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Regional report

Paropisthorchis caninus Stephens, 1912: Synopsis of identity and pathological findings due to spontaneous infection in the liver of a street dog in Assam, India

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ABSTRACT

The present study describes a small liver fluke recovered at post-mortem and the pathological alteration produced by the parasite in the liver of a street dog from Assam, India. The spatulate parasite measured 3–4 mm in length and 1.0–1.25 mm in width with spiny body surface and showed a grossly visible peduncle projecting from the ventral surface at the anterior portion. The eggs of the parasite contained well developed miracidia. The parasite was identified as *Paropisthorchis caninus*. Identity of the parasite is discussed in the light of available literature and found to be morphologically different from other reported species of dog *Opisthorchis.* Pathological lesions observed in the liver included degeneration of hepatic parenchyma, hyperplasia of bile duct epithelium, fibrous tissue proliferation and formation of pseudo lobule which were the characteristics of chronic proliferative inflammation.

1. Introduction

Opisthorchiids are small to medium sized trematode parasites that inhabit the hepato- biliary ducts, gall bladder and pancreatic ducts of piscivorous vertebrates with some species found in the digestive tract of birds and reptiles (Scholz, 2008). The parasites utilize aquatic snails and fresh water fish as their 1st and 2nd intermediate hosts respectively. Infection in the final host is acquired through consumption of raw or undercooked fish infected with the metacercariae stage of the parasite.

The so called small liver flukes under the genus *Opisthorchis* viz., *Opisthorchis viverrini, O. felineus* and *O. noverca* are known to infect man and other fish eating animals. *O. viverrini*, capable of producing cholangio-carcinoma in man is endemic in South –East Asian countries (Arimatsu et al., 2015). *O. felineus,* although known to be endemic in parts of Siberia, Kazakhstan and European countries, (Armignacco et al., 2008) has been sporadically recorded in dogs (Patnaik, 1959; Shastry and Patnaik, 1968), pigs (Gatne et al., 2008; Jawalagatti et al., 2016) and cats (Chandler, 1925; Chakrabarty and Sinha, 1960) from India. Natural infection with *O. noverca* has been recorded frequently in pigs (Sinha, 1968; Sahai and Srivastava, 1971, 1978; Sahu et al., 2015; John et al., 2017) and dogs (Mudaliar, 1943; Sahai, 1969; Sahai and Srivastava, 1971, 1978) with occasional reports of infection in man from India (Mc Connell, 1876, 1878). Besides infection with *O. noverca,* dogs and pigs from India are also known to be frequently infected with another species called *O. caninus* (Gupta and Pande, 1963; Rai, 1966; Srivastava and Shah, 1968; Ahluwalia et al., 1975). The parasite, *O. caninus* (Lewis & Cunningham, 1872) Barker, 1911 was originally described from the liver of Pariah dogs in Calcutta (India). Stephens (1912) examined parasite specimens collected from the liver of native dogs in India and found them to be identical to *O. caninus* but different from all other opisthorchids reported earlier. Based on valid differences, a separate genus *Paropisthorchis* was proposed by himself to accommodate the new species *P. indicus* as its type species, which along with *O. caninus* were together renamed as *P. caninus.* No further reports with specific descriptions of the parasite except one recorded from a cat (Bhatia et al., 1959) is available to date in the literature. The present communication reports the record of *P. caninus* along with the pathological findings in the liver of an affected street dog from Assam, India.

2. Materials & methods

2.1. History

An adult male street dog presented clinical signs of salivation, depression and off-feed was kept under observation with preliminary

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supportive treatment in a rescue home run by a non government organization "JBF" (Just Be Friendly) at Guwahati in the state of Assam (India). The animal died on the second day of initial treatment and the carcass was later brought for post-mortem.

2.2. Post-mortem examination

During the post–mortem examination, all the visceral organs were examined for gross abnormalities. Dark coloured bile with small sized parasites coming out from the incised liver was collected carefully in a petri dish, washed with physiological saline by centrifugation and the parasites separated out. The sediment was kept for microscopic examination and detection of parasite eggs.

