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Research Article

CONCURRENT INFECTION OF BILIARY AMPHISTOME, *GIGANTOCOTYLE EXPLANATUM* WITH TROPICAL LIVER FLUKE, *FASCIOLA GIGANTICA* IN BUFFALOES OF NORTH INDIA

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ABSTRACT: The helminths belonging to the family, *Fasciolidae* and Paramphistomidae *i.e Fasciola gigantica* and *Gigantocotyle explanatum*, respectively, have a usual tendency to exist together in the liver of the Indian water buffalo *Bubalus bubalis*, causing serious pathological damage to the vital organ, incurring huge economic losses. In the present study, a total of sixty liver samples were collected from slaughtered buffaloes of a local abattoir of Saharanpur in northern India. The study revealed that out of 25 animals which were found positive for the *Fasciola gigantica* infection (presence of adult flukes as a measure of being positive), 20 animals were positive for the *G. explanatum*. The overall abattoir prevalence of fasciolosis was 41.6 % and that of *G. explanatum* was 33.3% and the concurrency of these parasites was 80 %. The habitat preference by these worms may be a consequence of micro-environmental cues that guide these flukes to reach their habitat through different routes and establish a successful host-parasite relationship.

Key words: Digenetic trematodes, *Fasciola gigantica*, *Gigantocotyle explanatum*, Concurrent infection, *Bubalus bubalis*.

INTRODUCTION

The livestock sector is a major part of the economy of the developing countries like India, where it contributes about 16% to the income of marginal farmers, provide employment to about eight percent of country's population and in overall contributes 4.11% GDP and 25.6% of total Agriculture GDP (19 th Livestock Census 2012). The trematode infection in ruminants has been reported from almost all countries of the world resulting in huge economic losses both by direct or indirect means. The most important trematodes of ruminants in India are thought to be *Fasciola gigantica* and *Gigantocotyle explanatum* (Garg *et al*. 2009). An estimated loss of US $3.2 billion per annum has been accounted for due to fasciolosis in ruminants, caused by *Fasciola gigantica* (Spithill *et al*.1999). The disease not only causes significant losses due to mortality but also the infection that goes subclinical causes significant debt from lessened feed conversion ability (Mehra *et al*. 1999), reduced weight gains (Cawdery *et al*. 1977), milk production (Nizami *et al*. 1991, Charlier *et al*. 2008), carcass quality (Schweizer *et al*. 2005), decreased efficiency in draught animals and condemnation of liver during meat inspection at slaughterhouses (Vassilev and Jooste 1991). Alone in a state of India, *i.e*, Uttarakhand, an estimated annual milk loss of 90.41 crores has been estimated due to fasciolosis (Bowman 2014). The epidemiological data are still limited (Garg *et al*. 2009) despite the realization of this disease impact in India. The prevalent species in India is *F. gigantica*, although some reports of *F. hepatica*

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from temperate regions of the country is also there (Khan *et al*. 2016).

One of the most common and abundant groups of digenetic trematodes of domesticated livestock other than *Fasciola* spp. are the paramphistome fluke, *Gigantocotyle explanatum*, primarily a parasite of water buffaloes, found in the bile duct and gall bladder of buffaloes and sheep in India (Khan *et al*. 2016). The adult *G. explanatum* causes hemorrhage, pronounced periductal fibrosis and other hyperplastic changes (Kulasiri and Seneviratne 1956, Arora and Kalra 1971).

