Parasitology International 59 (2010) 599–605

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Sero-epidemiological survey of gnathostomiasis in Lao PDR

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ARTICLE INFO

Article history:
Received 9 March 2010
Received in revised form 20 August 2010
Accepted 24 August 2010
Available online 8 September 2010

Keywords:
Sero-prevalence
Gnathostomiasis
Gnathostoma spinigerum
Food-borne nematode
Immunoblot
Lao PDR

ABSTRACT

While human gnathostomiasis cases have been reported sporadically in Lao PDR since 1975, little is known about the disease in this country. We aimed to investigate sero-prevalence of gnathostomiasis and *Gnathostoma* species in Lao PDR. One village each in the north, central and south regions of Lao PDR was selected as the study sites. Overall, 125 (29.8%) of 420 sera from the randomly selected participants were sero-positive by immunoblot technique, with anti-*Gnathostoma* IgG antibody against the 24 kDa fraction. The sero-prevalence was high in the central (47.1%) and south (38.6%), but low (3.6%) in the north. Risk factor analyses revealed that the consumption of raw/undercooked fish was significantly associated with *Gnathostoma* sero-positivity (95% CI 1.05–17.05, $P=0.042$). The sero-positivity significantly increased with the age of the participants. Several fish, swamp eels, and frogs collected from central and southern Lao were infected with *G. spinigerum* advanced 3rd-stage larvae. *Channa limbata* (red-tailed snakehead fish) was identified as a natural second intermediate host of *G. spinigerum*. Eggs of *G. spinigerum* were found in dog feces collected in the south. Gnathostomiasis is endemic in central and southern Laos, so that preventive measures should be introduced for people living in these regions.

1. Introduction

Gnathostomiasis is a parasitic zoonosis, caused by several species of nematodes in the genus *Gnathostoma.* The most common clinical manifestation is intermittent subcutaneous migratory swelling or serpiginous creeping eruption, which may appear anywhere on the body surface. Other organs, such as the liver, eye, lung, genitourinary system, gastrointestinal tract, and central nervous system, may be involved in fatal cases [1,2].

The disease is endemic mainly in Asia [3–5] and Latin America [6,7]. Recently, gnathostomiasis cases have been reported sporadically in European countries among travelers who had visited endemic areas in Asia and Latin America [8,9], and more recently, Africa [10,11]. Nowadays, gnathostomiasis is no longer a local endemic disease but is spreading in non-endemic countries. Therefore, accurate epidemiological data in the presumed endemic areas is important not only for the local people but also for international travelers.

Humans acquire the disease mainly by ingesting raw or insufficiently cooked freshwater fish harboring advanced 3rd-stage larvae (AL3), or the infected meat of animal hosts (amphibians, reptiles, birds, and mammals) [1,2].

In many areas of Laos, raw or improperly cooked fish and raw animal meat are commonly consumed, often as tradition- or culture-related foods. In the neighbouring country, Thailand, where people also consume raw fish as Laotians do, thousands of gnathostomiasis cases have been reported [3–5]. Nevertheless, only a few cases of gnathostomiasis have been reported in Laos since the first record in 1975 [12]. In 1996–1997, two gnathostomiasis cases of Laotian women were reported one in Parkngeum and another one in Xaysetha district, Vientiane Capital; the worms were identified as *G. spinigerum* [Phoumindr N, personal communication]. An epidemiological survey for gnathostomiasis in Paksan District, Borikhamxay Province, revealed a prevalence of 0.66%, diagnosed by signs, symptoms, and eosinophilia >5% [13]. Gnathostomiasis cases among Laotian immigrants have been reported sporadically from the European Union and the United States [9,14,15]. However, a nationwide survey of gnathostomiasis has never been performed, and data for the possible prevalence/risks of gnathostomiasis is not available for Lao PDR. This study aimed to investigate the sero-prevalence of gnathostomiasis in 3 selected villages of Lao PDR using immunoblot assay, and to confirm the potential causative species of infection in animal hosts collected from those villages.

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1383-5769/$ – see front matter
doi:10.1016/j.parint.2010.08.007

2. Materials and methods

2.1. Study design and population

A cross-sectional study was performed in August 2008–September 2009. One village was selected from each of 3 geographical regions of the country, the north, central, and south, based primarily on a preliminary investigation of hospital records of gnathostomiasis/suspected cases (purposive sampling). A village where confirmed or suspected gnathostomiasis cases resided was selected in each region. In the north, no cases were found in the hospital records, so that we examined 27 donated serum samples from Houeixay Hospital, Bokeo Province, and found 3 sero-positive gnathostomiasis cases. They were originating from the same village; hence, this village was selected.

