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Subtle to severe hepatobiliary morbidity in *Opisthorchis viverrini* endemic settings in Southern Laos

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ABSTRACT

Evidence of severe hepatobiliary morbidity associated with *Opisthorchis viverrini* liver fluke infection including cholangiocarcinoma (CCA) is scarce in Laos although *O. viverrini* infection is highly prevalent. We assessed hepatobiliary morbidity using abdominal ultrasonography (US) in *O. viverrini* adult patients in Saravan province, Southern Laos. A random sample of 431 *O. viverrini* patients from 10 villages underwent abdominal US. Mild, moderate and markedly advanced periductal fibrosis was diagnosed in 7.0%, 66.5%, and 17.0% of patients, respectively. Normal liver parenchyma was seen in only 9.5% of patients. Presence of gall stones (13.2%), sludge (1.4%), gall wall thickening (1.2%), bile duct dilatation (1.6%), fatty liver (12.0%), kidney stones (8.6%) and cysts (7.9%) were diagnosed in considerable frequencies. In five patients (1.2%) hepatobiliary lesions suggesting CCA were diagnosed. Tumour markers, i.e. Interleukin-6, plasminogen activator inhibitor and carbohydrate antigen 19-9 were within normal range. The number of CCA suspected liver masses and hepatobiliary morbidity diagnosed among clinically asymptomatic adult patients in *O. viverrini* endemic area presents a major public health concern in Laos. However, definitive diagnosis of *Opisthorchis*-related severe sequelae including CCA is urgently needed to gauge the burden of this deadly disease in Laos.

1. Introduction

In Lao People's Democratic Republic (Lao RDP, Laos), information on morbidity due to *Opisthorchis viverrini* infection is scarce and absent for cholangiocarcinoma (CCA), a bile duct cancer associated with chronic *O. viverrini* infection. A recent study documented morbidity associated with liver flukes *O. viverrini* and *Schistosoma mekongi* infections in southern Laos (Sayasone et al., 2012). However, CCA cases and precursor lesions were not assessed. Infection prevalences of *O. viverrini* in southern Laos (Forrer et al., 2012; Phongluxa et al., 2013; Rim et al., 2003; Sayasone et al., 2007, 2011) suggest that hepatobiliary morbidity and CCA incidence in Laos are at least as high as or higher than in northeast Thailand, where similar *O. viverrini* infection prevalences are present.

CCA is a rare bile-duct cancer with a poor prognosis. Chronic *O. viverrini* liver fluke infection is a major risk factor for CCA (Sripa et al., 2012; Sithithaworn et al., 2014). *O. viverrini* has been classified as a carcinogenic agent (Bouvard et al., 2009). The highest CCA incidence worldwide is recorded in *O. viverrini* endemic areas in northeast Thailand (Sripa et al., 2011) where on average 119 CCA cases per 100,000 persons occur each year among adults aged

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35–64 (Blechacz and Gores, 2008) and approximately 5000 cases are diagnosed annually (Sripa et al., 2007). Medical and wage costs associated with CCA and *O. viverrini* fluke infection amount to an estimated USD 120 million in Thailand alone (Sripa, 2008).

Opisthorchiasis is a fish-borne trematode infection belonging to the group of tropical infections known as neglected tropical diseases (NTDs) (Keiser and Utzinger, 2009). Almost 67 million people are at risk of infection. An estimated 10 million infected persons live in Northeast Thailand and Laos. One third of the 5.5 million inhabitants of Laos are infected (Sithithaworn et al., 2012). In Laos, *O. viverrini* infections occur in all provinces but the highest prevalences are seen in the Central and Southern provinces, with rates reaching up to 90% of the population (Forrer et al., 2012; Sayasone et al., 2009, 2011). Although infection rates are very high, few studies have examined morbidity associated with the infection. Public health control activities are largely lacking.

We assessed hepatobiliary morbidity including lesions suggestive of CCA in infected adults in rural *O. viverrini* endemic communities of Southern Laos.

2. Population, materials and methods

2.1. Ethical considerations

Ethical approval was granted by the Lao National Ethics Committee for Health Research (NECHR, no. 278/NECHR) and by the Ethical Review Group of the World Health Organization-Western Pacific Region in Manila, Philippines to investigate hepatobiliary morbidity.

The study's objectives, procedures and potential risks and benefits were explained to village authorities and to all participating villagers in Lao and ethnic group languages. Informed written consent was obtained prior to enrollment. Patients were informed of any diagnosed infection and of ultrasonography (US) results and referred for treatment according to standard health care procedures of the Lao Ministry of Health (MOH, 2004). All patients with lesions suggestive of CCA were given additional counselling. A free follow-up investigation was proposed. All persons found to be stool positive for *O. viverrini* were treated with praziquantel (40 mg/kg, single oral dose).