In northern India, these two flukes of Indian water buffalo *i.e*, *Fasciola gigantica* and *Gigantocotyle explanatum*, have their abattoir prevalence of 31.14 and 80 %, respectively (Ambu 1978, Nizami *et al*. 1991, Garg *et al*. 2002). The economic losses data for *Fasciola* spp has been studied by a number of researchers whereas limited study related to the prevalence and economic impact of the *G. explanatum* has been conducted. The concurrency of these trematodes in the slaughtered buffaloes at the local abattoir indicates that there may be some association between the parasites that are causing these to flourish together. The interesting fact is that both have their strict preference for the liver as a final destination but the route is very different. The newly excysted juvenile (NEJ) of *Fasciola* secrete proteases like Cathepsins (Dalton and Heffernan 1989, Smith *et al*. 1993), penetrate through the intestinal wall and come out into the peritoneal cavity and move towards liver, burrow into the liver parenchyma leaving footprints behind in the form of fibrotic tunnels and reach permanent location i.e. bile ducts. Whereas after excystation within the duodenum the newly excysted juveniles of *G. explanatum* show backward migration from intestinal lumen through the bile duct opening in the duodenum and reach the upper region of the bile duct where they firmly attach to the epithelium leaving a hard knob like tissue plug at the site of attachment (Fig. 1). To determine the prevalence dynamics of these parasites, the regular monitoring of their intermediate host *i.e.* snail is necessary which ultimately will help to strategize the control measures.

In the present study, the liver samples were collected from the buffaloes brought in a local abattoir, Saharanpur, Uttar Pradesh, India and were inspected for the concurrence of these flukes. This study was conducted

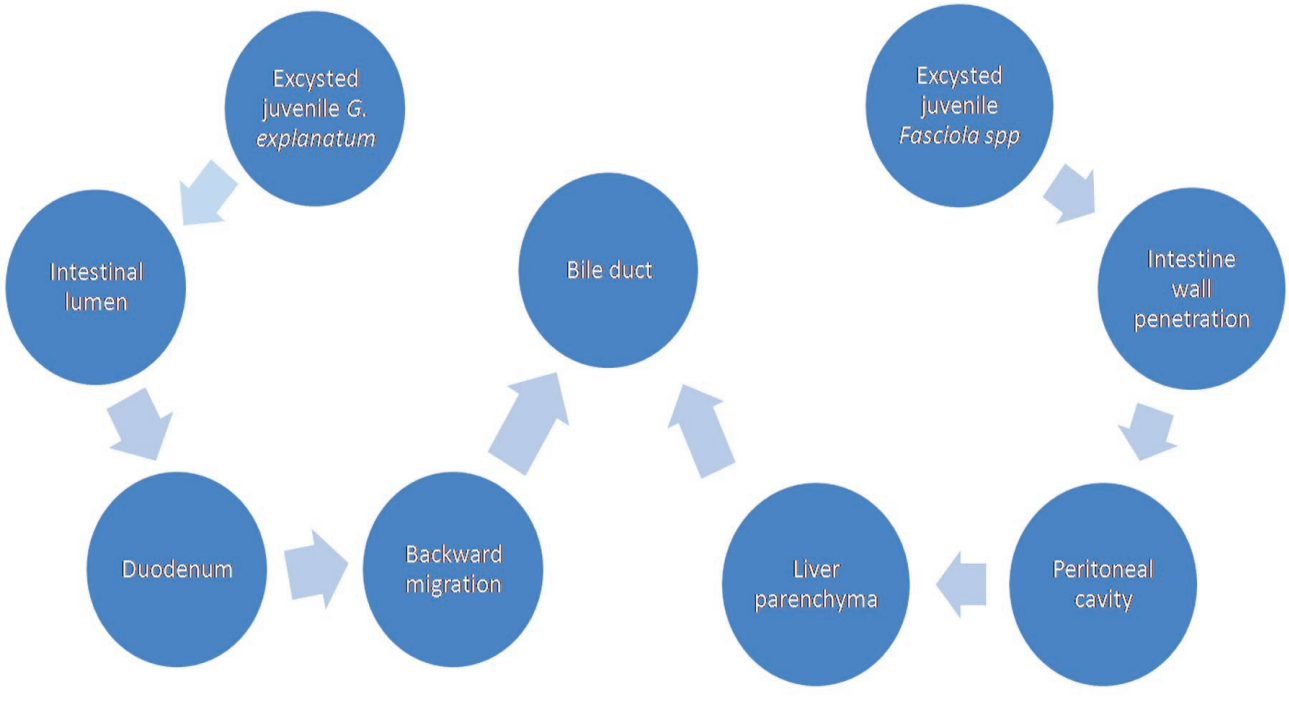

Fig. 1. The different migration route of the juvenile *G. explanatum* and *Fasciola* spp. (Soulsby 1982).

