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Short communication

High prevalence of anti-*Trichinella* IgG in domestic pigs of the Son La province, Vietnam

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ABSTRACT

Although several outbreaks of *Trichinella* infection among humans have occurred in northwestern Vietnam, no information is available on the circulation of *Trichinella* among domestic pigs. The objective of the present study was to estimate the seroprevalence of anti-*Trichinella* IgG in free-roaming pigs (*Sus scrofa*) in the Son La province of northwestern Vietnam, where a human outbreak of trichinellosis occurred in June 2008. Serum samples were collected from free-roaming pigs of four communes of the Bac Yen district (Son La province) and tested for *Trichinella* antibodies with a commercial ELISA kit using excretory/secretory antigens. Of 1035 pigs from which serum samples were collected, 206 were positive (19.9%). There was a significant difference in the prevalence among communes ($\chi^2 = 22.87$, 3 d.f., $p < 0.0001$). Muscle samples from 76 serologically positive pigs were tested by artificial digestion. *Trichinella* larvae were detected in 11 (14.5%) of them. The larvae were identified by multiplex PCR as *Trichinella spiralis*. This study provides the first data on the circulation of *T. spiralis* in domestic pigs reared in Vietnam, and the results are useful for evaluating the risk of infection for humans. The results indicate that pigs act as a reservoir and play an important role in the maintenance of the domestic cycle of *T. spiralis* in northwestern Vietnam.

1. Introduction

Nematodes of the genus *Trichinella* are zoonotic parasites, which show a cosmopolitan distribution (Pozio et al., 2009). In the past several decades, in many parts of the world *Trichinella* infections have been reported in new hosts, including reptiles, and with new epidemiological patterns (Pozio et al., 2009). Human infection (i.e., trichinellosis) is acquired by consuming raw or improperly cooked meat infected with larvae of *Trichinella* spp. (Dupouy-Camet and Bruschi, 2007; Gottstein et al., 2009). The increased occurrence of trichinellosis can be attributed to changes in consumer habits (e.g., an increased demand for meat), the importation of meat from areas where *Trichinella* infection is endemic, and inadequate veterinary controls, in part due to social upheaval resulting from political and economic changes (Murrell and Pozio, 2000; Gottstein et al., 2009). In Asia, *Trichinella* spp. infection has been documented in humans in 18 countries, in domestic animals (mainly pigs) in 9 countries, and in wildlife in 14 countries (Pozio, 2007). With regard to South East Asia, infection is endemic in both humans and animals in China and in the Lao Popular Democratic Republic (PDR), mainly in the regions bordering Vietnam, and in these two countries trichinellosis is almost exclusively attributable to the consumption of raw

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pork from backyard or outdoor pigs (Wang et al., 2006; Sayasone et al., 2006).

In Vietnam, although trichinellosis is considered to be rare, outbreaks have been reported. The first occurred in 1968 and was attributed to the consumption of pork imported from Lao PDR (Phan, 1997). From 1970 to 2004, three outbreaks occurred, involving 68 persons, with six deaths; these outbreaks occurred in the provinces of Yen Bai and Dien Bien (former Lai Chau province) (both in northwestern Vietnam) and were also attributed to the consumption of pork of unknown origin (Pozio, 2007). In June 2008, an outbreak involving 22 persons, with two deaths, occurred in the commune of Lang Cheu, in the Bac Yen district of the Son La province, which borders the two above-mentioned provinces; the epidemic was attributed to the consumption of a local dish known as "*lap*", which is made with raw pork, in this case from a local pig, and which had been served in a private home (NIMPE, 2008; Taylor et al., 2009). This outbreak showed that once again trichinellosis was re-emerging in Vietnam and suggested that *Trichinella* spp. parasites circulate among pigs in northwestern Vietnam. However, no information is available on the circulation or prevalence of *Trichinella* spp. in domestic pigs or other animals in Vietnam.

The objective of the present work was to estimate the prevalence of *Trichinella* infection in domestic pigs reared in the commune where the 2008 human outbreak occurred and in another three neighbouring communes of the Bac Yen district.

