CHAPTER V
DISCUSSION AND CONCLUSION

Many years ago, the definitive hosts of *Opisthorchis viverrini* had been addressed, there are dog (Schuster, 2007; Jessica et al., 2010), cats (Jessica et al., 2010). Recently, gerbil has been recognized for *O. viverrini* infection model (Boonmars et al., 2009; Wonkchalee et al., 2011). However, the previous reports had no the comparative studies infected hosts in many aspects such as pathological changes, parasite size including the reproductive organs. Thus, I am right now focusing on the comparative various pathologies which may be differences between both Syrian hamster and gerbil. The present study is the first report that addressed the comparative pathology of host specific for *O. viverrini* infection in gerbil and Syrian hamster and emphasized the new pathology, cirrhosis, which cause form *O. viverrini* which may occur in infected human. Moreover, we found the new novel that there was a different bile acid component between Syrian hamster and gerbil.

It is well known that the *O. viverrini* habitat in the definitive host or natural host is the hepatobiliary system, gallbladder, common bile duct, and intra/extrahepatic bile duct where the parasite promotes many pathologies (Adam et al., 1993; Sripa, Kaewkes, 2002; Boonmars et al., 2009; Boonjaraspinyo et al., 2011). The present study showed the pathological changes in gallbladder and common bile duct occurred as early as day 30 post-infection the gallbladder and common bile duct wall were slightly opaque while the fluke reached mature stage. The degree of opacity increased gradually with the duration of infection which are similar to the previous report (Sripa, Kaewkes, 2002; Sripa, 2003). Moreover, the present study is the first report, which addressed the histopathological changes after *O. viverrini* infection in gerbils by observation of liver, and biliary cirrhosis, which generated from fibrosis around the portal triad, the presence of granulomas and pooling of bile. Moreover, *O.viverrini* adults could be observed in the gallbladder, pancreatic duct, and common bile duct but not in the intra-hepatic bile ducts. In contrast, histopathological changes after

O. viverrini infection in hamster showed fibrosis around intra-hepatic bile duct, which contained *O.viverrini* adults, and increasingly fibrosis with the time manner. The severity of inflammation increase and reaches a maximum on days 30 of infection by proliferation of mononuclear cells, lymphocytes and eosinophils infiltrated around intrahepatic bile duct. The inflammations tend to decease after days 60 and 90 post-infection respectively. Thicken and dilated bile duct was a common feature similar to the previous reports (Bhamarapravati et al., 1978; Sripa, Kaewkes, 2002; Sripa, 2003). However, this is not in agreement with our present findings: in infected gerbils, adult worms were observed in the gallbladder and common bile duct (Figure 34) but not in the intrahepatic bile duct (Figure 35). Moreover, there are no existing reports on cirrhosis in gerbils caused by *O. viverrini* infection, this being the first. In this instance, cirrhosis may develop due to parasite obstruction at the cystic duct or common bile duct, as no parasites were observed at the pancreatic duct in animal with and without liver cirrhosis. Surprisingly, adults were observed in the pancreatic ducts in 85% of non-cirrhosis gerbils. This indicates that parasites may move to the junction of the pancreatic duct and common bile duct (Ampulla of Vater), resulting in normal bile flow even as the disease is progressing. Although liver and biliary cirrhosis could not be observed in this group, pancreatitis was often seen; this is in agreement with the findings of Sullivan, Koep (1980) that the liver fluke *C.sinensis* causes cholangiohepatitis. Therefore, this may be a reason why some gerbils did not develop cirrhosis. Moreover, some studies have found that primary biliary cirrhosis is correlated with ulcerative colitis (Koulentaki et al. 1999; Xiao, Liu 2003) however; we did not observe ulcerative colitis in any gerbils. Recently, Melling et al. (2009) suggested that primary biliary cirrhosis may be caused by opisthorchiosis. Histopathological changes and gross results both indicated that there were no parasites present in the intrahepatic bile ducts. All common bile ducts and gallbladders of infected gerbils were opaque and enlarged (Figure 33). Numerous adult parasites and/or those of large body size located at the cystic duct may cause an obstruction leading to gallbladder and common bile duct dilatation and inflammatory accumulation, known as cholecystitis, cystitis and longstanding can cause of cirrhosis. Obstruction of the biliary system is known to promote cholecystitis. Cholecystitis is the most common problem resulting from gallstones; it occurs when a stone blocks

the cystic duct. In this study, it occurred due to parasite obstruction of the cystic duct, which can impede the flow of bile from the gallbladder. This is in agreement with studies by Rohela et al. (2006); Lai et al. (2007) which found that *C. sinensis* caused acute cholecystitis. Following obstruction of the cystic duct, the gallbladder becomes hyperemic, edematous, tense, and distended. The initial inflammation induced by *O. viverrini* infection is an important feature of the established disease and its complications. During the first few days, bile appears macroscopically normal and is sterile; but as the inflammation progresses, absorption of pigments and bile salts takes place, and the contents then vary from a thin mucoid material to frank pus. The histological changes of the established condition involve the mucosa, fibromuscular wall and serosa and vary from mild to acute inflammation with transmural edema. This is in agreement with a general biliary pathophysiological condition (Krishnamurthy and Krishnamurthy 2000). A previous report (Flavell, 1982; Flavell et al., 1983; Boonmars et al., 2009) agreed with the present study that worm burden in hamster was 20-50% while in gerbils fluctuated, which corresponds to the present findings where worm recovery fluctuated from one to 25 worms; this may be attributed to the mucosal defense mechanism of the host by intestinal expulsion during development. The severity of pathology post-infection suggests that gerbil is not susceptible for *O. viverrini* infection. However, it is noteworthy that the adult worms in gerbils were all larger than those observed in Syrian hamsters. However, the adult size was all most bigger than that observed Syrian hamster these may cause from the different of bile acid compositions which that the amount of CA in bile fluid of gerbil was low or absent while compared with bile fluid of Syrian hamster as shows in Figure 29. However, what important of other TLC spots or each component are under investigated. CA is the major component in bile acid which play important role in anti microbial activities (Savage, Li, 2000; Lai et al, 2008). Thus, it is possible that gerbil bile fluid may have some of bacteria which may be the food for parasite so that is why *O.viverrini* form gerbils were larger than hamster. Actually we thought the amount of metacercariae infection may be the factor of the enlargement of the parasite size. Thus, I confirmed this hypothesis by using 10 metacercariae of *O.viverrini* for hamsters and gerbils infection and then sacrificed and collected the liver. Then the liver was divided in 4 parts; gallbladder, common bile duct, right and left lobe then

observed the parasite under stereomicroscope. Worm recovery was counted and I found nothing differences from the previous results such as worm recovery and worm size. Therefore, the number of metacercariae for infection was not involved in the parasite size. However, the really function of CA which involve in parasite development is still obscure.

The present studies I concluded that

1. Syrian hamster is susceptible for *O.viverrini* infection more than gerbil by observation of worm recovery even through the overall size of worm infected gerbil is quit larger.

2. 'The hepatobiliary pathological changes after *O.viverrini* infection in gerbil was some difference from those infected hamster such as i) more severity than hamster by observation of large common bile duct and cirrhosis ii) the location of worm in infected gerbil was observed at only large biliary cannel such as gallbladder, common bile duct, Ampulla of Vater and pancreatic duct. But for infected hamster, the worm was observed at gallbladder, common bile duct, intra-hepatic bile ducts and Ampulla of Vater.

3. The bile component of hamster and gerbil was difference. Gerbil bile acid has no CA by TLC detection but not hamster which may effect on the worm development.