MIGRATORY PATTERN OF *OPISTHORCHIS VIVERRINI* IN HAMSTERS

Sasithorn Kaewkes[1] and Banchob Sripa[2]

Departments of [1]Parasitology and [2]Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. The migratory pattern of *Opisthorchis viverrini* was studied by feeding each of 55 hamsters orally with 50 metacercariae collected from cyprinoid fish. The route of migration was followed from the gastrointestinal (GI) to biliary tracts for 3 months after infection at 11 durations: 1, 3, 6, and 12 hours, 1, 3, 7, and 14 days, 1, 2, and 3 months. After sacrifice, the stomach, small intestine, common bile duct, gallbladder, and 3 equal portions of each lobe (right, left, median, and caudal) of the liver were examined and counted for worm burden. The newly excysted worms of *O. viverrini* migrated from the duodenum into the common bile duct, gallbladder and proximal bile duct of the right lobe as early as 1 hour after infection. The farthest migratory distance, the distal bile ducts of all lobes, occurred with worms aged 14 days. After reaching maturity, the number of worms remaining in the extrahepatic bile ducts was lower than the intrahepatic bile ducts. The worm recovery rates in the right and left lobes were not significantly different, while those in the median and caudal lobes were significantly lower. Less than 5% of juvenile worms could migrate far to the distal part of the bile ducts, but these decreased when the worms became adult. This finding revealed the migratory pattern of *O. viverrini* in hamsters from the GI to the biliary system in detail, which should be useful for better understanding the host-parasite relationships along these routes of migration.

INTRODUCTION

Opisthorchis viverrini is a human liver fluke endemic in Thailand, Lao PDR, and Cambodia. It has been estimated that 6 million Thai people are infected with this parasite (calculated from overall 9.4% prevalence within the population in the year 2001) (Jongsuksuntigul and Imsomboon, 2003). The infection is associated with hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis, cholelithiasis and cholangiocarcinoma (Thamavit *et al*, 1978; Harinasuta *et al*, 1984; IARC, 1994; Vatanasapt *et al*, 1999). These pathologies occur mainly in the liver, extrahepatic bile duct and gallbladder in both infected humans and experimental animals (Bhamarapravati *et al*, 1978; Harinasuta *et al*, 1984; Riganti *et al*, 1989; Sripa and Kaewkes, 2000, 2002). Hosts become infected by ingestion of raw or inadequately cooked cyprinoid fish. After excystation in the duodenum, newly-excysted worms migrate up through the ampulla of Vater and common bile duct into the intrahepatic bile ducts and the gallbladder. Slightly higher numbers of worms were recovered in the right than the left lobes of human livers examined post-mortem, but the difference was not statistically significant (Sithithaworn *et al*, 1991). However, a few data show a relationship between the site of the host's organs and worm location, but no detailed information about the relationship between the route of migration and the duration of infection. The objective of this study was to investigate the migratory pattern of *O. viverrini* from the gastrointestinal to the biliary tracts of experimentally-infected hamsters during 3 months of infection.

Correspondence : Sasithorn Kaewkes, Department of Parasitology, Khon Kaen University, Khon Kaen 40200, Thailand.

MATERIALS AND METHODS

Opisthorchis viverrini metacercariae were obtained from naturally-infected cyprinoid fish from an endemic area in Khon Kaen Province, northeast Thailand, by pepsin-HCl digestion and infiltration. They were identified and collected under a stereomicroscope. Each of 55 male golden Syrian hamsters, aged 6-8 weeks, were infected with 50 metacercariae by intragastric intubation. Five animals were sacrificed at each period of 1, 3, 6 and 12 hours, 1, 3, 7 and 14 days, and 1, 2 and 3 months post-infection. After sacrifice, the position of ampulla of Vater and the terminal part of the common bile duct connecting to the cystic and intrahepatic bile ducts were tied to prevent the movement of worms from the normal position. The stomach, small intestine, common bile duct, gallbladder and 4 lobes (right, left, median, and caudal) of the liver were removed from the host. The cavity structures were opened and sedimented in normal saline, and the number of worms in each part was counted. Each lobe of the liver was divided into 3 portions; proximal, median, and distal, then each was separated into small pieces by 2 forceps, sedimented in normal saline and counted for worm burden per portion.

RESULTS

Migration of *O. viverrini* from GI to biliary tracts

At 1 hour post-infection, most newly-excysted worms (64.58%) were recovered in the first portion of the small intestine, and of the remainings, 14.58, 6.25 and 2.08% migrated into the common bile duct, gallbladder, and proximal bile duct of the right lobe, respectively. Some viable metacercariae (12.5%) still remained in the stomach. At the beginning of infection, juvenile worms were recovered mostly in the common bile duct and gall bladder and the worm recovery rates reached the peak in both organs (54.1 and 36.17%) at 3 and 6 hours post-infection, respectively (Fig 1). Thereafter, the number of worms recovered in these organs gradually decreased with the increase in the intrahepatic bile ducts. During 3 days to 3 months of infection, worms remained in the common bile duct and the gall bladder at rates lower than 20 and 10%, respectively, whereas more than 70% of the worms lived in the intrahepatic bile ducts.

