REVIEW

***Opisthorchis viverrini*: The carcinogenic human liver fluke**

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Abstract

Opisthorchiasis caused by *Opisthorchis viverrini* remains a major public health problem in many parts of Southeast Asia, including Thailand, Lao PDR, Vietnam and Cambodia. The infection is associated with a number of hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis and cholelithiasis. Multi-factorial etiology of cholangiocarcinoma, mechanical damage, parasite secretions, and immunopathology may enhance cholangiocarcinogenesis. Moreover, both experimental and epidemiological evidences strongly implicate liver fluke infection as the major risk factor in cholangiocarcinoma, cancer of the bile ducts. The liver fluke infection is induced by eating raw or uncooked fish products that is the tradition and popular in the northeastern and northern region, particularly in rural areas, of Thailand. The health education programs to prevent and control opisthorchiasis are still required in the high-risk areas.

Key words: *Opisthorchis viverrini*; Carcinogenic; Liver fluke; High-risk areas

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INTRODUCTION

History of Opisthorchis viverrini

Liver flukes are platyhelminth parasites of the class trematoda, and *Opisthorchis viverrini* (*O. viverrini*) is a member of the family *Opisthorchiidae*. *Opisthorchis* spp. are prevalent human parasites particularly in the Far East and South East Asia. *O. viverrini* is highly prevalent in Thailand and Laos while *C. sinensis* is endemic in South China, Japan, Korea and Taiwan, *O. felinus* is the prominent fluke in Eastern Europe^[1]. *O. viverrini* was first described in the post-mortem examination of two prisoners from a jail in Chiangmai, northern Thailand, in 1911 by Leiper^[2] who obtained specimens from Kerr. Kerr^[3] reported that 39 of 230 (17%) adult male prisoners examined in a prison in Chiangmai were infected with *O. felinus*. In 1927, Prommas^[4] identified the worms found at an autopsy of a 17-year-old Thai male residing in Roi-et, northeast Thailand, as *O. felinus*. Sadun EH^[5] commented that the liver fluke infection in Thailand was caused by *O. viverrini*, not by *O. felinus*^[5], and this was confirmed later in 1965 by Wykoff *et al*^[6].

Life cycle of O. viverrini

Fish-borne trematodes have a complicated life cycle with two intermediate hosts (Figure 1). Starting from a human host, the adult worms deposit fully developed eggs that are passed in the feces, the eggs from adult flukes are passed out with the faeces. The eggs must get into water in order to hatch and be able to infect their first intermediate host, a freshwater snail. After being ingested by a suitable snail, the eggs release miracidia which undergo in the snail several developmental stages: sporocysts, rediae, cercariae. The snail intermediate hosts are *Bithynia goniompharus*, *B. funiculata* and *B. siamensis*^[6,7]. Cercariae are released from the snail and then penetrate freshwater fish that are the second intermediate host (*Cyclocheilichthys* spp., *Puntius* spp., *Hampala dispa*), encysting as metacercariae in the muscles or under the scales. Cats, dogs, and various fish-eating mammals including humans are the definitive host^[8]. They become infected by ingesting undercooked fish containing infective metacercariae. In infected definitive host, the metacercaria excyst in the duodenum and ascend through the ampulla of Vater into the biliary ducts, where they attach and develop to adults, which lay eggs after 3 to 4

Figure 1 Life cycle of *O. viverrini*. Embryonated eggs are discharged in the biliary ducts and in the stool (1). Eggs are ingested by a suitable snail intermediate host (2). Each egg releases a miracidia (2a), sporocysts (2b), rediae (2c), and cercariae (2d). The cercaria is released from the snail and after a short period of free-swimming time in water, it penetrates the flesh of a freshwater fish, where it encysts as a metacercaria (3). Humans are infected through ingestion of undercooked or raw freshwater fishes (4). The metacercaria excysts in the duodenum (5) and ascends the biliary tract through the ampulla of Vater. Maturation to adulthood takes approximately one month (6). (Adapted from: <http://www.dpd.cdc.gov/DPDx/HTML/Opisthorchiasis.htm>).

Figure 2 Adult worm of human liver flukes: a: *O. viverrini*; b: *O. felinus*; c: *C. sinensis*. The average size of *O. viverrini* worm is 7.0 (5.4-10.2) x 1.5 (0.8-1.9) mm^[8]. (Adapted from Ash, 1997).

wk. The adult worms reside in the biliary system of the mammalian host, where they attach to the mucosa. The life span of *O. viverrini* in human is not known, however, it may be over 25 years as recorded in *C. sinensis*.

Morphology of *O. viverrini*

The *O. viverrini* are dorso-ventrally flattened, lancet-shaped, thin and transparent. The adult worms are monoecious and the average size is 7.0 (5.4-10.2) x 1.5 (0.8-1.9) mm. The oral sucker is subterminal and the ventral sucker at approximately anterior one-fifth of the body length. Two testes are deeply lobed, diagonal, situated near the posterior extremity (Figure 2)^[9]. *O. viverrini* is morphologically similar to *C. sinensis* but it is slightly smaller in size, the main difference from *C. sinensis*. The long, slightly coiled seminal vesicle terminates in the ejaculatory duct, which opens through the genital pore immediately in front of the ventral sucker. Cirrus sac and cirrus are absent. The multilobated ovary is in front of the anterior testes and nearby is the seminal receptacle and Laurer's canal. The vitellaria consist of numerous follicles disposed

Figure 3 Preparation of koi pla, pla som, pla ra and jaewbhong. koi pla is prepared with the fish-based meals with local herbs, spices, condiments, rice, and vegetables. Pla som is moderately fermented or stored for a few days to weeks. Pla ra is fermented fish sauce, popular in Northeastern Thai cuisine. It is made by combining fish, salt, and roasted rice, fermenting in a pot for one to three months. jaewbhong is made by combining pla ra and chili.

as several columnous groups in the lateral fields between ventral sucker and testes. Excretory bladder is long, sac-like tubed, S-shaped and runs between the two testes^[8].

