A Rare Abattoir Report on Heavy Infection of Pigs with *Opisthorchis tenuicollis*.

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ABSTRACT

This paper describes a rare observation of the parasitology and pathology of three cases of porcine opisthorchiosis encountered in an abattoir survey conducted at Mumbai. The load of parasites that were recovered was as high as 165 flukes from a mass of five grams of hepatic parenchyma. On the basis of morphometric analysis the flukes were identified as *Opisthorchis tenuicollis*. Predominant gross and histological lesions included extensive biliary cirrhosis as evident by fibrotic tracks on the surface as well as deeper in the substance of liver and dilatation of bile ducts with progressive cholangiohepatitis. Fibrotic liver or milk spotted liver encountered during necropsy of pigs should not be arbitrarily considered as a case of ascariosis as the gross lesions caused by *Opisthorchis tenuicollis* during massive infections may resemble the same picture.

Keywords: *Opisthorchis tenuicollis*; Pig; Liver Fibrosis, Pathology; Helminthology.

INTRODUCTION

Opisthorchiosis is a fish borne helminthic infection reported to occur in the bile ducts and less frequently in pancreatic ducts and intestine of dog, cat, fox, pig and man in eastern Europe (Poland, Germany, etc.) and Asian countries including India (1). The infection is acquired by consumption of raw or undercooked fish infected by metacercariae of the fluke. Various species of *Opisthorchis* spp. have been reported from different parts of the world (1-6). However information on its occurrence in India and its pathogenic effects in different hosts is far from adequate (7-9). It is worth to state here that in a previous abattoir study (10) on helminthosis conducted at Mumbai involving inspection of 501 pig carcasses did not reveal presence of *Opisthorchis* spp. The present report describes a rare observation of porcine opisthorchiosis associated with severe gross and histopathological changes in the bile ducts as well as in the hepatic parenchyma of three liver specimens encountered during post mortem meat inspection in an abattoir in Mumbai.

MATERIALS AND METHODS

During the process of evisceration of three carcasses of pigs slaughtered at an abattoir in Mumbai, livers with severe fibrotic lesions were discarded as suspected ascariosis. All the three non-descript country pigs brought from Rajasthan, India were reared in free range system. The discarded livers were brought to the laboratory on ice for further investigations.

At the laboratory, after incising the livers, flukes were recovered. The exudates oozing out from cut surfaces was cleared out by washing with physiological normal saline and intact flukes were collected in 10% formalin. Subsequently the medium was centrifuged at 2500 rpm for 5 min and sediment was examined for presence of eggs. A number of the flukes were subjected to acetic alum carmine staining and permanent mounts were prepared in Canada balsam. The flukes were identified by referring to the keys given by Yamaguti (11). A piece of liver weighing 5g was incised and the total fluke numbers was counted. Gross changes in the livers were noted and sections were sent for histopathological evaluation.

RESULTS

Macroscopic features

The livers were significantly enlarged showing white fibrotic lesions over the entire surface (milk spotted liver) and also in the parenchyma (Figure 1). Based on this picture the cases were considered to be that of severe ascariosis. However, when an incisions were made on the fibrotic surface, a thick viscous fluid oozed out containing an abundance of minute live flukes. Deeper dissection revealed dilated bile ducts (Figure 2) which were almost occluded with thick gelatinous jelly containing hundreds of rice particle sized white coloured flukes measuring 3.5 to 9 mm (Average 6.4 mm) in length (Figure 3). A total of 165 flukes in 5 g affected portion of the liver were counted.

Microscopic description of flukes

Based on morphological analysis of stained specimen (Figure 4) the flukes were identified as *Opisthorchis tenuicollis*. The integument was smooth. The suckers were closely placed. The oesophagus was short and the intestinal caeca reach almost to the posterior end. The testes were slightly lobed and diagonally placed in the posterior half of the body. A small ovary was located anterior to testes in the ventral midline. The vitellaria occupied the middle thirds of the lateral fields; they consisted of series of transversely arranged follicles. Transverse uterine coils occupied the space between the ventral sucker and the ovary.

Egg morphometry

Eggs were oval and embryonated (Miracidium was asymmetrical) measuring 9-12 x 26-32 µm. At the anterior end there was an operculum which fitted into a thickened rim of the shell (Figure 5). The egg morphometry recorded here were in correspondence with that described by Soulsby (1).

