Hydatid cysts of zebra shark (*Stegastoma fasciatum*): A case report

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ABSTRACT
We report the hydatid cysts in a Zebra Shark (*Stegastoma fasciatum*) captured in the public aquarium of Thailand. The carcass demonstrated 0.5-3 cm diameter uniloculated cysts in the spleen, with thick capsules. The liver showed enlargement with yellowish brown color. Other organs were normal upon gross examination. Microscopically, the splenic cyst consisted of several hydatid cysts, infiltrated with numerous crystalline particles called hydatid sand. The inner surface of these cysts appeared to be producing necrotic cell like bodies which accumulated in the cyst. The spleen was infiltrated with numerous inflammatory cells mainly eosinophils. Furthermore, the liver was infiltrated with numerous hydatid sand and macrophages. The hydatid sand also spread in to the gastrointestinal tracts. From pathological findings, the shark was infected by hydatid pathogen. This is the first report of hydatid cysts in a shark.

KEYWORDS : Zebra Shark, hydatid cysts, spleen

INTRODUCTION

Zebra shark (*Stegastoma fasciatum*) is monospecific in family *Stegastomatidae* (Last and Stevens, 1994). It is a primarily bottom dwelling elasmobranch that lives in warm, shallow, inland water of continental and island shelves, and frequently in coral reefs. It is distributed in the Indo-West Pacific (Red Sea and East Africa to New Caledonia, Japan, and Australia). Being a carnivore, it possesses rows of small teeth set in powerful jaws to feed on a wide variety of benthic vertebrates and invertebrates. It is a hardy shark and a popular species for public exhibition. (Kunze and Simmons, 2004)

The high urea concentration in tissues and body fluids of elasmobranches render a high specialized parasite fauna (Sanmartin *et al.*, 2000; Cislo and Caira, 1993). Presently, limited reports on parasite populations of rays and sharks have been published. Among them, hydatid cysts of the elasmobranches is extremely rare, it was reported as a zoonotic infestation caused by *Echinococcus granulosus* (Bownman, 1999). So far, only a few elasmobranches have been reported of hydatid cysts. This report on hydatid cysts in captured Zebra shark (*Stegastoma fasciatum*) would be beneficial to aquarium management and human heath concerns.

MATERIALS AND METHODS

A female adult zebra shark was reported sick in an aquarium in Thailand. Her body weight was 40 kilograms. The shark was clinically presented with anorexia over a month and then died 2 weeks after reported. The carcass was sent for necropsy at the Veterinary Medicial Aquatic Research Center (VMARC), Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. A necropsy was performed and samples of the visceral organs were collected and preserved in 10% formalin for histopathological examination.

RESULTS

The state of the carcass was fresh upon necropsy. The external appearance showed severe generalized hyperemia on the body surface that might be from the septicemia prior to death. Gross pathological examination of the visceral organs showed marked pathological lesions in the spleen, together with multiple unilocular cysts of 0.5-3 cm in diameter. The cysts were varied in size (Fig.1a) with fibrosed wall cystic content which was vicious yellowish creamy fluid-like pus. Bacterial culture of the cyst showed negative growth. The liver was hypertrophied with brownish yellow coloration. Other organs appeared normal.

Histopathologically, the splenic cyst was separated into lobules by complete connective tissue septa. The multiple unilocular cysts contains a lot of blood cells. (Fig.1b). The cyst wall consisted of three layers. The inner surface of the cyst wall was a single squamous

cells layer (germinal layer) producing numerous bodies which had accumulated in the cystic cavity and became necrotic tissue. The second wall layer was differentiated into thick non-cellular connective tissue in the intermediate layer (laminated layer). The outer layer was adventitious cell layer, formed by the host tissue reaction to the parasite. Each cyst contained cystic fluid. (Fig.1c)

Crystal-like particles were observed as "hydatid sand". The hydatid sand is the sand composed of free brood capsule and protoscolex in the bottom of the cyst. (Fig.1d). However, in some area of the tissue section, there was an atypical cell layer of single columnar plump cells between the germinal and laminated layer. (Fig.1e)

The tissue adjacent to the cyst was rather loose compared to the normal area of the spleen (Fig 1f). Numerous hydatid sand and inflammatory cells, predominately, eosinophils, were evident in the spleen, liver and GI tract. At higher magnification, numerous hydatid sand scattered in the liver parenchyma with several in macrophage infiltration were observed. (Fig.2). Hydatid sand was also observed in the lumen of the stomach along the distal portion of the intestine, as well, as in the esophagus (Fig.3a,b)

DISCUSSION

In this case, the hydatid cysts observed in the Zebra shark indicated previous infestation of tapeworm, *Echinococcus granulosus*. This cestode belong to the order Cyclophyllidea. which was reported to produce hydatid cyst in human with zoonotic potential (Calville, 1991).

