

PREVALENCE AND RISK FACTORS FOR *OPISTHORCHIS VIVERRINI* INFECTION AMONG CATS AND DOGS IN SIX DISTRICTS SURROUNDING THE UBOLRATANA DAM, AN ENDEMIC AREA FOR HUMAN OPISTHORCHIASIS IN NORTHEASTERN THAILAND

Surasit Aunpromma¹, Prapan Kanjampa¹, Pittaya Papirom¹, Sirikachorn Tangkawattana¹, Prasarn Tangkawattana², Smarn Tesana³, Thidarut Boonmars³, Apiporn Suwannatrai³, Suwit Uopsai², Peerapol Sukon² and Banchob Sripa⁴

¹Department of Pathobiology, ²Department of Anatomy, Faculty of Veterinary Medicine, Khon Kaen University; ³Department of Parasitology, ⁴Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract: *Opisthorchis viverrini* is a zoonotic liver fluke that causes opisthorchiasis among humans in endemic areas, such as northeastern Thailand. The objective of this study was to determine the prevalence and risk factors for *O. viverrini* infection among cats and dogs in six districts surrounding Ubolratana Dam. Fecal samples of 1,018 dogs and 249 cats were collected between 2008 and 2013 to examine for *O. viverrini* infection using a modified formalin-ether concentration technique. The prevalence of *O. viverrini* infection among cats (77 of 249, 30.92%) was higher than dogs (2 of 1,018, 0.20%). Age and the eating habits of cats were associated with *O. viverrini* infection. Cats aged >3 years were more likely than those aged <1 year [Odds ratio (OR)=2.96; 95% confidence interval (95% CI): 1.01-8.35; $p = 0.044$] of being infected. Cats that consumed raw fish were significantly more likely to be infected than those that consumed other foods (OR=1.82, 95% CI: 1.05-3.16; $p = 0.032$). Cats had a higher prevalence of *O. viverrini* infection than dogs and may play an important role in the transmission and maintenance of this disease in the study area.

Keywords: *Opisthorchis viverrini*, reservoir host, dog, cat, Ubolratana Dam, Khon Kaen, Thailand

INTRODUCTION

Liver fluke infection, opisthorchiasis in humans, is a major public health problem in the Greater Mekong sub-region

Correspondence: Surasit Aunpromma, Department of Pathobiology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

Tel: +66 (0) 43 202404; Fax: +66 (0) 43 202404
E-mail: asuras@kku.ac.th

countries including Thailand, Lao PDR, Vietnam and Cambodia (Kobayashi *et al*, 2000; Sripa *et al*, 2007). In Thailand, liver fluke infection due to *Opisthorchis viverrini* is found predominantly in the northeastern region and especially Khon Kaen Province. *O. viverrini* is considered by The International Agency for Research on Cancer as associated with human cancer (Sriamporn *et al*, 2004) and especially cholangiocarcinoma, a bile duct epithe-

lial cancer, a leading cause of death of people living in northeastern Thailand (Sriamporn *et al*, 2004; Sripa *et al*, 2007). The incidence of cholangiocarcinoma in Khon Kaen Province has been reported to be as high as 93.8-317.6/100,000 persons per year in Khon Kaen Province (Sriamporn *et al*, 2004) and 55.41/100,000 persons per year in Nong Bua Lam Phu Province, Thailand (Bureau of Epidemiology, Thailand), also in northeastern Thailand.

The life cycle of *O. viverrini* is associated with a number of species of animals that act as intermediate and reservoir hosts (Upatham and Viyanant, 2003; Sripa *et al*, 2007). The life cycle of *O. viverrini* starts when the parasite eggs are passed from the feces of infected villagers into the water (Aunpromma *et al*, 2012). The eggs are eaten by *Bithinia* snails. The eggs hatch and the free swimming parasites, cercariae, are released from the infected snails into the water. The cercariae seek their next intermediate hosts, cyprinid fishes. They enter the fish and encyst in the fins, skin and muscles of the fish and become metacercariae (Sithithaworn and Haswell-Elkins, 2003; Sripa *et al*, 2007). After the metacercariae of *O. viverrini* have developed in cyprinid fish, they have the potential to infect humans and other mammalian hosts. People become infected by eating raw, undercooked or fermented cyprinid fish containing viable metacercariae (Aunpromma *et al*, 2012). Cats and dogs can become infected with this liver fluke and may serve as reservoir hosts (Impand *et al*, 1983; Sripa *et al*, 2007). Previous studies have found the prevalence of *O. viverrini* infection to be higher in cats (22.6-35.51%) than dogs (0.4-1.9%) (Impand *et al*, 1983; Aunpromma *et al*, 2012). However, those were comparisons between studies. The risk factor associated with *O. viverrini* infection in cats and dogs

is unclear. We conducted this study to determine the prevalence of *O. viverrini* among potential reservoir hosts in areas endemic for human opisthorchiasis in northeastern Thailand.

