Scanning Electron Microscopy and Histological Morphology of *Cysticercus fasciolaris*, which induced Fibrosarcomas in Laboratory Rats

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ABSTRACT

*Cysticercus fasciolaris* is the larval stage of *Taenia taeniaeformis* and it is a feline tapeworm. The objectives of this study were to determine the *C. fasciolaris* morphology using scanning electron microscopy (SEM) and light microscopy (LM) and its effects on the laboratory rats’ liver. Methods: A total of 21 liver specimens of male Sprague-Dawley laboratory rats were collected and tissue specimen form infected liver that involved *C. fasciolaris* cysts was obtained for LM and SEM studies. Results: Gross pathology of the infected livers showed the harboured hepatic cysts as single or multicystic structures. Histopathological examination revealed the presence of multiple hepatic cysts, and the *C. fasciolaris* larva was surrounded by granulation tissue. Large and separated fibroblasts in different orientations with some neoplastic changes that suggest fibrosarcomas were seen in advanced hepatic cysticercosis. The SEM of mature *C. fasciolaris* bladder appeared contains small chamber that involved the larva and large chamber containing the small chamber and opalescent fluid. The scolex of the mature larva is distinctly large, bearing four lateral distinct suckers, a rostellum armed with double, and alternating rings of double circle of hooks. In conclusion, the *C. Fasciolaris* might induce hepatofibrosarcomas in the rats liver in advance cases.

Keywords: *Cysticercus fasciolaris*, Fibrosarcomas, SEM, Laboratory Rats

INTRODUCTION

*Cysticercus fasciolaris* is a larval and cystic stage of *Taenia taeniaeformis*. The intermediate hosts of *T. taeniaeformis* are mouse, rat, cat, muskrat, squirrel, rabbit, other rodent, bat, and human. The *C. fasciolaris* commonly found in a liver of intermediate hosts were infected through contaminated water or feed materials with infected cat faeces. There are some sporadic cases reported in human from Argentine, Czechoslovakia, Denmark and Taiwan (Ekanayake et al., 1995, Miyazaki, 1991). In an intermediate host, the *C. fasciolaris* cysts were found in different structured forms and they can be recognized by multiple hepatic cysts. Histopathological observations in advances cases showed plenty of fibroblasts with neoplastic characterizations as a fibrosarcomas (Hanes and Stribling, 1995). Mature *C. fasciolaris* showed obvious scolex, long neck (strobila 3-4 cm) and pseudo-segmentation, larva revealed armed rostellum characterized by double rows of hooks and four suckers which were clearly evident (Mahesh et al., 2006). There are no more documents yet about the histology of the *C. fasciolaris* and scanning electron microscopy studies showed the presence of *C. fasciolaris* within the infected liver. Therefore, the objectives of this study were to determine the *C. fasciolaris* morphology using scanning electron microscopy (SEM) light microscopy (LM) and its effects on the laboratory rat liver.

MATERIALS AND METHODS

Specimens Collecting and Histopathology Processing

Twenty-one liver specimens of male Sprague-Dawley laboratory rats were collected and inspected from animal units of University Sains Malaysia/ Health Campus. These cases were collected from another research where the animals fed accidently on contaminated food by cat’s faeces that were infected by *Taenia taeniaeformis*. The rats were killed by inhalation of CO₂ in the
laboratory with animal ethic number PPSG/07(A) 044. They were dissected and the visceral of abdominal cavity included the gastrointestinal tract, liver and kidney were checked grossly for *C. fasciolaris* cysts.

Tissues specimens from infected liver that involved *C. fasciolaris* cysts were collected and fixed overnight in 10% neutral buffered formalin. The specimens were sent to histopathology laboratory for further processing. The specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin stain. The slides were observed under the light microscope.

**Scanning Electron Microscopy (SEM) Procedure**

Liver specimens that involved cysts were collected and washed in 1% phosphate buffered saline (PBS). The cysts along with liver lobes were opened, a viable larva with white to opalescent fluid was observed. The larva was washed in 1% PBS and then entire specimen were fixed in 2.5% glutaraldehyde, dehydrated through a graded ethanol series, immersed in hexamethyldisilazane (HMDS) for 5 minutes 3 times. Finally with the removed as much of the HDMS’ the specimen was air-dried. The treated samples were then ready to mount and sputter coated with gold for scanning electron microscopy (Al-Jashamy and Islam, 2007).

**RESULTS**

**Gross Pathology Findings**

Infected livers with *C. fasciolaris* showed macroscopically visible solitary cysticercus granulomas and seizures or multicystic structures with 2-2.5 mm in size, most of them in the right lobe of the liver, with encysted larvae of whitish to yellow colour. The large cysts surrounded by slightly pale zone, while other areas of the liver showed normal morphological structures (Fig. 1).

