

# PREVALENCE OF *OPISTHORCHIS VIVERRINI* INFECTION IN THE CANINE AND FELINE HOSTS IN THREE VILLAGES, KHON KAEN PROVINCE, NORTHEASTERN THAILAND

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**Abstract.** To determine the current reservoir status and prevalence of *Opisthorchis viverrini* infection in endemic areas, 78 dogs and 22 cats were sampled for fecal examination in 3 villages along the Chi River in Khon Kaen Province, northeastern Thailand. Sex, age, color, identifiable markings, subjective body condition scores (BCS) and diet were recorded and analyzed. Fecal samples were evaluated using direct smear and modified formalin-ethyl acetate fecal assays. All parasites found were identified. In dogs and cats, the prevalences of *O. viverrini* infection were 3.8% and 36.4%, respectively. The highest prevalences of parasitic infection in both dogs and cats were *Ancylostoma* spp, in 64.1% and 77.3%, respectively. These results provide insight into the role of the canines and felines in maintaining the presence of *O. viverrini* eggs in the environment. If similar patterns occur on a broader scale, the zoonotic role of dogs and cats in the epidemiology of this disease should be considered in the development of improved control and education programs.

**Key words:** *Opisthorchis viverrini*, canine and feline hosts, Thailand, formalin-ethyl acetate assays

## INTRODUCTION

*Opisthorchis viverrini*, a liver fluke, is an important zoonotic trematode in Thailand and in Southeast Asian countries where humans traditionally consume infective metacercariae in raw or under cooked cyprinoid fish. Up to 95% of natu-

ral cyprinoid fish have been found to harbor *O. viverrini* metacercariae (Kuper *et al*, 2000; Sithithaworn and Haswell-Elkins, 2003; Upatham and Viyanant, 2003; Chai *et al*, 2005; Macpherson, 2005). *O. viverrini* has a predilection for the intrahepatic bile ducts causing mechanical, chemical and immunological irritation. Infection is mainly associated with the liver, gall bladder, bile ducts and kidney, and the severity depends largely on intensity and duration of infection. Severe, complications, such as cholangitis, cirrhosis, pancreatitis and most importantly, promotion of cholangiocarcinoma, can occur (Mairiang

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and Mairiang, 2003; Sripa, 2003; Chai *et al*, 2005). The International Agency for Research on Cancer has determined *O. viverrini* as an agent associated with human cancer. Cholangiocarcinoma has been reported to be a leading cause of death in northeastern Thailand (Kuper *et al*, 2000; Khurana *et al*, 2005). *O. viverrini* has been estimated to infest 9 million people globally with as many as 7 million in Thailand (Kuper *et al*, 2000; Sithithaworn and Haswell-Elkins, 2003; Chai *et al*, 2005).

Canines and felines are recognized as reservoir hosts for *O. viverrini*. Studies of felines as reservoir hosts in Lao PDR revealed the prevalence of infection was 20-36% (Ditrich *et al*, 1990; Giboda *et al*, 1991; Scholz *et al*, 2003). No recent studies of felines or canines have been reported in Thailand. Research has concentrated on the role of transmission and re-infection as being largely attributed to humans. The frequency of infection in reservoir hosts, such as canines and felines has been proposed to have little association with human opisthorchiasis. Further studies need to be carried out before this statement can be accepted. The role of canines and felines as a reservoir has not been well studied in Thailand. Animal reservoirs may have important implications for mass drug treatment control programs if only humans are targeted (Sithithaworn and Haswell-Elkins, 2003; Chai *et al*, 2005). This would play an important role in environmental sanitation which has been proposed as a major strategy for liver fluke control (Jongsuksuntigul and Imsomboon, 2003).

This study was done to determine the potential contribution of domestic canine and feline hosts to the persistence of this important zoonotic parasite in Thailand and to better understand what further studies and control programs may need to be implemented. It is hypothesized ca-

nines and felines are currently major reservoirs for *O. viverrini* in northeastern Thailand, and this high prevalence of infection contributes to its persistence in the environment, and that control programs will remain unsuccessful unless measures are taken to decrease the prevalence of *O. viverrini* infection in canine and feline reservoirs.

