



Seasonal Variation of *Opisthorchis viverrini* Metacercarial Infection in Cyprinid Fish from Southern Cambodia

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Abstract

Small liverfluke infections are caused by fish-borne trematodes, which remain the major causative agent of cholangiocarcinoma in the Greater Mekong Subregion. In this paper, we describe variations of metacercarial infection with *Opisthorchis viverrini* among cyprinid fish caught in endemic areas of southern Cambodia, during the period 2008-2009. One thousand, eight hundred and seventy-four fish (1,874), comprising 17 different species, were collected from 8 sites in Kandal Province. The pectoral fins, tail fins and muscles of the fish were examined by tissue-compression technique. Opisthorchid metacercariae were found among all species of fish tested, ranging from 5.6% (1/18) in *Crossocheilus reticulatus* (Cambodian name – Trey Changwa Chunh Chuak) to 100% (10/10) in *Amblyrhynchichthys truncatus* (Trey Kambot Chramos). The prevalence of *Opisthorchis viverrini* among fish in Kandal Province was 34.3% (642/1,874). The highest level of infection found was in fish caught from O'Roley Bridge, with a rate of 57.2% (214/374). Metacercariae appeared throughout the entire examination period – except for August 2009, when the area was dry and fish could not be caught – with different positive rates; the average and range were 34.3% and 2.0-76.2%, respectively. Numbers peaked in March and were lowest in July. The prevalence of *O. viverrini* metacercariae in fish was significantly ($p < 0.05$) negatively correlated with the water level in Lake 500, as recorded monthly during the study period.

Keywords: *Opisthorchis viverrini*, metacercaria, cyprinid fish, southern Cambodia, seasonal variation

Introduction

Small liverfluke infection is a significant public health problem in the Mekong Basin, with an estimated 10 million people infected with

Clonorchis sinensis or *Opisthorchis viverrini* [1]. *O. viverrini* is the primary causative agent, with heavy, chronic infections inducing the development of cholangiocarcinoma [2]. Opisthorchiasis is distributed in countries right along the Mekong River, from Central and Southern Laos, to Northeastern Thailand, Southern Cambodia and Central Vietnam, all with high prevalence [3-7].

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A low prevalence of opisthorchiasis was also reported in Stung Treng and Kampong Cham provinces, northeastern Cambodia [8,9]. Information about fish intermediate hosts in Cambodia, however, is still limited. Recently, *O. viverrini* metacercariae were reported in 10 species of cyprinid fish (17.5%) collected from Lake 500, Kandal Province, southern Cambodia [10], and *Opisthorchis* metacercariae were also found infecting the cyprinid fish, *Puntioplites proctozystron*, in Takeo Province [11].

Seasonal variations of metacercarial infection in fish have been reported for several species of trematode, including small liverflukes and related species of heterophyid intestinal flukes. Variation patterns differed by species of parasite and by country region [12-14]. Reports have previously focused on the relationship between abundance of parasites and the physical attributes of the water where they are found [15,16].

This study attempted to investigate seasonal variations of *O. viverrini* metacercarial infection in cyprinid fish in Lake 500, Kandal Province, southern Cambodia, and to analyze the relationship between the Lake's water level and rates of metacercarial infection in its cyprinid fish.

Materials and methods

Eight sites in Kandal Province around Lake 500 – reported as an endemic area in our previous study from 2006-2007 [10] – were selected for fish collection, during the period September 2008-August 2009 (Fig 1). On the 3rd Saturday of every month, a fisherman was hired to catch cyprinid fish using a cast net. Fish were immediately packed in an ice box and transported to Bangkok. Two days after being caught, the fish arrived at the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University. They were then identified by species, via taxonomic reference book [17] and a poster of freshwater fishes in the Kingdom of Cambodia (Part I). Two examination methods were performed to record numbers of positive fish: first, by tissue-compression technique of the pectoral fin, tail fins, and body muscles, which

were examined under a stereomicroscope [10]; second, by digestion method, where metacercariae were isolated from positive portions of each fish – metacercariae were then identified by morphology [18-20]. Species of liver fluke metacercariae from this region have previously been confirmed as *O. viverrini*, using animal experimental infection as well as COI sequencing [10]. In this study, all metacercariae displaying morphological features of the liver fluke were identified as *O. viverrini*, and only *O. viverrini* metacercariae were recorded and analyzed.

Positive rates of opisthorchid metacercaria were analyzed descriptively by fish species and collection site. Variations in *O. viverrini* positive rates over the year of examination were expressed as mean \pm standard error. Correlations between water depth and prevalence of liver flukes in fish caught from Lake 500 were observed using Pearson's correlation test.

