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Potential risk of a liver fluke *Opisthorchis viverrini* infection brought by immigrants from prevalent areas: A case study in the lower Northern Thailand

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ABSTRACT

Considering the long lifespan of the liver fluke *Opisthorchis viverrini*, human mobility from prevalent regions to other neighboring areas has the possibility to disperse carriers and complicate the opisthorchiasis problem. To evaluate this, mass screening of the fluke infection was conducted in nine communities of lower Northern Thailand, combined with a questionnaire survey to distinguish the participant's origin. The liver fluke infection was found in 70 individuals (7.2%) of the examined 971 stool samples from seven communities, with light intensity providing small numbers of eggs in the examined stool. Prevalence in the positive communities varied from 2.1% to 28.7%. As a result of generalized linear mixed models fitting, regional origin and raw-fish eating habits were stably selected as variables affecting the parasite infection while occupation and educational background were secondary ones. Majority of the infected cases (64.3%) were found from the immigrants of northeastern Thailand (the fluke prevalent region), providing 2.28–2.42 times higher infectious risk on average against the local residents. Daily consumption of raw fish averaged a 3.12–3.60 times higher risk compared to those with no raw-fish eating habit. Our findings suggest that people's origin and moving history deserve further attentions in health promotion programs including education for safe eating.

1. Introduction

Animal and human movements are accompanied by disease risks (Wilson 1995; Daszak et al., 2000; Gushulak and MacPherson, 2006). Much empirical evidence is available of emerging infections having been spread to potential candidates due to pathogens translocated from a prevalent area to new sensitive ones (Tourchin et al., 2002; Woolhouse and Gowtage-Sequeria, 2005; Farrer et al., 2011; Warnecke et al., 2012; Lymbery et al., 2014). Human activities, promoting local development and globalization, are unavoidable causes of cultivating these disease problems (Tatem et al., 2006; Lindahl and Grace, 2015), especially in developing countries or regions where the targeted people are still suffering from neglected endemic diseases. Social backgrounds, including mobility, are thus worth considering in epidemiological surveys.

A liver fluke *Opisthorchis viverrini* is an endemic parasite causing opisthorchiasis throughout South-East Asia, mainly in Lao PDR, Cambodia, Vietnam and Thailand (Andrews et al., 2008; Sripa et al., 2010; Sithithaworn et al., 2012). Human infections are induced from consumptions of traditional raw or undercooked dishes (koi pla and pla som) made from cyprinoid fish harboring infective metacercariae as a second intermediate host (Sadun 1955; Upatham et al., 1984; Sithithaworn and Haswell-Elkins, 2003; Sripa et al., 2011; Prasongwatana et al., 2013). Most cases of the infection are asymptomatic with light burden (Elkins et al., 1991; Sithithaworn et al., 1991a,b; Sithithaworn and Haswell-Elkins, 2003; Armignacco et al., 2008), but chronic or heavy cases can lead to hepatobiliary diseases including hepatomegaly, cholangitis, cholecystitis, gallstones and

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cholangiocarcinoma (Sithithaworn and Haswell-Elkins, 2003; Sripa et al., 2007; Sripa et al., 2011).

In Thailand, originally, a near 100% prevalence and high intensity of *O. viverrini* infection has been reported in the Northeast region (Sadun 1955; Upatham et al., 1982; Upatham et al., 1984; Upatham et al., 1985). The nationwide prevalence has subsequently been decreasing as a result of helminthiasis control. Health development project continuously conducted in some high-risk areas is a national policy (Sripa et al., 2015), and has eventually succeeded in decreasing its level to 9.6% in 2001 (Jongsuksuntigul and Imsomboon, 2003). However, human infections are still remaining in the Northeast (16.6% in prevalence), North (10.0%), the Central (1.3%) and the South (0.01%) (Sithithaworn et al., 2012). Therefore, further efforts are being explored (Sithithaworn et al., 2012; Sripa et al., 2015; Sripa et al., 2017).

Considering the long lifespan of this parasite, up to 10 years (Sithithaworn and Haswell-Elkins, 2003), human translocation from prevalent areas to other neighboring sensitive areas and vice versa also deserves further attention, as a potential agent to prevent fluke control (Stauffer et al., 2004). To evaluate the significance of this effect on the infection risk, therefore, we conducted a mass screening of *O. viverrini* infection in the communities of lower Northern Thailand, with combining a questionnaire survey to determine the subjects' origin. Incidentally, the targeted region (i.e. the lower Northern) is included in an important drainage system harboring a specific lineage of *O. viverrini* distributed in the North (Saijuntha et al., 2007; Andrews et al., 2008). With the exception of one report by Radomyos et al. (1998), spatial epidemiological data is less available in this area. That is, the present status in the lower Northern is uncertain compared to those in the other regions, despite the peoples' lives being at risk. In addition, the lower northern area consists of a number of high risk communities where a part of population has migrated from *O. viverrini* highly infected region, the northeastern region of Thailand.