MATERIALS AND METHODS

Study area

This epidemiological survey was conducted between 2008 and 2013 in 79 villages, 4 districts (Ubolratana, Nong Ruea, Phu Wiang and Nong Na Kham) of Khon Kaen Province and 2 districts (Non Sang and Si Bun Rueang) of Nong Bua Lam Phu Province, in northeastern Thailand. These areas have a high prevalence of human opisthorchiasis (Sriamporn *et al*, 2004). All the villages are along the water reservoir of Ubolratana Dam (Fig 1). Its catchment area is 410 km² with a population of 361,179 in 2013 in the six districts (Department of Provincial Administration, Minister of Interior, Thailand).

Fecal sample collection and examination

The study animals were physically examined before collecting fecal samples. Owners were also asked to provide information for the survey form. Fecal samples were collected from 1,018 dogs and 249 cats after being given a rectal enema. The fecal samples were examined with the modified formalin-ether concentration technique (Elkins *et al*, 1986). The number of eggs per gram (EPG) in the studied samples was recorded and classified as: light (<50 EPG), moderate (50-500 EPG), heavy (501-1,500 EPG) and very heavy (>1,500 EPG) infections (Elkins *et al*, 1986; Aunpromma *et al*, 2012).

Statistical analyses

The prevalence of opisthorchiasis was calculated and presented using descriptive statistics. Logistic regression analysis was used to determine the odds ratio (OR)

Table 1
Prevalence of *O. viverrini* infection among cats (n=249) and dogs (n=1,018) in the study area.

Province	District	Number of villages in district with <i>O. viverrini</i>	Cats		Dogs	
			Infected no. (%)	Mean EPG of <i>O. viverrini</i> (range)	Infected no. (%)	Mean EPG of <i>O. viverrini</i> (range)
Khon Kaen	Phu Wiang	17/29	37/99 (37.4)	315 (1 - 2,292)	1/363 (6.3)	12
	Nong Na Kham	1/6	2/10 (20.0)	143 (65 - 221)	0/79 (0.0)	0
	Nong Ruea	3/8	4/16 (25.0)	14 (7 - 19)	0/119 (0.0)	0
	Ubolratana	1/3	1/16 (6.3)	15	0/29 (0.0)	0
	Subtotal	22/46	44/141 (31.2)	274 (1 - 2,292)	1/590 (0.2)	12
Nong Bua Lam Phu	Non Sang	12/27	23/88 (26.1)	842 (4 - 17,504)	1/354 (0.3)	26
	Si Bun Rueang	5/6	10/20 (50.0)	1,596 (5 - 5,240)	0/74 (0.0)	0
	Subtotal	17/33	33/108 (30.6)	1,070 (4 - 17,504)	1/428 (0.2)	26
Total		39/79	77/249 (30.9)	615 (1 - 17,504)	2/1,018 (0.2)	19 (12-26)

EPG, egg per gram of feces.

and its 95% confidence interval (CI) for each associated risk factor. A *p*-value < 0.05 was considered significant. Statistical analysis was carried out using SPSS for Windows, version 17 (SPSS, Chicago, IL).

Ethical considerations

The protocol for this study was approved by the Animal Ethics Committee of Khon Kaen University, Thailand (No. AEKKU 09/2551, 40/2556). This approval included a survey form asking about specific risk factors, such as age, sex and the eating habits of the animals. The animal owners were asked to sign a consent form before being studied.

