Chapter – 3

Hepatic Histopathological studies in metacestode and *Capillaria*- infected rodents
INTRODUCTION

Importance of wild rats as reservoir of helminth infection has long been known (Firlotte, 1948). Researchers have used rodent species infected with these parasites as potential animal models for the studies of hepatic fibrosis and cirrhosis and parasitic carcinogenesis. However, there is scanty information and little scientific literature related to rat-borne liver infection.

Both *Capilaria hepatica* and *Taenia taeniaeformis* have been reported as a common parasite of rodents worldwide. Frequent occurrence of *C. hepatica* has been reported from Asia; the prevalence of infection ranges from as low as < 20% to as high as 100% (Seong et al., 1995; Namue and Wongsawad, 1997; Claveria et al., 2005; Tung et al., 2009). As high as 80% prevalence was reported from the city of Baltimore, Maryland, USA (Farhang-Azad, 1977; Conlogue et al., 1979; Easterbrook et al., 2007). In Africa a low prevalence of infection was reported from Norway rats in Egypt (El-Nassery et al., 1991) and in black rats in Ethiopia (Farhang-Azad and Schlitter, 1978). With regard to Europe, a low prevalence (> 2.9%) of *C. hepatica* infection was reported from Norway rats in Croatia (Stojcevic et al., 2002) and considerably higher prevalence (41-44%) in England (Webster and MacDonald, 1995), France (Davoust et al., 1997) and Italy (Ceruti et al., 2001).

*T. taeniaeformis* has been reported from the black rats in Asia (Seo et al., 1964; Seo et al., 1968; Seong et al., 1995; Claveria et al., 2005; Singla et al., 2008), in Norway rats in Canada (Firlotte, 1948), the USA (Andrews and White, 1936) and Europe (Webster and MacDonald, 1995; Battersby et al., 2002) and in both black and Norway rats in New Zealand (McKenna, 1997).

For both the parasites wild common rats act as reservoir of infection and through predation help to spread the infections. Till date 38 cases of human infection with *C. hepatica* have been recorded (Nabi et al., 2007) and children under 5 years of age are at particular risk of developing the disease (Berger et al., 1990; Spratt and Singleton 2001); human infections with *T. taeniaeformis* are rather rare and only a few cases have been reported (Hoberg, 2002).
*T. taeniaeformis* is a taeniid cestode found in the intestine of cats and other felines and carnivores as definite hosts. Rodents serve as the intermediate host, in which the larval form or the metacestode, *Cysticercus fasciolaris*, develops in the liver and other organs as a fluid-filled bladder worm inducing fibroplasia and progressive inflammation that may eventually progress to form fibrosarcoma (Hanes 1995). Sarcomas of the rat liver due to the presence of Cysticercii have long been known; several experiments using the bladder worm to induce malignant growth of the connective tissues in the liver of rats were successfully conducted as early as the first quarter of the twentieth century (Bullock and Curtis 1920, 1924, 1926, 1928). Liver fibrosarcoma due to the presence of *C. fasciolaris* was suggested to be an appropriate model for studying parasitic carcinogenesis and pathogenesis in wild rats (Tucek et al., 1973).

*C. hepatica* is a zoonotic nematode found in the liver of rodents and other lagomorphs that can also parasitize man (Cochrane et al., 1957; Cislaghi & Radice, 1970; Berger et al., 1990; Choe et al., 1993) and which has been the most frequently encountered species in wild and house rodents (Junker et al., 1998; Seong et al., 1998). A high prevalence of this parasite in wild rats was observed in northern part of India (Mittal 1980; Gupta and Trivedi 1988; Somvanshi et al., 1995; Chahota et al., 1997). *C. hepatica* has a direct life cycle which takes place in the liver of a single host (Calle, 1961); both male and female worms are found within the liver parenchyma where they live and unembryonated eggs are released only after the host dies and its liver decays or when the host is eaten by a predator carnivore. Under favourable conditions the ova embryonate and become infective, the larvae hatch in the intestine and migrate via the portal vein into the liver where they become mature and mate. The female worms of *C. hepatica* die soon after laying eggs and disintegrate inside the liver freeing thousands of eggs in the liver, forming focal necro-inflammatory lesion that heals by encapsulation, calcification and resorption (Luttermoser, 1938; Ferreira and Andrade, 1993; Gotardo et al., 2000). Several studies have been carried out on the pathogenesis of *C. hepatica* and its effect on the liver tissue. In laboratory mice, the infection can reduce the reproductive output or even cause death of the host (Luttermoser 1938; Singleton and Spratt 1986).
In view of the frequent occurrence of the two parasites in the rodents collected during the bamboo flowering period in Mizoram, the present studies aimed to find out the effect of the metacestode and *C. hepatica* on the liver parenchyma of the hosts and to ascertain their potential as a tool for biological control of rodents. For the purpose a histopathological approach was adopted.