Epidemiology of *O. viverrini*

In Thailand, at least 15 species of cyprinoides' fish, the freshwater fish, are able to serve as intermediate hosts of *O. viverrini* metacercariae. The tradition of eating raw or uncooked fish products is the main reason that liver fluke is a problem in Thailand. This is very popular in the northeastern and northern region, particularly in rural areas. In the northeast, three types of preparations contain uncooked, small and medium-sized, fish: (1) koi pla which is eaten soon after preparation; (2) moderately fermented

Figure 4 Prevalence of *O. viverrini* infection in Thailand^[15], OV: Percentage of *O. viverrini* infection.

pla som which is stored for a few days to weeks; and (3) *pla ra* which is extensively fermented, highly salted fish, and stored for at least 2-3 mo^[5,10] (Figure 3). The consumption frequency of *koi pla* in the population of some communities who ate it every week was approximately 80%^[11]. Studies found the highest prevalence of liver fluke infection in the northeasterners who ate *koi pla*^[12,13]. The frequencies of *koi pla* consumption have declined and are generally confined to special social occasions, while other under-cooked fish preparations like *pla som* and other moderately preserved fish are generally eaten several times a week^[10]. *Pla ra* and *jaewbhong*, fully preserved fishes, are an important staple food and consumed daily by 60%-98% of northeasterners and lowland Laotians^[11,14]. Studies have reported that *Koi pla* is probably the most infective, followed by the fish preserved for < 7 d, then *pla ra* and *jaewbhong* in which viable metacercariae are rare^[10].

A small scale helminthiasis control program started in 1950 included the opisthorchiasis control in some high-risk areas. Following a number of studies and trial projects of the Faculty of Tropical Medicine, Mahidol University, the National Liver Fluke Control Program has been developed and operated under several National Public Health Development Plans. Presently, the program is being operated in some provinces of the Central, and all provinces of the northeast and north of Thailand^[15]. The main liver fluke control strategies comprise of three interrelated approaches, namely stool examinations and treatment of positive cases with praziquantel for eliminating human host reservoir; health education for promotion of cooked fish consumption to prevent infection; and the improvement of hygienic defecation for the interruption of disease transmission^[15]. The prevalences of *O. viverrini* infection in the north (19.3%), the northeast (15.7%), the central (3.8%) and the south (0%) have recently been reported (Figure 4)^[5,15]. The decline in the northeast from 34.6% in 1981 to current level^[16,17] is

attributed to intensive and continuous control activities^[15]. Sithithaworn *et al*^[10] performed a cross-sectional survey of intestinal helminthes infections by examining the fecal samples collected from 332 rural northeast Thais from 3 communities during October-November 2000, and they found that the average prevalence of *O. viverrini* infection was 14.2% (range 8.6%-19.4%)^[10]. Similarly, the prevalence of *O. viverrini* infection in Khon Kaen Province, northeast Thailand was found to be 24.5%, which was rather higher in men (27.6%) than in women (21.4%)^[18]. A total of 479 stool specimens were collected from rural communities of Ubon Ratchathani Province, Thailand and examined by using two techniques: the modified Kato thick smear and the direct smear. The prevalence of *O. viverrini* was 14.8%, being much higher than other intestinal parasite infections^[19]. *O. viverrini* is still prevalent and a serious health problem in some parts of Thailand. Therefore, the health education and promotion is still more required.

Pathology and pathogenesis of *O. viverrini*

Most *O. viverrini* infection is asymptomatic. Mild symptoms include dyspepsia, abdominal pain, constipation or diarrhea. With chronic infection, the symptoms can be more severe, and hepatomegaly and malnutrition may be present. In rare cases, cholangitis, cholecystitis, and cholangiocarcinoma (CCA) may develop. The severity of the pathology appears to be associated with both intensity and duration of infection. In humans, *O. viverrini* inhabits mainly in the intra- and extrahepatic bile ducts and, rarely, in the gall bladder and pancreatic duct. The infection can induce several pathologic changes in the liver, gall bladder and extrahepatic bile ducts. Its infection can enlarge the liver and the weight of the liver may be more than the normal up to 3000-3500 g or more in heavily infected cases. The subcapsular bile ducts of heavily infected patients are usually dilated and show prominent fibrotic wall^[20-22]. Microscopically, the intrahepatic lesions of opisthorchiasis are confined to the biliary tree, particularly to the large and medium-sized bile ducts where the flukes are harboured^[21]. The typical histologic changes of intrahepatic bile ducts in liver fluke infection include inflammation, epithelial desquamation, goblet cell metaplasia, epithelial and adenomatous hyperplasia and periductal fibrosis^[20-23].

Periportal and periductal fibrosis appeared as the most prominent histologic feature in chronic *O. viverrini* infection in hamster. This fibrosis correlates with the marked increase in synthesis of hepatic content of type I and, particularly, type III collagen as demonstrated in long-term *O. viverrini* infected hamsters. Periductal inflammatory cell infiltration of lymphocytes, monocytes, macrophages, eosinophils and plasma cells was also seen both in infected hamsters^[24,25] and in human^[21]. Granulomatous inflammation in response to the entrapped parasite eggs in the periductal tissue and liver parenchyma was occasionally observed in human^[26] but frequently seen in animal model^[24]. Histologically, the changes seen in the gall bladder and extrahepatic bile duct in opisthorchiasis are adenomatous hyperplasia, epithelial hyperplasia and chronic inflammation^[21,27,28]. However, the studies of 189 cholecystectomy specimens in Khon Kaen revealed that

Figure 5 Proposed mechanisms of *Opisthorchis*-derived CCA initiation. (Adapted from Sripana *et al.*^[30]).

fibrosis of the gall bladder wall the most common finding, among several other histopathologic features, associated with opisthorchiasis as evidenced by parasite-specific antibody. For the extrahepatic bile duct, thickened cystic duct has been noted in autopsies with opisthorchiasis. Hamsters infected with *O. viverrini* also showed heavy inflammatory cell infiltration and periductal fibrosis^[29].