Histopathological findings

The cross section of affected portions of the liver showed typical lesions of biliary fibrosis with abundant extension in the periductal hepatic parenchyma. The bile ducts were enlarged with large numbers of migratory flukes in the lumen (Figure 6). In few foci degenerating flukes were also noticed. There was adenomatous hyperplasia of biliary epithelium and thickening of the duct wall with connective tissue proliferation. Papillary projections of biliary epithelium were seen protruding into the lumen (Figure 6). Due to extensive proliferation of the fibrous connective tissue, liver parenchyma appeared to be divided into number of pseudo-lobules (Figure 7). Multifocal haemorrhages were also seen. Capsular cirrhosis was present but not consistently. There were foci of hepatocytes showing degenerative changes (cloudy swelling) as well as localised inflammatory infiltration consisting primarily lymphocytes and eosinophils.

Figure 1: Gross liver showing white fibrotic spots

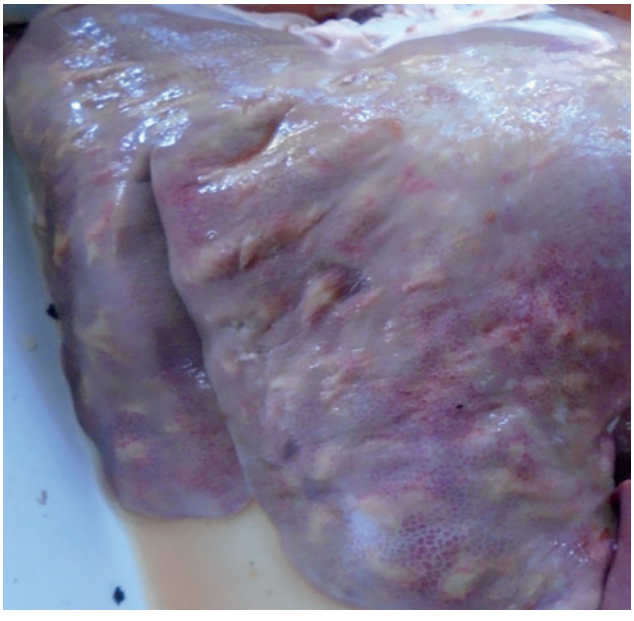


Figure 2: Bile ducts occluded with flukes & exudates

DISCUSSION

In domestic animals, two distinct species *viz. O. viverrini* and *O. tenuicollis* have been recognised (1). The available literature on the taxonomy of genus *Opisthorchis* is complex and to some extent ambiguous, however in the present communication the specimens recovered were identified as *Opisthorchis tenuicollis* since *O. viverrini* has not been reported to occur in India and not described in pigs (1). Further morphometric analysis of the flukes corresponded with the description of *O. felineus* which is now known as *O. tenuicollis* (11).

This paper thus attempts to throw some light on opisthorchiosis in animals and in this case report pigs. Apart from the information available in texts, the occurrence and prevalence and disease entity of opisthorchiosis in animals has not been described in detail. The gross and microscopic pathological lesions reported here are in general agreement with the observations of James and McGavin (12) who reported pronounced chronic cholangitis, dilated intrahepatic bile ducts with hyperplastic adenamatous biliary epithelium often projecting into the lumen, thickened fibrotic duct walls and mild to moderate inflammatory infiltrate. In contrast, the macroscopic picture of episode of porcine opisthorchiosis reported earlier by Gatne *et al*. (9) was principally different since in the latter case there was a table tennis sized thick walled swelling of the bile duct on the surface of liver which revealed thousands of flukes after dissection in the laboratory and the rest of liver parenchyma was apparently normal and did not revealed fibrotic changes.

In fact the case was suspected as hydatidosis at the time of collection as the remaining portion of bile duct, apart from the dilatation, was embedded in the substance of the liver. This time as well, *prima fascia*, the liver was suspected for

Figure 3: Gross appearance of flukes

Figure 4: Morphological features of *Opisthorchis tenuicollis*

Figure 5: Microphotograph of eggs of *Opisthorchis tenuicollis* (100X)

