

## High Prevalence of *Opisthorchis viverrini* Infection in a Riparian Population in Takeo Province, Cambodia

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**Abstract:** *Opisthorchis viverrini* infection was found to be highly prevalent in 3 riverside villages (Ang Svay Chek A, B, and C) of the Prey Kabas District, Takeo Province. This area is located in the southern part of Cambodia, where the recovery of adult *O. viverrini* worms was recently reported. From May 2006 until May 2010, fecal examinations were performed on a total of 1,799 villagers using the Kato-Katz thick smear technique. In the 3 villages, the overall positive rate for helminth eggs ranged from 51.7 to 59.0% (av. 57.4%), and the percentage positive for *O. viverrini* was 46.4-50.6% (47.5%). Other helminths detected included hookworms (13.2%), echinostomes (2.9%), *Trichuris trichiura* (1.3%), *Ascaris lumbricoides* (0.6%), and *Taenia* spp. (0.06%). The prevalence of *O. viverrini* eggs appeared to reflect a lower infection in younger individuals (<20 years) than in the adult population (>20 years). Men (50.4%) revealed a significantly higher ( $P=0.02$ ) prevalence than women (44.3%). The Ang Svay Chek villages of the Prey Kabas District, Takeo Province, Cambodia have been confirmed to be a highly endemic area for human *O. viverrini* infection.

**Key words:** *Opisthorchis viverrini*, opisthorchiasis, trematode, prevalence, Cambodia (Takeo)

Five species of small liver flukes belonging to the family Opisthorchiidae are known to infect humans around the world, *Clonorchis sinensis*, *Opisthorchis viverrini*, *Opisthorchis felineus*, *Metorchis conjunctus*, and *Metorchis orientalis* [1,2]. Among them, *C. sinensis* and *O. viverrini* are highly important in clinical and public health points of view. They are capable of causing chronic inflammation in the bile duct leading to cholangitis, and cirrhosis of the liver, and may also be a predisposing factor for cholangiocarcinoma [1,3]. *O. viverrini* is highly prevalent among people in the Indochina peninsula, including Thailand, Lao PDR, and Vietnam [4]. Cambodia is also included in this list based on the discovery of small trematode eggs (20-32  $\mu$ m in length) in the feces of Cambodian people [3,5-8], and also af-

ter recovery of adult *O. viverrini* flukes from 6 human cases who resided in the Prey Kabas District [9]. Thus, Takeo Province, Cambodia appears to be an endemic area for human opisthorchiasis [9]. However, no detailed data on its prevalence and endemicity have been reported.

From May 2006 until May 2010, we conducted stool surveys in riverside villages of the Ang Svay Chek area in the Prey Kabas District, where we found a high occurrence of *O. viverrini* eggs. Here, we report that *O. viverrini* is highly endemic in riparian areas of Takeo Province, a southern part of Cambodia, through which the Mekong River flows.

The surveyed regions included 3 villages (Ang Svay Chek A, B, and C) in the Prey Kabas District, an area approximately 45 km south of Phnom Penh (Fig. 1). Nearly 45,000 people reside in this District. Most villagers are farmers or fishermen. During the actual survey times (May 2006, November 2008, and May 2010), a total of 1,799 fecal samples were collected from the subjects (aged 1 though 94 years), 1 sample per each person, and transported to the Centre for Parasitology, Ento-

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mology, and Malaria Control in Phnom Penh, Cambodia. The Kato-Katz thick smear technique was used to detect helminth eggs. Fecal examinations of the villagers were sanctioned by the Ministry of Health, Cambodia, under the agreement of the Korea-Cambodia International Collaboration on Intestinal Parasite Control in Cambodia (2006-2011). Prevalence differences between men and women were statistically determined using the z-test.

The overall positive rates for helminth eggs, for 1 or more species, were found to be high but not significantly different between each of the 3 villages (A, B, and C), which was in the range of 51.7-59.0% (av. 57.4%) (Table 1). The most commonly observed eggs were those of *O. viverrini*/minute intestinal flukes (MIF) (most of these here were regarded as *O. viverrini*), with the prevalence ranging from 46.4 to 50.6% by village (av.



**Fig. 1.** Map showing the surveyed area (Ang Sway Chek villages, Prey Kabas District) in Takeo Province, southern Cambodia.