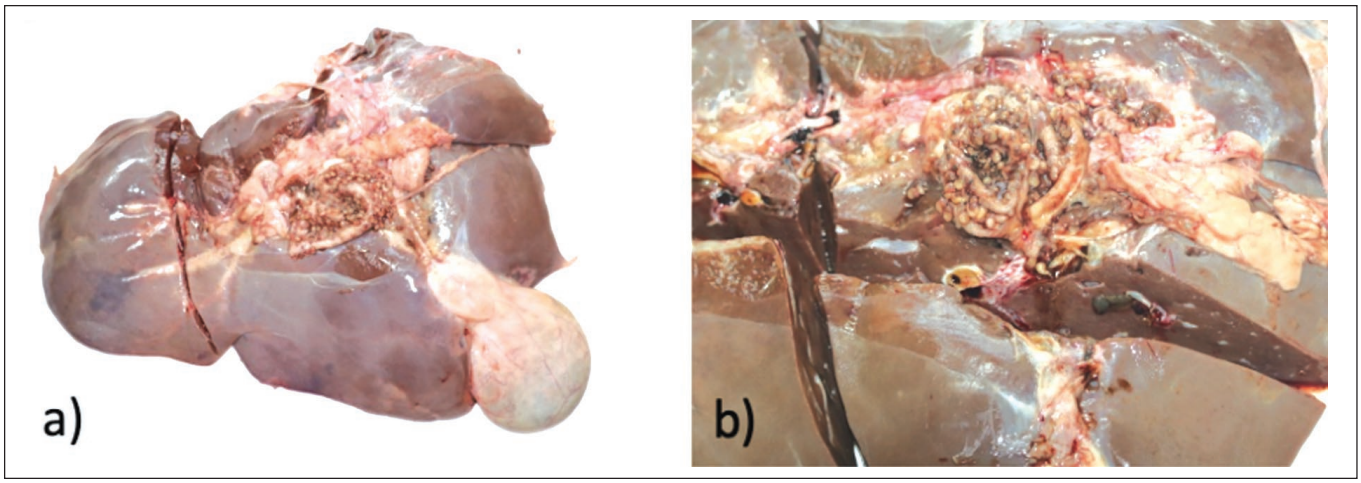

Fig. 2 (a and b). Gross lesions on the liver samples depicting the infection of *Fasciola gigantica* and *Gigantocotyle explanatum.*