The microscopic characters of the *E. granulosus* infestation are unilocular cyst, structure and a composition of three membrane layers with accumulation of hydatid sand similar to the observation in this report. The host tissue layer or adventitious layer was formed by the host tissue reaction to the parasite. The laminated and germinative layer was produced by the parasite (Bowman *et al.*, 2003). The inflammatory cell observed were eosinophils and macrophages which indicated parasitic infestation and chronic infections, respectively (Dellmann, 1993).

Generally, the adult minute tapeworm *E. granulosus* was usually found in the intestines of dog and other canidae. Two different cycles - pastoral and sylvatic and a number of different strains have been recognized. The intermediate host in the pastoral cycle are primarily herbivores. The most important intermediate host is sheep, but in some areas *E. granulosus* is a common parasite of goats, swine, cattle, and horse. In North Africa, Iraq and Iran, hydatid cysts frequently occurred in the lungs of camels. Human infection is principally seen in the sheep-raising areas of the world, including endemic in domestic animal raising regions of the world such as Central Europe, the Mediterranean, the Middle East, South America, Australia, New Zealand and South Africa. The sylvatic cycle occurs in wolves. Hydatid cyst was reported in wild ungulates (moose and reindeer) in Alaska, Canada, Scandinavia, and northern Eurasia. In Australia, the wallaby-and-dingo strain is more significant than the sheep and dog strain. Humans are less commonly infected as an accidental host in the sylvatic cycle (John and Petri, 2006). In both cycles, human infection may be acquired by ingesting the parasitic eggs when handling the feces of infected carnivores (Bowman *et al.*, 2003).

However, hydatid cyst involvement of the elasmobranches or any other lower vertebrate has been extremely rare. From research, only one study reported the liver lesion produced by hydatid-like cyst in the electric ray (Smith and Little, 1969). The shark is possibly an intermediate or accidental host like human, which was infected by ingesting contaminated and uncooked meat or poor hygiene (John and Petri, 2006). Pathogenesis of hydatid cysts suggests that, adult tapeworm in the definitive host pass proglottids or eggs into the feces. When ingested by intermediate or accidental host, the egg hatches in the intestine. The oncospheres (hexacanths) penetrate the gut wall, enter the portal vein and reach the liver, which is the first capillary filter for the larvae and subsequently into the lungs, which are the second capillary filter. The remaining oncospheres may reach other organs via the arteries. In all these organs, hydatid cysts (bladder worm) can be developed. There are large cysts which were filled with fluid and tapeworm heads (protoscolices) in the hydatid cyst which gradually develop over several months. They are generally 5-10 cm in diameter and contain fluid. The hydatid cyst is composed of a thick outer, concentrically laminated membrane (Bowman, 1999), which is similar to the appearance of the cyst in this report. About 5 months after infection of brood capsules, each containing several protoscolices develop from the inner part or germinal membrane of the capsule. The brood capsules, which contained several protoscolices developed from the germinal membrane. The brood capsules may become detached and float in the cystic fluid, being in term of "hydatid sand" (Bowman, 1999). In this case, there were only two types of cyst. The sterile cyst which is the cyst that failed to produce the brood capsule, and acephalocyst which is the cyst that failed to produce protoscolex. (Bowman, 1999). We can infer that shark was not a normal host of this parasite.

In this case, the spleen showed severe inflammatory reactions. The spleen was congested with numerous hydatid cysts embedded in the tissue. This coincided with the lesion in other domestic animals (Bowman, 2003). Several types of blood cells were present, especially, eosinophils, due presumably to parasitic infestation (Dellmann, 1993). The inner surface of all

cysts appeared to be producing the cell-like bodies, which accumulated in the cystic lumen and became necrotized. The other organs involved in this case were the liver, and gastrointestinal tracts. Since liver is the first capillary filter of the larva migration from the portal vein, therefore, numerous hydatid sand and macrophages had accumulated there. Some hydatid sand also presented in the esophageal, gastric and intestinal lumen, due to suspected hematogenous spreading.

