60% buffaloes slaughtered at the local abattoirs had *Gigantocotyle explanatum* infection (GoI, 2011-2012). Adult parasite remained attach to the epithelium of the bile duct of domestic ruminants where they impose severe damage. The common pathological lesions caused by *G. explanatum* are proliferation of connective tissues, hemorrhage and hyperplasia in the bile duct of the liver (Malik *et al.*, 2017).

In the present study, gross and microscopic findings of the natural infections in the liver of water buffalo caused by *Gigantocotyle* spp. parasite have been described.

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## MATERIALS AND METHODS

A total of 12 adult buffaloes presented for the post-mortem examination over a period of 6 months to the Department of Veterinary Pathology, CGKV, Anjora, Durg, Chhattisgarh were included in the present study. Detailed systemic post-mortem examination of all the animals was carried out and gross pathological lesions were recorded carefully.

Liver samples infected with *Gigantocotyle spp.* parasite were collected in 10% formalin for the histopathological examination. Dehydration and clearing of the tissue samples was done by using acetone and benzene, respectively followed by paraffin wax impregnation and sectioning at 5 $\mu$  thickness. Hematoxylin and eosin staining performed as per standard procedure described by Slaoui and Fiette (2011).

## RESULTS AND DISCUSSION

### Prevalence

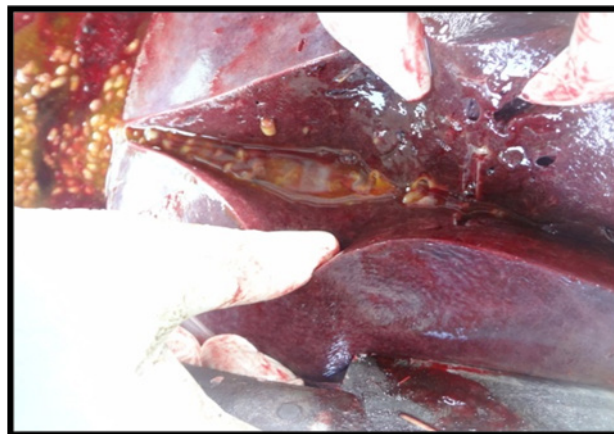
Results of the present study revealed that 2 out of total 12 water buffaloes were found to be infected with the parasite of *Gigantocotyle spp.* Liver of the one animal was found to be severely infected with this parasite; while other had moderate infection. The prevalence of parasite in the present study was found to be 16.67% (2/12).

The results of the present findings were in accordance with Haque *et al.* (2011). They have observed severe infection of adult parasite in the bile ducts and intrahepatic ductules with a prevalence rate of 18% in water buffaloes.

Nath *et al.* (2016) reported 28.10% prevalence of all types of amphistomes infection in the water buffaloes of Madhya Pradesh, which is a neighbor state of Chhattisgarh and shares significant agro-climatic similarities.

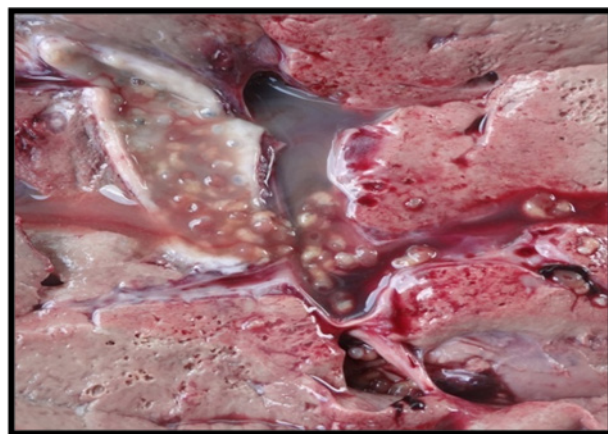
### Gross lesions

Gross examination of liver of the infected animals showed hepatitis and petechial haemorrhages along with multiple necrotic foci on its surface. Moderate fibrosis and mucosal thickening of bile duct has also been observed. On cut section, partially to entirely occluded bile ducts and ductules of the liver with the adult parasites and moderate icterus were observed (Fig. 1).



**Fig. 1:** Liver of buffalo infected with *Gigantocotyle spp.* showing occluded bile ductule along with moderate icterus.

Lumen of the bile duct found to be filled with the white color parasites along with necrotic debris (Fig. 2), making duct partially to fully inactive for the physiological mechanism. The black color nodules were observed at the site of attachment of the parasites due to strong sucking action by the parasite (Fig. 2).



**Fig. 2:** Liver of buffalo infected with *Gigantocotyle spp.* showing presence of white color parasite in the bile duct along with black color nodule (Arrow head).

Grossly, the parasites were conical shape which was slightly convex from the dorsal side. Parasites were measured around 5-6 mm in length and 3-4 mm in width. They were white to pink in color. The ventral sucker of the parasites was larger than the oral sucker.

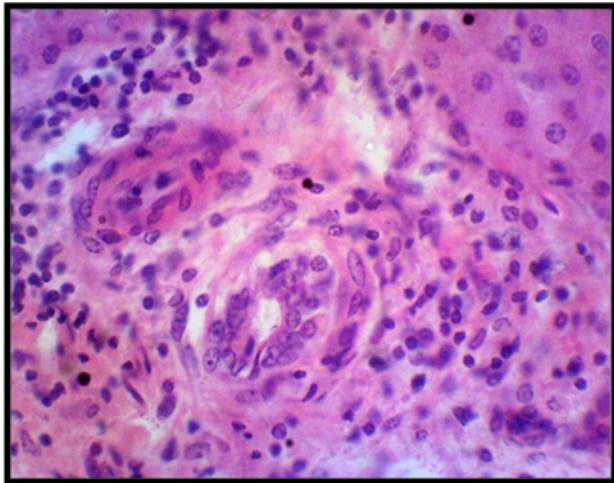
The present findings were closely related with Gamit *et al.* (2017). Grossly, they have observed atrophied and

inflamed infected liver specimens. Bile ducts were partially or completely occluded due to mucosal attached parasites as well as free parasites in the lumen. They also observed thickening and fibrosis of mucosa and submucosa of ducts along with bluish black color nodules in it.