Multi-factorial etiology of CCA, mechanical damage, parasite secretions, and immunopathology may enhance cholangiocarcinogenesis (Figure 5)^[30,31]. The pathogenesis of *O. viverrini*-mediated hepatobiliary changes may be due to mechanical irritation caused by the liver fluke suckers and/or its metabolic products^[18,24]. However, several authors suggest that immunopathological process may contribute to the hepatobiliary inflammation and damage^[22,24,26,27]. Many pathological features in the hamster model reveal evidence of such mechanism, e.g. periportal infiltration of lymphocytes and macrophages. Acute damage may be induced by parasite factors, but the progressive changes are consistent with immunopathologic mechanisms^[22]. During liver fluke infection, inflammation, periductal fibrosis, and proliferative responses, including epithelial hyperplasia,

goblet cell metaplasia, and adenomatous hyperplasia, may represent predisposing lesions that enhance susceptibility of DNA to carcinogens^[32-35]. Several N-nitroso compounds and their precursors occur at low levels in fermented food, such as preserved mud fish paste, *pla ra*, a condiment that is a ubiquitous component of the cuisine of northeastern Thailand and Laos^[30]. Some authors hypothesised that N-nitroso compounds are a primary carcinogen leading to CCA in humans in northeastern region^[36,37]. Jinawath *et al.*^[38] showed the selective up-regulation of genes involved in xenobiotic metabolism in Thai patients with CCA, implying that these genes may be involved in the detoxification of possible carcinogens, such as nitrosamines. In hamster experimentally infected with *O. viverrini*, CCA was induced by exposure of sub-carcinogenic doses of nitrosamine^[34]. Liver fluke infection can induce the endogenous nitrosation both in humans and experimental animals. Infected hamster showed nitric oxide synthase (NOS) expression by immune effector cells in the inflamed areas and increased endogenous nitrosation of thiazolidine-4-carboxylic acid (thioprolin)^[39]. In human studies, infected cases showed a higher endogenous

nitrosation than uninfected cases^[40,41]. Infected cases had increased levels of plasma and urinary nitrate and nitrite compared to the uninfected cases^[39]. Praziquantel treatment and co-administration of ascorbic acid with proline showed enhanced immune responses to *O viverrini* and an increase of endogenous nitrosation^[42]. Thus the aforementioned findings clearly demonstrate both exogenous and endogenous nitrosation may lead to DNA alkylation and deamination in predisposed and inflamed tissues^[43-45].

Molecular biology of *O viverrini*

Information regarding the molecular biology of *O viverrini* is limited in comparison of other known trematode parasites. The ribosomal DNA of *O viverrini* has been identified, showing the total length of approximately 13 kb consisting of 4.2 kb of large subunit rDNA, 2.0 kb of small subunit rDNA, 1.0 kb of transcribed spacer DNA and 5.8 kb of non-transcribed + external transcribed spacer DNA. The rDNA units represent 6.1% of the total genomic DNA. The small subunit gene is 1992 nucleotides long and has a G + C content of 50.94%. The use of the rRNA gene sequence comparison for determination of phylogenetic classification of *O viverrini* is comparable to conventional taxonomic classification^[46,47]. The mitochondrial cytochrome c oxidase subunit I (CO I) gene and the second internal transcribed spacer region (ITS II) gene of *O viverrini* were compared among *O viverrini* from various areas in northeast Thailand. In the nucleotide sequences of partial CO I gene (417 bp), the intraspecific variations were classified into 5 patterns but no area-specific pattern was observed. Nucleotide sequences of a region of the *O viverrini* ITS II gene (296 bp) from different areas were different from those of *C. sinensis*, *Haplorchis taichui*, *H. pumilio*, *F. gigantica*, *Echinostoma malayanum* and *Centrocestus* sp.^[48]. DNA methylation and demethylation processes are thought to be taken place in *O viverrini*. The *O viverrini* genome contains a highly repeated DNA element which is very specific to the organism^[49]. The construction of DNA probe to detect genomic DNA of the parasite, for diagnosis of suspected patients or identification of parasites in suspected specimens from endemic areas was developed. The construction of specific DNA probe *O viverrini* was achieved using a unit of highly repeated DNA element (334 base pairs)^[50]. The specific DNA probe was characterized and used for detecting the presence of eggs DNA in stool samples^[51,52]. Specific primers were designed and used for PCR-based detection. The PCR detection was highly specific and 100% positive. Further studies on molecular biology of *O viverrini* are needed to better understanding of the parasite and host-trematode relationship. Several other molecular studies of *O viverrini* have been reported, including cloning and expression of glutathione S-transferase genes^[53,54].

Laha *et al*^[55] reported the gene discovery effort for *O viverrini* that should expedite molecular studies of cholangiocarcinogenesis and accelerate research focused on developing new interventions, drugs and vaccines, to control *O viverrini* and related flukes. Approximately 5000 randomly selected cDNAs from the adult stage of *O viverrini* were characterized and accounted for 1932 contigs, representing

about 14% of the entire transcriptome, and, presently, the largest sequence dataset for any species of liver fluke. Twenty percent of contigs were assigned GO classifications. Abundantly represented protein families included those involved in physiological functions that are essential to parasitism, such as anaerobic respiration, reproduction, detoxification, surface maintenance and feeding. A total of 164 *O viverrini* contigs contained ORFs with signal sequences, many of which were platyhelminth-specific, such as convergent evolution between host and parasite secreted/membrane proteins identified were homologues of vaccine antigens from other helminths. Finally, ORFs representing secreted proteins with known roles in tumorigenesis were identified, and these might play roles in the pathogenesis of *O viverrini*-induced CCA^[55]. One of candidate gene, cysteine protease was studied by Kaewpitoon *et al* (unpublished data). By using specific fluorogenic substrates, majority of *O viverrini* protease was cysteine protease cathepsin L whereas serine and aspartic protease showed very low activities. Molecular cloning of cysteine protease encoding gene by RACE-PCR using degenerated oligonucleotide primers derived from conserved active site obtained a full-length mRNA of 981 bp encoding for 326 amino acids (GenBank, accession number AY821800). The cDNA of the novel *O viverrini* cysteine protease was designated *Ov-cp-1*. Sequence analysis showed high homologies with cysteine proteases of other trematodes, such as *Clonorchis sinensis* (85%) and *Paragonimus westermani* (55%). *Ov-cp-1* cDNA expression was observed in various developmental stages of *O viverrini* including the metacercariae, juvenile (1, 2, 3 wk), adult worms, and eggs. Polyclonal antibody to *Ov-CP-1*, expressed protein, showed strong reaction with the gut epithelium, testes, vitelline glands and eggs in the uterus of the adult worm. Further functional study revealed that *Ov-CP-1* could digest human hemoglobin and the activity was inhibited by the addition of E-64. The results suggest that the recombinant *Opisthorchis* cysteine protease *Ov-CP-1* is potential for development of host-parasite relationship.

