



Trichinella infection in wild boars and synanthropic rats in northwest Vietnam



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ABSTRACT

Trichinellosis is an emerging parasitic zoonosis in North Vietnam. In this survey, hunted and farm-bred wild boars as well as synanthropic rats were sampled in two provinces of northwest Vietnam where outbreaks of trichinellosis have recently occurred. Evidence of *Trichinella* infection was studied by parasitological, serological and molecular methods. The results showed relatively low prevalence of *Trichinella spiralis* in hunted wild boars (2/62 (3.2%; 95% CI: 0.8–4.8)) and rats (23/820 (2.8%; 95% CI: 13.7–32.3)). Parasite burdens in the muscle tissues were between 0.1 and 0.03 larvae/g, and 0.1 and 7 larvae/g in wild boars and rats, respectively. Seroprevalence in farm-bred wild boars was negative. The findings of *Trichinella*-infected rats in 7 of the 20 districts of Dien Bien and Son La provinces suggest that the parasite is circulating in these regions. These results indicate that the local population and health centers should be made aware of the risks of eating raw or undercooked meat dishes prepared from wild animals.

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1. Introduction

In southeast Asia, trichinellosis is a food-borne parasitic zoonosis causing several outbreaks each year, especially in the mountainous regions of Cambodia, southwest China, Lao PDR, north Thailand, and northwest Vietnam (Sayasone et al., 2006; Barennes et al., 2008; Cui et al., 2011; Nguyen et al., 2012). In these areas, the disease is associated with the consumption of traditional raw or undercooked meat dishes, mostly during communal wedding, funeral or New Year celebrations. Pork is the main source of infection, but some outbreaks were caused by consumption of game (Kaewpitoon et al., 2006; Nguyen et al., 2012) and there is concern on the presence of *Trichinella* sp. in dogs, from which the meat is also consumed in the region (Vu Thi

et al., 2013; Srikitjakarn et al., 1981). *Trichinella spiralis* is the most common species associated with human disease in the region, but cases of infection with non-encapsulated species (*T. pseudospiralis*, *Trichinella papuae*) were also described in Thailand (Jongwutiwes et al., 1998; Kusolsuk et al., 2006). The synanthropic life cycle is maintained by the scavenging behavior of free roaming pigs and the feeding of these animals with kitchen leftovers. Data on the occurrence of *Trichinella* sp. in wildlife in the region are scarce.

In Vietnam, reports of trichinellosis outbreaks are a rather recent phenomenon. During 1997–2012, five outbreaks of human trichinellosis were described in mountainous provinces of north Vietnam (Pozio, 2007; Taylor et al., 2009; Nguyen et al., 2012). These outbreaks were characterized by severe clinical signs and a relatively high case-fatality ratio (Nguyen et al., 2012). All but one of these outbreaks could be attributed to the consumption of raw pork dishes; in the last outbreak the probable source was

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wild boar meat. An investigation in a village where an outbreak occurred showed high prevalence of trichinellosis in the pig population (Vu et al., 2010); but also outside outbreak villages in Dien Bien and Son La provinces, serological evidence of *Trichinella* infection was found in pigs and dogs (Vu Thi et al., 2013). The main problems associated with trichinellosis in Vietnam are the limited awareness on the disease and diagnostic capacities of the local health centers. This leads to underreporting, delayed diagnosis and inappropriate treatment.

In order to better inform the local population on the risks of infection, a better knowledge of the local epidemiology is required. This includes information on the synanthropic and sylvatic reservoirs of *Trichinella*. The objectives of the present research were (1) to study the occurrence of *Trichinella* infection in hunted and farm-bred wild boar and in synanthropic rats in Dien Bien and Son La provinces in Northwest Vietnam, by using parasitological and serological methods and (2) to identify the parasite species isolated from these animals by molecular methods.