47.5%). Other helminths included *Ascaris lumbricoides* 0.4-0.7% (av. 0.6%), hookworms 1.9-16.0% (av. 13.2%), *Trichuris trichiura* 1.0-2.8% (av. 1.3%), *Taenia* spp. 0.0-0.07% (av. 0.06%), and echinostomes 2.6-3.9% (av. 2.9%). Eggs of *Enterobius vermicularis*, *Hymenolepis nana*, and *H. diminuta* were also noted each in a few cases (Table 1).

*O. viverrini* showed differential prevalences according to age and sex (Table 2). Younger individuals (<20 years) showed lower prevalences (27.4-40.0%) than the adult population (>20 years) (59.3-68.1%). Men revealed a significantly higher ( $P=0.02$ ) prevalence (50.4%) than women (44.3%). For another parasite, *A. lumbricoides*, all 9 positive cases occurred in children and young adults under 20 years of age. Hookworm infections appeared to be age independent until the age 60, when the prevalence increased.

The present study confirmed, for the first time, the high endemicity of human *O. viverrini* infections in a southern area of Cambodia. The prevalence was assessed as high as almost 50% among the surveyed people. Previous reports occasionally suggested the occurrence of *O. viverrini* infection in Cambodia based on recovery of small trematode eggs in the feces from the population. For example, from 1981 through 1982, 2 of 102 Cambodian refugees in the USA were found to be positive for *C. sinensis*, but which was probably *O. viverrini* eggs [5]. A small number of cases were later confirmed positive for eggs in Kracheh in 1999 [6], Kampongcham in 2002 [7], and Stung Treng in 2003 [8]. Subsequently, in 2010, adult *O. viverrini* flukes have been detected in 6 human cases in the Prey Kabas District of Takeo Province [9].

Small trematode eggs may result from infections by various species of hepatic and intestinal flukes [1]. In Thailand, Viet-

**Table 1.** Prevalence of intestinal helminths among people in Ang Sway Chek villages, the Prey Kabas District, Takeo Province, Cambodia as determined by the Kato-Katz fecal examination from May 2006 to May 2010

Village Code <sup>a</sup> (Ang Sway Chek villages)	No. of people exam.	No. positive (%) for helminth eggs							
		<i>Opisthorchis viverrini</i> /MIF <sup>b</sup>	<i>Ascaris lumbricoides</i>	Hookworms	<i>Trichuris trichiura</i>	<i>Taenia</i> spp.	Echino-stomes <sup>c</sup>	Others <sup>d</sup>	Total <sup>e</sup>
A	251	127 (50.6)	1 (0.4)	19 (7.6)	7 (2.8)	0 (0.0)	9 (3.6)	9 (3.6)	148 (59.0)
B	207	96 (46.4)	1 (0.5)	4 (1.9)	3 (1.4)	0 (0.0)	8 (3.9)	3 (1.4)	107 (51.7)
C	1,341	632 (47.1)	9 (0.7)	215 (16.0)	14 (1.0)	1 (0.07)	35 (2.6)	20 (1.5)	778 (58.0)
Total	1,799	855 (47.5)	11 (0.6)	238 (13.2)	24 (1.3)	1 (0.06)	52 (2.9)	32 (1.8)	1,033 (57.4)

<sup>a</sup>Examined in May 2006 (A), November 2008 (B), and May 2010 (C).

<sup>b</sup>May include minute intestinal fluke (MIF) eggs of 24-32  $\mu$ m in length, but in 6 treated and purged cases only adult flukes of *O. viverrini* were recovered [9].

<sup>c</sup>Some of these turned out to be *Echinostoma revolutum* (to be published).

<sup>d</sup>Include eggs of *Enterobius vermicularis*, *Hymenolepis nana*, and/or *Hymenolepis diminuta*.

<sup>e</sup>Total no. of villagers positive for 1 or more helminth species.

