



## Parasitic pneumonia caused by *Paragonimus* spp. in a wild Royal Bengal Tiger, Mysuru, South India

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**Abstract** Parasitic pneumonia induced by genus *Paragonimus* involves many species, which affects both humans and animals and it is a food borne zoonotic disease. In this report, we have described the gross and histopathological findings of *Paragonimus* fluke infection in lungs of tiger. The postmortem examination of sub adult male wild tiger (*Panthera tigris tigris*) died in captivity was conducted, earlier which was rescued by Forest Department, Mysuru, Karnataka, India. External examination of carcass revealed pale oral and conjunctival mucous membranes with sunken eye balls. During necropsy, moderate congestion, consolidation and numerous transparent to dark encysted lesions were found in the parenchyma of all lobes of lungs visible grossly on pleural surface. Lungs were hemorrhagic with necrotic foci around the cysts. The incision of encysted lesions revealed the presence of flukes (2–3 in numbers) in each cyst with brownish exudate. The lung tissues with lesions were collected in 10% formalin and haematoxylin and eosin staining was done for histopathological evaluation. The flukes were identified as *Paragonimus* spp. based on the morphology and micrometry. The histopathological examination revealed presence of longitudinal sections of flukes in bronchial lumen (in pair) with tegument and

tegumental spines surrounded by connective tissue capsule as cystic encapsulation and numerous eggs in adjacent lung parenchyma. Necrosis and moderate fibrosis of lung parenchyma with infiltration of polymorphonuclear and mononuclear inflammatory cells were observed around fluke as well as eggs. The squamous cell metaplasia of lining bronchial epithelium and atelectasis of alveoli were also prominently seen.

**Keywords** *Paragonimus* spp. · Pneumonia · Royal Bengal Tiger · Metaplasia · Mysuru

### Introduction

Lung paragonimiasis is caused by several flukes of *Paragonimus* genus, among these *Paragonimus westermani* is an imperative species which is involved in causation of potential food borne parasitic zoonotic disease and it has been reported to affect around 22 million people across the world (Seco et al. 2011; Arellano et al. 2006). *Paragonimus* spp. is geographically distributed in Siberia, Japan, Korea, China, Taiwan, Philippines, Indonesia, Malaysia, Thailand, Sri Lanka and India (Dharanisha et al. 2017). Human beings get infection by eating raw or undercooked crayfishes (*Cambaroides*) and fresh water crabs (*Sinopotamon*, *Candidiopotamon*, *Sundathelphusa*, *Parathelphusa*, *Geohelphusa*, and *Ranguna*), which harbor encysted metacercariae in their muscle and gills and serve as second intermediate host in the life cycle of this fluke (Liu et al. 2008). The immature flukes from intestine enter the abdominal cavity and cause mechanical injury to intestinal wall, liver, diaphragm and become adult in lungs. Even aberrant migration to ectopic locations like brain and myocardium occurs (Yokogawa et al. 1960; Madarame

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et al. 2009). Pigs and some smaller rodents have been shown to act as paratenic hosts (Miyazaki and Hirose 1976). The wild tigers may get infection by predated on wild pigs or other animals which host this fluke and also by ingestion of second intermediate hosts which harbor metacercariae. The epidemiological investigation in Sumatra, Indonesia, among 10 infected tigers revealed 1596 flukes in lungs of one tiger (Kwo and Miyazaki 1968). In India, most cases of pulmonary paragonimiasis were reported more frequently from humans rather than in animals which was misinterpreted as tuberculosis and majority were recorded in north eastern states compared to southern India with very less cases particularly in wild animals (Singh et al. 2009). In south part of the country, lung fluke infection was reported in dogs from Coorg in Madras Presidency way back in Rao (1935). Recently, pulmonary paragonimiasis in a female wild Royal Bengal tiger (*Panthera tigris tigris*) has been reported from Mysuru, Karnataka, India by Dharanesh et al. (2017).