Immunity to *O viverrini* infection

O viverrini infection can induce both cellular and humoral host immune responses. Humoral immune response has been reported in both humans and animal model. The parasite antigens were detected in the epithelial lining of intrahepatic and extrahepatic bile ducts as early as d 3 after hamsters were infected with *O viverrini* and stimulated the host immune system^[25]. The antibody levels in the patients with severe opisthorchiasis were significantly higher than those with mild infection. The infected hamster serum contained a component, possibly specific immunoglobulin(s) capable of reacting with adult, juvenile, excretory-secretory (Es) and egg antigens^[56-61]. Antibody response to both somatic and Es antigens were detected^[60,62]. The antibody responses in hamsters were first detected as early as 7-14 d post-infection. During early infection, hamsters infected with 50 or 100 metacercariae (Mc) showed higher antibody levels than those infected with 25 Mc. Then, the antibody level increased rapidly to a plateau at approximately 2 mo. The antibody levels to egg and somatic, but not to Es antigens, were significantly higher in the hamsters infected with 25 Mc than in those

infected with 50 or 100 Mc. These antibody responses, particularly to egg and Es antigens, did not correlate with worm burden or egg output^[59]. Secretory IgA antibody reacted with both egg and Es antigens also appeared in the bile of infected hamsters. IgG, IgA and IgE antibodies in serum and bile have been reported in patients with opisthorchiasis^[61-64]. Analysis of bile and serum from individual patients showed that while IgG was the predominant in the serum of all patients, IgA was present at approximately the same level as IgG or higher in the bile, while IgE antibody could be detected in 50% of the bile samples^[64]. The IgG levels to the somatic antigen were most markedly elevated in disease cases and were closely associated with gall bladder size and dysfunction, and also significantly correlated with opisthorchis egg counts^[32]. The antibody response to *O viverrini* decreased after treatment^[65,66]. Immunoblot analysis revealed a 91/93-ku metacercaria homogenate doublet which was recognized only in sera of individuals with severe liver disease on ultrasound findings, including cholangiocarcinoma^[64,67,68].

Cell-mediated immune responses (CMIR) to *O viverrini* infection have been demonstrated. Granulomatous inflammatory response to adult flukes and eggs during chronic infection is one of the typical CMIR. The inflammatory granuloma is surrounded by eosinophilic precipitates, neutrophils, eosinophils, mononuclear cells and macrophages after 6 wk of infection^[24]. Similarly, patients infected with *C sinensis* exhibited egg-induced granuloma, both in the gall bladder and in the liver portal area^[69,70]. Although both cellular and humoral immunity to *O viverrini* have been reported, there exists a striking difference in the antigenic recognition pattern of T and B cells which may be important for selecting antigen molecules for immunological studies of this trematode infection in man^[71]. The molecular weight of the main immunogenic fractions for T cells ranged from 28 to 46 ku, whereas those recognized by antibodies were 45, 52, 56, 59, 65, 69, 75 and 81 ku. The exposure to *O viverrini* depressed the egg and egg reduction could be due to biliary obstruction and not necessarily to immunological damage of the reproductive system of the flukes. Biliary obstruction is unlikely to be the major factor as it is known to be uncommon in experimental opisthorchiasis^[24,72]. Infection of hamsters with *O viverrini*, whether or not eliminated with praziquantel before the time of the re-challenge, failed to confer any protective immunity to reinfection^[73].

An attempt to potentiate acquired immunity in hamsters with prior infection by immunizing them with aqueous somatic extract of adult worms resulted in a 30% reduction of worm^[74]. IgG, IgA and IgM were found significantly higher levels to adult worm homogenate and metacercaria homogenate in stool of the egg-negative residents in the *O viverrini*-endemic area comparing to stool of the egg-positive group, thereby indicating some protective evidence of opisthorchiasis^[75]. The lack of protective immunity is perhaps due to suppression of both cellular and humoral immune responses to unrelated antigens by *O viverrini* as shown by reduced phytohemagglutinin-induced lymphoproliferation and response to sheep red blood cell stimulation^[68,75]. Moreover, when specific antibody was

measured in the infected animals, the titer was found to be depressed during the late stage of infection, particularly in heavily infected group^[77]. The parasites may be resistant to immune damage or able to evade the defense system of the host. The inability to kill the worms by serum from infected animals or from patients with opisthorchiasis, and the resistance to immune damage may be related to tegumental shedding and repair^[68,76]. The role of T cells and cytokines in protective immunity and pathogenesis of opisthorchiasis is also unclear. The set of cytokines produced by these different T helper responses, in turn, can exert opposing effects on the parasite, resulting in either control of infection or promotion of disease. *O viverrini* may induce the Th2 response as found with other flukes, such as *Scistosoma mansoni*. However, information regarding the significance of immune responses generated during liver fluke infections is limited^[68].

Diagnosis of *O viverrini* infection

Diagnosis of the infection is basically based on the standard fecal examination of the fluke's eggs. However, in cases with bile duct obstruction or in a light infection, eggs may not be detected in the feces^[77]. Detection of fecal *Opisthorchis* antigen has been reported^[78,79], though it is not so convenient. There is a need for a reliable diagnostic tool to solve the clinical and parasitological diagnosis problems in opisthorchiasis. Immunodiagnostic method is one of highly sensitive techniques in diagnosis of *O viverrini* infection. Several serologic tests for the diagnosis of opisthorchiasis have been reported^[58,65,78-83]. However, some cross-reactions with other parasitic infection are still a problem. The recombinant cysteine proteinase-based ELISA was used to diagnose the infected hamsters and humans. The use of *Op*-CP-1 as antigens showed that humoral immune responses in hamsters infected with 50 Metacerciae of *O viverrini* occurred as early as d 15 post-infection and rapidly increased at one month before reaching a plateau at mo 2, which is in agreement with that reported by using *O viverrini*-somatic extract, excretory-secretory product or egg antigens^[58]. In human cases, the *Op*-CP-1-based ELISA did not cross-react with sera from patients with hookworm, minute intestinal fluke, *S. stercoralis*, *Taenia sp.*, *G. lamblia* and *E. coli* infection. The sensitivity and specificity of the test were 94.66% and 95.55%, respectively. This is even higher than conventional indirect ELISA using *O viverrini* somatic extracts or excretory-secretory product antigen^[63,64,79,81]. The sensitivity and specificity are similar to other studies using ELISA based on recombinant trematode cysteine protease, such as *C sinensis* (sensitivity: 81.3%-96%; specificity: 92.6%-96.2%)^[84,85] and *P. westermani* (sensitivity: 86.2%-93%; specificity: 93%-98%)^[86,87]. It seems that the more specific purified antigen used, the more sensitivity and specificity obtained. Recently, Ruangsittichai *et al.*^[54] reported a high sensitivity and specificity of a recombinant eggshell protein (ESP) as a potential serodiagnosis of human opisthorchiasis. However, detection of *O viverrini* DNA is expensive and requires skillful professional personnel. This overall result suggests that *Op*-CP-1 has potential as an antigen for ELISA-based immunodiagnosis of opisthorchiasis.