Results

In this study, only cyprinid fish were examined from September 2008 to August 2009. A total of 1,874 fish were collected. Numbers varied from 0 to 343 fish per month, while no fish were caught in August; most fish were collected in November (Fig 2). The total number of fish positive for liverfluke metacercariae was 624. The lowest number of positive fish was 1 in July, while the highest was 160 fish in March; the mean number of positive fish \pm standard error was 53.5 ± 14.9 . The greatest prevalence occurred from November to March. The average (range) of monthly positive rates was 34.3% (2.0-76.2%); mean positive rate per month \pm standard error, 28.5 ± 6.4 .

A total of 1,874 cyprinid fish encompassing 17 different species were examined. Every species was found to harbor liverfluke metacercaria. The average (range) percentage was 34.3% (5.6-100%), while the mean rate \pm standard error was 38.3 ± 6.6 (Table 1). *Henicorhynchus siamensis*, *Barbonymus brevis*, *Cyclocheilichthys lagleri*, *Labiobarbus siamensis*, *Puntioplites proctozystron*, and *Hampala dispar* were the most common fish collected. Over 50% of collected *C. lagleri*, *Amblyrhynchichthys*

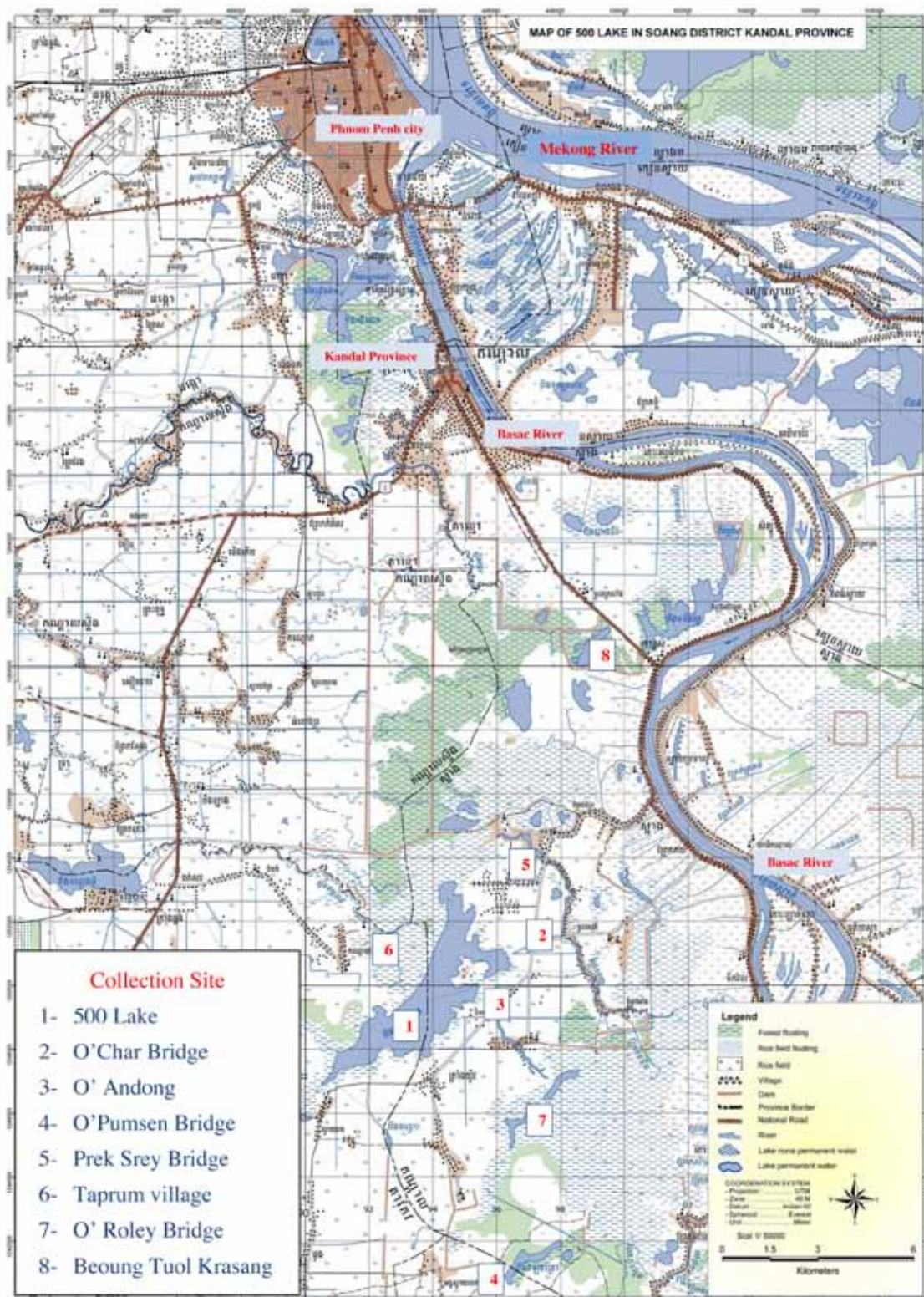


Fig 1 Map of 8 fish collection sites.