RESULTS

Prevalence, intensity and clinical signs of opisthorchiasis

The prevalences and intensities of opisthorchiasis among the cats and dogs in each studied village in the six districts surrounding Ubolratana Dam are shown in Table 1 and Fig 1. The overall prevalence of *O. viverrini* among studied cats (77 of 249; 30.9%) was much higher than among studied dogs (2 of 1,018; 0.196%), but were similar between the 2 study provinces (31.2% and 30.6% among cats and 0.2% and 0.2% among dogs in Khon Kaen and Nong Bua Lam Phu Province, respectively). The cats with *O. viverrini* infection tended to be from the villages on the northern and western sides of the dam: with 50.0% in Si Bun Rueang District, 37.4% in Phu Wiang District, 26.1% in Non Sang District, 25.0% in Nong Ruea District, 20.0% in Nong Na Kham District and 6.3% in Ubolratana District. The intensities of in-

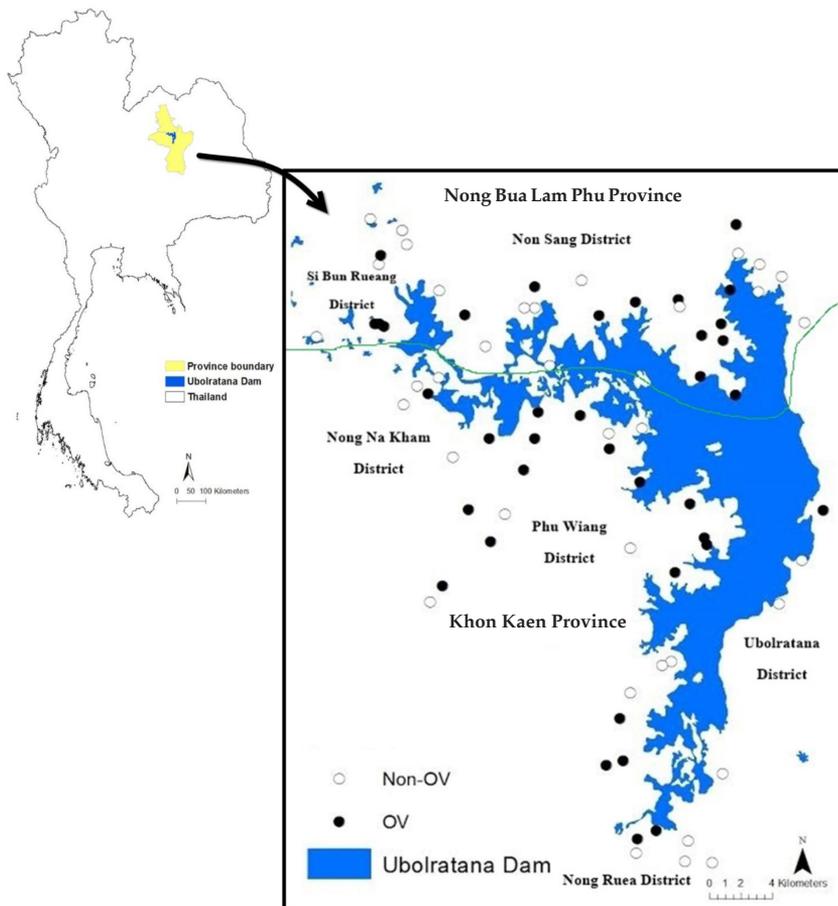


Fig 1—Geographical distribution of opisthorchiasis among studied animals in Khon Kaen and Nong Bua Lam Phu Provinces.

fection among cats varied: light (35 cats), moderate (28 cats), heavy (9 cats) and very heavy infection (5 cats). Si Bun Rueang District had the highest average of EPG of *O. viverrini* infection among studied cats. Most infected animals had no signs, but those with very heavy infection had lethargy and ocular and nasal discharge.

Factors associated with opisthorchiasis

Since only 2 out of 1,018 dogs studied were positive for *O. viverrini* infection, we could not determine any associations with *O. viverrini* infection among dogs in this study. The factors found to be associated

with *O. viverrini* infection among cats are shown in Table 2. Two factors (age and eating habits) were significantly associated with *O. viverrini* infection in cats. The prevalence of *O. viverrini* infection was greater among older cats (aged >3 years) than among younger cats (aged <1 year) (OR=2.90; 95% CI: 1.01-8.35). The prevalence of *O. viverrini* infection was greater among cats that ate raw fish than among cats that did not (OR=1.82; 95% CI: 1.05-3.16). Most cats in the endemic area were feral or stray cats; only 249 cats were considered domesticated. None

Table 2
Association between *O. viverrini* infection and various factors among studied cats.