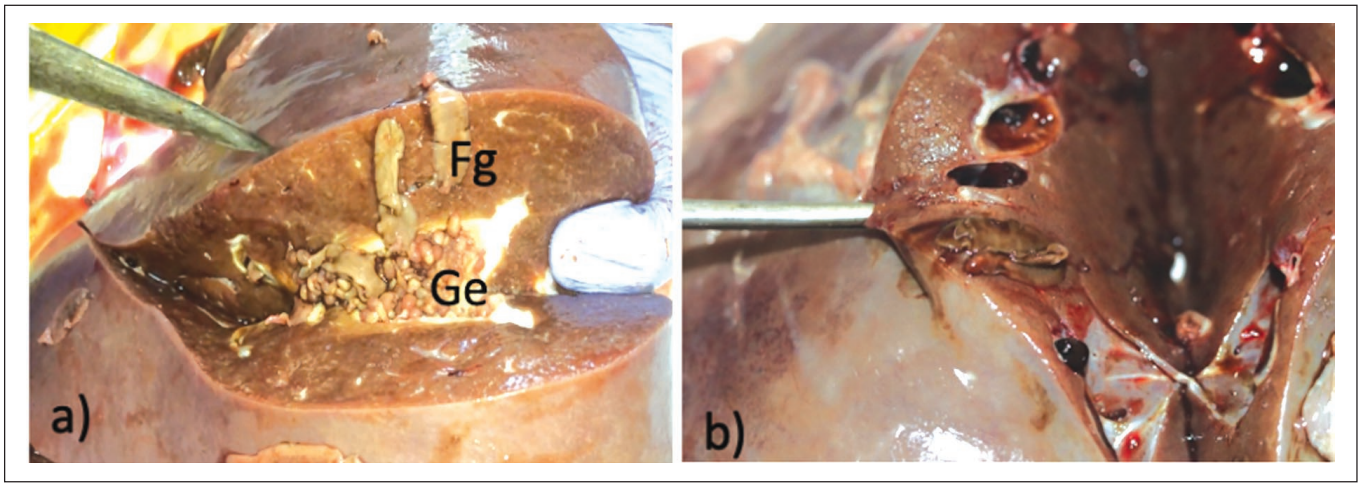

Fig. 3. Concurrently infected liver of Indian water buffalo with *Fasciola gigantica* and *Gigantocotyle explanatum.* [Leaves like parasites are the *F. gigantica* (Fg) and bulb like *G. explanatum* (Ge)].

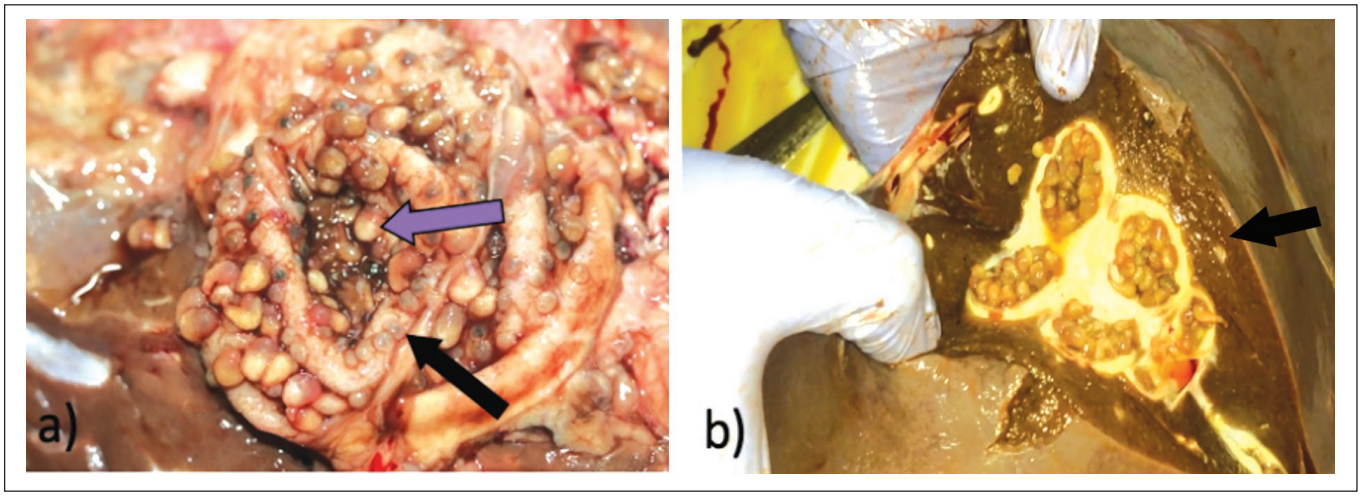

Fig. 4. Arrow black points to the granulomatous nodules, the point of attachment of parasites and arrow purple indicates numerous adult flukes attached throughout the luminal surface of the bile duct which has been cut open.