2. Materials and methods

2.1. Study area

The Son La province is located in northwestern Vietnam and has low per capita income in the country (www.sonla.-gov.vn/sonla/Vietnam/VH/DT/index2.htm). The province's Bac Yen district is a highland area (average altitude: 700 m above sea level) located in the northern part of the province; it is characterized by a continental climate, with an average annual temperature of 21 °C (IMH, 2008). In Bac Yen, the human population is characterized by seven ethnic minorities, with the H'Mong ethnic group representing 50% of the total population. This ethnic group lives in 11 of the district's 16 communes, and most people are illiterate (www.sonla.-gov.vn/sonla/Vietnam/VH/DT/index2.htm). In these communes, all pigs are free-roaming or kept in crudely constructed pens. The H'Mong people often consume raw pork (*lap*) (Taylor et al., 2009) (Fig. 1).

2.2. Study design and sampling

The study area consisted of four communes in the Bac Yen district: Lang Cheu (where the 2008 outbreak occurred), Chim Van, Phieng Ban, and Ta Xua (Fig. 1). The survey was conducted on all of the free-roaming pigs in these communes. A blood sample (2–3 ml) and muscle sample (50 g in total of the diaphragm, tongue and masseter) were collected from each pig, from December 9, 2008 to April 30, 2009.

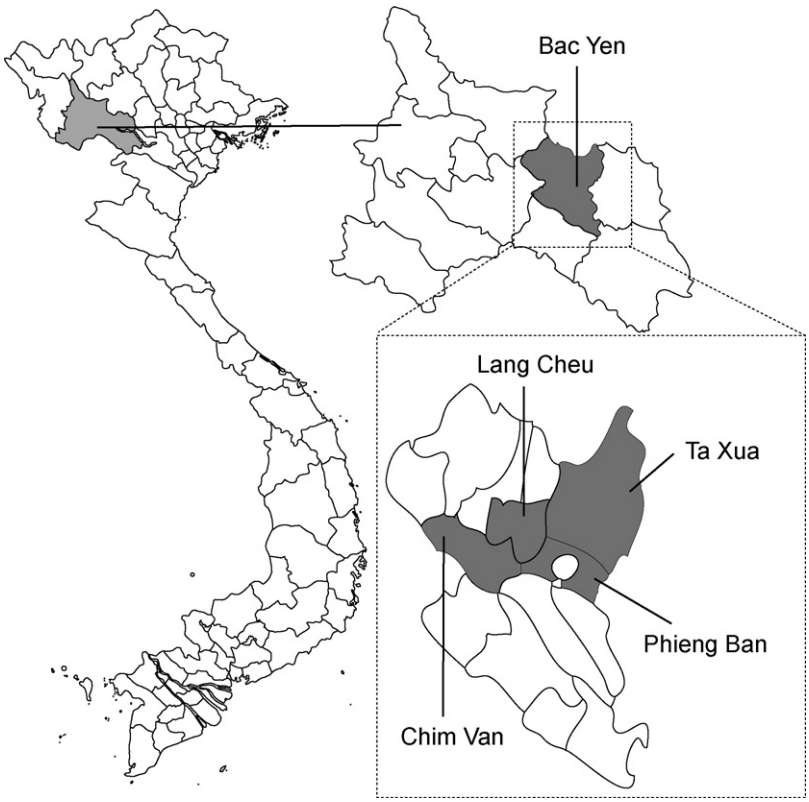

Fig. 1. Map of the communes of pig origin (Chim Van, Lang Cheu, Phieng Ban and Ta Xua) of the Bac Yen district, Son La province, Vietnam.

The blood sample was collected from the heart during evisceration, stored in ice boxes, and immediately transported to the National Centre for Veterinary Diagnosis in Hanoi, where it was centrifuged to separate the serum; the serum samples were stored at −20 °C until analysis. Muscle samples were preserved in ice boxes, sent to Hanoi, stored at 2–8 °C, and tested within 7 days of collection.