Sample size was calculated using the standard normal distribution formula with 50% sero-prevalence and a 5% level of desired accuracy, with 95% confidence level. The total study population was determined to be 420 from three villages (140 people/ village). Before starting, the villagers were informed of the study objectives and process. The ages of the villagers ranged from 10 to 70 years, and none declared to be pregnant. The participants were randomly selected from the villagers.

This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM 2008-028-01) and the National Ethics Committee for Health Research, Ministry of Health, Lao PDR (No 184/NECHR). All procedures were explained to the participants before starting any medical activities. The consent and assent forms were presented to the participants for signature prior to enrollment. All sero-positive cases were treated with albendazole 400 mg daily for 21 consecutive days [16].

2.2. Study sites

One village from Bokeo, Vientiane capital, and Champasack province was selected in the northern, central and southern parts of the country, respectively. Phibounthong Village, Houeixay District, Bokeo is about 900 km north of Vientiane Capital, and has 601 inhabitants (299 females:302 males). Naxon Village, in Parkngeum District, Vientiane Capital is in the central Laos and has 1676 inhabitants (776 females:900 males). Nongtearnoy Village, in Phonthong District, Champasack Province is about 700 km south of Vientiane Capital; and has 977 inhabitants (510 females:467 males) (Fig. 1).

2.3. Collection of serum samples and interview

Blood samples (3 ml each) were collected from all participants in the 3 villages. The sera were separated and packed in ice and transported to the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, in Bangkok, for serological analysis. The randomly selected participants were interviewed using the questionnaire which was developed and pre-tested. The questions addressed demographic information and the following risk factors: (i) eating raw/undercooked meats, (ii) having cats and/or dogs in the household, (iii) knowledge about gnathostomiasis, (iv) handling raw fish, (v) suffering from migratory swelling/itchy eruption, and (vi) experiencing worms coming out of the skin or a wound.

2.4. Collection of animal hosts

As potential 2nd intermediate hosts, fish were purchased at local markets in each village, and examined using the compression technique to detect *Gnathostoma* larvae [17]. The excreted stools of the definitive hosts, dogs, were collected in each village, and examined in the field by Kato–Katz thick smear [18], to detect *Gnathostoma* eggs.

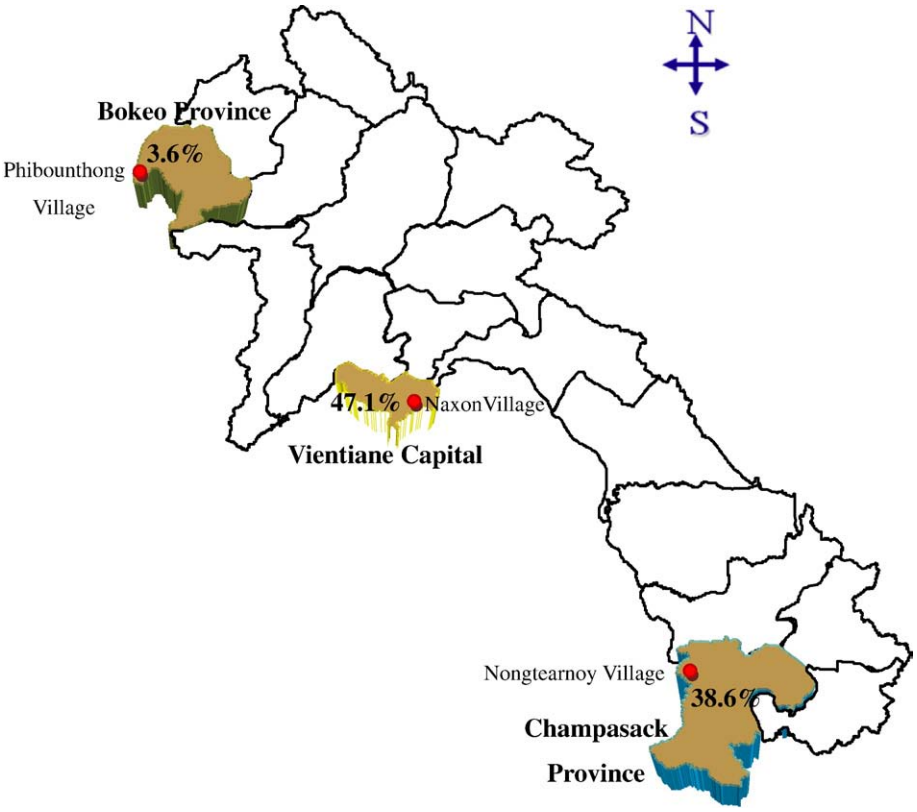

Fig. 1. Sero-prevalence of *Gnathostoma* infection in three studied villages of Lao PDR.