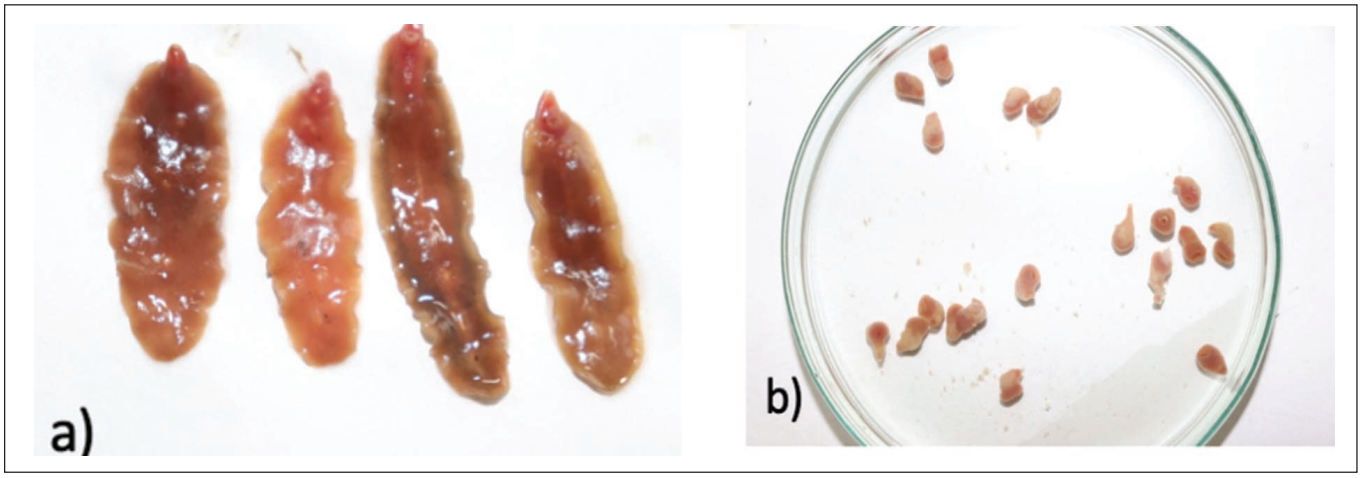

Fig. 5. Gross adult Fluke procured from the liver samples a) *F. gigantica* b) *G. explanatum.*

Fig. 6. Snails (Intermediate host) a) *Lymnaea auricularia* (*F. gigantica*), b) *Gyraulus convexiculus* (*G. explanatum*).

so as to avoid the negligence toward the huge economic losses resulted due to paramphistome *G. explanatum* and to increase the productivity of animals.

MATERIAL AND METHODS

The study area

A study was conducted in the abattoir of ALM industries, Village Harora, District Saharanpur, Uttar Pradesh to detect the concurrency of the *Fasciola gigantica* (liver flukes) with *G. explanatum* in slaughtered buffaloes. Saharanpur city is a part of a geographical doab region and is located at 29.97°N latitude; 77.55°E longitude. ALM industries abattoir slaughters about 60-80 buffaloes daily.

Samples collection

A total of sixty liver samples were collected from slaughtered buffaloes of a local abattoir (ALM industries, village Harora, District Saharanpur, UP) located in the Tarai region of north India. The animals in the abattoir are usually brought from the states of Uttar Pradesh, Haryana and Uttarakhand.

Samples preparation for postmortem inspection

The livers were brought to the laboratory of the abattoir on ice and thoroughly examined for the presence of mature and immature flukes. Liver and gall bladder were inspected by making multiple cuts and sub-cuts of about

1 cm thickness to check the presence of *Fasciola gigantica* and *G. explanatum*. Identification of the species based on the morphological features of the agent was made based on Soulsby (1982).

RESULTS AND DISCUSSION

It was observed that out of 25 animals which were found positive for the *Fasciola* gigantica infection (presence of adult flukes as a measure of being positive), 20 animals were positive for the *G. explanatum* as well, which clearly justify the coexistence of these parasite in our study. The overall abattoir prevalence of fasciolosis was 41.6% and that of *G. explanatum* was 33.3% and the concurrency of these parasites was 80% (Fig. 2 to Fig. 5). Although the study involved only 60 samples from a single abattoir, a detailed study is required taking a large number of samples from different slaughter units to strengthen the outcome of the present study.

The liver fascioliasis condemnation numbers revealed that buffalo's fascioliasis is higher during the rainy season followed by winter and autumn. In this study, the macroscopic postmortem inspection revealed that infected liver was very hard and has numerous injuries with congestion, enlargement with very hard fibrosis. The examination also revealed an intact liver showing the presence of different sizes (1.4-2.5 cm) of *Fasciola* spp. impeded on the hepatic tissue with characteristic grey or creamy color.