2.3. Serodiagnosis

Serum samples were tested at the National Centre for Veterinary Diagnosis with a commercial ELISA kit using excretory/secretory (ES) antigens (PrioCheck® *Trichinella* Ab ELISA, Prionics, Schlieren-Zurich, Switzerland), in accordance with the manufacturer's instructions. Serum samples were tested only at a 1:40 dilution according to the manufacturer's instructions. The microtiter plates were read at 450 nm within 15 min. Positive and negative control sera were those supplied by the manufacturer. The ELISA results were expressed as the ratio sample/positive control (S/P) (%) by comparing the optical density (OD) of the sample to the mean OD of the positive control serum, tested in duplicate. The result was considered to be valid when the mean OD of the positive controls was >1.0, the mean percentage of positivity (PP) of the weak positive controls was >35%, and the mean OD of the negative controls was <0.2. If these criteria were not met, the results were considered to be not valid and the samples were tested again. The cut-off of the ELISA was 15 PP. Serum samples were considered to be positive when the PP was equal to or greater than the cut-off, whereas serum samples were considered to be negative when the PP value was below the cut-off.

2.4. Parasitological diagnosis and parasite identification

For economic reasons, the parasitological diagnosis was performed on a randomly selected sample of 76 pigs that were positive for anti-*Trichinella* IgG. For each of these pigs, 50 g in total of muscle tissue from the diaphragm, tongue and masseter were tested for the presence of *Trichinella* sp. larvae by artificial digestion, in accordance with the Commission Regulation No. 2075/2005 of the European Union (European Commission, 2005).

Trichinella sp. larvae collected after digestion were preserved in 2 ml conical vials with 90% ethanol and forwarded to the Community Reference Laboratory for Parasites, Istituto Superiore di Sanità, Rome, Italy, for the species identification of single larvae by multiplex PCR, in accordance with a previously published protocol (Pozio and La Rosa, 2003).

2.5. Data analysis

The Chi square test was used to analyze the prevalence of infection among pigs, by commune of pig origin, sex and age. A p value of <0.05 was considered to be significant.

3. Results and discussion

Serum and muscle samples were collected from all 1035 free-roaming pigs (41.6% males and 58.4% females). The age distribution of these pigs was as follows: 89 (8.6%) younger than 2 months, 406 (39.2%) between 2 and 8 months, 441 (42.6%) between 9 and 36 months, and 99 (9.6%) older than 36 months. Of the 1035 pigs, 206 (19.9%) were seropositive, and seropositive pigs were found in all four communes. The highest serological prevalence (25.7%) was detected in the Lang Cheu commune (117 of the 456 pigs tested), where the human outbreak of trichinellosis had occurred in 2008. In the other three communes (Chim Van, Ta Xua and Phieng Ban), the seroprevalence was, respectively, 20% (27/135), 16.8% (46/274), and 9.4% (16/170), with significant differences among the communes ($\chi^2 = 22.87$, 3 d.f., $p < 0.0001$).

The seroprevalence increased with the age of the pigs: in particular, it was 1.1%, 1.2%, 32.9% and 55.6% for, respectively, pigs younger than 2 months, between 2 and 8 months, between 9 and 36 months, and older than 36 months. There was no significant difference in the seroprevalence between pigs younger than 2 months and those 2–8 months of age ($p > 0.05$), whereas there was a significant difference between pigs up to 8 months of age and those older than 8 months ($p < 0.0001$). The overall prevalence of infection did not significantly differ by gender ($\chi^2 = 1.92$, 1 d.f., $p = 0.165$).

Muscle samples from the 76 randomly selected seropositive pigs (which ranged in age from 7 months to 10 years) were examined by artificial digestion to confirm infection. The randomly selected pigs were from the communes of Phieng Ban, Chim Van, and Lang Cheu. *Trichinella* larvae (from 2 to 19 larvae) were detected in only 11 (14.5%) animals, which originated from the communes of Chim Van and Lang Cheu. The age of these 11 pigs ranged from 24 to 120 months. All larvae were identified as belonging to *Trichinella spiralis*.

The domestic pig is one of the main reservoirs of *T. spiralis* worldwide, and it was the means through which *T. spiralis* colonized most of the continents, acting as a sort of Trojan horse (Rosenthal et al., 2008), together with the wild boar (*Sus scrofa*), from which the domestic pig originates (Larson et al., 2005). The present study clearly shows for the first time that *T. spiralis* is widespread in northwestern Vietnam and explains the occurrence of human outbreaks of trichinellosis. Northwestern Vietnam is located between northern Lao PDR and southeastern China (Yunnan province), which are two areas with a high prevalence of *T. spiralis* infection in domestic pigs (Barennes et al., 2008; Wang et al., 2006). Furthermore, the H'Mong people, who make up a large part of this area's population, often consume raw pork, and they are unaware of the basic means for preventing the transmission of foodborne diseases.