## Methods

A male wild tiger (*Panthera tigris tigris*), aged about 18 months was rescued from snare (trap) at Bekkesodluru adjoining Lakshmanthirtha by Forest Department, Ponnampet Range, Virajpet Division in the month of January, 2017. The tiger died 2 days later in captivity at Chamundi Animal Conservation, Rescue and Rehabilitation Centre, Koorghalli, Mysuru, Karnataka, India. The necropsy was conducted as per the standard procedure and systematic examination was carried out. The lungs were congested, emphysematous with discrete raised transparent to dark encysted lesions. Lung cysts were incised, flukes were removed and collected in phosphate buffered saline (PBS) for identification. From the affected part of lungs, tissue slices of 5–10 mm thickness were collected in 10% neutral buffered formalin (NBF) for histopathological examination. About 5–10 g of fecal material from intestine was also collected in a container. The formalin fixed tissues were processed, sectioned at 4–5 micron thickness and stained with haematoxylin and eosin (H&E) for histopathological evaluation.

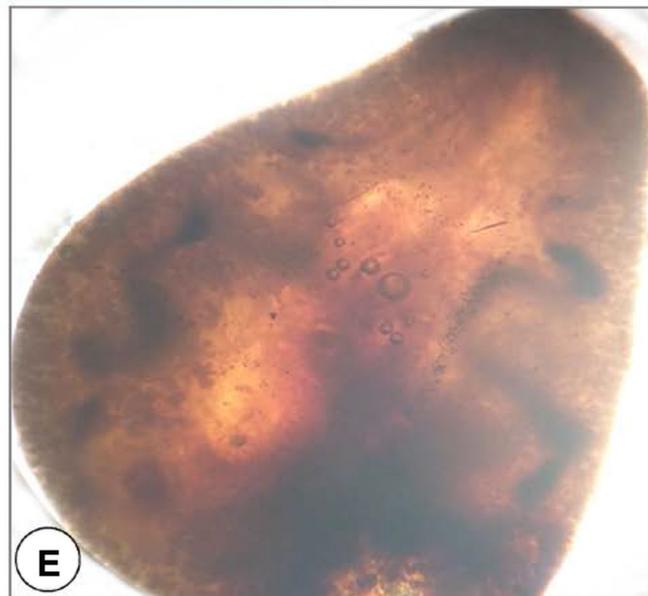
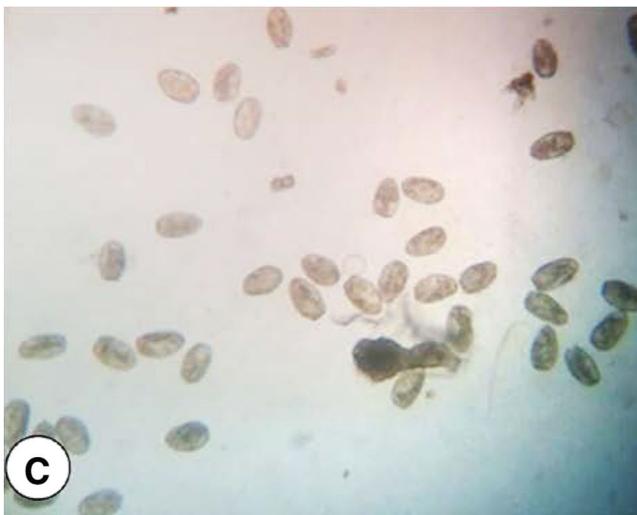
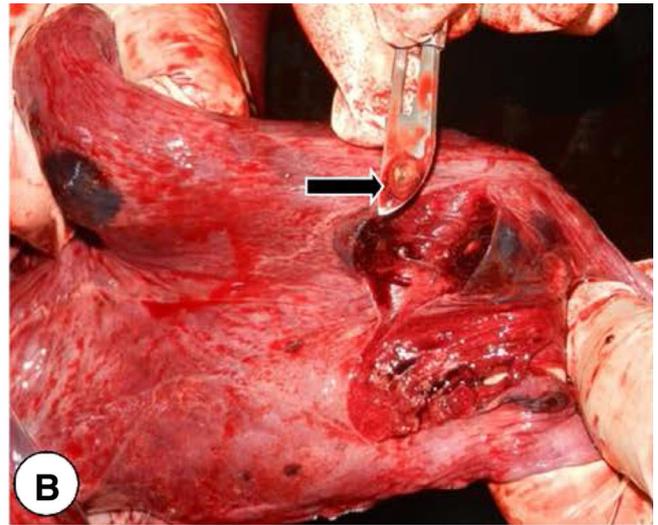
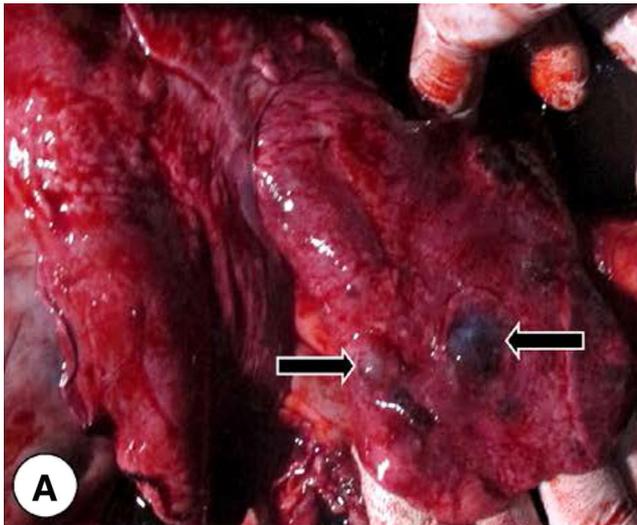
## Results and discussion