**MATERIALS AND METHOD**

As mentioned in the preceding chapters, a total of 280 rodents belonging to 9 species representing 6 genera that are commonly prevalent rodent species in the region were collected from 12 different locations.

Upon necropsy of the host animals, the hepatic parenchyma was observed to be studded with cream-coloured soft cysts, which upon opening were found to contain viable creamy white metacestode, i.e., *Cysticercus fasciolaris*. Presence of *C. hepatica* was also observed by gross examination of the liver tissue and the nematode was easily recognized externally by the presence of irregular white or yellowish white nodules containing the eggs or adult worms and scattered all over the surface of the liver.

The liver tissue containing both the parasites was separated and washed with PBS and stored at -40°C for frozen sections. The fresh frozen tissues of the infected and control (uninfected) liver were sectioned in cryostat (Model No LEICA CM 1850) at 14μm thickness at -20°C, and stained. For histopathological studies, alteration if any in collagen, lipids and eosinophils were used as the parameters, for detection of which Masson trichrome, Hematoxylin and eosin (H&E) and Sudan black respectively were used following Pearse (1968).
RESULTS

In 22.3% of the rodent hosts creamy white-coloured cysts were observed mostly in the caudal and lateral lobes of the liver (Fig. 3.1). The number of cysts collected from a single host varied from 1-15. Morphological examination of the stained parasites revealed typical characteristics of taeniid cestodes with the presence of an armed rostellum having two rows of large and small hooks and four prominent suckers on the scolex, a long neck and pseudo segmentation of the entire body length with a terminal bladder, which is in consistence with the larvae of *T. taenideaformis*.

In 24.4% of the hosts examined, granulomatous lesions associated with eggs or portion of damaged adult nematode worms, consistent with *Capillaria hepatica*, were detected either scattered on the liver surface or localized in a single lobe. Prevalence of the metacestode was found to be highest in the rodent species *R. norvegicus*, whereas *R. nitidus* showed highest prevalence of *C. hepatica*.

Histopathological studies of the infected liver revealed distortion of the normal morphology of the liver parenchyma and inflammation due to the presence of both the parasites (Fig. 3.3). The presence of metacestode was revealed inside a well defined fibrous tissue capsule. The cells appeared spindle shaped and clustered together with abnormal nuclei in and around the area where the metacestode occurs; in some area the cells seemed to be larger as compared to normal and a large numbers of them were multinucleated and the normal architecture of the liver cells seems to be altered (Fig. 3.3A). As revealed with Masson’s trichrome stain, numerous neoplastic cells were observed with an abundant deposition of blue coloured collagen sheath (Fig. 3.3B).

In the *C. hepatica* infected liver; the lipid content was found to be more than the uninfected tissue, more so on the surface of the *C. hepatica* eggs as compared to the hepatic parenchyma (Fig. 3.3C). Partially calcified worm debris and collections of immature and mature eggs were found in the area where *C. hepatica* worms occurred and disintegrate. Granulomatous lesions surrounding the eggs of *C. hepatica* were detected (Fig. 3.3D); sometimes the lesion contained only a calcified core. Besides these inflammatory lesions there also occured septal formations within the infected
liver (Fig. 3.3E). The clusters of *C. hepatica* eggs on the liver parenchyma were clearly visible as having an ovoid structure with bipolar caps. Abundant eosinophilic cytoplasm was observed in the region where the metacestode of *Taenia* and *C. hepatica* occurred red adjacently (Fig. 3.3F).
Fig. 3.1. *Taenia* sp. cysts attached to the liver lobe (black arrow) and irregular white streak (blue arrow) showing the presence of *C. hepatica*.