2.3. Parasite identification

Counting of parasites, their gross appearance and measurement were noted. Some of the specimens were flattened and fixed in 10% formalin. A few of them were put subsequently in lactophenol for clearing (Chai et al., 1986) to a desired level and temporary whole mount preparation, while other fixed specimens were stained with alcoholic borax carmine, processed through ascending grades of alcohol, cleared in creosote and mounted in DPX medium. Parasites in temporary and permanent mounts and their eggs were examined under microscope (4× and 10× objectives) for detailed morphological study. Species level identification of the parasite specimens was done using the keys given by Yamaguti (1958, 1971).

2.4. Pathological examination

Affected liver was subjected to gross pathological observation followed by histopathology in 5–6 µm thick cut sections of liver tissues processed by routine histological methods and staining with haematoxylin and eosin (H&E).

3. Results

3.1. Identification of parasite specimens

The fresh parasite specimens (20) were pinkish with a spatulate body, anterior end drawn out like a small neck, mid region broader and round and the posterior end tapering to a point. The ventral surface looked concave at the anterior portion with a very small whitish knob like projection; the peduncle was visible without magnification (Fig. 1). The parasites measured 3–4 mm in length and 1.0–1.25 mm maximum

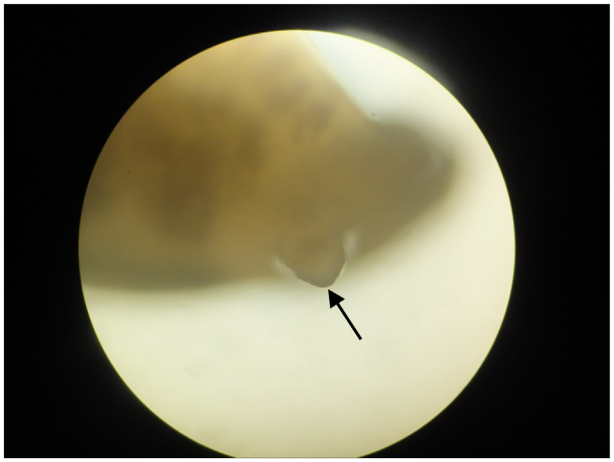

Fig. 1. Ventral surface showing peduncle in lactophenol cleared parasite specimen (*Paropisthorchis caninus*).

Fig. 2. Anterior portion of spatulate parasite showing different internal organs including the peduncle with the acetabulum and common genital pore on it.

breadth at the mid region. Microscopic examination of lactophenol cleared and stained whole mount specimens revealed the following: the body surface was covered with scaly spinules; oral sucker was larger than the acetabulum; a broad pharynx and short oesophagus; presence of a groove with a cylindrical peduncle broader at its base and provided with the acetabulum followed by the common genital pore at its summit; intestinal caeca were simple and reaching nearly the posterior end with deep inward curvature at the testicular zone; uterus, filled with many eggs, loosely coiled and inter caecal in position extending from the ovary up to the level of anterior vitelline acinus; ovary 3 lobed, pre-testicular and median in position; testes oval, entire and situated in a diagonal position posterior to the ovary; excretory tube sigmoid and passing between the two testes to open at the posterior end; and vitelleria consisting of 8 bunches of grape like acini, extracaecal on either side in the mid region from the level behind the peduncle to the level of ovary and upper margin of the anterior testis (Fig. 2). Eggs measured on average 28.5 µm × 11.40 µm in size with an operculum at the thickened rim of the shell and a small knob like structure at the opposite pole. Eggs contained well developed miracidia. Considering the host species involved, body organ affected, previous description from a geographical region and present morphological findings on the specimens and its eggs, the parasites were identified as *Paropisthorchis caninus.*

3.2. Pathological findings

All the visceral organs except liver were found to be apparently normal. The liver was enlarged, pale and hard. Upon incision, the cut surfaces showed marked thickening of the bile ducts (Fig. 3) with a viscid and blackish bile along with the small sized parasites. The

Fig. 3. Cut surface of affected liver showing thickening of the bile ducts.