**Histopathology Findings**

Histopathological examination showed the multiple hepatic cysts and tract of migration *C. fasciolaris* larvae that were surrounded by a thick inflammatory zone infiltrated with inflammatory cells including polymorphonuclear cells, kupffer cells, eosinophils (Fig. 2a). Granulation tissue with congested blood vessels, large and separated fibroblasts in different orientations were seen in advanced hepatic cysticercosis. The fibroblasts are spindle shaped cells, while the neoplastic cells appeared as pleomorphic cell with hyperchromatic bizarre nuclei, and abundant eosinophilic cytoplasm which was separated by collagen as intercellular matrix, and there were scanty of mitotic figures, and no metastases were observed. These neoplastic changes were suggested of fibrosarcoma (Fig. 2b). The hepatic cyst appeared to contain two chambers, the small one ((Larva...
chamber (LC)) directly surrounded and covered the larva, which is very thin with membranous structure. On other hands, the large chamber that was filled with larval fluid as an opalescent fluid (fluid chamber (FC)) which externaly contained both the small chamber and larval fluid. Larvae were visualized attached to the hepatic tissue (Fig. 2c).

**Scanning Electron Microscopy**

The scolex of the mature larva was distinctly large, bearing four lateral distinct suckers and the rostellum armed with double circlet and alternating rings of hooks (Figs 3a & b). Top surface of the rostellum had micro-papillae (Fig 3c). The SEM of the opened *C. fasciolaris* cyst showed two chambers which involved a small larval chamber (LC) and a large chamber as a fluid chamber (FC) that was filled with opalescent fluid (Fig. d).

**DISCUSSION**

The results of this study showed the laboratory rat livers infected with multiple hepatic cysts. Some of these cystic structures are found in diameter of less than 5mm, liver with these size of cysts are suspected in age of less than one month of infection and the larva scolex did not developed yet. In cases of more than one month of infection, the cysts were biggest and clearest, seen with developed scolex, which bears four lateral distinct suckers and a rostellum armed with double and alternating rings of hooks. The mature larva shows complete morphological features
with four suckers and hooks in both of double circllet rings of the scolex. These structures will be developed when the larvae will be adult as *T. taeniaeformis* in cats’ intestine as a definitive host, these suckers and hooks play roles of fixation of the young *T. taeniaeformis* in the host’s intestinal mucosa (Al-Jashamy and Islam, 2007; Yohei et al., 2003).

The microscopic observations showed that there are two stages of inflammatory reactions according to the cystic age which include acute and chronic inflammations. When the hepatic cysts are within 5mm in diameter, the suspected infection period appears to be within less than one month. In this period of infection, the inflammatory reaction surrounded the cyst and it has recognized inflammation which showed polymorphonuclear cells, scanty of Kupffer cells and eosinophils. In case of chronic stage, the hepatic cysts with a diameter of more than 5mm, which were appear to be infected by more than one month and the inflammation is recognized by presence of zone of inflammation around the cyst, including granulation tissue which are infiltrated with Kupffer cells, scanty lymphocytes and fibroblasts. In advance cases, where the hepatic cysts are more than three months old, and the long standing larvae may induce fibrosarcomas in the liver tissue (Al-Jashamy et al., 2004; Armando et al., 2007; Jithendran and Somvanshi 1995). Fibrosarcoma is a malignant tumor, derived from fibrous connective tissue. The rough surface of *C. fasciolaris* might cause irritation of the hepatic tissue, which surrounds the cyst. This may induce the inflammation in the hepatic tissue (Al-Jashamy et al., 2004; Jithendran and Somvanshi 1995). The long standing of larvae in the hepatic tissue can give a long period of irritation between the rough surface of the larvae and the hepatic tissue. The irritation might stimulate and promote

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**Fig. 3**: Scanning electron microscopy micrographs of *C. fasciolaris* scolex showing: 3 a) the scolex is a visible shape with four lateral distinct suckers (SU). 3b) The scolex is distinctly larger, bearing rostellum armed with double and alternating rings of eruption hooks (RH), 3c ) the top surface of the rostellum with micro-papillae (MP). 3d) *C. Fasciolaris*' s cyst contents of Larva chamber (LC) and Fluid Chamber (FC).
the hepatic cells around the cyst to develop carcinogenic behaviour (Al-Jashamy et al., 2004), and the chemical reactions between the larvae and hepatic tissue may induce the cellular changes which finally develop into fibrosarcomas. If there is no treatment given, the fibrosarcomas may cause the failure of liver functions. The development of sarcomas in rats induced by Taenia sp. is thought to be attributable to the chronic inflammatory reaction of the capsule. There are parallels between these and other tumors occurring in mice and rats with suggested chronic inflammatory aetiologies (Mahesh et al., 2006). No metastasis was observed in other systemic organs (Mahesh et al., 2006). The presence of C. fasciolaris in laboratory animals could lead to misinterpretation of result for biological experiments (Jithendran and Somvanshi 1995). In conclusion, C. fasciolaris might induce hepato-fibrosarcomas in the rats in advance cases.

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REFERENCES