## MATERIALS AND METHODS

### Village selection

Three villages along the Chi River (Fig 1) in northeastern Thailand were selected for this study based on four main criteria: a high prevalence of human cholangiocarcinoma, the number of households in the village, location along the Chi River, and accessibility. The prevalence of human cholangiocarcinoma in the study villages is a subject of current epidemiological investigations (Sripa B, personal communication). Prevalence calculations were estimated based on the current population and a 12-20 year accumulated record of cholangiocarcinoma case data. The size of the villages was  $\leq 100$  households for this preliminary study. The three study locations were selected from villages along the Chi River, an area of high prevalence of cholangiocarcinoma: one village is on the west side of the river, one on the east side, and one a few kilometers to the north of the other 2 villages (*ie*, Ban Chot, Ban Tha Khoi, and Ban Wang Woen, respectively) (Table 1).

### Animal samples

No data was available regarding animal population in the selected villages. A subjective estimate of the dog and cat population sizes was determined upon arrival by consultation with villagers and concurrent enumeration on travel through the villages. This estimate was then used

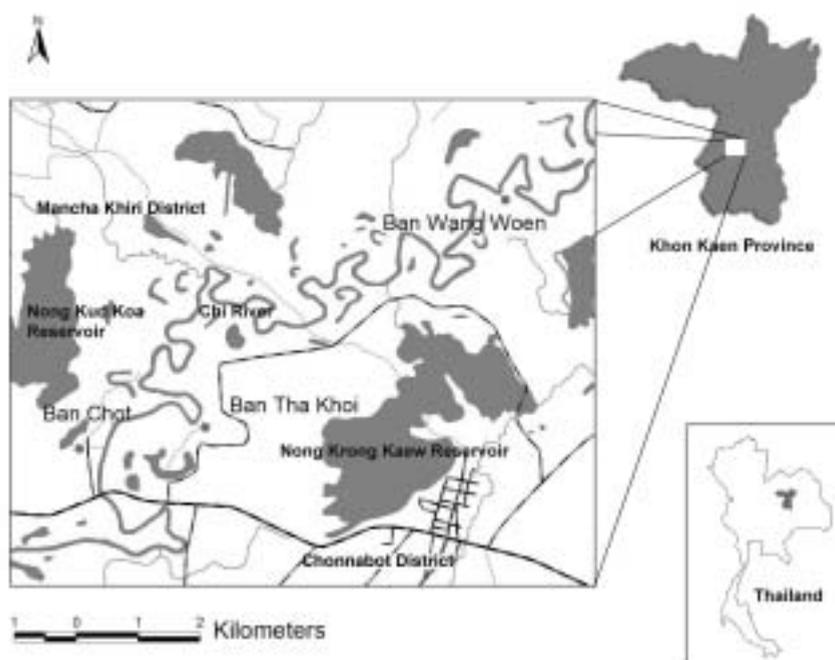


Fig 1—Schematic map of sampling areas in three villages along the Chi River, Khon Kaen Province, northeastern, Thailand.

Table 1  
Percentage of cholangiocarcinoma cases in humans, number of households, and the estimated population of canines and felines in three villages.

Village no.	Cholangiocarcinoma (%)	No. household	Estimated no.	
			Dogs	Cats
1. Ban Chot	2.94	222	300	30
2. Ban Tha Koi	3.98	45	60	30
3. Ban Wang Woen	2.60	98	100	15

to establish the number of animals to sample according to the sample size formula of Cannon and Roe (1982) at a 95% confidence interval. The criteria for the specific dogs or cats selected for sampling were as follows: 1)  $\geq 3$  months old to allow for time to wean from the mother and begin eating solid food, as well as 1 month for the parasite to complete the pre-patent

period and shed eggs; 2). animals should be sampled when available at random. The entire village area was covered with all animals available being sampled.