PCR-based amplification of parasite DNA from stools has been documented for its high specificity. Compared with that of formalin-ether and Stoll's egg count, the sensitivity of the PCR was found to be 100%, 68.2%, and 50% in the fecal specimens containing > 1000, 200-1000, and < 200 eggs per gram of feces, respectively. Human fecal materials showed a strong reduction in PCR sensitivity of detection^[51,52]. Recently, an adult stage *O viverrini* cDNA library was constructed and was isolated a glutathione S-transferase (GST) cDNA. The cDNA has a size of 893 bp and encodes a GST of 213 amino acids (OV28GST). The OV28GST RNA was detected in the parenchymal cells of adult parasites. Both purified recombinant and purified native OV28GST were resolved as 28-ku proteins. Molecular analysis of OV28GST is helpful in ongoing development of diagnostic applications for *O viverrini*^[54].

O viverrini is a medically important food-borne trematode in some part of Southeast Asian countries, including Thailand. The opisthorchiasis has been studied for more than 50 years, a major break through in pathology and pathogenesis of opisthorchiasis was the discovery of *Opisthorchis* antigens in the biliary epithelium and its associated intense inflammatory response in certain infected animals^[25]. The infection is associated with cholangiocarcinoma and other hepatobiliary diseases. The epidemiology is found in some rural areas because of the tradition of eating raw or uncooked fish products. The health education programs to prevent and control opisthorchiasis are still required in the high-risk areas.

