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Research Article

Human Trichinosis in rural area Mountainous Provinces in Vietnam in 2015-2016

Abstract

Trichinellosis is an important food borne parasitic zoonosis caused by nematodes in the world. From 1967 to 2013, six outbreaks of trichinellosis have been documented in four mountainous provinces of North Vietnam. This study aims to estimate the magnitude of association of individual factors with current human Trichinellosis in endemic areas. Baseline cross-sectional data collected between May 2015 and June 2016 from a large community randomized-control trial were used. We interviewed a total of 4,362 individuals who provided serum samples to assess ELISA assay to detect anti-Trichinella immunoglobulin G. The association between individual factors and the prevalence of current infection with Trichinellosis was analysis by Stata 12.0. The results obtained suggest that increasing age, being male and consuming pork as well as a larger proportion of roaming pigs, are at higher risk of infection. Furthermore, consuming pork at another village market had the highest increased prevalence odds of current infection. A survey of trichinellosis seroprevalence in these five districts showed the disease to be associated with consuming raw pork (OR=2.84, p<0.05). Seroprevalence was estimated with 95% confidence and was in the range 0% - 10.5%. For control of trichinellosis to be improved, the factors identified as influencing its maintenance in the study areas must be communicated to the local administrative organizations and veterinary and public health offices.

Introduction

Trichinellosis, a zoonotic disease caused by the ingestion of larvae of Trichinella nematodes, occurs globally and has commonly been reported in Southeast Asia [1]. Trichinosis is a disease caused by the larvae, 'trichinae', of a small nematode worm (Trichinella spiralis), which can affect many species including humans. People can become infected by eating raw, undercooked or processed meat from pigs, wild boar, horses or game that contain the trichinae. The infection commonly causes symptoms such as diarrhoea, abdominal cramps and malaise. It can progress, causing fever, muscle pain and headaches and in severe cases may affect the vital organs possibly leading to meningitis, pneumonia or even death [1,2]. The disease is related to lack of understandings, habit of eating raw or undercooked meat; in additions, slaughtering animals for food not controlled by food safety and hygiene controls or livestock grazing are important factors that help infection of the diseases. Trichinella is found in domesticated animals (mostly pigs) in 43 countries (21,9%) and in wildlife animals in about 66 countries (33,3%) [3]. Human trichinosis is documented in 55 countries (27,8%) in the world [2]. There are reported 8 species of Trichinella (T. spiralis, T. nativa, T. britovi, T. murrelli, *T. nelsoni*, *T. pseudospiralis*, *T. papure* và *T. zimbabwensis*) and 4 genotypes (T6, T8, T9, T12). All of those species and genotypes are classified into 2 major groups based on whether musclestage larvae is encapsulated or not [1].

In Asia, *Trichinella* spp. infection has confirmed in humans in 18 countries, domestic animals (mainly pigs) in 9 countries, and wildlife in 14 countries [4]. *T. spiralis* has a regional distribution [4] with the majority of outbreaks recorded in the ethnically diverse regions of central and northern Laos, northern Thailand and northwest Vietnam where consumption of uncooked pork is common [5–7].

In Vietnam, *Trichinella* sp. was detected for the first time in 1923 in two (0.04%) of 4,952 pigs tested in Hanoi [8]. Trichinellosis was diagnosed among six soldiers in Saigon, two of whom died, in 1953 [9]. During 1967–2013, human trichinosis caused by *Trichinella spiralis* is documented in at least 6 outbreaks with 134 who was diagnosed patients, of which 8 (6.1%) died in mountainous provinces in the North region with four provinces as Yen Bai, Son La, Dien Bien and Thanh Hoa. All infected people had consumed raw pork, it means Lap food, from backyard and roaming pigs or wild boar at wedding, funeral, or New Year parties [10]. More recently, T.

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spiralis larvae were detected in free-roaming pigs [10] and the source of infection was a wild pig, rat [11,12] where an outbreak of trichinellosis occurred.

The aim of this work was to evaluate on the status of human Trichinella infection in four mountain provinces in the North Vietnam: Yen Bai, Son La, Dien Bien and Thanh Hoa where outbreaks of human trichinosis occurred; and to find out possible risk factors related to Trichinella infection among habitat communities.

Study Methods

Study area and population

The Cross-sectional study was conducted from May 2015 to June 2016 in four mountain province in the North of Vietnam which differed in terms of climate, topography, farming systems, range of ethnicities and low socioeconomic status (e.g. sanitation, drinking water and education). There were individuals (06 years old and above), irrespective of ethnicity, gender, occupation living in the selected research places In addition to discussion with local partners, a report by the National Institute of Malariology, Parasitology and Entomology (NIMPE) which detailed geographic differences of indicators of socioeconomic status was consulted to ensure variation in risk factors for the pathogens investigated.

There were 20 villages in four endemic provinces: Yen Bai, Son La, Đien Bien and Thanh Hoa. Each province: including 1 village had an outbreak in the past and four villages around that have not had any outbreaks in this time period.

