BRIEF REPORT

Severe Morbidity Due to *Opisthorchis viverrini* and *Schistosoma mekongi* Infection in Lao People's Democratic Republic

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We assessed morbidity due to *Opisthorchis viverrini* and *Schistosoma mekongi* infections in 243 individuals in Lao People's Democratic Republic. Morbidity was associated with *O. viverrini* infection intensity. Coinfection with *S. mekongi* resulted in excess risk of liver fibrosis and left liver lobe enlargement. The high public health impact of opisthorchiasis warrants control.

Food-borne trematodiasis is of public health importance in Southeast Asia [1]. More than 8 million people are parasitized by the liver fluke *Opisthorchis viverrini*, and an estimated 76 000 individuals have severe clinical manifestation, causing a global burden of 70 000 disability-adjusted life-years [2]. *Opisthorchis viverrini* infection is associated with hepatobiliary morbidity, such as hepatomegaly, jaundice, cholecystitis, cholangitis, and cholangiocarcinoma, a fatal bile duct cancer [3].

In Lao People's Democratic Republic (PDR), >70% of the resident population lives in areas where the prevalence of *O. viverrini* exceeds 5%, and almost 2 million people are infected [4]. The highest prevalence rates occur in central-southern Lao PDR [5]. However, detailed information on intestinal and hepatobiliary morbidity due to *O. viverrini* infection is lacking. Furthermore, the effect of coinfection with the blood fluke *Schistosoma mekongi*, endemic in the southern Champasack province [6], has never been assessed.

Opisthorchis viverrini is acquired by consumption of insufficiently cooked fish dishes. The adult parasite lives and reproduces in bile ducts. In contrast, *S. mekongi* cercariae penetrate human skin when exposed to infested water. The adult parasite reproduces in the portal vein. Both parasites lead to hepatobiliary pathologies. The most widely used diagnostic approach is the detection of parasite eggs in stool. The objective of this study was to assess the severity of morbidity associated with *O. viverrini* and concurrent *S. mekongi* infection in adults in Lao PDR.

METHODS

Ethics Statement

The study was approved by the Ethics Committee of Basel, Switzerland, and the National Ethics Committee, Vientiane, Lao PDR. Written informed consent was obtained from all participants. Head of households signed for minors (aged <18 years). People infected with *O. viverrini* and/or *S. mekongi* were treated with praziquantel (40 mg/kg, single oral dose). Soil-transmitted helminth infections were treated with albendazole (400 mg, single oral dose) [7]. Antispasmodic treatment and oral rehydration were provided to purged patients with abdominal pain and diarrhea.

Field and Laboratory Procedures

Cross-sectional surveys were carried out in hospitals and villages. Hospital investigations were done at the infectious disease ward of Mahosot (largest hospital in the capital city of Vientiane) and Savannakhet provincial hospital (referral hospital for central provinces of Lao PDR) in September–October 2005. Village surveys were conducted in *O. viverrini*–endemic areas in June 2006 (Champhone district, Savannakhet province; and Saravane district, Saravane province), in *O. viverrini*–*S. mekongi* coendemic areas (Mounlapamok and Khong districts, Champasack province), and in an area where neither fluke infection is present (Paksong district, Champasack province) between March and May 2006.

Hospital patients and villagers (aged ≥15 years) who submitted a stool sample for parasitological examination, which revealed the presence of *O. viverrini*–like eggs, were selected and invited to participate in the study. Three stool specimens collected over a period of 5 days were subjected to the

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Kato-Katz method (single 41.7-mg-thick smear per specimen) [8]. Helminth eggs were enumerated under a microscope and recorded for each species separately.

The number of *O. viverrini* flukes was quantified by purgation. Study participants were treated with praziquantel (40 mg/kg) after dinner. Albendazole (400 mg) was added if the patient was coinfected with soil-transmitted helminths. The following morning a purgative (45 mL of monosodium-sulphate solution, Swiff, Berlin Pharmaceutical Industry Co, Ltd, Berlin, Germany) was administered. All stools produced within 24 hours (usually 6–8 bowel movements) were collected and quantitatively examined for adult *O. viverrini* worms (length 5.5–9.5 mm, width 0.7–1.6 mm).