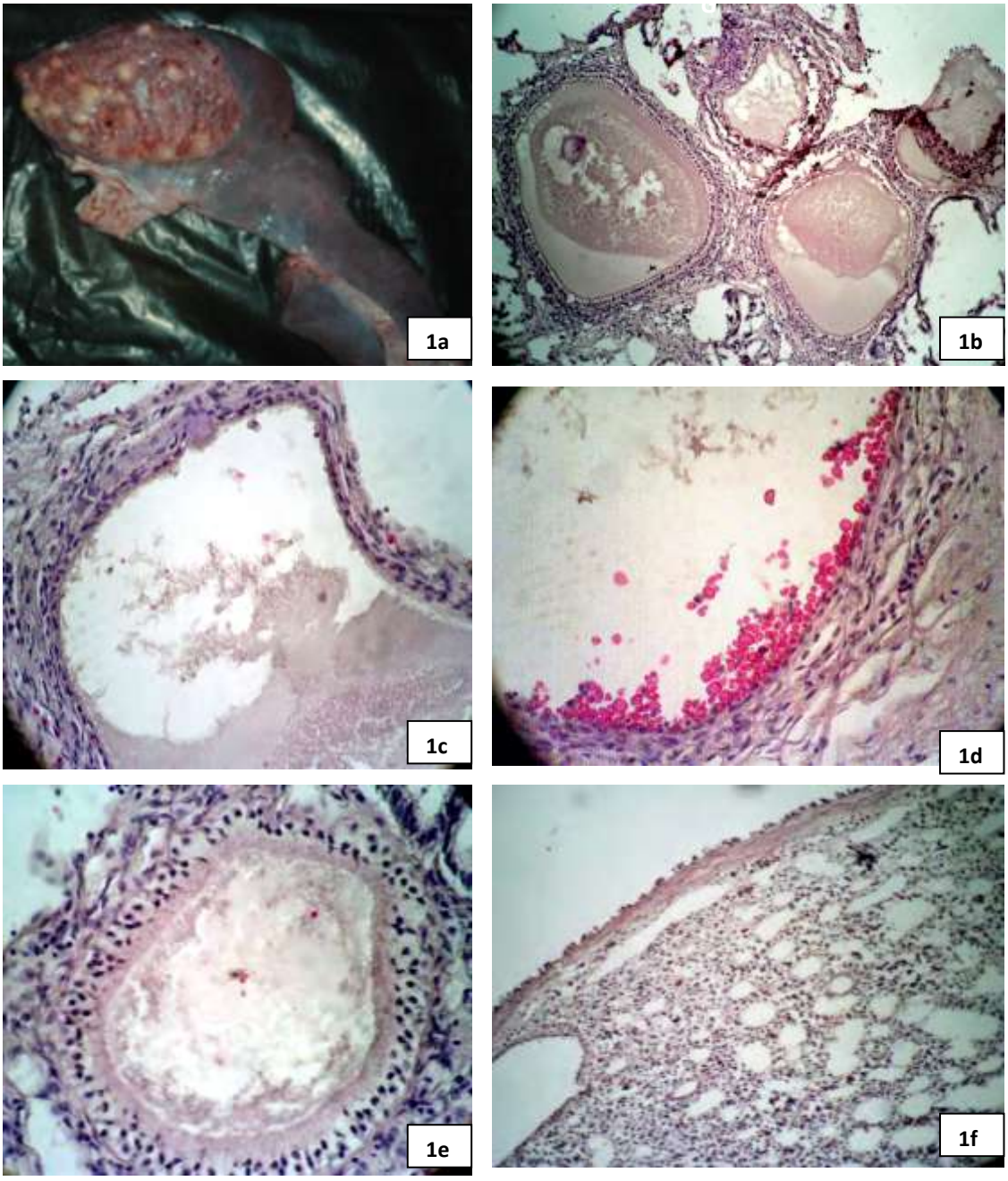

Fig.1 The spleen of a zebra shark *Stegastoma fasciatum* showed numerous creamy yellowish multiple cysts. (a) Under microscopic examination, there were multiple unilocular fluid filled cysts (40x) (b) The capsule of each cyst had three layers called, host tissue layer (*H*), laminated membrane layer (*L*) and germinal layer (*G*), (100x) (c) The crytal-like particle or hydatid sand in the cystic cavity. (100x) (d). A typical cell layer between germinal and laminated cyst wall layers. These cells were single columnar, plump cells. (100x) (e). The loosed interstitial tissue adjacent to the cyst in the spleen (10x) (f).