Variable	Infected no. (%)	Odds ratio (95% CI)	p-value
Age (years)			
< 1 (reference)	9/47 (19.2)	1	-
1-3	57/175 (32.6)	2.04 (0.92-4.51)	0.074
> 3	11/27 (44.4)	2.90 (1.01-8.35)	0.044
Consuming raw fish			
No (reference)	42/160 (26.3)	1	-
Yes	35/89 (39.3)	1.82 (1.05-3.16)	0.032
Feed			
Left over human food	51/158 (32.3)	1.91 (0.61-5.99)	0.268
Owner- prepared food	22/71 (31.0)	1.80 (0.54-6.00)	0.337
Other (reference)	4/20 (20.0)	1	-

CI, confidence interval.

of the cats had a history of a veterinary visit, vaccination or anthelmintic drugs. Seventy-one point four percent of cat owners (120/168) had a history of eating raw or undercooked fish, 14.3% (24/168) had a history of being diagnosed with a liver fluke infection, and 1.2% (2/168) had a history of a person in their family with cholangiocarcinoma. Cats ate left over human food more often than other food.

DISCUSSION

In this study, we evaluated *O. viverrini* infection among cats and dogs in an area highly endemic for human opisthorchiasis and cholangiocarcinoma (Sripa *et al*, 2007, 2008) in northeastern Thailand. The overall prevalence of *O. viverrini* among cats (77/249, 30.9%) was much higher than among dogs (2/1,018; 0.20%). This finding is similar to previous studies among cats in Lao PDR (20-36%) (Giboda *et al*, 1991; Scholz *et al*, 2003), Pitsanulok and Khon Kaen Provinces, Thailand (22.6%) (Impand *et al*, 1983), three villages in Chonnabot and Mancha

Khiri Districts in Khon Kaen, Thailand (36.4%) (Enes *et al*, 2010) and 29 villages in Ban Haet, Ban Phai, Chonnabot and Mancha Khiri Districts, Thailand (35.5%) (Aunpromma *et al*, 2012). The intensity of *O. viverrini* infection (in EPG) among our study animals was higher among cats than dogs, similar to previous studies (Enes *et al*, 2010; Aunpromma *et al*, 2012). These results support the conclusion cats are an important reservoir host for *O. viverrini* infection among humans in the study area.

The age and eating habits of cats in our study were associated with *O. viverrini* infection. Prevalences of *O. viverrini* infection were higher among older cats and among cats that ate raw fish, similar to a previous study (Enes *et al*, 2010). Our findings suggest cats contract their infections by eating raw cyprinid fish where they become infected by the metacercariae.

The Ubolratana Dam reservoir area in the northeastern Thailand has a wide area of freshwater wetlands environmentally suitable for the reproduction of the first host for *O. viverrini* infection, the snails of

the genus *Bithinia*, and the secondary intermediate hosts for *O. viverrini* infection, cyprinid fishes. The metacercariae, found in the flesh of the cyprinoid fish and are transmitted to people by consuming raw or undercooked fish, fermented fish or fermented sour fish, commonly consumed by people in this area. Cats and dogs also become infected by ingesting raw fish with metacercaria (Enes *et al*, 2010; Aunpromma *et al*, 2012); domestic and feral animals in this area feed on leftovers of human meals (Enes *et al*, 2010).

The National Control Program for Human Opisthorchiasis (NCPHO) in Thailand has been trying to promote stool examinations, treatment and health education (Jongsuksuntikul and Imsomboon, 2003). However, the prevalence of human opisthorchiasis in this area remains unsatisfactorily high (Jongsuksuntikul and Imsomboon, 2003; Sripan *et al*, 2007). This program did not consider cats and dogs would act as reservoir hosts for *O. viverrini* infections, being a potential source for human infection (Aunpromma *et al*, 2012). The villages on the northern and western sides of the dam had higher prevalences of *O. viverrini* infection among cats and dogs than the other sides of dam. There are also higher prevalences of human *O. viverrini* infections among districts in these areas (Sriamporn *et al*, 2004). Our findings suggest the NCPHO needs to manage *O. viverrini* infections among cats and dogs in these areas as well. Cats and dogs can be treated with praziquantel at a dose of 40 mg/kg (Nissen *et al*, 2014).

In this study, the number of feral cats was far greater than the number of cats with owners. The number of feral cats sampled was limited due to the difficulty in trapping these animals. The prevalence of *O. viverrini* infection in this study repre-

sents primarily cats with owners. Because the population of cats with owners is relatively smaller, the factors associated with infection are only specific for this cohort and might vary if more feral cats were sampled.

In conclusion, the high prevalence of *O. viverrini* infection among cats in our study confirm that cats may be an important reservoir host for human opisthorchiasis in the villages around the Ubolratana Dam. Further studies are needed to determine the prevalence of *O. viverrini* infection among feral and wild cat species in this area and any associated factors.

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