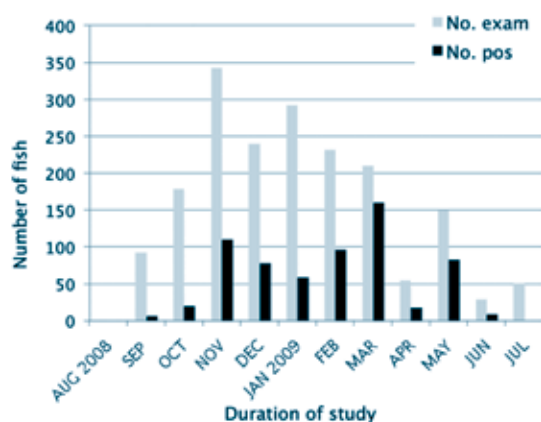


Fig 2 Numbers of cyprinid fish presenting with *Opisthorchis viverrini* metacercariae, and numbers of fish collected in Kandal Province from September 2008 to August 2009.

truncates, *Barbonymus schwanenfeldii*, *C. armatus*, *L. siamensis*, and *Thynnichthys thynnoides* were positive for liverfluke metacercariae. The results showed *C. lagleri* (Trey Srawka Kdam) and *L. siamensis* (Trey Ach Kok) both play a leading role in opisthorchiasis transmission in southern Cambodia.

Forty-four to 579 cyprinid fish were collected from 8 sites in Kandal Province in the vicinity of Lake 500. The numbers of positive fish from each collection site varied from 0 to 214 fish. The average (range) of positive percentages was 34.3% (0-57.2%), and mean \pm standard error was 26.9 ± 8.6 (Table 2). The infection rates among fish collected from O'Roley Bridge and Lake 500 were $> 50\%$. For the duration of the study period, fish were able to be collected from Lake 500 more frequently than from the other sites. Scatter plots and trend lines were made to show the relationship between water level and prevalence of liverfluke metacercaria in cyprinid fish at Lake 500. Infection rates in fish increased when the water level decreased. Water depth demonstrated a significant negative correlation with prevalence ($r = -0.7$, $p < 0.05$) (Fig 3).

Discussion

Our study area was the floodplain along the Bassac River in southern Cambodia. The rainy season starts every year in either May or June; it is not heavy rain and there is no water flow at this point. From mid-September, the water comes in from upcountry and water from the Bassac River flows in to fill Lake 500 and its floodplains. Cyprinid fish move to the floodplain where they spawn, then move back to the Lake when the water recedes from the river around the end of November. During the migration season, fish are easily caught. In 2008, however, it rained very little and water flowed into the Lake later than usual. The numbers of fish collected varied from site to site, and in this study high infection rates appeared from November to March, and were low from April to October. However, the rate increased in May 2009 – unexpectedly differing from our previous study, which found fish to be negative for *O. viverrini* metacercariae in May 2007 [10].

Seasonal variation of the small liverfluke, *O. viverrini*, in Cambodia was different from that reported in Thailand. Sithithaworn *et al* [13] found seasonal variation in liverfluke metacercaria to be widespread in northeastern Thailand, in both high (Khon Kaen Province) and low (Maha Sarakham Province) endemic areas. High metacercarial burdens occurred in the late rainy season and in winter (July to January), with low burdens occurring during summer (March to June).

Seasonal variations in *C. sinensis* infection were reported in Korea in 1983. *Pseudorasbora parva*, the most suitable fish host, was collected from hyperendemic areas of human clonorchiasis in spring (March to May), summer (June to July), autumn (September to November), and winter (February). The highest metacercarial infection rates were found in May-September, which then decreased from November through February; the lowest rates occurred around March to April [14]. It can be concluded that seasonal variations in fish with small liverfluke metacercarial infections, as well as high and low infection durations, differ by place and year of study.

Table 1 Percentage of cyprinid fish positive with *Opisthorchis viverrini* metacercaria examined during September 2008 to August 2009.