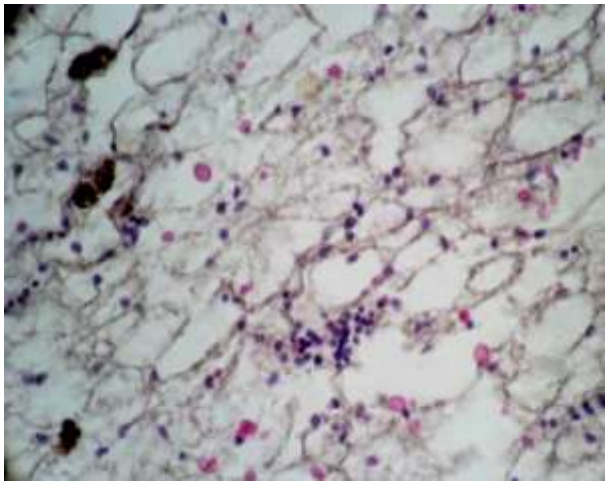

Fig.2. Numerous hydatid sands scattered in the liver parenchyma with numerous macrophages infiltration. (100x)

The hydatid sand appeared to be structural cell bodies rather than protoscolices or brood capsules. These bodies may represent germination attempt of larval cestode in the accidental host. Such cysts are, therefore, sterile (John and Petri, 2006). However, the appearance of the cyst content suggested that the rapid proliferating bodies became necrosis and generated the hydatid sand, filling in the cyst. Pathologic effects of hydatid cysts were pressure atrophy to the surrounding tissues and allergic response due to hydrated fluid leakage. Rupture of a fertile hydatid cyst may scatter the germinative membrane, scolices, and brood capsules spreading throughout the peritoneal cavity. Multiple hydatidosis can be recognized when the host dies. (Bowman *et al.*, 2003). In this case, the decrease in spleen functions for defense mechanism, due to generation and depletion of lymphopoitic tissue may result in immunosuppressant leading to higher susceptibility to secondary bacterial infection septicemia and death.

CONCLUSION

This is the first report of hydatid cyst in a shark. From this study, due to the possibility of *E. granulosus* infection in shark. The diagnosis of hydatid cysts should be included in the differential diagnosis upon necropsy. Since indigenous fish species can be another source of *E. granulosus* that may cause infestation in human, care should be taken when consuming unprocessed fish product.

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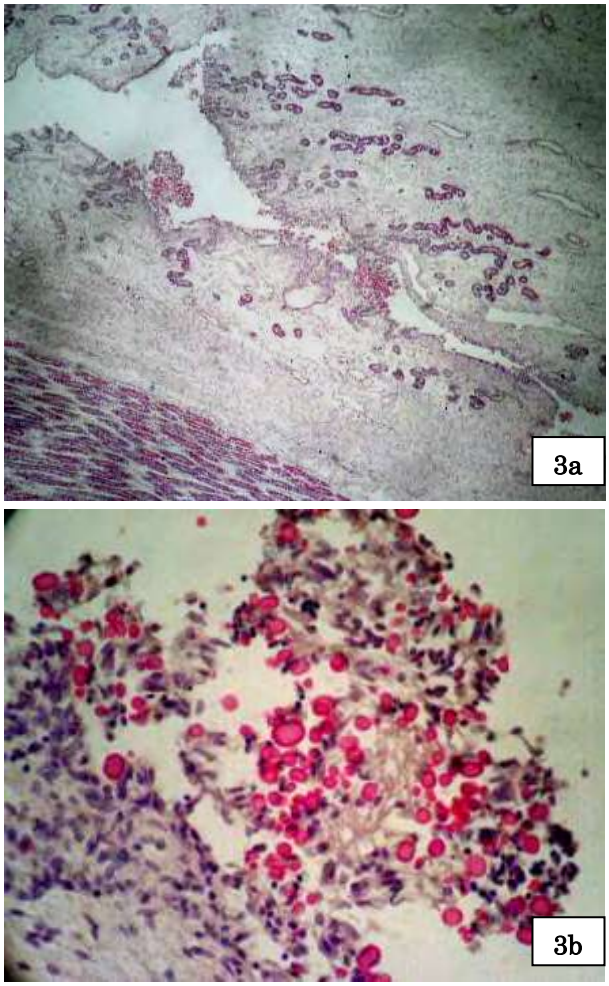

Fig.3. Hydatid sand in the lumen of the stomach (10x) (a). The hydatid sand with necrotic cells. (100x) (b)

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