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Field survey focused on *Opisthorchis viverrini* infection in five provinces of Cambodia

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ABSTRACT

Background: Opisthorchiasis is endemic in Thailand and Lao People's Democratic Republic and constitutes a major public health problem throughout the Mekong Basin. Although Cambodia is located in the Mekong Basin, the status of *O. viverrini* infection in that country was not previously clarified. This research was conducted to document the extent and distribution of *O. viverrini* infection in Cambodia.
Methods: Surveillance was conducted in 55 villages in five Cambodian provinces. Research tools included stool examination using the Kato–Katz thick-smear technique, identification of intermediate hosts, and interviews covering factors related to *O. viverrini* infection. Some larvae and egg-positive stool samples were examined using PCR to detect *O. viverrini* DNA.
Results: A total of 16,082 stool samples from the 55 villages were examined, of which 1232 were egg positive. In 15 villages with egg-positive rates of greater than 10%, eggs were found in 998 of 3585 stool samples, for an egg-positive rate of 27.8%. PCR analysis showed that 30 of 33 samples were positive for *O. viverrini* DNA from five villages in Kampong Cham and Kampong Thom provinces. The first intermediate host *Bithynia siamensis siamensis* was identified in the target areas of Takaev, Kandal, and Kampong Cham provinces. Cercariae were identified morphologically as *O. viverrini* and some were confirmed using PCR. Metacercariae of *O. viverrini* were identified by morphologic observations, animal experiments, or PCR in six species of fish in the target areas.
Discussion and conclusions: Four Cambodian provinces were identified as endemic areas of *O. viverrini* infection. Careful planning is necessary for effective field surveys, because complex environmental factors might be involved in the distribution of *O. viverrini* infection-endemic areas in Cambodia. Many problems remain to be resolved regarding the status of *O. viverrini* infection in Cambodia, and a nationwide baseline survey is necessary.

1. Introduction

Opisthorchis viverrini is one of the most important food-borne liver flukes in Southeast Asia. It is endemic in Thailand and Lao People's Democratic Republic (Lao PDR) and constitutes a major public health problem in the entire Mekong Basin [1–13]. Although Cambodia is located in the Mekong Basin, previous reports only referred to the country as a whole and did not break down infection rates by province or district [1,4–10]. Before the present research started in 2006, seven manuscripts on *O. viverrini* infection were found in the Pub Med/1980–2005 database, and only one published in 2002 was a parasitological survey focusing on Cambodia. A Korean team using an unspecified technique reported that the *Opisthorchis* spp. infection rate was 4.0% in primary schools in Kampong Cham [14]. Thus, *O. viverrini* infection was suspected to be a prevalent yet neglected tropical disease in Cambodia.

When we conducted research in 2005 on anemia in pregnant women in Takaev province, the results suggested that some areas of Cambodia had a high prevalence of *O. viverrini* infection. The National Center for Malaria Control, Parasitology and Entomology (CNM), Ministry of Health (MOH), Cambodia, became interested in cooperating with an epidemiological survey. We prepared the first research plan in February 2006 and began the survey in March. Stool samples were examined using the Kato–Katz technique in two villages in the Prey Kabas district, Takaev province, in the first field research focusing on *O. viverrini* infection in Cambodia, which is continuing. This paper is a summary of the results obtained from March 2006 to August 2012.

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1.1. Purpose of the research

The investigation focused on confirming that *O. viverrini* infection is endemic in several provinces in Cambodia.

2. Materials and methods

2.1. Study areas

This study was conducted in 55 villages in five provinces located in central and south Cambodia from March 2006 to August 2012 (Table 1, Fig. 1). The five provinces are located in the Mekong River basin and tributary areas. The target villages were located near streams, lakes, or ponds formed after flooding in the rainy season. The survey began in Takaev province, where two villages in which high endemic rates of opisthorchiasis were found in March 2006. It continued in Kandal province to the north of Takaev, then moved to adjoining Prey Veng and Kampong Cham provinces, and finally to Kampong Thom bordering on Kampong Cham (Fig. 1).

2.2. Methods

The survey included the collection of stool specimens for examination along with intermediate host surveillance; in addition, interviews were conducted on basic living conditions, and the environmental conditions of villages thought to be related to *O. viverrini* infection were noted.

2.2.1. Stool sample examinations using the Kato–Katz technique

Single stool samples from one man and one woman over 20 years of age were collected from each target family within the surveyed villages each morning and then immediately examined by technicians from the CNM. The Kato–Katz thick-smear technique was used to detect helminth eggs. Initially, all target village households were surveyed, but in 2010 the protocol was changed to quasi-random sampling in order to cover more areas of Kampong Cham and Kampong Thom provinces.