Study design and sampling

The sample size calculation used a seroprevalence of 10% as little prior information was available and was sufficient to estimate human seroprevalence with 5% precision. In total, 20 clustes were randomly selected (5 clustes in each province) using probability proportional to human population. In each village, 17 households were randomly selected regardless of pig ownership during a village-wide meeting. Within these households, one household member over 6 years of age was randomly selected to be sampled and interviewed, resulting in a total of 4,362 human participants. A questionnaire for humans, developed in consultation with local health authorities, gathered information on socio-economic factors, pigfarming practices, cooking and eating behaviour, sanitation facilities and hygiene practices. Questionnaires were administered by health station officials belonging to several ethnic groups and were conducted in native languages of the villagers. Knowledge dissemination to participating villages consisted of a summary of results and information regarding prevention of these diseases in pigs and humans.

Serological survey of pathogens

Finger finger samples and serum samples were collected from field participants. All laboratory testing was performed in Vietnam at the National Institute of Malariology, Parasitology and Entomology (NIMPE), Ministry of Health. Finger blood samples were collected for all participants and to make copies of blood drills in the field. The samples were transferred to the laboratory and then as a 7% staining technique and eosinophil counts [13].

Blood vein samples were collected in plain vacutainers. Samples were refrigerated and then placed on ice until arrival at the laboratory in NIMPE, where they were stored at -20°C before testing. Human serum samples were tested for the presence of antibodies against *T. spiralis* using the following commercial diagnostic kits: *T. spiralis* IgG ELISA (IBL International, Germany and reported sensitivity of 95% and specificity of 94.8%). Manufacturers' instructions were followed when conducting and interpreting these kits [14]. The positive serum samples with anti-ELISA IgE Trchinella were subjected to Western blot (WB) for confirmation. Antibodies against T. spiralis antibodies using the Priocheck Trichinella Ab ELISA (Prionics, Switzerland. Sensitivity: 97.1–97.8% and specificity: 99.5–99.8%) [14], were detected using an protocol as per Maria Angeles Gomez et al. [15].

Statistical analysis

Data management: All questionnaire and serological data were entered into a questionaire survey design and management application. This application was designed with Vietnamese language display features so that entry and data checking could be undertaken by a team member in their native language. Data cleaning and descriptive statistical analysis were conducted in Microsoft Excel. The remainder of statistical analyses were carried out in Stata (v. 12.0).

Exploratory data analysis: Seroprevalence of zoonotic pathogens were estimated for humans at the NIMPE and chi-squared tests were performed using the stats package to assess whether seroprevalence in humans seropositive differed significantly between Provinces. Risk factor variables included in the analysis were: water sources, pork consumption and food preparation habits (including consumption of raw pork, herbs, wild vegetables and pigs' blood) and contact with pigs including presence of pigs in the household, involvement in pig husbandry and participation in pig slaughtering. General population characteristics e.g. gender, age occupation and province were included as supplementary variables.

Risk factor analysis: Risk factor analysis was performed to assess whether the participants increased the risk of testing seropositive for *T. spiralis* pathogens. Village was included as a random effect to control for the correlation of humans within villages. Age and gender were included as fixed effects to control for potential confounding effects of these variables. Gender was subsequently removed from any models where it was associated with the outcome with a p-value > 0.05. Chi-squared tests were used to assess the associations with variables of interest and the pathogens.

Research ethics

Approved by Medical Ethics and Scientific Committee of NIMPE, MoH Vietnam.

Results

Population structure

A total of 4,362 persons from 324 households in 20 communities in four provinces were eligible to participate in this survey. The result show in table 1.

The final survey population consisted of 2,239 Thai (51.3% compliance), 1,623 Hmong (37.2%), 296 Kinh (6.8%) and 204 Muong (4.7%). Male population was 34% and female 66%. Most participants were aged 16–59 years (87.0%) and living in poverty (67.6%). Some (1.6%) who get sick in the outbreak before also involved in this investigation. The rate of positive Anti-ELISA Trichinalle IgG was low in general communities with 5.1%. Mean of eosinophils higher than 6% was 10.1 + 3.3% (rang 7–23) (Figure 1) (Table 2).

Female positive Trichinella is higher than male, (75.1% vs 24.8%; p<0.05). Age of 16–59 group positive Trichinella is higher than others (80.4% vs 8.4% & 11.2%; p<0.05). Thai ethnic group positive Trichinella is highest with 51.4%, follow Mong (44.1%0, Kinh (3.2%) and Muong lowest with 1.4%, this difference was significant (p<0.05) (Table 3).

Positive Trichinella eating wild meet is two times higher than that among negative group (p<0.05; RR: 2.47); and eating raw pork is three times higher than that among negative group (p<0.05; RR: 2.83). Eating raw pork with Lap traditional food is two times higher than that among negative group (p<0.05; RR: 2.22).