The incision on the liver showed thick wall fibrosis by fascioliasis tunnels. The opening of this tunnel exerted leaf-like liver flukes from the infected liver. Most larvae of *F. gigantica* enter the liver from the abdominal cavity by penetrating the capsule (Soulsby 1982). The severe haemorrhages were no doubt the result of the greater ability of the larger flukes to damage the tissue. Similar lesions were observed by authors in Bangladesh (Alim *et al*. 2005) and in Egypt (Khan *et al*. 2016).

Adult amphisomes like *G. explanatum* sp. have been found in the bile duct causing severe damage leading to fibrosis in the bile duct and liver (Singh 1958). *Explanatum* sp. is attached by their acetabulum in the liver and produces granulomatous nodules that are infiltrated by numerous inflammatory cells (Ahmedullah *et al*. 2007, Haque *et al*. 2011). The *G. explanatum* fluke gains entry to the bile ducts from the intestine via the common bile duct (Ghafoor 1970, Arora and Kalra 1971).

In the case of *G. explanatum*, bile may be chemotactic for the immature migrating flukes, attracting them from the duodenum into the bile ducts. There is a morphological similarity with fasciola infection, *i.e.* the enlarged, thickened bile ducts found with both infections. However, there were numerous unique changes which may be used to distinguish them. The presence or absence of parenchymal lesions was the major distinguishing feature. Parenchymal lesions associated with damage caused by the migration of immature flukes through the parenchyma, prior to their entry into the bile ducts, was a prominent feature of infection with *F. gigantica*, whereas there are no parenchymal changes in the liver of buffaloes infected with *G. explanatum.*

In our study, we found both the infections manifesting their lesion concurrently similar to a study conducted by Copeman (Copeman 1983, Khan *et al*. 2016), where he speculated that some of the pathological changes in the liver ascribed to *F. gigantica* may be partly owing to infection with *G. explanatum*. Recently, Khan (2016) also reported tropical liver fluke, *Fasciola gigantica,* and biliary amphistome, *Gigantocotyle explanatum*, concurrent infection in *Bubalus bubalis.*

The intermediate host the *F gigantica* is *Lymnaea auricularia* while that of the *G. explanatum*, it is *Gyraulus convexiculus* (Fig. 6). These are primarily the aquatic snails or freshwater snails which usually live in streams, irrigation channels, and marshy swamps. They usually flourish during the rainy season and in highly damp pastures soil (Bowman 2014).

The abattoir involved in the present study usually procures animals from the belt of Madhya Pradesh, Uttar Pradesh, and Uttarakhand. These states have the optimum conditions for the prevalence of these snails thus making conditions favorable for these flukes. These snails can be grossly differentiated from each other (Fig. 6). The control strategies of these snails are different from that of the mud snails *Gigantocotyle (Lymnaea) truncatula*, which are the intermediate host of *F. hepatica*.

The repeated treatment of the animal with anthelmintics to prevent patency is mostly followed because treatment of local water bodies with molluscicides is usually unrealistic as it may infect fish and other creatures. The best measure is to provide piped water supply to the animal troughs and prohibiting animal access to natural water.

High level of *Gigantocotyle explanatum* buffalos in the present study represents a high rate of infection and immense economic losses to the country. In accordance with this finding, it is suggested that livestock owners who rear buffaloes ought to improve arrangement of feeds to their animals so they may have great body condition that may resist the infection of both the infections. Plus, they ought to have the option to consistently treat their

animals with the suitable anthelmintics and awareness ought to be made on the prevention and control techniques for these helminths. To make an effective control plan against gastrointestinal helminth a year-round epidemiological study covering all the buffalo rearing areas of the country with proper diagnosis is warranted.

CONCLUSION

The results show that there is concurrency of the both these flukes *i.e*, *Fasciola gigantica* and *Gigantocotyle explanatum* in the liver of water buffaloes. There is a need for detail study to confirm our studies but the fluke (*G. explanatum*) shall not be neglected and a proper control strategy is required.

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