External examination of sub adult male tiger showed fair body condition, weighed around 64.9 kg, pale oral and conjunctival mucous membranes with sunken eye balls. External examination of the carcass revealed deep necrosed wound accumulated with pus in left and right forelimb (near carpal joint) along with bruises and edema due to

snare. The large contusion wound with hemorrhages on left inner thigh at stifle joint was also noticed. Grossly, lungs showed congestion, consolidation, hemorrhage, areas of atelectasis and emphysematous changes. Trachea and bronchi were moderately congested. Numerous transparent to dark encysted lesions were found in the parenchyma of all lung lobes which were visible on pleural surface (Fig. 1a). The extent of lesion was severe and all the lobes in both right and left lungs were affected. The incised cyst showed oozing of brownish exude and presence of flukes 2–3 in number (Fig. 1b). The coffee bean shaped flukes collected from cystic lesions were identified as *Paragonimus* spp based on the morphological characters and micrometry (Fig. 1d). The flukes were 9.5 mm long and 4.5 mm wide. The oral and ventral suckers were almost same sized placed in anterior aspect of the body. The intestinal caeca extended up to the posterior end. The lobed testes were adjacent from each other located at the posterior end, and the lobed ovaries were placed in median aspect and slightly posterior to acetabulum (Fig. 1e). The eggs were 94 µm long and 60 µm wide in morphometry. The yellowish brown colored eggs with oval shape, thick shelled and often asymmetrical ends, one end slightly flattened and the other end with operculum were found in fecal sample (Fig. 1c). These findings were similar to morphological characters described by Soulsby (1982). The pericardial cavity showed increased pericardial fluid of turbid nature. The epicardial surface showed petechial hemorrhages. Liver was severely congested and gall bladder was engorged with bile. The cortex of both the kidneys and spleen were congested. The focal hemorrhages and ulcers in the mucosa of small and large intestines were noticed.

The histopathological examination revealed presence of paired adult flukes in the bronchial lumen (Fig. 2a) as fibrous encapsulation and characterized by hemorrhage, loss of cilia, desquamation and proliferation of lining epithelium, transmural and peribronchial infiltration of inflammatory cells, mainly mononuclear cells like macrophages and plasma cells (Fig. 3a). The flukes had characteristic serrated tegument with spines (Fig. 2b). The squamous metaplasia of lining bronchial epithelium was conspicuous and intriguing histopathological finding (Fig. 3c, d). Alveoli were filled with inflammatory exudate, hemorrhage with hyperplasia of lining alveolar cells, thickening of septal connective tissue and alveolar atelectasis were evident (Fig. 3b). Adjacent to fluke, yellowish eggs were found as large aggregations surrounded by fibrous encapsulation, mononuclear cell infiltration and fibrous tissue proliferation (Fig. 2c, d).