Distribution of *O. viverrini* in intrahepatic bile ducts

A newly-excysted fluke was first found in the proximal bile duct of the right lobe within 1 hour post-infection. At 6 hours, the worms in the right and left lobe migrated far to the middle bile duct while those in the median lobe were found only in the proximal part, and none in any bile duct of the caudal lobe. Worms in all lobes could migrate farthest into the distal bile ducts at 14 days (Fig 2). Less than 5% of overall juvenile worms could migrate to the distal part of the bile ducts, and these decreased when worms became adult. The number of adult worms in the right and left lobes was not significantly different, while those in the median and caudal lobes were significantly fewer.

DISCUSSION

The present study has demonstrated for the first time that newly-excysted *O. viverrini* can migrate from the duodenum into the common bile duct, gallbladder, and proximal bile duct of the right lobe, as early as 1 hour after infection with metacercariae by intragastric intubation. The farthest distance of migration occurs at 14 days post-infection. The worms at this age are approximately 1.5 mm long or approximately 5 times smaller than adult worms (Kaewkes, unpublished data). Between 14 to 30 days worm growth is very rapid. The size of these mature worms may be too large to live in the small distal bile ducts, causing backward migration into the larger bile duct, proximal and middle parts. A similar finding was also detected in rats infected with *Clonorchis sinensis* (Hong *et al*, 1993). They divided four lobes of rat livers into two portions, proximal and distal, and examined for the recovery and the proliferation pattern of the biliary mucosal epithelial cells at 1, 2, 5, and 15 weeks post-infection with 100 *C. sinensis* metacercariae each. The flukes were found in the distal bile ducts only at 2 week post-infection and they lived mainly in the common bile duct and the right and left hepatic bile ducts. Epithelial hyperplasia was mainly developed by direct and local stimulation of the worms (Hong *et al*, 1993). In a human autopsy study, the distribution of *O. viverrini* in the right and left lobes of livers was not significantly different, and also in the proximal and middle bile duct of both lobes (Sithithaworn *et al*, 1991). Recently, Sripa

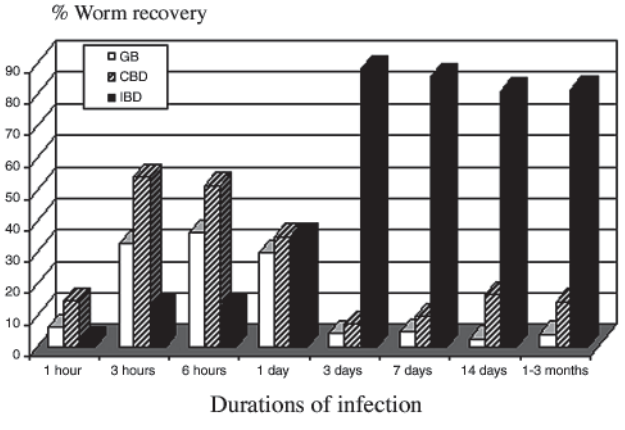

Fig 1- Migratory pattern of *O.viverrini* in the extrahepatic and intrahepatic bile ducts of hamster livers. GB: gallbladder, CBD: common bile duct, IBD: intrahepatic bile duct.

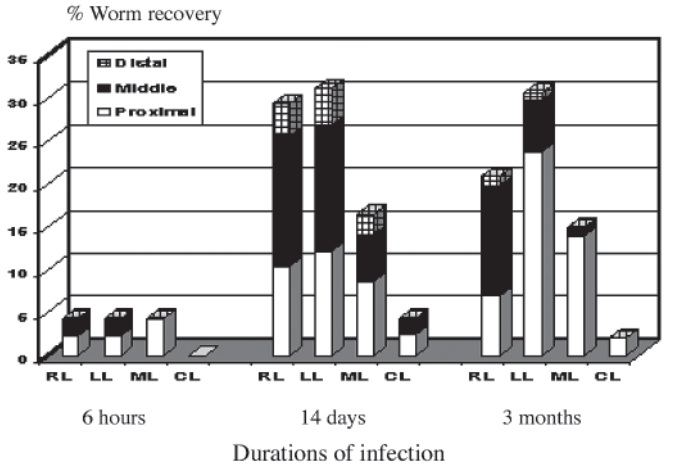

Fig 2- Distribution of *O.viverrini* in the intrahepatic bile ducts of hamster livers. RL: right lobe, LL: left lobe, ML: median lobe, CL: caudal lobe.

and Kaewkes (2000) studied the localization of liver fluke antigens in hamsters and demonstrated that *O. viverrini* antigen was detected in the epithelium of both large and small bile ducts, which are not normally inhabited by adult flukes. The recovery of juvenile flukes in the distal bile duct in the current report probably supports the contention that the small bile duct epithelium may absorb antigens from the excretory and/or secretory products of the flukes before the worms move back into the larger bile duct.

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