2.2. Study area and population

From January to April 2011, a cross-sectional study was carried out in adults (aged ≥ 20 years) in 10 *O. viverrini* endemic villages in Saravan district, Saravan province, southern Laos. Approximately 350,000 inhabitants live in the province (9 districts, 168 villages). Parasitological studies in the Saravan district documented a high *O. viverrini* infection prevalence (>50%) in the general population (Sayasone et al., 2007). In addition, in-depth parasitological investigations, including demonstration of adult food-borne trematodes in human stool showed that almost all *O. viverrini* infected individuals were co-infected with minute intestinal flukes (MIF), in particular *Haplorchis taichui* (Sayasone et al., 2009).

For this study, 840 participants from randomly selected households were screened for *O. viverrini* infection (Fig. 1). Of these 85.0% had an *O. viverrini* infection. Abdominal US examination was performed in adults aged 20 years and older (431, 51.3%).

2.3. Laboratory analysis of stool and blood

Stool examinations followed a standard procedure. In brief, each participant provided two fresh stool samples on consecutive days. Stool containers were transferred to the laboratory the same morning. From each sample, two Kato-Katz thick smears were prepared using standard 41.7 mg templates and examined under a light microscope (100× magnification) (Katz et al., 1972). The number of *O. viverrini* eggs per slide was recorded. Slides were read within 30–45 min after preparation. Ten per cent of all smears were re-examined for quality control (Soukhathammavong et al., 2011). An *O. viverrini*-positive patient was defined as one with at least one *O. viverrini* egg present in at least one of the four Kato-Katz faecal thick smears.

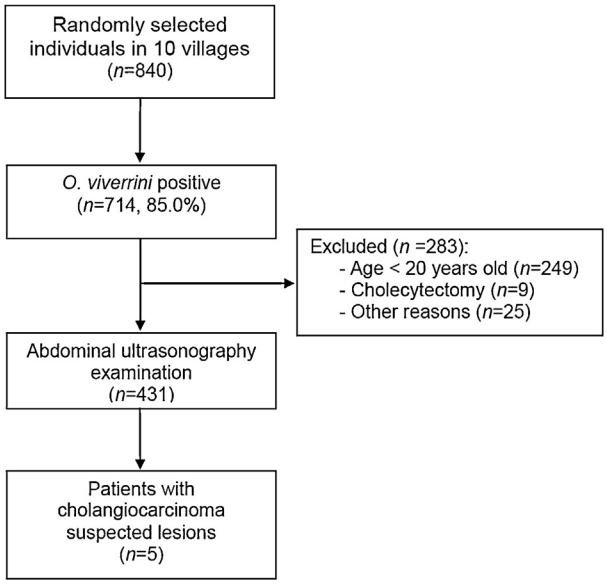

Fig. 1. Study flowchart: study participants in 10 villages, Saravan district, Saravan province, Laos.

From patients with CCA suspected lesions, a 30 ml venous blood sample was drawn. Tumour markers, i.e. carbohydrate antigen 19-9 (CA 19-9), Interleukin-6 (IL-6) and plasminogen activator inhibitor (PAI) were assessed in Khon Kaen University's reference laboratory (Sripa et al., 2009).

For each patient, demographic data were recorded (i.e. sex, age, place of residence, contact details and occupation, number of years of school attendance) and information on abdominal symptoms, raw fish consumption and the presence and utilization of latrine was obtained.

2.4. Assessment of hepatobiliary morbidity

Abdominal US examinations were performed in the study village using a portable US machine (SSD-500, Aloka, Tokyo, Japan) with a 3.5 MHz convex abdominal transducer. Patients were asked to fast 8 h before US examination. Liver parenchyma fibrosis was assessed using an adapted examination protocol from Niamey (Niamey Working Group, 2000) in combination with the standard protocols used earlier in community-based studies in Khon Kaen, Thailand (Mairiang et al., 2012). Liver parenchyma patterns were graded as normal or no echoes (=0), starry sky (=1+), rings and pipe stems (=2+), or highly echogenic 'patches' extending to peripheral areas (=3+). Patients were grouped into those with "none or mild advanced periportal fibrosis" and "advanced fibrosis" according to the US grade (equal ≤1 *versus* ≥2). Gallbladder was examined before and 30 min after consumption of a fatty meal (a sterilised milk of 250 mL and two boiled eggs).

US examiners (PAS, VR) were blinded to the laboratory results. Images were recorded on a DVD (Sony DVD recorder RDR-HX780) by senior radiologists (Oroth Rasphon, Mahosot Hospital; Dr. Eimorn Mairian, Khon Kaen University, Khon Kaen) for quality control.