The fluke-provoked lung lesions could be due to direct mechanical injury during migration in lung before encystation, toxic metabolites, also by eggs and damage induced

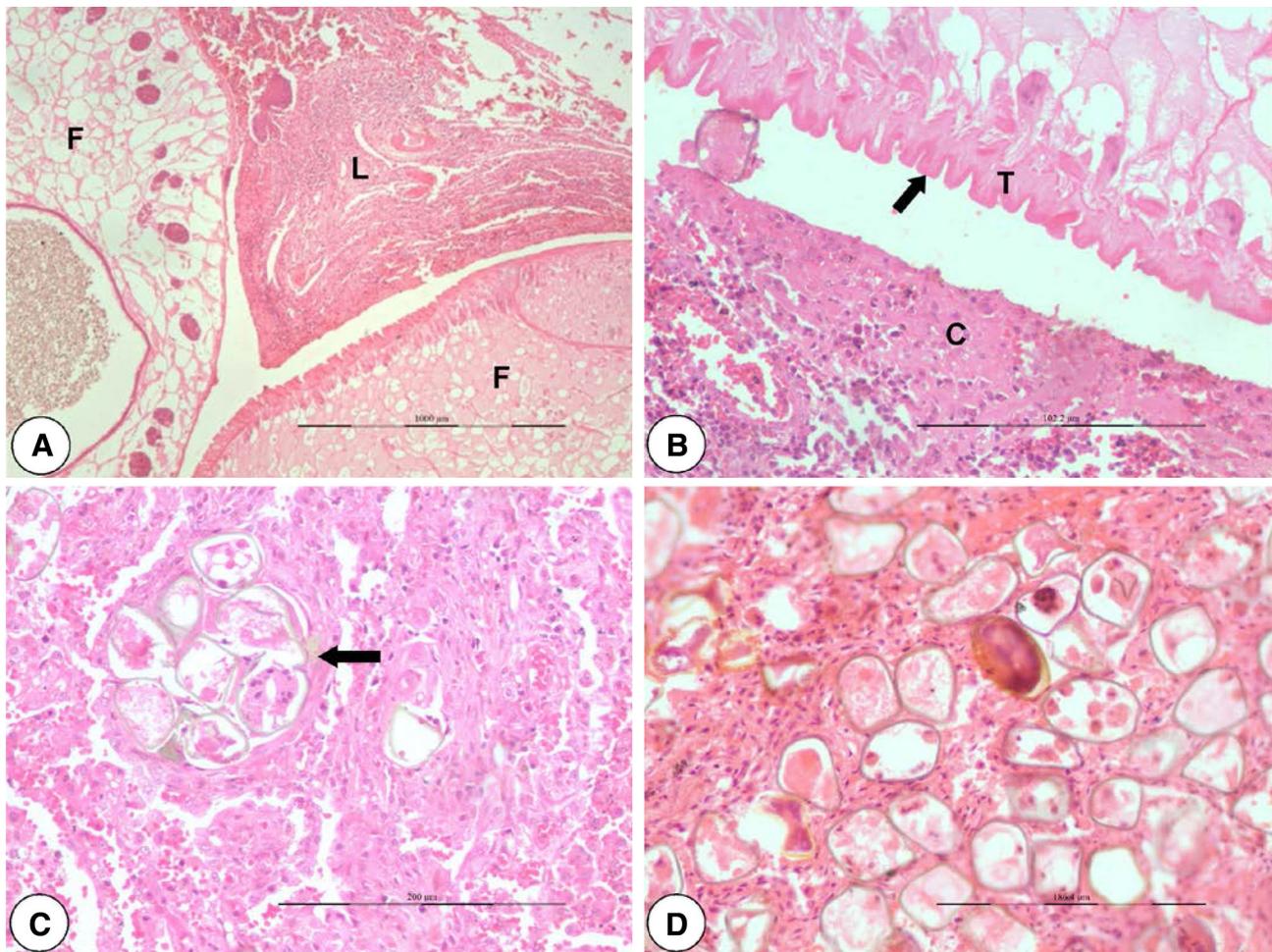


**Fig. 1** **a** Lungs congested, emphysematous, and pneumonic with discrete raised transparent to dark encysted lesions (arrow). **b** Incision of encysted lesions contained fluke (arrow) along with haemorrhage, necrosis and brownish exudate. **c** The yellowish brown colored, oval shaped, and operculated eggs in fecal sample ( $\times 10$ ). **d** The *Paragonimus* spp. of flukes appeared as coffee bean shape. **e** The fluke showed the oral, ventral suckers, intestinal caeca and gonadal organs ( $\times 10$ )

by host immune mechanism (Blair et al. 1999). Further consequences such as, secondary complications to fluke infections like abscess formation and subsequently fibrosis were observed. The excretory and secretory products of adult *Paragonimus* spp. contain cysteine protease enzymes responsible for invoking immunological process during infection (Lee et al. 2006; Na et al. 2006). In this study, the