Morbidity data were obtained from each participant by interview (ie, right upper quadrant [RUQ] pain and abdominal discomfort [ADC]) and by physical examination (ie, hepatomegaly, jaundice, splenomegaly, and RUQ pain at palpation), conducted by a general physician. Examining the level of left liver lobe (hepatomegaly) and of spleen was done in the standing position from xiphoid to liver or spleen edge. Additionally, an experienced radiologist performed an abdominal ultrasonography in all enrolled persons to assess left liver lobe enlargement measuring from cranial to the caudal margin, destruction surrounding intrahepatic bile duct (periductal fibrosis), common bile duct dilatation (CBD), and intrahepatic bile duct dilatation (IHBD). The radiologist was unaware of stool examination results.

Statistical Analysis

Data were double-entered in EpiData (www.epidata.ch) and analyzed using Stata software (version 10.1). Data were summarized using proportions (categorical variables) and arithmetic mean (worm counts). Negative binomial regression was used to calculate the intensity rate ratio (IRR) and to associate adult worms counts with the clinical indicators (presence or absence). Multivariate logistic regressions were used to compare clinical outcomes of noninfected individuals with categories of infected individuals. Left liver lobe size was classified into normal (size $\leq$2 SD) and enlarged (size >2 SD) using height-adjusted measures from a Chinese reference population [9]. A P value of <.05 was considered statistically significant.

RESULTS

Two hundred forty-three individuals were enrolled: 15 from hospitals, 80 from *O. viverrini*–endemic areas, 49 from *O. viverrini*–*S. mekongi* coendemic areas, and 99 from an area where neither parasite is endemic (uninfected). One hundred twenty-three participants (50.6%) had an *O. viverrini* infection, whereas 21 individuals (8.6%) were concurrently infected with *O. viverrini* and *S. mekongi*. All individuals with *S. mekongi* infections were restricted to the Kong and Mounlapamok districts, and they were all coinfected with *O. viverrini*. There were 117 females (48.2%); median age was 38 years (range, 15–81 years).

Most participants reported ADC (65.8%), whereas one-third reported RUQ pain (13.6%). Upon physical examination, jaundice was diagnosed in 9 patients (3.7%). Ultrasonography revealed CBD (4.1%), any liver fibrosis (10.7%), and gallbladder stones (7.3%). IHBD was diagnosed in 3 patients (1.2%).

Opisthorchis viverrini infection intensity was significantly higher in males than in females (IRR, 1.9; 95% confidence interval [CI], 1.1–3.5) and increased with age (IRR, 1.2; 95% CI, 1.1–1.5). Positive associations were observed between morbidity and *O. viverrini* infection intensity, the latter quantified by the number of adult *O. viverrini* worms after purging (Table 1). Patients with RUQ pain (IRR, 2.6; 95% CI, 1.1–6.3), ADC (IRR, 2.7; 95% CI, 1.4–5.2), and jaundice (IRR, 2.3; 95% CI, 1.1–5.9) had an almost 3 times higher *O. viverrini* worm burden than those without. Hepatobiliary pathology was positively associated with *O. viverrini* infection intensity. The strongest association was found in patients with IHBD (IRR, 12.9; 95% CI, 5.2–20.2).

Association between morbidity and *O. viverrini* infection (infected vs noninfected) and coinfection with *S. mekongi* showed strong associations but distinctly different patterns. Whereas patients with a single *O. viverrini* infection had a highly increased risk for RUQ pain (odds ratio [OR], 3.6; 95% CI, 1.5–8.3) and ADC (OR, 6.3; 95% CI, 3.4–11.5) compared with their noninfected counterparts, patients coinfected with *O. viverrini* and *S. mekongi* had an increased risk for liver pathology. Compared with the uninfected control group, they had an almost 12-fold (OR, 11.7; 95% CI, 4.3–31.7) and 14-fold (OR, 13.6; 95% CI, 3.9–47.7) higher risk for liver fibrosis and left liver lobe enlargement, respectively.

DISCUSSION

This report highlights the severe intestinal and hepatobiliary morbidity that may be associated with *O. viverrini* and *S. mekongi* coinfection. We investigated patients from different settings of central and southern Lao PDR and found severe morbidity associated with *O. viverrini* single infection and with *O. viverrini*–*S. mekongi* coinfection. Strikingly, the *O. viverrini* worm burden, as determined after purging, is strongly associated with self-reported morbidity and with clinical and ultrasound investigations. Purging is the only direct method of worm burden assessment, but this procedure is rarely performed [10]. Hence, this investigation provides new evidence that *O. viverrini* infection causes substantial morbidity in Lao

Table 1. Prevalence and Association of Morbidity With *Opisthorchis viverrini* Infection Intensity (Mean Worm Burden) and Infection Status (*O. viverrini* Infection and Coinfection Between *O. viverrini* and *Schistosoma mekongi*) Among Study Participants (N = 243)