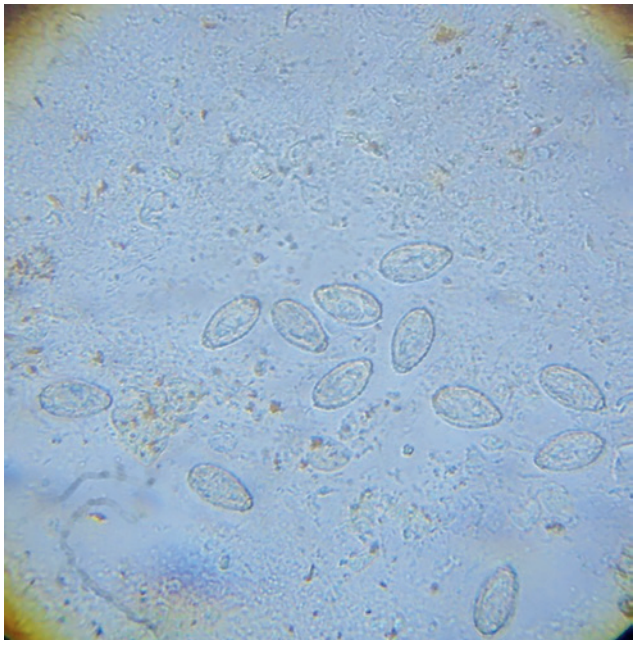


Figure 6: Histopathology showing presence of fluke *Opisthorchis tenuicollis* (100X) in dilated bile duct and protruded papillary projections (H&E 1000X)

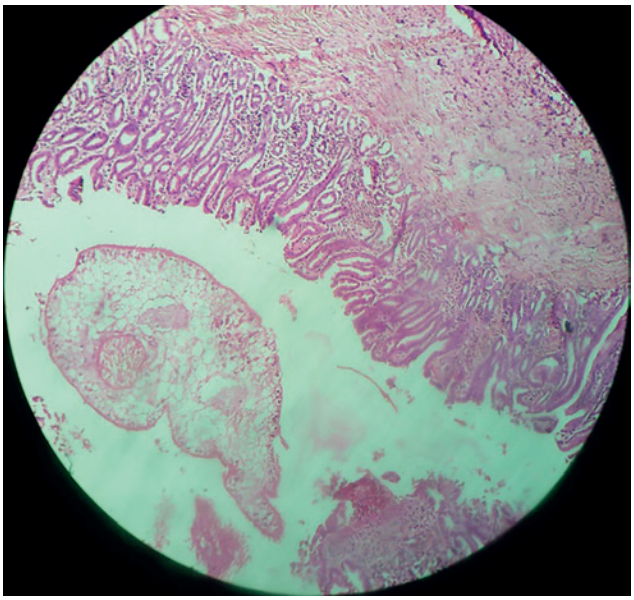
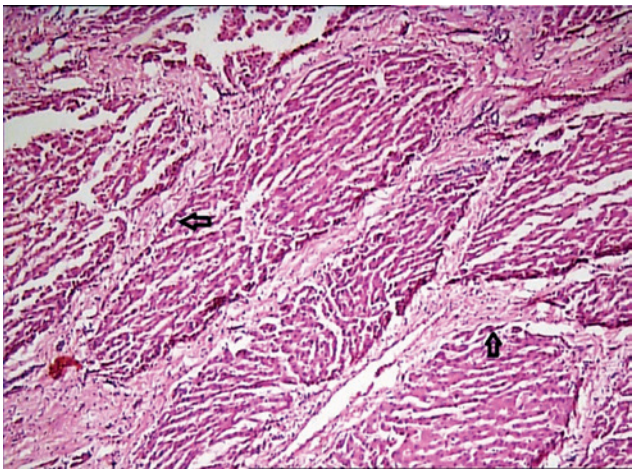


Figure 7: Liver parenchyma showing number of pseudolobules (H&E x100)



ascariosis owing to fibrotic lesions with whitish spots and patches on the surface. However, on dissection the flukes were found in almost all the parts of all the three livers suggesting massive infection involving secondary and tertiary bile ducts. This finding highlights the need for frequent laboratory investigations for precise identification of etiological agents. Furthermore there is every reason to believe that occurrence of the disease in pigs must be higher than that projected in the literature since the possibility of missing mildly infected cases due to absence of gross lesions does exist. Such a heavy infection also indicates substantial infections in fresh water cypriniid fish with metacercarial stages, a common source of infection for animals and man. Although in the present study the location/region from where the three pigs were brought to the abattoir could not be ascertained due to sequential involvement of chain of animal transporters, it could be assumed that the animals were from the region where fresh water fish are bred and consumed on large scale.

Thus the present findings point out a potential alarming situation as far as public health significance is concerned. Nonetheless extensive hepatic damage might also lead to suboptimal meat production owing to albumin associated hypoproteinamia. Hepatic adenocarcinomas have been reported to be associated with *Opisthorchis tenuicollis* infection in cats and humans (1). However in the present study adenocarcinomatous changes were not encountered during histopathological analysis of the sections from all the three livers, in spite of heavy infections.

The overall analysis of the episode warrants systematic study on opisthorchiosis in animals and implementation of corrective measures to curb the prevalence of the liver fluke and associated public health hazards, particularly in the regions where fresh water fish are bred and consumed. Creating awareness about the disease and advocating thorough cooking of fish are the important keys to achieve this goal.

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