Haque *et al.* (2011) reported presence of mature flukes and yellowish green necrotic material in the lumen of bile duct. Bile ductules as well as the main bile ducts showed thickened wall with chronic inflammation involving mucosa and submucosa. Ghosh *et al.* (1982) observed that the common bile and the hepatic duct were distended and lumen of the bile duct was embedded with numerous small parasites. They also found haemorrhagic spots on the surface of liver and bile duct.

### Microscopic lesions

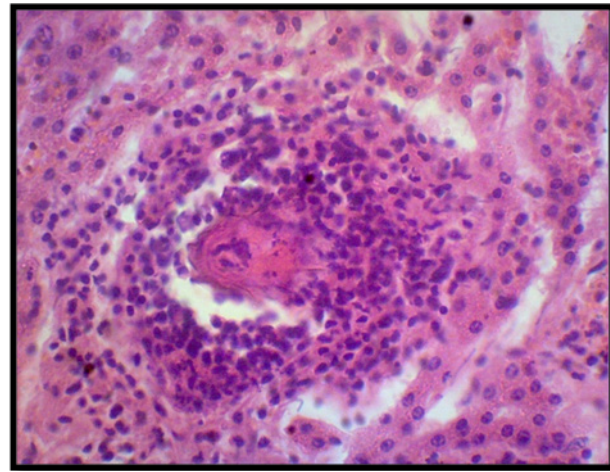
Histopathological examination of infected liver revealed presence of many adult *Gigantocotyle spp.* parasites, haemorrhages in parenchyma, bile duct hyperplasia (Fig. 3), moderate fibrosis and formation of granulomatous nodules (Fig. 4).



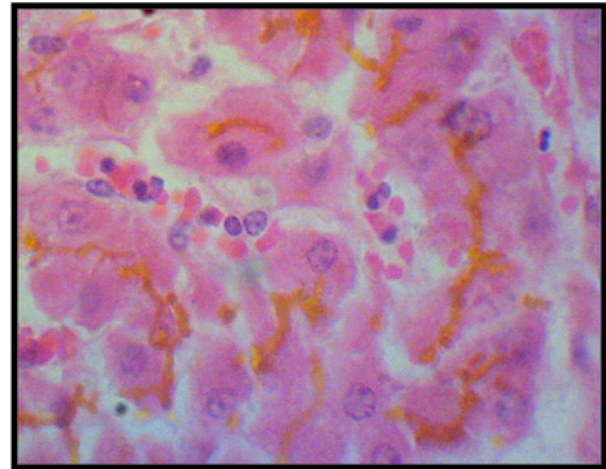
**Fig. 3:** Microscopic section of liver of buffalo infected with *Gigantocotyle spp.* showing bile duct hyperplasia and cellular infiltration. H&E  $\times$  400

Granulomatous nodule formed at the site of parasite attachment were characterized by intense infiltration of inflammatory cells such as macrophages, lymphocytes, eosinophils and plasma cells with deposition of significant amount of fibrous connective tissues. Liver with icterus

revealed presence of bile inside the hepatocytes as well as in the sinusoidal spaces (Fig. 5).



**Fig. 4:** Microscopic section of liver showing granulomatous nodule characterized by infiltration of various inflammatory cells along with a cross section of parasite located in the centre of granuloma. H&E  $\times$  400



**Fig. 5:** Microscopic section of icteric liver showing bile deposition in hepatocytes and sinusoidal spaces. H&E  $\times$  400

Photomicrograph of a granulomatous nodule showed presence of acetabulum of the adult parasite in an anchoring position which was tightly attached with mucosa of bile duct.

The present findings were closely corroborated with Haque *et al.* (2011). They reported that granulomatous nodules were intensely infiltrated with inflammatory





cells such as lymphocytes, macrophages, plasma cells, eosinophils as well as fibroblast, which were indicative of the chronic nature of infection. They found lymphocytes as predominant amongst all inflammatory cells which might be due to generation of strong immune response of the host against parasite.

Ahmedullah *et al.* (2007) observed thickening of bile ducts with moderate fibrosis along with infiltration of reactive cells and marked proliferation of mucosal gland with hyperplasia of mucosal epithelium.

Haque *et al.* (2011) reported presence of numerous whitish pink color trematode parasites, measuring on an average 6 x 4 mm, being attached throughout the luminal wall of bile duct. On microscopic examination, granulomatous nodule with the trematode *Explanatum explanatum* showed acetabulum of the fluke attached on the host tissue.

Singh (2003) discussed the morphology of the parasite *Gigantocotyle spp.* and observed conical shape with the convex dorsal side with a measurement of 8-13 mm in length and 3.6-4.3 mm in width. Moreover parasite had oral and ventral sucker in which ventral sucker was about 5 times of diameter of oral sucker facing the ventral side.

## CONCLUSION

The present study revealed the prevalence and pathology of the parasite *Gigantocotyle spp.* in the water buffaloes. This may cause serious economic loss to the dairy farmers as it damages the liver which indirectly plays major role in the productivity of the animal. It needed prompt prevention policies in terms of cleaning and management of natural habitat of water buffalo as snail acts as an intermediate host for the transmission of the parasite. Time to time dewormer has to be given to the animal as preventive measure.

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