2. Materials and methods

2.1. Ethical approvals

The research was approved by the National Ethics Committees, Naresuan University, Phitsanulok, Thailand (IRB 068/57). All participants were informed about research objective, procedures, potential risks and benefits of the study, and signed the consent form. For the infected participants found, an anthelminthic remedy (i.e. oral administration of praziquantel for 40 mg/kg) was provided.

2.2. Study area

The study area covered lower northern Thailand, consisting nine provinces (Fig. 1). Those provinces are further composed of small districts and communities. For the survey, we selected a representative community consisting of residents who were originally born and raised there and immigrants who had moved from other localities including the northeastern region for more than 10 years.

2.3. Stool sampling and questionnaire survey

During January to October 2014, stool samples were collected from original residents and immigrants (n = 1005) who had been living in the selected communities more than 10 years. The minimum duration of immigrants living in selected communities was 17 years and the maximum was 54 years. For an evaluation of infection status in this study, only those who provided complete responses in the following questionnaire survey were used. Guidance for the participants and sample collection were conducted by research team members and public health staffs at Health Promoting Hospital in each community. All participants submitted their stool samples (10–20 g) in a 30 ml plastic container, with a questionnaire. All samples were kept in an ice box and transported within 1–2 days to the Parasitology Laboratory, Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University for microscopic examination and DNA extraction.

Question contents were demographic information (i.e. 1: gender [male/female] and 2: age [unknown/≤20/21-40/41-60/over 60]), 3: original hometown (local/immigrant/unknown origin), 4: occupation (farmer/other type of jobs), 5: educational background (primary school only/higher background), 6: consumption of raw dishes, koi pla or pla som (yes/no), 7: proper latrine use (yes/no), 8: stool test experience (yes/no) and 9: antihelminthic treated experience (yes/no). The completely answered data was used to evaluate risk factors of the fluke infection.

2.4. Microscopic and molecular examinations

The stool samples were divided for microscopic examination and DNA extraction for PCR analysis. For microscopic examination, two grams of individual stool samples were processed by the quantitative formalin ethyl acetate concentration technique (Elkins et al., 1991). The infection intensity was calculated as egg number counted per gram of feces (EPG) (Maleewong et al., 1992).

For molecular analysis, two grams of stool samples containing Opisthorchis-like eggs were washed with the phosphate-buffered saline (PBS)-ethyl acetate and allowed to sediment (Duenngai et al., 2008). Lysis buffer (400 µl) was added to sediments (200 µl) and underwent three freeze-thaw cycles. DNA was extracted with a QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction. Since other minute intestinal flukes and *O. viverrini* are often co-endemic, purified DNA was amplified by PCR using *O. viverrini* specific primers, OV-6F and OV-6R (Wongratanacheewin et al., 2002). The PCR was carried out in 25 µl of total volume containing PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 105 mM MgCl2), 200 µM of each dNTPs, 1 µM of each primers, 1.5 unit of Taq DNA polymerase and 3 µl of DNA sample. Microscopic examination and DNA extraction were performed at the Parasitology Laboratory, Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Thailand. PCR analysis was done at the National research center for Protozoan diseases, Obihiro, Japan.

2.5. Statistical analysis

Descriptive statistics were calculated to summarize the overall prevalence of *O. viverrini* infection. The prevalence by origins, gender, age groups, occupation, dietary habit, latrine use, stool test experience, and antihelminthic experience, with providing intensity of EPG from faecal egg count, were also calculated.

To elucidate relationships between the infection risk and living conditions, generalized linear mixed models (GLMMs) with logit link function were fitted for the prevalence data in the province where the parasite infection was detected. In this analysis, nine candidate factors were tested as explanatory variables, with community and individual differences considered random effects. The candidate models were ranked by Akaike's information criterion (AIC) (Burnham and Anderson, 2002). The difference in AIC value (ΔAIC) between a constructed model and an optimal model with the lowest AIC value was checked for the model selections; as a rule of thumb, a model with $\Delta AIC < 2$ is substantially supported and received consideration in making data inference (Burnham and Anderson, 2002). In all models with $\Delta AIC < 2$, relative coefficient values of the selected variables were estimated including two standard deviations, i.e. about 95.45% confidence interval (CI). The above analysis was performed by using R 3.1.2 (R Core Team, 2015), with the lme4 package and coefplot package for CI estimation in the selected variables.