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Opisthorchis viverrini infections and associated risk factors in a lowland area of Binh Dinh Province, Central Vietnam

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ABSTRACT

Opisthorchiasis caused by *Opisthorchis viverrini* is a major public health problem in the Mekong Basin in South East Asia. It is associated with cholangiocarcinoma, a fatal cancer of the bile duct, which is very common in some areas of Thailand and Lao PDR. Although there is evidence of opisthorchiasis in the central and Southern provinces of Vietnam, data are scarce and Vietnam is often not considered an opisthorchiasis endemic area in the international literature. We conducted a cross-sectional survey in June 2015 in a lowland rural area of Binh Dinh Province in Central Vietnam to investigate the apparent prevalence of *O. viverrini* infection in the population and the associated risk factors. A total of 254 stool samples were collected and examined by the Kato Katz method. Consenting people shedding *Opisthorchis*-like eggs with their stools were treated with praziquantel and $MgSO_4$ and adult worms were collected from stools for morphological and molecular identifications. Risk factors were studied with a structured questionnaire and the association with infection was evaluated by univariate and multivariate Firth's logistic regression analysis. The apparent prevalence in the investigated population determined by stool examination was 11.4% (CI: 8–16%). Infection with *O. viverrini* was confirmed in all 11 individuals consenting to receive praziquantel treatment and subsequent worm recovery from stools. The mean number of worms recovered after treatment/purgation was 14.5 (range 2–44). Male gender and the consumption of dishes prepared from raw small wild-caught freshwater fish (*Carassius auratus*) were found to be significant risk factors associated with opisthorchiasis in the area. These findings confirm the presence of *O. viverrini* infection in Central Vietnam related to the consumption of raw fish dishes. Awareness campaigns and control programs should be implemented in the region to combat this potentially fatal fluke infection.

1. Introduction

Opisthorchis viverrini, a trematode belonging to the family Opisthorchidae causes opisthorchiasis, a potentially fatal fish-borne zoonotic infection in humans and carnivores in the Mekong Basin in the South East Asian region (Andrews et al., 2008; Sithithaworn et al., 2012). More than 8 million people are infected with this parasite in Thailand, mainly in the northeastern region; and 98% of Lao PDR's population is at risk of infection because of the habit of eating raw fish dishes (Sripa et al., 2007, 2011; World Health Organization, 2008). A strong association was found between opisthorchiasis and cholangiocarcinoma (CCA), or bile duct cancer. In Thailand it was found that the reported number of liver cancer cases in men in the opisthorchiasis endemic areas was 20 times higher than in non-endemic areas (World Health Organization, 2011; Sripa et al., 2012). In that country, economic losses resulting from this disease account for 120 million USD annually (Andrews et al., 2008).

While *O. viverrini* and opisthorchiasis are well documented in Thailand (Kaewpitoon et al., 2012; Chaiputcha et al., 2015; Chudthaisong et al., 2015), Lao PDR (Sripa et al., 2011; Forrer et al., 2012) and Cambodia (Tai-Soon et al., 2012), which are considered *O. viverrini* endemic areas in the international literature, Vietnam

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is usually not mentioned as an endemic area in the region because "no data are available" (Andrews et al., 2008). More attention in this country has gone to the related *Clonorchis sinensis* that is endemic in the northern part and has a very similar lifecycle and epidemiology, and to fish-borne intestinal flukes (Do et al., 2007; De and Le, 2012; Nguyen et al., 2015). However, there are some local reports on the presence of *O. viverrini* infections in Central Vietnam (Nguyen et al., 2009; http://www.impe-qn.org.vn/impe-qn/vn/portal/InfoDetail.jsp?area=58&cat=1068&ID=724). A lack of knowledge on *O. viverrini* infection in Vietnam may hinder prevention and control programs of this fluke. Therefore, a cross-sectional survey was conducted in My Tho commune, in the lowland area of Binh Dinh Province, in Central Vietnam where the local population has maintained the culinary habit of eating raw fish dishes. The objective of this study was to investigate the prevalence and associated risk factors of *O. viverrini* infections, aiming at confirming the opisthorchiasis endemic status in this part of Vietnam.

2. Materials and methods

2.1. Study area

Binh Dinh Province is located in the South central coast region of Vietnam and is composed of 11 districts. The province is divided into the highland region along the western border, and lowlands in the center and along the coast (Fig. 1). Most of the population of the province lives near the coast. The annual average temperature and rainfall are 26 °C and 1935 mm, respectively, with a dry season from January to August, and a rainy season from September to December, with mostly serious flooding in December as a result of tropical storms. People in the province mainly live on agriculture, including rice cultivation, raising of livestock and poultry, and fish production by sea fishing and aquaculture. Fresh water in the province mainly comes from four big rivers including: the Kon River, Lai Giang River, Ha Thanh River and Latinh River, of which three rivers support fresh water for the lowlands. In addition, 49 artificial freshwater reservoirs support freshwater requirements during the long dry season in the province.