**Table 2.** Age and sex prevalence of intestinal helminths among people in Ang Svay Chek village C, the Prey Kabas District, Takeo Province, Cambodia as determined by the Kato-Katz fecal examination (May 2010)

Age group & sex	No. of people examined	No. positive (%) for helminth eggs							Total <sup>d</sup>
		<i>Opisthorchis viverrini</i> /MIF <sup>a</sup>	<i>Ascaris lumbricoides</i>	Hookworms	<i>Trichuris trichiura</i>	<i>Taenia</i> spp.	Echino-stomes <sup>b</sup>	Others <sup>c</sup>	
0-9	318	87 (27.4)	2 (0.6)	46 (14.5)	2 (0.6)	0 (0.0)	7 (2.2)	8 (2.5)	126 (39.6)
10-19	450	180 (40.0)	7 (1.6)	72 (16.0)	8 (1.8)	0 (0.0)	19 (4.2)	6 (1.3)	249 (55.3)
20-29	201	127 (63.2)	0 (0.0)	33 (16.4)	0 (0.0)	0 (0.0)	4 (2.0)	2 (1.0)	140 (69.7)
30-39	116	75 (64.7)	0 (0.0)	14 (12.1)	2 (1.7)	0 (0.0)	2 (1.7)	3 (2.6)	84 (72.4)
40-49	113	67 (59.3)	0 (0.0)	22 (19.5)	1 (0.9)	0 (0.0)	3 (2.7)	0 (0.0)	76 (67.3)
50-59	72	49 (68.1)	0 (0.0)	8 (11.1)	1 (1.4)	0 (0.0)	0 (0.0)	1 (1.4)	52 (72.2)
60 & over	69	46 (66.7)	0 (0.0)	20 (29.0)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	50 (72.5)
Unknown	2	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)
Males	643	324 (50.4) <sup>e</sup>	2 (0.3)	127 (19.8)	10 (1.6)	0 (0.0)	8 (1.2)	9 (1.4)	402 (62.5)
Females	696	308 (44.3) <sup>e</sup>	7 (1.0)	88 (12.6)	4 (0.6)	1 (0.1)	27 (3.9)	11 (1.6)	376 (54.0)
Total	1,341	632 (47.1) <sup>e</sup>	9 (0.7)	215 (16.0)	14 (1.0)	1 (0.07)	35 (2.6)	20 (1.5)	778 (58.0)

<sup>a</sup>May include MIF eggs but most are regarded as *O. viverrini*.

<sup>b</sup>Some of these turned out to be *Echinostoma revolutum* (to be published).

<sup>c</sup>Include eggs of *Enterobius vermicularis*, *Hymenolepis nana*, and/or *Hymenolepis diminuta*.

<sup>d</sup>Total no. of villagers positive for 1 or more helminth species.

<sup>e</sup>Significantly higher ( $P=0.02$ ) in men than in women as analyzed by the z-test.

nam, and Lao PDR, *O. viverrini*, *C. sinensis*, and MIF which include *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchis yokogawai*, *Prosthodendrium molenkampii*, and *Phaneropsolus bonnei*, may be responsible for this type of eggs [10-13]. In Lao PDR and Thailand, mixed infections with *O. viverrini* and MIF are a common finding [10-13]. However, the relative prevalence of each fluke species varies by locality. For example, in Lao PDR, *O. viverrini* was the predominant species in Vientiane Municipality, whereas *H. taichui* predominated in Saravane Province [10]. In a mountainous area of Phongsaly Province, Lao PDR, no *O. viverrini* worms were recovered from 10 villagers examined, while many *H. taichui* and *H. yokogawai* worms were recovered [13]. Therefore, in Cambodia, presence of human infections with MIF, including *Haplorchis* spp. and leicthodendriids (*P. molenkampii* and *P. bonnei*), here in Takeo Province or elsewhere, cannot be completely ruled out.

Data on fish intermediate hosts supporting the endemicity of *O. viverrini* in Takeo Province have been reported. During 2007-2008, *O. viverrini* metacercariae were detected in various species of freshwater fish, including *Hampala dispar* and *Puntius brevis*, caught in the vicinity of Lake 500, a water reservoir located near the border of Takeo and Kandal Provinces [14]. The metacercariae were identified through both cytochrome c oxidase subunit 1 sequencing and confirmation of adult flukes after experimental infection to hamsters [14]. Another fish study

was performed in Kandal Province which borders Takeo Province; here, the metacercariae of *O. viverrini* were identified by detecting the mitochondrial *nad1* gene [15]. In our previous study on fish hosts caught near the Ang Svay Chek villages, we confirmed the presence of *O. viverrini* metacercariae in *Puntiolites proctozysron* [9]. In that study, average 10 metacercariae per fish were collected, and the metacercariae were confirmed by recovery of adult flukes from infected hamsters. Because this fish species is popularly eaten by the villagers, it is suspected as a source of human infections.

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