Fig. 3.2. Uninfected liver (Haematoxylin & Eosin)
Fig. 3.3 A. Spindle shaped cells aggregating near the area where the parasite occurs (haematoxylin).
B. Infected liver tissue, showing nuclei (black), Cytoplasm (red) and large deposition of collagen sheath (blue)- Masson’s trichrome stain.
C. Presence of lipids in the liver tissue infected with C. hepatica- Sudan black.
D. Granulomatous liver lesion with clusters of C. hepatica eggs (H&E).
E. A thin fibrous septa appears across the liver parenchyma (Haematoxylin)
F. Haematoxylin and cosin stain shows fibrous tissue encapsulation of the metacstode and abundant eosinophilic cytoplasm around the area where both the parasites occurs adjacenty (H&E).
DISCUSSION

The presence of the metacestode of *Taenia* and *C. hepatica* altered the normal morphology of the liver of rats. Hepatic sarcoma associated with *Teania* sp. in wild rats has been reported (Tucek et al., 1973). A granulomatous reaction in the liver is a common histopathological finding in naturally infected rats (Davous et al., 1997; Ceruti et al., 2001). These focal inflammatory lesions are determined by the presence of live, dying or dead worms and their eggs. In consistence with the present findings spindle-shaped pleomorphic neoplastic invading liver parenchyma and the cells separated by collagen have been reported earlier in Sprague Dawley rats (Hanes, 1995; Wohrmann and Teredesai, 2002); in addition infiltration of eosinophils, plasma cells and macrophages was also reported (Kumar et al., 2006). Lethargy, weigh loss, anoerexia and sudden death are the clinical signs that have been associated with the presence of the metacestode in the liver of rats (Tucek et al., 1973; Hanes, 1995). In case of *C. hepatica* infection, focal encapsulating fibrous response caused by the dead worms eventually progresses to form septal fibrosis and leads to cirrhosis (de Souza et al., 2000). Septal fibrosis is a frequent morphological type of hepatic fibrosis, which is represented by thin and straight fibrous septa that dissect the liver parenchyma. Its pathogenesis is a matter of considerable interest (Bhunchet and Wake, 1992; Bhunchet and Fujeida, 1993; Onori et al., 2000). Experimental data obtained after *C. hepatica* egg infection have shown the presence of focal lesions, which are necessary for the development of septal fibrosis (Santos et al., 2001). Although focal lesions and septal fibrosis run on independent pathways, the induction of the latter is triggered during early infection (Gomes et al., 2006). Consistent with the present findings, Oliveira and Andrade (2001) reported the occurrence calcified adult worms and immature eggs within the focal lesion delimited by fibrous capsule and also the formation of mild septal fibrosis. Recently, Yi et al. (2010) reported the presence of both *C. hepatica* and *T. taeniaeformis* in 31.5% of the wild rats captured in South Korea and also observed that the lesion caused by the parasite were closely associated with pulmonary arteriolar hypertrophy, which may represent the effects of pulmonary hypertension. These authors suggested that *C. hepatica* infected rats might be useful as an animal model for the studies of portopulmonary hypertension. Kataranovski et al. (2010) also reported the presence and characteristics of *C. hepatica* and *T.*
*taeniaeformis* liver infection in wild *Rattus norvegicus* from Belgrade, Serbia; fibrous inflammatory and granulomatous reactions surrounding the *C. hepatica* eggs were reported, an observation which is corroborated by the present findings as well. Considering the effects of both the parasites on the liver of the rodent hosts it would be worthwhile to investigate further and ascertain the possibility of using these parasites as a potential biological control tool.