Three villages, Chanh Trach 1 (14° 13′ 46″ N, 109° 10′ 25″ E), Chanh Trach 2 (14° 13′ 44″ N, 109° 10′ 39″ E) and Chanh Truc (14° 13′ 06″ N, 109° 10′ 07″ E) of My Tho Commune (14° 13′ 23″ N, 109° 9′ 16″ E), a typical lowland area of Binh Dinh Province, were included in the study. These villages are located northeast of the Freshwater Reservoir in the basins of the Latinh River. These villages were selected based on, (i) previous records on *O. viverrini* infections diagnosed at the IMPE Quy Nhon hospital; (ii) representativeness of typical Central Vietnamese lowland villages characterised by the proximity of irrigation canals from which freshwater fish is caught; (iii) maintenance of the tradition of eating raw fish dishes, which is typical for rural areas in lowland Central Vietnamese provinces (ranging from Quang Tri to Nha Trang and Phan Rang Provinces).

A cross-sectional survey was conducted in June 2015 to investigate the *O. viverrini* infection status in the area and to determine the associated risk factors of the disease.

2.2. Sample size and sampling

The sample size was calculated based on an expected prevalence of *O. viverrini* infection of 8% (Nguyen et al., 2009), a confidence level of 95%, a desired absolute precision (d) of 0.05 and using the following formula: $n = p(1-p)\ 1.96^2/d^2$ (Thrusfield, 2005). Since individuals have similar raw fish consumption habits within villages, a correction factor of magnitude 2 (Wagner and Salman, 2004) was applied to account for the clustering of individuals within villages. In addition, contingencies were adjusted for by adding another 15% of individuals leading to a total of 254 persons to be faecal sampled. A questionnaire was administered to all individuals to determine the potential risk factors associated with the disease.

The sampling frame was the list of all administrated households in the 3 villages (total: n = 1015): Chanh Trach 1, 165 households, Chanh Trach 2, 239 households, and Chanh Truc, 611 households; from which 254 households were randomly selected by the proportionate stratified sampling of households in each village. One randomly selected member of each household, older than 7 years of age and with the ability to understand and respond to the study requirements was invited to participate in the study. Each study participant provided one stool sample for coprological examination.

A questionnaire was then administered to the selected members in each household. Researchers in the study read the questions and invited the participants to answer, and to sign the form upon completion of the questionnaire. The questionnaire addressed the following points: name, address, gender (male/female), age (<18 years/18–60years/>60 years), occupation (agriculture/others), knowledge of *O. viverrini* (know *O. viverrini*: yes/no; know *O. viverrini* transmission: yes/no; know *O. viverrini* harmfulness to human health: yes/no), eating raw fish (yes/no), how often (usually/sometimes/rarely/never), which type of fish prepared for raw fish dish (small wild caught fresh water fish/sea fish/both), where the fish dish was consumed (at home/restaurant/relative & friend home/both), history of examination and treatment for *O. viverrini* infection (examination or dewormed before: yes/no). All collected information was used for the evaluation of risk factors associated with *O. viverrini* infection.

2.3. Stool examination

Stool samples were examined within 12 h after collection by the Kato-Katz technique as described by World Health Organization, (1991). In brief, two Kato-Katz smears were prepared from each stool sample. Then, both faecal slides were examined by light microscopy (×100) by the same examiner. Opisthorchis-like eggs were identified and enumerated. The average number of counted eggs from two slides was multiplied by 24 to obtain the number of eggs per gram (EPG) of faeces. Intensity of *O. viverrini* infection was scaled as follows: light infection: EPG < 1000, moderate infection: EPG from 1000–10,000 and heavy infection: EPG > 10,000 (Maleewong et al., 1992). Prevalence of *O. viverrini* infection is considered low (<10%), moderate (10–15%) or high (>15%) (Sithithaworn et al., 2012).

2.4. *Adult* O. viverrini *expulsion*

Eleven persons who were positive for Opisthorchis-like eggs at faecal examination and who were willing to participate in the procedure of worm expulsion were treated under the responsibility of local physicians. The patients were asked to eat a light liquid meal in the evening before treatment. The following morning they were given oral praziquantel 400 mg (Distocide®, Shin Poong Pharmaceutical Co. Ltd., Seoul, Korea) at a dosage of 40 mg/kg; 1 h later, they were given a solution of 30 g of $MgSO_4$ dissolved in 100 ml pure water. Subsequently, 3–4 consecutive post-treatment stools were collected. Worms were recovered by a series of washing steps (Chai et al., 2005; Do et al., 2007). Patients who did not consent to the procedure of adult worm expulsion were given a free praziquantel treatment.