2.2.2. Detection of O. viverrini DNA from stool samples using PCR

After identifying villages in which *O. viverrini* infection was endemic in Kampong Cham and Kampong Thom provinces, five villages that had not yet undergone deworming with praziquantel administration were selected. After the initial Kato–Katz screening, 33 villagers whose egg per gram (EPG) values of *O. viverrini* were high agreed to provide additional samples. Approximately 3 ml of feces was placed in a test tube containing 6 ml of ethanol (70–80% density) and mixed thoroughly [15,16]. Nested PCR was performed at Dokkyo Medical University in Japan with the primers for *O. viverrini* according to the method of Lovis et al. [17].

2.2.3. Surveillance of intermediate hosts

Snails and fish known to be the first and second intermediate hosts of *O. viverrini* were examined in infection-endemic villages to confirm the existence of vectors. Village leaders, residents, and fishermen cooperated to provide information concerning types of fish normally eaten raw and to collect cyprinid fish and *Bithynia* spp. snails from all endemic areas. Those samples came from ponds, small lakes, rice fields, and streams near the villages. All the fish were photographed, and some were fixed in formalin for morphological classification by a specialist. After removal of the intestines, the fish samples were cooled on ice, transferred to the laboratory of the CNM in Phnom Penh or Dokkyo Medical University in Japan, and then examined.

Snail shells were crushed, and the presence of cercariae was determined using a stereoscopic microscope. Cercariae of *O. viverrini* from snails were identified morphologically using a biological microscope. The fins, scales, and/or muscles of fish were observed using a stereoscopic microscope. Muscles were examined with the compressing and/or digestion methods. Suspected metacercariae were collected and identified morphologically using a biological microscope [1]. Some metacercariae were used for experimental infection in hamsters. Fourteen male Syrian hamsters were infected with 10–60 metacercariae by intragastric inoculation. Two to 3 months after infection, the animals were killed, and *O. viverrini* worms were collected from the bile ducts. In addition to morphological identification, PCR was also performed using ethanol-fixed cercariae and metacercariae specimens based on the method of Lovis et al. [17].

2.2.4. Adult worm collection

Adult worms were collected from patients in one Kandal province village that had a high egg-positive rate. Seven patients who had high egg density but no other health problems were chosen to participate after giving informed consent. These patients received a single dose of praziquantel 20 mg/kg, which is half the dose regularly administered by the CNM, early in the morning before breakfast to acquire as many complete adult worms as possible. After 3–4 h, magnesium sulfate ($MgSO_4$) 10–15 g (10 g for patients weighing <50 kg and 15 g for those weighing >50 kg) was administered with as much water as possible under the supervision of a Cambodian physician. After 1–2 h, defecation occurred, and the total feces were collected. One month later, the patients began to receive regular praziquantel treatment from local healthcare workers. The adult worms obtained were fixed

Table 1
Surveillance times and areas.

Time[a]	Province	District	No. of communes	No. of villages	No. of *O. viverrini*-positive villages (>10%)		No. of stool samples examined (positive)	
Mar 2006	Takeo	Prey Kabas	1	2	2	(2)	807	(352)
Nov 2006–Dec 2007	Takeo	Prey Kabas	7	16	2	(0)	10,422	(21)
	Takeo	Angkor Borei	6	12	0	(0)		(0)
Apr–May 2008	Kandal	S'ang	1	2	2	(2)	797	(161)
	Kandal	Preaek Ambel	1	2	2	(0)	615	(11)
Aug–Nov 2010	Kampong Cham	Kampong Cham	1	1	1	(1)	379	(108)
	Kampong Cham	Kang Meas	2	11	10	(5)	1,358	(322)
	Prey Veng	Ba Phnom	1	1	0	(0)	643	(0)
Jun–Jul 2012	Kampong Cham	Kang Meas	1	1	1	(1)	105	(22)
	Kampong Cham	Kampong Siem	1	1	1	(1)	126	(82)
	Kampong Cham	Srei Santhor	2	3	2	(1)	427	(53)
	Kampong Thom	Baray	1	1	1	(1)	183	(89)
	Kampong Thom	Santuk	1	1	1	(1)	104	(11)
	Prey Veng	Ba Phnom	1	1	0	(0)	116	(0)
Total no.	5 provinces	11 districts	27	55	26	(15)	16,082	(1,232)

[a] Intermediate host surveillance was conducted in endemic areas during additional time periods.