REFERENCES

- 1 **Infection with liver flukes (*Opisthorchis viverrini*, *Opisthorchis felinus* and *Clonorchis sinensis*)**. IARC Monogr Eval Carcinog Risks Hum 1994; **61**: 121-175
- 2 **Leiper RT**. Notes of the occurrence of parasites presumably rare in man. *J London School Trop Med* 1911; **1**: 16-19
- 3 **Kerr AFG**. Intestinal parasites in northern Siam. *Trans Soc Trop Med* 1916; **9**: 82-89
- 4 **Prommas C**. Report of case of *Opisthorchis felinus* in Siam. *Ann Trop Med Parasitol* 1927; **21**: 9-10
- 5 **Sadun EH**. Studies on *Opisthorchis viverrini* in Thailand. *Am J Hyg* 1955; **62**: 81-115
- 6 **Wykoff DE**, Harinasuta C, Juttijudata P, Winn MM. *Opisthorchis viverrini* in Thailand-the life cycle and comparison with *O. felinus*. *J Parasitol* 1965; **51**: 207-214
- 7 **Brandt RAM**. The non-marine aquatic mollusca of Thailand. *Arch Moll* 1974; **105**: 1-423
- 8 **Kaewkes S**. Taxonomy and biology of liver flukes. *Acta Trop* 2003; **88**: 177-186
- 9 **Ash LR**, Oreil TC. Ash&Orihel's Atlas of Human Parasitology. 5th ed. Chicago: ASCP Press, 2007: 330-331
- 10 **Sithithaworn P**, Haswell-Elkins M. Epidemiology of *Opisthorchis viverrini*. *Acta Trop* 2003; **88**: 187-194
- 11 **Migasena S**, Egoramaiphon S, Tungtrongchitr R, Migasena P. Study on serum bile acids in opisthorchiasis in Thailand. *J Med Assoc Thai* 1983; **66**: 464-469
- 12 **Kurathong S**, Lerdverasirikul P, Wongpaitoon V, Pramoolsinsap C, Kanjanapitak A, Varavithya W, Phuapradit P, Bunyaratvej S, Upatham ES, Brockelman WY. *Opisthorchis viverrini* infection and cholangiocarcinoma. A prospective, case-controlled study. *Gastroenterology* 1985; **89**: 151-156
- 13 **Upatham ES**, Viyanant V, Kurathong S, Rojborwonwitaya J, Brockelman WY, Ardsungnoen S, Lee P, Vajrasthira S. Relationship between prevalence and intensity of *Opisthorchis viverrini* infection, and clinical symptoms and signs in a rural community in north-east Thailand. *Bull World Health Organ* 1984; **62**: 451-461
- 14 **Changbumrung S**, Migasena P, Supawan V, Juttijudata P, Buavatanana T. Serum protease inhibitors in opisthorchiasis, hepatoma, cholangiocarcinoma, and other liver diseases. *Southeast Asian J Trop Med Public Health* 1988; **19**: 299-305
- 15 **Jongsuksuntigul P**, Imsomboon T. Opisthorchiasis control in Thailand. *Acta Trop* 2003; **88**: 229-232
- 16 **Jongsuksuntigul P**, Imsomboon T. The impact of a decade long opisthorchiasis control program in northeastern Thailand. *Southeast Asian J Trop Med Public Health* 1997; **28**: 551-557
- 17 **Anantaphruti MT**, Jongsuksuntigul P, Imsomboon T, Nagai N, Muennoo C, Saguankiat S, Pubampen S, Kojima S. School-based helminthiasis control: I. A baseline study of soil-transmitted helminthiasis in Nakhon Si Thammarat Province, Thailand. *Southeast Asian J Trop Med Public Health* 2002; **33** Suppl 3: 113-119
- 18 **Sriamporn S**, Pisani P, Pipitgool V, Suwanrungruang K, Kamsa-ard S, Parkin DM. Prevalence of *Opisthorchis viverrini* infection and incidence of cholangiocarcinoma in Khon Kaen, Northeast Thailand. *Trop Med Int Health* 2004; **9**: 588-594
- 19 **Tungtrongchitr A**, Chiworaporn C, Praewanich R, Radomyos P, Boitano JJ. The potential usefulness of the modified Kato thick smear technique in the detection of intestinal sarcocystosis during field surveys. *Southeast Asian J Trop Med Public Health* 2007; **38**: 232-238
- 20 **Hitanant S**, Trong DT, Damrongsak C, Chinapak O, Boonyapisit S, Plengvanit U, Viranuvatti V. Peritoneoscopic findings in 203 patients with *Opisthorchis viverrini* infection. *Gastrointest Endosc* 1987; **33**: 18-20
- 21 **Riganti M**, Pungpak S, Sachakul V, Bunnag D, Harinasuta T. *Opisthorchis viverrini* eggs and adult flukes as nidus and composition of gallstones. *Southeast Asian J Trop Med Public Health* 1988; **19**: 633-636
- 22 **Pairojkul C**, Shirai T, Hirohashi S, Thamavit W, Bhudhisawat W, Uttaravicien T, Itoh M, Ito N. Multistage carcinogenesis of liver-fluke-associated cholangiocarcinoma in Thailand. *Princess Takamatsu Symp* 1991; **22**: 77-86
- 23 **Sripa B**. Pathobiology of opisthorchiasis: an update. *Acta Trop* 2003; **88**: 209-220
- 24 **Bhamarapravati N**, Thamavit W, Vajrasthira S. Liver changes in hamsters infected with a liver fluke of man, *Opisthorchis viverrini*. *Am J Trop Med Hyg* 1978; **27**: 787-794
- 25 **Sripa B**, Kaewkes S. Localisation of parasite antigens and inflammatory responses in experimental opisthorchiasis. *Int J Parasitol* 2000; **30**: 735-740
- 26 **Viranuvatti V**, Stitnimankarn T. Liver fluke infection and infestation in Southeast Asia. In: Popper H, Schaffner F, editors. Progress in Liver Disease. New York: Grune and Stratton, 1972: 537-547
- 27 **Tansurat P**. Opisthorchiasis. In: Marcial-Rojas RA, editor. Pathology of Protozoal and Helminthic Diseases. Baltimore: Williams and Wilkins, 1971: 536-545
- 28 **Harinasuta T**, Riganti M, Bunnag D. *Opisthorchis viverrini* infection: pathogenesis and clinical features. *Arzneimittelforschung* 1984; **34**: 1167-1169
- 29 **Sripa B**, Kaewkes S. Gall bladder and extrahepatic bile duct changes in *Opisthorchis viverrini*-infected hamsters. *Acta Trop* 2002; **83**: 29-36
- 30 **Sripa B**, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, Pairojkul C, Bhudhisawasdi V, Tesana S, Thinkamrop B, Bethony JM, Loukas A, Brindley PJ. Liver fluke induces cholangiocarcinoma. *PLoS Med* 2007; **4**: e201
- 31 **Flavell DJ**. Liver-fluke infection as an aetiological factor in bile-duct carcinoma of man. *Trans R Soc Trop Med Hyg* 1981; **75**: 814-824
- 32 **Flavell DJ**, Flavell SU. *Opisthorchis viverrini*: pathogenesis of infection in immunodeprived hamsters. *Parasite Immunol* 1986; **8**: 455-466
- 33 **Haswell-Elkins MR**, Sithithaworn P, Mairiang E, Elkins DB, Wongratanacheewin S, Kaewkes S, Mairiang P. Immune responsiveness and parasite-specific antibody levels in human hepatobiliary disease associated with *Opisthorchis viverrini* infection. *Clin Exp Immunol* 1991; **84**: 213-218