The results of this study reveal that *T. spiralis* is widespread among free-roaming pigs, with a prevalence ranging from 9.4% to 25.7%. It is likely that the parasite was introduced from Lang Cheu (the commune with the highest prevalence) to the other communes. For the outbreaks of human infection in the Yen Bai province in 1970 and in the Dien Bien province in 2001 and 2004 (both of which border the Son La province), we do not know whether the outbreaks were due to the consumption of local pigs or of illegally imported pigs or pork products from Lao PDR or China.

The overall prevalence of infection among pigs was 19.9% (206/1,035), yet only 11 (14.5%) of the 76 pigs tested had *Trichinella* larvae. There are several plausible reasons for this discrepancy. In particular, the artificial digestion test could be insufficiently sensitive for detecting larvae in muscle tissues with a low worm burden (e.g., <1–3 larvae/g); this is consistent with the very low number of larvae (from 2 to 19) detected in 50 g of positive samples. Furthermore, the specificity of the ELISA kit could be lower than expected, even though ES antigens were used. A recent serological investigation on human serum samples using ELISA and ES antigens revealed a high number of cross-reactions with other human pathogens (Gómez Morales et al., 2008), suggesting that the specificity of the ES antigens may be questionable. Finally, the ELISA kit used in the present study was evaluated with serum samples from European pigs, and it is well known that the immune response can differ by breed (World Organisation for Animal Health, 2008).

Although serologically positive pigs were found for all four age categories, most of them were older than 36 months. This is not surprising, since *Trichinella* infection shows a typical cumulative effect. In fact, sows and boars live longer than fattening pigs and thus have an increased risk of exposure to *Trichinella* infection.

In the Bac Yen district, the hygienic conditions of breeding are very poor, which is one of the major factors in the circulation of parasitic diseases in both humans and pigs. In fact, *Trichinella* infection in pigs is consistently associated with poor hygienic conditions and breeding, in which pigs, particularly free-roaming pigs, are allowed to eat *Trichinella*-infected pork scraps scattered in the environment after slaughtering, to prey on rodents or other animals, and to eat carcasses of dogs and cats, mainly when the pig diet is poor in protein (Liu and Boireau, 2002; Pozio and Murrell, 2006; Sapkotal et al., 2006).

The high number of infected pigs in the investigated area may represent a risk for human infection, and it is possible that the documented outbreaks are only the tip of the iceberg. Actually, in endemic areas with poor sanitary conditions, inhabitants may have difficulties in accessing healthcare facilities. Moreover, trichinellosis can be clinically mild and resemble other diseases, in that it does not have pathognomonic signs or symptoms, and in endemic areas, if people frequently eat *Trichinella*-infected meat, they can develop an asymptomatic form of the disease (Owen et al., 2005). For these reasons, diagnosis should be based on several clinical and laboratory features, as suggested by the algorithm proposed by Dupouy-Camet and Bruschi (2007).

The high serological prevalence among pigs in the Bac Yen district suggests that investigations in neighbouring districts and provinces should be performed, despite the discrepancy between the serological and parasitological results of our study, which should also be investigated. The occurrence of *Trichinella* infection in both pigs and humans indicates that the people living in this area should be made aware of the risk of this disease and be encouraged to adopt adequate livestock-breeding practices.

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References

Barennes, H., Sayasone, S., Odermatt, P., De Bruyne, A., Hongsakhone, S., Newton, P.N., Vongphrachanh, P., Martinez-Aussel, B., Strobel, M., Dupouy-Camet, J., 2008. A major trichinellosis outbreak suggesting a high endemicity of *Trichinella* infection in northern Laos. Am. J. Trop. Med. Hyg. 78, 40–44.

Dupouy-Camet, J., Bruschi, F., 2007. Management and diagnosis of human trichinellosis. In: Dupouy-Camet, J., Murrell, K.D. (Eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis, 1st ed. World Organisation for Animal Health, Paris, France, pp. 37–68.