#### Field collection

A Patar NaCl enema of 20 ml for dogs and 10 ml for cats was administered using petroleum jelly lubricant. Fecal samples were stored on ice in a styrofoam

chest until processed at the laboratory. Each sample was labeled with a number specific for the village and the animal. The age, sex, subjective body-condition score (LaFlamme, 1997), diet, color, identifying markings and GPS location were recorded for each animal to prevent repeat sampling. The animal was physically restrained by the owner unless it was passive and restraint was not needed. When the enema was completely emptied into the rectum, restraint of the animal was loosened or released. The dogs were kept on a leash for the length of time thought necessary to allow the enema to take effect. Cats were kept in cages until defecation was observed. After defecation, the sample was collected from the ground or from the newspaper in the cage using latex gloves and collection sticks to prevent cross contamination and to maintain sanitation. Observations recorded on collection included gross visual parasite findings (eg tapeworm segments), consistency, and whether the sample was collected from a possibly contaminated location, such as a cow stall where other fecal material may have been present. The samples were stored on ice in the field, transferred, and processed in the laboratory during the same day or refrigerated overnight.

### Fecal assays

The fresh fecal samples were assayed using a direct smear with saline and Lugol's iodine. The intensity of parasitic infection was estimated by quantitative formalin ethyl acetate sedimentation modified from Smith and Malone (2006) using 2 grams of feces. The formalin ethyl acetate assay was expressed as eggs per gram after two drops were examined and averaged. Specific emphasis was placed on the identification of *O. viverrini* eggs based on morphology and size. The morphologic

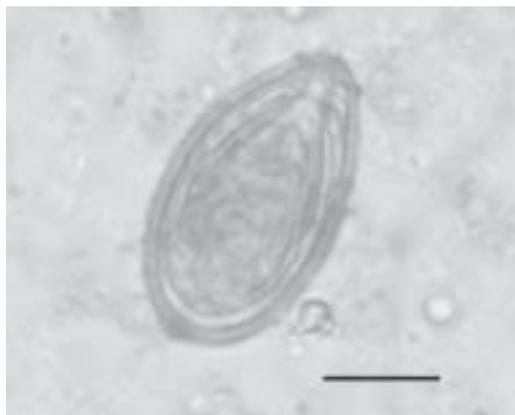


Fig 2—Photograph of *O. viverrini* egg from cat feces (bar=10  $\mu$ m).

characteristics used were a singly operculated egg with a prominent shoulder, an obvious knob at the opposite end to the operculum, a rough outer eggshell and embryonated with a fully developed miracidium. These special characteristics differentiated *O. viverrini* eggs from similar minute intestinal flukes eggs (Kaewkes *et al*, 1991; Tesana *et al*, 1991). All *O. viverrini* positive samples were examined twice to ensure proper identification and to take photographs. Eggs per gram of feces were also calculated and recorded.

### RESULTS

The number of households weakly correlated with the number of dogs in the village (Table 1). Villagers provided food for their animals from their meal leftovers including raw or fermented cyprinoid fish, and discarded heads and fins of fish during fish dish preparation. Three dogs out of 78 sampled were positive for *O. viverrini* with an average prevalence of infection of 3.8% (Table 2). Canine *O. viverrini* eggs per gram calculated from the modified formalin ethyl acetate sedimentation assay

Table 2  
Number and percentage of dogs infected with parasites in three villages.

Parasite	Ban Chot (N=29)	Ban Tha Khoi (N=24)	Ban Wang Woen (N=25)	Total (N=78)(%)
<i>O. viverrini</i>	0	1	2	3 (3.8)
<i>Ancylostoma</i> spp	22	6	22	50 (64.1)
<i>Capillaria</i> sp	0	1	0	1 (1.3)
Coccidian	1	1	0	2 (2.6)
<i>Giardia</i> sp	1	1	0	2 (2.6)
MIF	3	1	1	5 (6.4)
<i>Physaloptera</i> sp	0	1	0	1 (1.3)
<i>Spirocerca</i> sp	1	1	0	2 (2.6)
<i>Strongyloides</i> sp	1	0	0	1 (1.3)
<i>Taenia</i> sp	1	0	0	1 (1.3)
<i>Trichuris</i> sp	1	1	1	3 (3.8)
No parasite found	5	15	3	23 (29.5)

MIF- minute intestinal fluke

Table 3  
Number and percentage of felines infected with parasites in three villages.