- 34 **Thamavit W**, Bhamarapravati N, Sahaphong S, Vajrasthira S, Angsubhakorn S. Effects of dimethylnitrosamine on induction of cholangiocarcinoma in *Opisthorchis viverrini*-infected Syrian golden hamsters. *Cancer Res* 1978; **38**: 4634-4639
- 35 **Kim YI**. Liver carcinoma and liver fluke infection. *Arzneimittelforschung* 1984; **34**: 1121-1126
- 36 **Migasena P**, Reaunsuwan W, Changbumrung S. Nitrates and nitrites in local Thai preserved protein foods. *J Med Assoc Thai* 1980; **63**: 500-505
- 37 **Srisraluang T**, Boriboon P. Nitrosamines in meat products. *J Nutr Assoc Thai* 1988; **21**: 163-185
- 38 **Jinawath N**, Chamgramol Y, Furukawa Y, Obama K, Tsunoda T, Sripa B, Pairojkul C, Nakamura Y. Comparison of gene expression profiles between *Opisthorchis viverrini* and non-*Opisthorchis viverrini* associated human intrahepatic cholangiocarcinoma. *Hepatology* 2006; **44**: 1025-1038
- 39 **Srianujata S**, Tonbuth S, Bunyaratvej S, Valyasevi A, Promvanit N, Chaivatsagul W. High urinary excretion of nitrate and N-nitrosoproline in opisthorchiasis subjects. *IARC Sci Publ* 1987; **84**: 544-546
- 40 **Srivatanakul P**, Ohshima H, Khlat M, Parkin M, Sukaryodhin S, Brouet I, Bartsch H. *Opisthorchis viverrini* infestation and endogenous nitrosamines as risk factors for cholangiocarcinoma in Thailand. *Int J Cancer* 1991; **48**: 821-825
- 41 **Satarug S**, Haswell-Elkins MR, Sithithaworn P, Bartsch H, Ohshima H, Tsuda M, Mairiang P, Mairiang E, Yongvanit P, Esumi H, Elkins DB. Relationships between the synthesis of N-nitrosodimethylamine and immune responses to chronic infection with the carcinogenic parasite, *Opisthorchis viverrini*, in men. *Carcinogenesis* 1998; **19**: 485-491
- 42 **Oshima Y**, Kobayashi K, Hidaka C, Izu S, Imada N. Differences in the drug-metabolizing enzyme activities among fish and bivalves living in waters near industrial and non-industrial areas. *Bull Environ Contam Toxicol* 1994; **53**: 106-112
- 43 **Miwa M**, Stuehr DJ, Marletta MA, Wishnok JS, Tannenbaum SR. Nitrosation of amines by stimulated macrophages. *Carcinogenesis* 1987; **8**: 955-958
- 44 **Oshima M**, Tsuchiya M, Yagasaki M, Orita T, Hasegawa M, Tomonoh K, Kojima T, Hirata Y, Yamamoto O, Sho Y. cDNA clones of Japanese hepatitis C virus genomes derived from a single patient show sequence heterogeneity. *J Gen Virol* 1991; **72**: 2805-2809
- 45 **Nguyen T**, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA* 1992; **89**: 3030-3034
- 46 **Korbsrisate S**, Mongkolsuk S, Haynes JR, England D, Sirisinha S. Nucleotide sequence of the small subunit ribosomal RNA-encoding gene from *Opisthorchis viverrini*. *Gene* 1991; **105**: 259-261
- 47 **Korbsrisate S**, Mongkolsuk S, Haynes JR, Wong Q, Sirisinha S. Cloning and characterization of ribosomal RNA genes from *Opisthorchis viverrini*. *Parasitology* 1992; **104** (Pt 2): 323-329
- 48 **Ando K**, Sithithaworn P, Nuchjungreed C, Tesana S, Srisawangwong T, Limviroj W, Chinzei Y. Nucleotide sequence of mitochondrial CO I and ribosomal ITS II genes of *Opisthorchis viverrini* in northeast Thailand. *Southeast Asian J Trop Med Public Health* 2001; **32** Suppl 2: 17-22
- 49 **Sermeswan R**, Mongkolsuk S, Sirisinha S. Characterization of the *Opisthorchis viverrini* genome. *J Helminthol* 1991; **65**: 51-54
- 50 **Sermeswan R**, Mongkolsuk S, Panyim S, Sirisinha S. Isolation and characterization of *Opisthorchis viverrini* specific DNA probe. *Mol Cell Probes* 1991; **5**: 399-407
- 51 **Wongratanacheewin S**, Pumidonming W, Sermeswan RW, Maleewong W. Development of a PCR-based method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. *Parasitology* 2001; **122**: 175-180
- 52 **Wongratanacheewin S**, Pumidonming W, Sermeswan RW, Pipitgool V, Maleewong W. Detection of *Opisthorchis viverrini* in human stool specimens by PCR. *J Clin Microbiol* 2002; **40**: 3879-3880
- 53 **Eursittichai V**, Viyanant V, Vichasri-Grans S, Sobhon P, Tesana S, Upatham SE, Hofmann A, Korge G, Grams R. Molecular cloning and characterization of a glutathione S-transferase encoding gene from *Opisthorchis viverrini*. *Asian Pac J Allergy Immunol* 2004; **22**: 219-228
- 54 **Ruangstittichai J**, Viyanant V, Vichasri-Grans S, Sobhon P, Tesana S, Upatham ES, Hofmann A, Korge G, Grams R. *Opisthorchis viverrini*: identification of a glycine-tyrosine rich eggshell protein and its potential as a diagnostic tool for human opisthorchiasis. *Int J Parasitol* 2006; **36**: 1329-1339
- 55 **Laha T**, Pinlaor P, Mulvenna J, Sripa B, Sripa M, Smout MJ, Gasser RB, Brindley PJ, Loukas A. Gene discovery for the carcinogenic human liver fluke, *Opisthorchis viverrini*. *BMC Genomics* 2007; **8**: 189
- 56 **Sirisinha S**, Chawengkirttikul R, Sermeswan R. Immunodiagnosis of opisthorchiasis. *Southeast Asian J Trop Med Public Health* 1991; **22** Suppl: 179-183
- 57 **Sirisinha S**, Tuti S, Vichasri S, Tawatsin A. Humoral immune responses in hamsters infected with *Opisthorchis viverrini*. *Southeast Asian J Trop Med Public Health* 1983; **14**: 243-251
- 58 **Sirisinha S**, Chawengkirttikul R, Sermeswan R, Amornpant S, Mongkolsuk S, Panyim S. Detection of *Opisthorchis viverrini* by monoclonal antibody-based ELISA and DNA hybridization. *Am J Trop Med Hyg* 1991; **44**: 140-145
- 59 **Sripa B**, Kaewkes S. Relationship between parasite-specific antibody responses and intensity of *Opisthorchis viverrini* infection in hamsters. *Parasite Immunol* 2000; **22**: 139-145
- 60 **Akai PS**, Pungpak S, Chaicumpa W, Kitikoon V, Ruangkunaporn Y, Bunnag D, Befus AD. Serum antibody responses in opisthorchiasis. *Int J Parasitol* 1995; **25**: 971-973
- 61 **Wongratanacheewin S**, Chawengkirttikul R, Bunnag D, Sirisinha S. Analysis of *Opisthorchis viverrini* antigens by immunoprecipitation and polyacrylamide gel electrophoresis. *Parasitology* 1988; **96**: 119-128
- 62 **Tanvanich S**, Doungchawee G, Boonpucknavig S, Thamavit W. A characteristic immunoblotting pattern for Opisthorchiasis sera with metacercarial antigens. *Clin Exp Immunol* 1988; **74**: 355-358
- 63 **Wongratanacheewin S**, Sirisinha S. Analysis of *Opisthorchis viverrini* antigens: physicochemical characterization and antigen localization. *Southeast Asian J Trop Med Public Health* 1987; **18**: 511-520
- 64 **Wongratanacheewin S**, Bunnag D, Vaeusorn N, Sirisinha S. Characterization of humoral immune response in the serum and bile of patients with opisthorchiasis and its application in immunodiagnosis. *Am J Trop Med Hyg* 1988; **38**: 356-362
- 65 **Thammapalerd N**, Tharavanij S, Nacapunchai D, Bunnag D, Radomyos P, Prasertsiriroj V. Detection of antibodies against *Opisthorchis viverrini* in patients before and after treatment with praziquantel. *Southeast Asian J Trop Med Public Health* 1988; **19**: 101-108
- 66 **Wongratanacheewin S**, Charupatana C, Bunnag D, Sirisinha S. Effect of praziquantel treatment on antibody levels and lymphoproliferative responses in patients with opisthorchiasis. *Southeast Asian J Trop Med Public Health* 1988; **19**: 109-116
- 67 **Akai PS**, Pungpak S, Kitikoon V, Bunnag D, Befus AD. Possible protective immunity in human opisthorchiasis. *Parasite Immunol* 1994; **16**: 279-288
- 68 **Wongratanacheewin S**, Sermeswan RW, Sirisinha S. Immunology and molecular biology of *Opisthorchis viverrini* infection. *Acta Trop* 2003; **88**: 195-207
- 69 **Sun T**. The non-antigenicity of intact ova of *Clonorchis sinensis*. *J Med Microbiol* 1969; **2**: 358-360
- 70 **Sun T**. Clonorchiasis: a report of four cases and discussion of unusual manifestations. *Am J Trop Med Hyg* 1980; **29**: 1223-1227
- 71 **Wongratanacheewin S**, Good MF, Sithithaworn P, Haswell-Elkins MR. Molecular analysis of T and B cell repertoires in mice immunized with *Opisthorchis viverrini* antigens. *Int J Parasitol* 1991; **21**: 719-721
- 72 **Flavell DJ**, Pattanapanyasat K, Lucas SB, Vongsangnak V. *Opisthorchis viverrini*: liver changes in golden hamsters maintained on high and low protein diets. *Acta Trop* 1980; **37**: 337-350
- 73 **Akai PS**, Pungpak S, Chaicumpa W, Viroj K, Bunnag D, Befus AD. Serum antibody response to *Opisthorchis viverrini* antigen