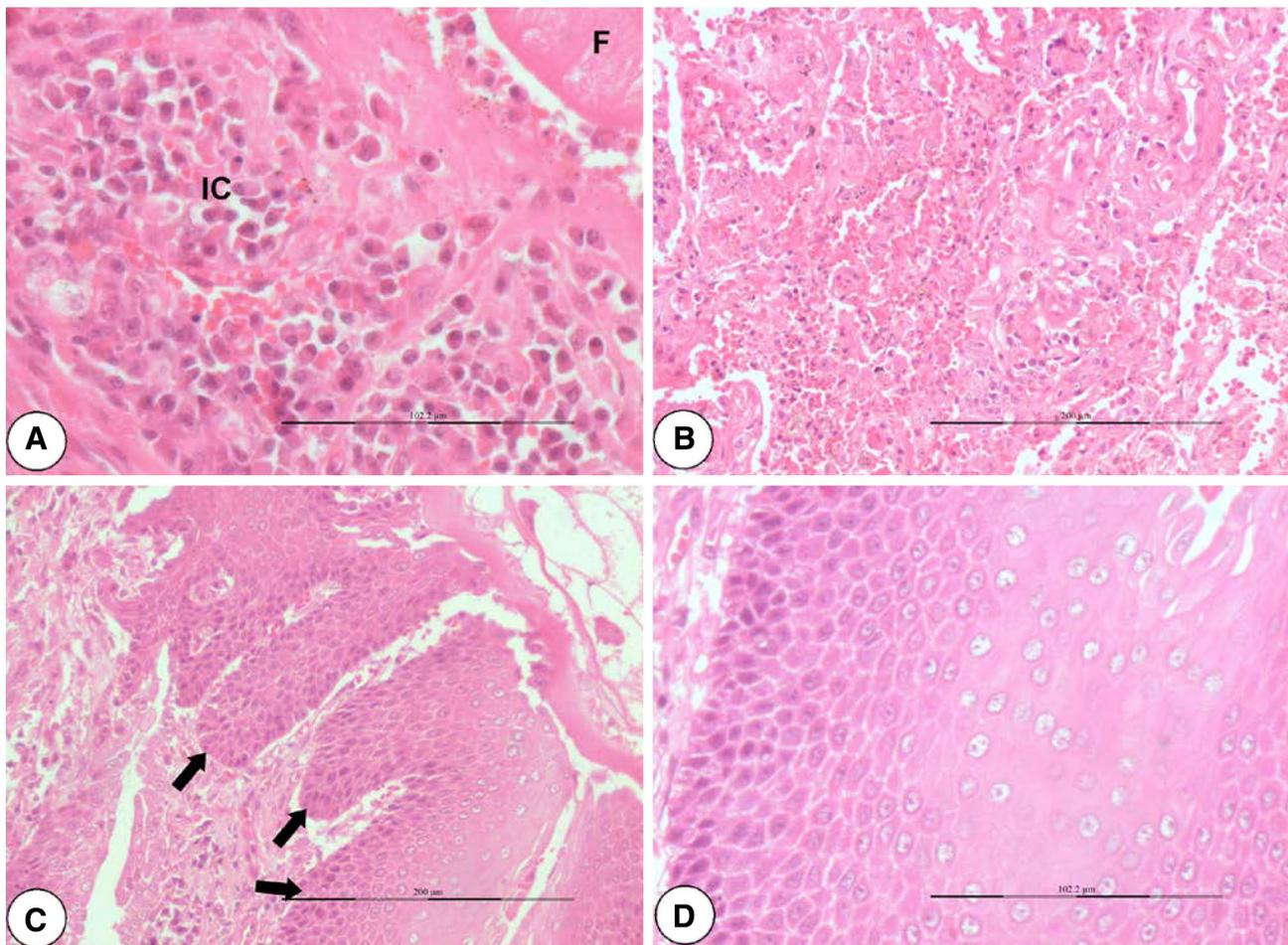
gross pathological findings in lungs such as numerous cystic lesions with congestion, hemorrhage, necrosis, atelectasis, emphysema, paired flukes and brownish exudates within the cysts were identical to earlier reports (Singh et al. 2012). The histopathological studies revealed similar changes which were described earlier such as presence of paired flukes in bronchial lumen, damage to lining epithelium, transmural and peribronchial inflammatory cell infiltration (Lee 1979; Madarame et al. 2009; Dharanesha et al. 2017). The chronic inflammatory changes around fluke eggs were in consensus with earlier reports (Lee 1979; Madarame et al. 2009; Dharanesha et al. 2017).