European Commission, 2005. Specific rules on official controls for *Trichinella* in meat no. 2075/2005. Off. J. Eur. Union 338, 60–82.

Gómez Morales, M.A., Ludovisi, A., Amati, M., Cherchi, S., Pezzotti, P., Pozio, E., 2008. Validation of an ELISA for the diagnosis of human trichinellosis. Clin. Vaccine Immunol. 15, 1723–1729.

Gottstein, B., Pozio, E., Nockler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22, 127–145.

Institute of meteorology and hydrology, 2008. www.imh.ac.vn/a_gioi_thieu?set_language=en&cl=en.

Larson, G., Dobney, K., Albarella, U., Fang, M., Matisoo-Smith, E., Robins, J., Lowden, S., Finlayson, H., Brand, T., Willerslev, E., Rowley-Conwy, P., Andersson, L., Cooper, A., 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307, 1618–1621.

Liu, M., Boireau, P., 2002. Trichinellosis in China: epidemiology and control. Trends Parasitol. 18, 553–556.

Murrell, K.D., Pozio, E., 2000. Trichinellosis: the zoonosis that won't go quietly. Int. J. Parasitol. 30, 1339–1349.

National Institute of Malariology Parasitology and Entomology, 2008. http://nimpe.vn/newsdetail.asp?nld=425.

Owen, I.L., Gomez Morales, M.A., Pezzotti, P., Pozio, E., 2005. *Trichinella* infection in a hunting population of Papua New Guinea suggests an ancient relationship of *Trichinella* with human beings. Trans. R. Trop. Med. Hyg. 99, 618–624.

Phan, L., 1997. Parasite and Veterinary Parasitic Diseases. Hanoi Agriculture Publishing, Hanoi, 260 pp. (in Vietnamese).

Pozio, E., 2007. World distribution of *Trichinella* spp. infections in animals and humans. Vet. Parasitol. 149, 3–21.

Pozio, E., Hoberg, E., La Rosa, G., Zarlenga, D.S., 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infect. Genet. Evol. 9, 606–616.

Pozio, E., La Rosa, G., 2003. PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. Methods Mol. Biol. 216, 299–309.

Pozio, E., Murrell, K.D., 2006. Systematics and epidemiology of *Trichinella*. Adv. Parasitol. 63, 367–439.

Rosenthal, B.M., La Rosa, G., Zarlenga, D.S., Dunams, D., Chunyu, Y., Mingyuan, L., Pozio, E., 2008. Human dispersal of *Trichinella spiralis* in domesticated pigs. Infect. Genet. Evol. 8, 799–805.

Sapkotal, R., Horchner, F., Srikitjakarn, L., Kyule, M.N., Baumann, M.P., Nockler, K., 2006. Seroprevalence of *Trichinella* in slaughter pigs in Kathmandu Valley, Nepal. Southeast Asian J. Trop. Med. Public Health 37, 1078–1082.

Sayasone, S., Odermatt, P., Vongphrachanh, P., Keoluangkot, V., Dupouy-Camet, J., Newton, P.N., Strobel, M., 2006. A trichinellosis outbreak in Borikhamxay Province, Lao PDR. Trans. R. Soc. Trop. Med. Hyg. 100, 1126–1129.

Taylor, W.R., Tran, G.V., Nguyen, T.Q., Dang, D.V., Nguyen, V.K., Nguyen, C.T., Nguyen, L.T., Luong, C.Q., Scott, T., Thach, D.T., Ninh, T.T., Nguyen, T.D., Pham, K.T., Fox, A., Horby, P., Wertheim, H., Nhan, D.H., Nguyen, H.H., Trinh, L.M., Nguyen, T.V., Nguyen, K.V., Nguyen, D.H., 2009. Acute febrile myalgia in Vietnam due to trichinellosis

following the consumption of raw pork. Clin. Infect. Dis. 49, e79–e83.
Wang, Z.Q., Cui, J., Xu, B.L., 2006. The epidemiology of human trichinellosis in China during 2000–2003. Acta Trop. 97, 247–251.
World Organisation for Animal Health, 2008. Principles of validation of diagnostic assays for infectious diseases. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, World Organisation for Animal Health, Paris, France (chapter 1.1.4).