2. Materials and methods

2.1. Study site

The study was conducted in Dien Bien and Son La provinces located in northwest Vietnam (20°39'–22°33' north latitude, 102°10'–105°20' east longitude (IMH, 2010)), where outbreaks of trichinellosis occurred in the last decade (Pozio, 2007; Taylor et al., 2009; Nguyen et al., 2012) (Fig. 1). The area has a subtropical climate with distinctive rotation of four seasons and an average annual temperature of 21–23 °C (IMH, 2010). These mountainous provinces have a forest cover of 37% of the total land area (SRVN (Socialist Republic of Viet Nam, Government portal), 2013).

2.2. Sampling design

Two samplings were organized in the framework of this study. First, restaurant owners and hunters were asked to collect skeletal muscle samples (diaphragm, masseter, and legs) (Kapel, 2001) from hunted wild boars during 2010–2013. A total of 62 wild boar muscle samples could be collected. Except for the hunting site, which was recorded, other information such as, sex and age data could not be recorded systematically. In addition, 261 serum samples from farm-bred wild boars raised in seven farms located in the same provinces were collected. Sex and age data of these animals were recorded systematically. Second, 820 rats were trapped in rice fields in the vicinity of villages. The traps used were made locally from steel grid, and measure approximately 10 to 30 cm; a bait was put inside to attract the rats. The sample size for each province was based on a conservative 50% estimated prevalence (95% CI, 5% error) (Branscum et al., 2006). In both provinces, care was taken to collect an equal number of rats from each of the 20 districts. The latitude and longitude of the origin of rats were recorded, but the rat species was not determined.

2.3. Magnetic stirrer artificial digestion

Wild boar and rat muscle samples were stored at –20 °C before being subjected to the magnetic stirrer artificial digestion method (Gamble et al., 2000) to determine the presence of *Trichinella* larvae. Individual digestions of 37 ± 15 g skeletal muscles were done for each wild boar. In rats muscle samples of 5 g from 10 animals were pooled before being subjected to digestion. In the case of a positive result, individual muscle samples were retested. The recovered larvae were stored in 90% ethyl alcohol until species identification.

2.4. Molecular identification

DNA was extracted from single larvae according to the guideline of the European Union Reference Laboratory for Parasites (EURLPISS, 2013). Single larvae from International Trichinella Reference Centre (ITRC) isolates *T. spiralis* (code ISS599) and *T. pseudospiralis* (code ISS13) were used as controls.

A multiplex PCR protocol was carried out in a final volume of 30 µl (Zarlenga et al., 1999). A volume of 10 µl crude DNA from a single larva was amplified in 20 µl of 5 units of master mix (Promega code M7505) containing 1 µl Taq polymerase, 25 µl 5 × PCR buffer, 8 µl MgCl₂, 1 µl dNTPs, 61 µl pure water and 4 µl of primers (*I–V* primer sets) (10 pmol/µl of each primer). Amplification consisted of 39 cycles, each consisting of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C. The fragments amplified from purified DNA were separated on a 1.5% agarose gel (Eurogentec, code EP-0010-05) with TAE buffer (Promega, code V4271), stained with syb safe (Invitrogen, code S33102) and visualized under a blue light transilluminator (Safe imager™ 2.0, Invitrogen).

2.5. Enzyme-linked immunosorbent assay

Serum samples from wild boars were tested to detect anti-*Trichinella* IgG by ELISA using excretory/secretory antigens, according to a published previous protocol (Vu Thi et al., 2013). The cut off on each plate was calculated based on the optical densities (OD) of eight negative pig samples using a Student's *t*-test at a probability of $p < 0.001$. Serum samples from experimentally *Trichinella* infected pigs were used as positive controls.

2.6. Data analysis

Exact binomial 95% confidence intervals for proportions were calculated using STATA software package, version 11.0 (Stata Corp., College Station, TX, USA).

3. Results

Trichinella sp. larvae were found in two (3.2%; 95% CI: 0.8–4.8) of the 62 hunted wild boars. These two wild boars had been shot in the same commune, Ta Xua in Bac Yen district, Son La province (Fig. 1). The parasite burdens in the muscle tissues were 0.1 and 0.03 larvae/g, respectively. Five larvae collected from the two infected animals (four

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