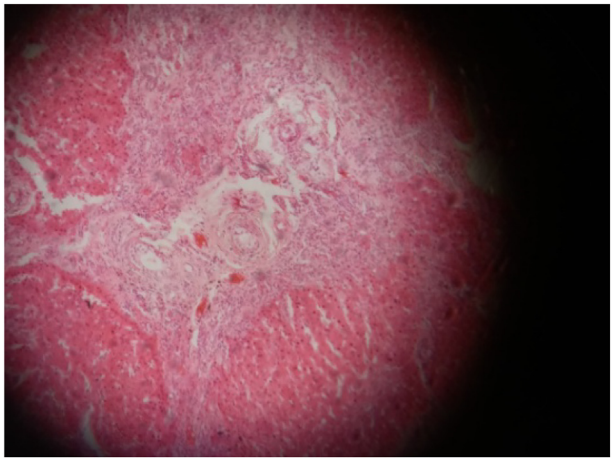

Fig. 4. Cirrhosis of liver and formation of pseudolobules due to *P. caninus* infection.

hepatocytes, in histopathology sections of the affected liver, showed varying degrees of degenerative changes like cellular swelling, hydropic degeneration and necrosis. There was massive hyperplasia of biliary epithelial cells forming new acini, which were much dilated. Infiltration with mononuclear and polymorphonuclear cells was observed in the peri-biliary fibrous connective tissue. The fibrous tissue in the peri-portal areas proliferated towards the hepatic lobules resulting in the replacement of hepatocytes and formation of pseudolobules (Fig. 4). There was presence of dark brown pigments in the cytoplasm of Kupffer's cells. The portal vein, central vein and sinusoids were congested.