Parasite	Ban Chot (N=10)	Ban Tha Khoi (N=6)	Ban Wang Woen (N=6)	Total (N=22)(%)
<i>O. viverrini</i>	5	2	1	8 (36.4)
<i>Ancylostoma</i> spp	9	5	3	17 (77.3)
<i>Capillaria</i> sp	0	1	0	1 (4.5)
Coccidian	1	1	3	5 (22.7)
<i>Giardia</i> sp	1	0	0	1 (4.5)
Echinostomes	0	1	0	1 (4.5)
MIF	4	1	1	6 (27.3)
<i>Physaloptera</i> sp	2	0	0	2 (9.1)
<i>Spirometra</i> sp	1	1	0	2 (9.1)
<i>Taenia</i> sp	2	0	0	2 (9.1)
Unidentified parasite	0	2	0	2 (9.1)
No parasite found	0	0	1	1 (4.5)

MIF- minute intestinal fluke

ranged from 16.5 to 256.5. Other canine parasites were observed, such as coccidian oocysts, *Giardia* spp cysts and eggs of *Ancylostoma* spp, *Capillaria* spp, minute intestinal flukes (MIF), *Physaloptera* spp, *Spirocerca* spp, *Taenia* spp, *Trichuris* spp and *Strongyloides* larvae. No parasites

were found in 23 of 78 dogs (Table 2). Eight cats out of 22 sampled were positive for *O. viverrini* (Fig 2) and the average prevalence of infection was 36.4% (Table 3). The feline *O. viverrini* eggs per gram ranged from 4.7 to 484. Other feline parasites were *Ancylostoma* sp, *Capillaria* sp, coccidian,

*Echinostoma* sp, *Giardia* sp, minute intestinal flukes, *Physaloptera* sp, *Spirometra* sp, *Taenia* sp and other unidentified flukes. The highest prevalence of parasitic infection in both dogs and cats was *Ancylostoma* spp with 64.1% and 77.3%, respectively. Only 1 cat out of 22 was negative for parasitic infection.

Most animals had a body condition score of 3 or 4 on a scale of 9 with 1 being emaciated, 9 grossly obese, and 3 and 4 being very thin and thin, respectively.

## DISCUSSION

The results of the present study provide clear evidence canines and felines are currently reservoirs for *O. viverrini* in northeastern Thailand. There was little difficulty in differentiating between minute intestinal flukes and *O. viverrini* using the methods described previously. In the three villages studied, the average prevalence was low in dogs (3.8%) but high in cats (36.4%). Similar prevalences of infection in felines in Lao PDR were reported (20-36%) (Ditrich *et al*, 1990; Giboda *et al*, 1991; Scholz *et al*, 2003). The intensity of *O. viverrini* (eggs/gram) was generally higher in cats than dogs. Cats live closely with their owners. Cats prefer to eat fish, including the heads and fins, more than dogs. Felines, in general, had a greater number of parasites than canines; only one cat out of 22 had no evidence of parasites by fecal examination compared to 23 out of 78 canines. It is interesting, yet speculative, to note the three canines found positive for *O. viverrini* were 6 months old or younger, suggesting a possible level of natural resistance or a clearance mechanism in adult canines or diet preference differences between young and old dogs. Since we did not examine extremely young animals (<3 months old) in the present

study, we may have missed some infections in both canines and felines. Animals in the villages were observed consuming solid food much younger than expected.

The villages were found to be very traditional in customs, food consumption habits and lifestyles. The animals were fed leftover food from the human's meals. This may play a major role in transmission of the parasite, possibly more than other sources of transmission such as eating live-caught or dead fish around water bodies. Interviews with villagers revealed they rarely sought care for dogs and cats, and administration of anthelmintics was not common. Most animals had a body condition score of 3 or 4 on a scale of 9 with 1 being emaciated, 9 grossly obese and 3 and 4 being very thin and thin respectively (LaFlamme, 1997). A correlation between infection and body condition score could not be made.