- as a marker for opisthorchiasis-associated cholangiocarcinoma. *Trans R Soc Trop Med Hyg* 1994; **88**: 471-474
- 74 **Sirisinha S**, Wongratanaheewin S. Immunization of hamsters against *Opisthorchis viverrini* infection. *Southeast Asian J Trop Med Public Health* 1986; **17**: 567-573
 - 75 **Sirisinha S**, Rattanasiriwilai W, Puengtomwatanakul S, Sobhon P, Saitongdee P, Koonchornboon T. Complement-mediated killing of *Opisthorchis viverrini* via activation of the alternative pathway. *Int J Parasitol* 1986; **16**: 341-346
 - 76 **Sakolvaree Y**, Ybanez L, Chaicumpa W. Parasites elicited cross-reacting antibodies to *Opisthorchis viverrini*. *Asian Pac J Allergy Immunol* 1997; **15**: 115-122
 - 77 **Sithithaworn P**, Tesana S, Pipitgool V, Kaewkes S, Pairojkul C, Sripa B, Paupairoj A, Thaiklar K. Relationship between faecal egg count and worm burden of *Opisthorchis viverrini* in human autopsy cases. *Parasitology* 1991; **102**: 277-281
 - 78 **Chaicumpa W**, Ybanez L, Kitikoon V, Pungpak S, Ruangkunaporn Y, Chongsa-nguan M, Sornmani S. Detection of *Opisthorchis viverrini* antigens in stools using specific monoclonal antibody. *Int J Parasitol* 1992; **22**: 527-531
 - 79 **Sirisinha S**, Chawengkirttikul R, Haswell-Elkins MR, Elkins DB, Kaewkes S, Sithithaworn P. Evaluation of a monoclonal antibody-based enzyme linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. *Am J Trop Med Hyg* 1995; **52**: 521-524
 - 80 **Srivatanakul P**, Viyanant V, Kurathong S, Tiwawech D. Enzyme-linked immunosorbent assay for detection of *Opisthorchis viverrini* infection. *Southeast Asian J Trop Med Public Health* 1985; **16**: 234-239
 - 81 **Wattanakuppanich D**, Waikagul J, Anantaphruti MT, Dekumyoy P. Evaluation of *Bithynia funiculata* snail antigens by ELISA-serodiagnosis of human opisthorchiasis. *Southeast Asian J Trop Med Public Health* 1997; **28**: 593-598
 - 82 **Wongsaroj T**, Sakolvaree Y, Chaicumpa W, Maleewong W, Kitikoon V, Tapchaisri P, Chongsa-nguan M, Cross JH. Affinity purified oval antigen for diagnosis of *Opisthorchiasis viverrini*. *Asian Pac J Allergy Immunol* 2001; **19**: 245-258
 - 83 **Waikagul J**, Dekumyoy P, Chaichana K, Thairungroje Anantapruti M, Komalamisra C, Kitikoon V. Serodiagnosis of human opisthorchiasis using cocktail and electroeluted *Bithynia* snail antigens. *Parasitol Int* 2002; **51**: 237-247
 - 84 **Nagano I**, Pei F, Wu Z, Wu J, Cui H, Boonmars T, Takahashi Y. Molecular expression of a cysteine proteinase of *Clonorchis sinensis* and its application to an enzyme-linked immunosorbent assay for immunodiagnosis of clonorchiasis. *Clin Diagn Lab Immunol* 2004; **11**: 411-416
 - 85 **Zhao QP**, Moon SU, Lee HW, Na BK, Cho SY, Kong Y, Jiang MS, Li AH, Kim TS. Evaluation of *Clonorchis sinensis* recombinant 7-kilodalton antigen for serodiagnosis of clonorchiasis. *Clin Diagn Lab Immunol* 2004; **11**: 814-817
 - 86 **Yun DH**, Chung JY, Chung YB, Bahk YY, Kang SY, Kong Y, Cho SY. Structural and immunological characteristics of a 28-kilodalton cruzipain-like cysteine protease of *Paragonimus westermani* expressed in the definitive host stage. *Clin Diagn Lab Immunol* 2000; **7**: 932-939
 - 87 **Yang SH**, Park JO, Lee JH, Jeon BH, Kim WS, Kim SI, Yun KJ, Jeong ET, Lee KW, Kim YM, Lee MH, Park H. Cloning and characterization of a new cysteine proteinase secreted by *Paragonimus westermani* adult worms. *Am J Trop Med Hyg* 2004; **71**: 87-92

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