4. Discussion

4.1. Identification of parasite specimens

On the basis of morphological characters, the parasite specimens were found indistinguishable from *P. caninus* described earlier by Stephens (1912). Morphological description of the parasite also agreed to that reported by Seuerian (1919). The peduncle of the parasite was visible to the naked eye in all the fresh specimens before processing. However, the same was not seen in some of the permanent mounts when examined under microscope, rather the acetabulum looked to be sessile one with a dark shadow surrounding it. Such a variation might be related to the flattening and fixation procedures performed for permanent mounting. Stephens (1912) recorded the peduncle to be a retractile one and in that situation there was presence of a spherical structure around the acetabulum. According to Scholz (2008), the presence of peduncle in a retractile position could easily be recognized, being separated from the body by a deep groove. The flattened parasite specimens of the present study also showed a groove on the body surface around the protruding peduncle. This organ in question was never observed in any of the described parasites under the genus *Opisthorchis* (Scholz, 2008) where the acetabulum was a sessile one and embedded in body parenchyma. This might have led Stephens (1912) to propose further division of the genus *Opisthorchis* and creation of a new genus *Paropisthorchis* accommodating *P. caninus* (*O. caninus*) as its genotype in the line of creation of genera *Amphimeras* and *Clonorchis* to place *O. noverca* as *A. noverca* and *O. sinensis* as *C. sinensis* respectively on valid grounds. Identification of present parasite specimens was performed as per the keys provided by Yamaguti (1958, 1971) who recognized taxonomical validity of the genus *Paropisthorchis* created by Stephens, 1912. Therefore, identification of *P. caninus* on the basis of peduncle present and body spination in contrast to the type species of *Opisthorchis* genus is considered to be valid and this agrees with the documentation in different literature (Bowman et al., 2002; Scholz, 2008). Several researchers (Gupta and Pande, 1963; Ahluwalia et al., 1975) from India except Bhatia et al. (1959) have preferred to retain the name *O. caninus* for the parasite recorded from dogs and pigs. Saleh and Ahmed (1965) considered *O. caninus* and *P. caninus* as two distinct species recorded from dogs in Pakistan. Mehra (1980) while describing mammalian opisthorchids of India and adjacent countries mentioned three species (*O. tenuicollis*, *O. noverca* and *O. sinensis*) under the genus *Opisthorchis* (Syn: *Amphimeras*, *Paropisthorchis* and *Clonorchis*) in which *O. noverca* (Syn. *O. caninus*, *Distoma conjunctum*, *P. indicus*) was characterized by the presence of retractile peduncle. However, Sahu et al. (2015), performing morphology based identification of *O. noverca* collected from pigs in North East India, the same region of the present study, did not mention the peduncle but identified them by a break in the arrangement of vitelline glands and confirmed the parasites as a distinct and valid species through molecular analysis. Cai et al. (2014) using Real time PCR and high resolution melting (HRM) analysis also claimed rapid and specific identification of *C. sinensis* (*O. sinensis*) and *O. viverrini* which were usually difficult to be distinguished by other molecular methods because of high genetic homology (Park, 2007). Morphological description of parasite specimens of the present study also does not conform to those provided for *O. tenuicollis* (*O. felineus*) (Soulsby, 1982; Jawalagatti et al., 2016), *O. noverca* (Sinha, 1968; Sahai and Srivastava, 1978; Sahu et al., 2015), *O. viverrini* (Mehra, 1980; Mas-Coma and Bargues, 1997; Bowman et al., 2002) and *O. sinensis* (Mehra, 1980; Soulsby, 1982; Bowman et al., 2002). It is apparent that, even if all these species are grouped under the one genus *Opisthorchis*, they are considered different from each other on valid morphological grounds. King and Scholz (2001), in a review of the trematodes of the family Opisthorchiidae, described *Amphimeras* and *Paropisthorchis* as distinct and valid genera. Thus the parasite specimens of the present study showed remarkable morphological differences by the existence of a peduncle bearing on its summit the acetabulum and the common genital pore which leads to the identification of *P. caninus* as a taxonomically valid species similar to *Amphimeras noverca* (Mas-Coma and Bargues, 1997). This record of *P. caninus* for the first time from Assam adds to its previous record in dogs and cats by Stephens (1912) and Bhatia et al. (1959) respectively and further supports to its prevalence record in India.

Besides India, *P. caninus* has been recorded in dogs from Pakistan (Saleh and Ahmed, 1965). The epidemiology of infection in dogs is not fully known. The *Melanoides* snails and ophiocephalid spp. of fresh water fish were found to be naturally infected with different opisthorchids (Sahai and Srivastava, 1978; Sachdeva, 1990) in India. Ingestion of raw or rotten fish thrown away in the unorganized market premises might be the common source of infection to the stray dogs in which 24.67% were found with infections by. Gupta and Pande (1963).

4.2. Pathological findings

The present findings of gross pathological changes in liver and bile duct due to *Paropisthorchis caninus* infection support the observations of Sinha (1968) and Ahluwalia et al. (1975) in pig livers infected with *O. noverca* and *O. caninus* respectively. In the histopathology, cholangiocarcinoma, as observed with *O. viverrini* (Waikagul and Thaenkham, 2014) infection in man, was not found in the present study. Thus, the resultant picture in the liver was that of a chronic proliferative inflammation characterized by hyperplasia of fibrous connective tissue and bile duct epithelium and formation of pseudolobules which were characteristic of cirrhosis as described earlier in pigs and dogs infected with different *Opisthorchis* spp. (Shastry and Patnaik, 1968; Sahai and Srivastava, 1971; Ahluwalia et al., 1975; Jawalagatti et al., 2016; John et al., 2017).

5. Conclusion

In conclusion, the present study confirms that *P. caninus* causing cirrhosis in liver of a dog is a distinct and valid species recorded from Assam (India) after over 100 years of its first description by Stephens (1912) from the country. Further, molecular studies are warranted to define the genetic variations among the different species of *Opisthorchis*, *Amphimeras*, *Paropisthorchis* and *Clonorchis*.

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