In the current report, squamous cell metaplasia of lining bronchial epithelium is an interesting finding. The squamous metaplasia, usually contain more than one layer of



**Fig. 2** **a** The section of paired adult *Paragonimus* spp. (F) in bronchial lumen, severe transmural and peribronchial infiltration of mononuclear cells with hemorrhage, and fibrosis in the surrounded lung parenchyma (L) (H&E  $\times 40$ ). **b** The fluke in the bronchial lumen showing serrated tegument (T) with spines (arrow), surrounded by fibrous tissue and inflammatory cells (C) (H&E  $\times 400$ ). **c** Thick

shelled ovoid egg aggregates with fibrous encapsulation (arrow), hemorrhage, thickening of alveolar septa and infiltration of inflammatory cells especially mononuclear cells (H&E  $\times 200$ ). **d** Numerous yellowish, thick shelled, ovoid, distorted eggs of fluke were surrounded by fibrosis and mononuclear cells infiltration (H&E  $\times 400$ )



**Fig. 3** **a** The section of *Paragonimus* spp. (F) in bronchial lumen with severe transmural and peribronchial infiltration of mononuclear cells (IC) especially plasma cells and macrophages in lung parenchyma (H&E  $\times$  400). **b** Alveoli were filled with inflammatory exudate, mononuclear cell infiltration, hemorrhage with hyperplasia of lining alveolar cells and thickening of septal connective tissue

(H&E  $\times$  200). **c** The squamous metaplasia (arrow) of lining bronchial epithelium and desquamation (H&E  $\times$  200). **d** Higher magnification of squamous metaplasia of lining bronchial epithelium showed five layers of epidermis (H&E  $\times$  400)

cells and it is generally presumed to be caused in response to chronic irritation or injury or inflammation of bronchial epithelium and considered as adoptive change to resist further damage. Although, consequently all squamous metaplasia may not have given rise to neoplasia but some time it is considered as preneoplastic (Renne et al. 2009). Few authors coated squamous metaplasia of bronchial epithelium by experimental infection with metacercariae of *Paragonimus* spp in dog and cat (Choi 1984; Weina and England 1990) and a similar finding was reported in naturally affected mink (Presidente and Ramsden 1975). This is the first report of squamous metaplasia of bronchial epithelium recorded in wild tiger lung due to lung fluke as of our knowledge is concerned. It can be predicted that squamous metaplasia in the bronchial epithelium could be caused due to chronic irritation by serrated tegument of

*Paragonimus* spp and many other factors may play role in inducing metaplasia, but this need further elucidation.

In the present study, cause of the death of tiger was parasitic pneumonia due to obstruction of air passages by flukes leading to hypoxia and respiratory failure. Further, deep necrosed wounds resulted in septicemia which was evidenced by petechial hemorrhages on the epicardial surface could also be responsible for death.

This study highlights the occurrence of *Paragonimus* spp in wild tiger at Mysuru, Karnataka, Southern India and also its involvement in causation of lung pathology. The lungs are vital organs involved in respiration, when there is severe damage to parenchyma by *Paragonimus* could cause impairment of normal gaseous exchange and affects the routine hunting process in the wild by reducing its prey chasing ability. To overcome dominating predators competing with its food and to shun infighting among other

healthy tigers, affected one may lurk into the human habitat adjoining the forest in search of domestic animals as weak prey for food source and this may culminate in man-wild animal conflict. This report also reiterates the need for considering this parasite as a potentially danger for wild tigers. Further, prevalence survey of metacercarial infection of this trematode in cray fish and fresh water crabs in water reservoirs in these areas is necessary.

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**Authors' contribution** DNK conducted necropsy, written manuscript and interpreted histopathology slides; SM reviewed and edited the paper; MP edited paper; RKR supervised necropsy; GP and BSM provided resources; AKJ analysed of fecal and parasite samples.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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