		Intensity of Infection			Association			
		No. of Adult *O. viverrini* Flukes in Stool (N = 243)			No. Infection (n = 99)	*O. viverrini* Infection (n = 123)	*O. viverrini*–*S. mekongi* Coinfection (n = 21)	
Indicator Measurements	Prevalence (N = 243) % (No.)	Mean	IRR (95% CI)	*P* Value	OR (95% CI)	OR (95% CI)	OR (95% CI)	*P* Value
Self-report morbidity								
Right upper quadrant pain								
No	86.4 (210)	87	1.0		1.0	1.0	1.0	
Yes	13.6 (33)	225	2.6 (1.1–6.3)	.022	0.2 (.1–.6)	3.6 (1.5–8.3)	1.1 (.3–3.8)	.002
Abdominal discomfort								
No	34.2 (83)	50	1.0		1.0	1.0	1.0	
Yes	65.8 (160)	135	2.7 (1.4–5.2)	.004	0.2 (.1–.4)	6.3 (3.4–11.5)	0.5 (.2–1.3)	<.001
Physical examination								
Jaundice								
No	96.3 (234)	103	1.0					
Yes	3.7 (9)	235	2.3 (1.1–5.9)	.011	NA	NA	NA	NA
Ultrasound examination								
Intrahepatic bile duct dilation								
No	98.8 (240)	92	1.0					
Yes	1.2 (3)	1188	12.9 (5.2–20.2)	.003	NA	NA	NA	NA
Common bile duct dilation								
No	95.9 (233)	94	1.0					
Yes	4.1 (10)	383	4.1 (1.1–19.1)	.026	NA	NA	NA	NA
Periductal liver fibrosis								
No	89.3 (217)	87			1.0	1.0	1.0	
Yes	10.7 (26)	265	3.1 (1.1–8.2)	.012	0.2 (.1–.6)	0.9 (.4–2.2)	11.7 (4.3–31.7)	<.001
Size of left liver lobe								
Normal ≤2 SD	53.5 (130)	140	1.0		1.0	1.00	1.0	
Enlarged >2 SD	46.5 (113)	67	0.5 (.3–.9)	.023	0.3 (.1–.5)	1.7 (.9–2.8)	13.6 (3.9–47.7)	<.001

P values were obtained from the likelihood ratio test.

Abbreviations: CI, confidence interval; IRR, intensity rate ratio calculated from negative binomial regression model; NA, not applicable; OR, odds ratio obtained from logistic regression model; SD, standard deviation.

PDR. The widespread distribution of this liver fluke in Lao PDR and in northeastern Thailand and adjacent countries of Southeast Asia, and the high morbidity it causes, calls for concerted action [3, 5, 11, 12].

Ultrasonographic examinations revealed that individuals with concurrent infections with *O. viverrini* and *S. mekongi* were frequently diagnosed with liver fibrosis and hepatomegaly. However, the liver fibrosis frequency reported (10.7%) was considerably lower than in the endemic area in northeast Thailand (23.6%) [13]. *Schistosoma mekongi* can cause liver (eg, advanced liver fibrosis and portal hypertension) and spleen morbidity [6]. The current study included 1 group of patients coinfected with *S. mekongi* and *O. viverrini*. Our findings suggest that concurrent infection with both flukes aggravates morbidity, compared with single infection, supporting earlier reports suggesting that multiple helminth infections, even at low-intensity levels, may elicit important morbidity [14].

In future research, it will be crucial to compare the current findings with a patient group infected with *S. mekongi* alone. Other infection factors related to hepatic morbidity, such as viral hepatitis, might have contributed to morbidity, and this needs further scientific inquiry.

We adhered to a rigorous diagnostic approach. Study participants had multiple stool samples examined with the quantitative Kato-Katz technique, supplemented with purging to determine the worm burden. Usually, the number of eggs found in the stools is taken as proxy for the number of adult parasites. Purging allows direct counting of adult *O. viverrini* worms and enables differential diagnosis with intestinal fluke infections, also prevalent in Lao PDR. We showed that

substantial hepatobiliary pathologies are induced by *O. viverrini* infection in Lao PDR; it is therefore conceivable that morbidity attributable to *O. viverrini* is currently underestimated [2]. Community-based investigations, including the assessment of precursors of cholangiocarcinoma, are warranted. In particular, *S. mekongi*–induced morbidity is of importance and needs urgent attention and public health action.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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