Observations noted an abundance of dogs in the villages while cats were present in considerably smaller numbers; 78 dogs were sampled as compared to 22 cats. It was difficult to estimate the cat population due to their natural roaming behaviors. However, results provided here represent preliminary data that strongly suggests the need for further studies of the role of cats and possibly dogs in the epidemiology and transmission dynamics of *O. viverrini* in Thailand. Correlations between breed, sex, body condition score, distribution of infection using GPS and diet could not be made based on the limited scope of the study. The results of a current human *O. viverrini* prevalence survey are pending. The results may then be examined to evaluate a correlation between the prevalence in humans and reservoir hosts. This study demonstrates that alongside established control programs in humans, programs may also need to be de-

veloped to treat and control *O. viverrini* infections in canines and felines.

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#### REFERENCES

- Cannon RM, Roe RT. Livestock disease surveys: a field manual for veterinarians. Canberra: Australian Bureau of Animal Health, 1982.
- Chai JY, Murrell KD, Lymbery AJ. Fish-borne parasitic zoonoses: Status and issues. *Int J Parasitol* 2005; 35: 1233-54.
- Ditrich O, Scholz T, Giboda M. Occurrence of some medically important flukes (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir, Laos. *Southeast Asian J Trop Med Public Health* 1990; 21: 482-8.
- Giboda M, Ditrich O, Scholz T, Viengsay T, Bouaphanh S. Current status of food-borne parasitic zoonoses in Laos. *Southeast Asian J Trop Med Public Health* 1991; 22 (suppl): 56-61.
- Jongsuksuntigul P, Imsomboon T. Opisthorchiasis control in Thailand. *Acta Trop* 2003; 88: 229-32.
- Kaewkes S, Elkins D, Sithithaworn P, Haswell-Elkins M. Comparative studies on the morphology of the eggs of *Opisthorchis viverrini* and Lecithodendriid trematodes. *Southeast Asian J Trop Med Public Health* 1991; 22: 623-30.
- Khurana S, Dubey ML, Malla N. Association of parasitic infections and cancers. *Indian J Med Microbiol* 2005; 23: 74-9.
- Kuper H, Adami H-O, Trichopoulos D. Infections as a major preventable cause of human cancer. *J Int Med* 2000; 248: 171-83.
- LaFlamme DP. Development and validation of a body condition score system for dogs. *Canine Pract* 1997; 22: 10-5.
- Macpherson C. Human behaviour and the epidemiology of parasitic zoonosis. *Int J Parasitol* 2005; 35: 1319-31.
- Mairiang E, Mairiang P. Clinical manifestation of opisthorchiasis and treatment. *Acta Trop* 2003; 88: 221-7.
- Scholtz T, Uhlířová M, Ditrich O. Helminth parasites of cats from the Vientiane Province, Laos, as indicators of the occurrence of causative agents of human parasitosis. *Parasite* 2003; 10: 343-50.
- Sithithaworn P, Haswell-Elkins M. Epidemiology of *Opisthorchis viverrini*. *Acta Trop* 2003; 88: 187-94.
- Smith PH, Malone JB. Parasitology veterinary diagnostic laboratory manual 2005-2006. Baton Rouge: Louisiana State University, 2006: 6.
- Sripa B. Pathobiology of opisthorchiasis: an update. *Acta Trop* 2003; 88: 209-20.
- Tesana S, Srisawangwonk T, Keawkes S, Sithithaworn P, Kanla P, Arunyanart C. Eggshell morphology of the small eggs of human trematodes in Thailand. *Southeast Asian J Trop Med Public Health* 1991; 22: 631-46.
- Upatham ES, Viyanant V. *Opisthorchis viverrini* and opisthorchiasis: a historical review and future perspective. *Acta Trop* 2003; 88: 171-6.