Fish species	Combodian name	Number examine	Number positive	%
<i>Amblyrhynchichthys truncatus</i>	Kambot Chramos	10	10	100
<i>Barbonymus altus</i>	Kahe Kror horm	20	6	30.0
<i>Barbonymus gonionotus</i>	Chhpin Prak	93	15	16.1
<i>Barbonymus schwanenfeldi</i>	Kahe	53	35	66.0
<i>Crossocheilus reticulatus</i>	Changwa Chuhn Chuak	18	1	5.6
<i>Cyclocheilichthys apogon</i>	Srawka Kdam	84	27	32.1
<i>Cyclocheilichthys armatus</i>	Pka Kor	39	33	84.6
<i>Cyclocheilichthys lagleri</i>	Srawka Kdam	268	168	58.2
<i>Hampala dispar</i>	Khmann	115	36	31.3
<i>Henicorhynchus lobatus</i>	Riel Angkam	3	1	33.3
<i>Henicorhynchus siamensis</i>	Riel Top	338	37	10.9
<i>Larbiobarbus siamensis</i>	Ach Kok	199	102	51.3
<i>Osteochilus hasselti</i>	Kros or Kros Memay	66	4	6.1
<i>Puntioplites sproctoysron</i>	Chra Keng	135	62	45.9
<i>Puntius brevis</i>	Angkat Prak	292	49	16.8
<i>Systemus orphoides</i>	Ampil Tum	69	25	36.2
<i>Thynnichthys thynnoides</i>	Linh	72	43	59.7
Total (mean \pm SE)		1,874	642	34.3 (38.3 \pm 6.6)

Table 2 Percentage of cyprinid fish positive with *Opisthorchis viverrini* metacercaria from 8 Collected sites in Kandal Province during September 2008 to August 2009.

Collected sites	Number examined	Number positive	%
1. Lake 500	410	207	50.5
2. O' Char Bridge	80	1	1.3
3. O' Andong Bridge	158	67	42.4
4. O' Pumsen Bridge	158	71	44.9
5. Prek Srey Bridge	44	0	0
6. Taprum village	579	78	13.5
7. O' Roley Bridge	374	214	57.2
8. Tuol Krasang Lake	71	4	5.6
Total (mean \pm SE)	1,874	642	34.3 (26.9 \pm 8.6)

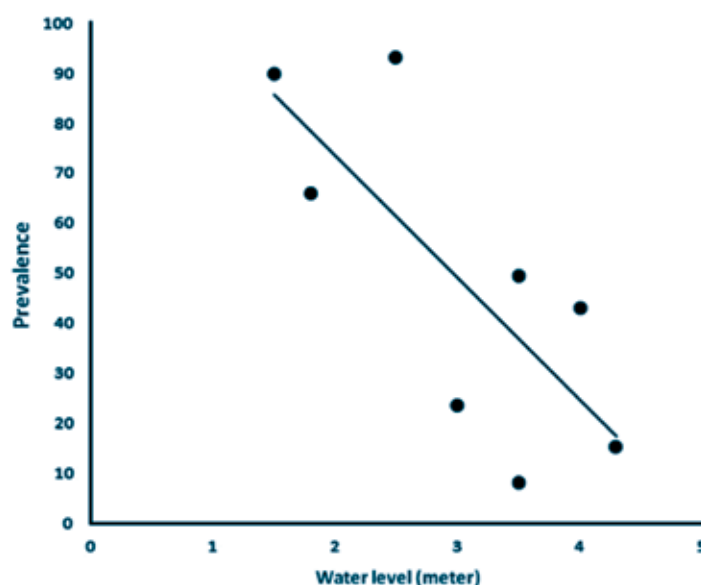


Fig 3 Scatter plot of infection rates of *Opisthorchis viverrini* metacercaria in cyprinid fish, and water levels at Lake 500 at time of collection.

A study of seasonal variations in bucephalid metacercariae among freshwater fish in the Yodo River, central Japan, showed a relationship between abundance of parasites and water-condition factors; mean water temperature positively correlated with mean metacercarial abundance, while mean water level and flow rates correlated negatively with mean metacercarial intensity among fish [15]. A study on the effects of water quality and helminth infection in cyprinid fish was carried out in 3 lakes in the Kashmiri Himalaya. The results showed parasite infection significantly positively correlated with water temperature; however, it was significantly negatively correlated with water depth and dissolved oxygen [16].

In this preliminary study, analysis of *O. viverrini* metacercarial prevalence and water levels at Lake 500 (our fish collection site) demonstrated a significant negative correlation. This finding agrees with the 2 previous studies mentioned above, which focused on the relationship between different parasite infection rates/intensity and connected water levels. The relationship between liverfluke metacercarial infection in fish and water

conditions/environment should be the focus of further inquiries, so we can better understand the dynamics of the transmission factors involved. These can then be applied to develop improved control measures for liverflukes in future.

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