Discussion

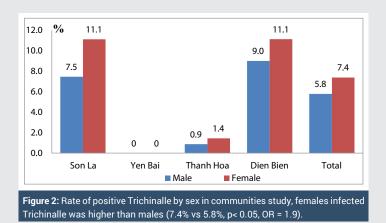
Arccoding to Dupouy-Camet et al., 2002, human infected Trichinella occurs via the consumption of encysted larvae in the muscle of infected animals and involves an enteral phase associated with excystment, sexual maturation, reproduction and larval penetration of the intestinal wall and a parenteral phase associated with the migration of larvae, via lymphatic and blood vessels, to striated muscles where they encyst in a nurse cell complex. Clinical symptoms in humans are related to the number of viable larvae consumed and are typically associated with the parenteral phase. Humans are a dead-end host and not involved in perpetuating the lifecycle [1].

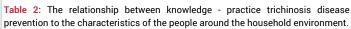
Over the past 30 years, sporadic outbreaks of the disease usually occur at festivals or funerals and the largest reported in 4 provinces: Yen Bai, Thanh Hoa, Dien Bien and Son La with large uspected human cases [10,16]. However, in fact the many outbreaks have so much in the provincial epidemiological community by eating habits and preventive hygiene practices is limited. The first trichinellosis outbreak in Vietnam was reported in 1967 in Mu Cang Chai, Nghia Lo (now Yen Bai province) in the Northern Region [17] and since then outbeaks have been continually documented [18,19]. The continual reporting of cases through the national surveillance system led to Vietnam being named as one of the trichinellosis endemic countries in Asia and Sourthen East Asia [2,10]. The clinical epidemiological surveys carried out from 2010 to 2012 revealed statistically significant associations of myalgia and facial edema with a Trichinella-positive serology (by both ELISA and Western blot) in persons from villages, whereas a statistically significant association of myalgia with a Trichinella-positive serology was observed in persons sampled in hospitals [18]. The high prevalence (1.6-3.5%) of anti-Trichinella IgG in persons from Vietnamese provinces where Trichinella spiralis is circulating in pigs strongly supports the need to develop control programs to eliminate the infection from pigs and for consumers' education and protection [20].

The results suggest that in local study sites, where a trichinellosis outbreak occurred before and neighborhoods this investigation who had eaten raw meat dishes prepared from the same wild boar had trichinellosis. Diagnosis was

Site study Variable		Son La	Yen Bai No.(%)	Thanh Hoa	Dien Bien	Total	X²	Р
		No.(%)		No.(%)	No.(%)	No.(%)		
No. participants		1,033	1,135	1,175	1,019	4,362		
Trichinella infectio	n	101(9.8)	0(0.0)	17(1.4)	107(10.5)	225(5.1)	206.59	< 0.
Age	6-15	88(8.5)	52(4.6)	40(3.4)	65(6.4)	245(5.6)	78.26	< 0.0
	16-59	833(80.6)	1,006(88.6)	1,030(87.7)	920(90.3)	3,789(86.9)		
	60-92	112(10.8)	77(6.8)	105(8.9)	34(3.3)	328(7.5)		
Ethnicity	Kinh	73(7.1)	5(0.4)	203(17.3)	15(1.5)	296(6.8)	3600	< 0.0
	Hmong	646(62.5)	972(85.6)	0(0.0)	5(0.5)	1,623(37.2)		
	Muong	170(16.5)	0(0.0)	32(2.7)	2(0.2)	204(4.7)		
	Thai	144(13.9)	158(13.9)	940(80.0)	997(97.8)	2,239(51.3)		
Wealth status	Patient	17(1.7)	0(0.0)	38(3.2)	14(1.4)	69(1.6)	30.68	< 0.0
	Normal	1,019(98.3)	1,129(99.5)	1,137(96.8)	1,008(98.9)	4,293(98.4)		
Sex	Male	429(41.5)	465(41.0)	344(29.3)	246(24.1)	1,484(34.0)	106.45	< 0.0
	Female	604(58.5)	670(59.0)	831(70.7)	773(75.9)	2,878(66.0)		
income (\$/per./month)	<50	703(68.1)	899(79.2)	735(62.6)	610(59.9)	2,947(67.6)	178.03	< 0.
	50-100	264 (25.6)	197(17.4)	302(25.7)	364(35.7)	1,127(25.8)		
	>100	66 (6.4)	39(3.4)	138(11.7)	45(4.4)	288(6.6)		

made based on eosinophilia, and on ELISA followed by WB; no biopsies could be taken in this study for confirmation. Because anti-Trichinella IgG antibodies can persist for many years after infection/exposure to the parasite [21], it cannot be ruled out that the positive serological results were from older infections. People live in Dien Bien and Son La infected Trichinella higher than Thanh Hoa and Yen Bai (10.5% and 9.8% vs 1.45 and 0%). This result is consistent with the fact that outbreaks have occurred more frequently in Dien Bien, Son La than in Thanh Hoa and Yen Bai. In Dien Bien have occurred three outbreaks of human Trichinella disease in 2001, 2004 and 2013 [22-24]. In Son La have one outbreak with 22 pateints and 2 of them died in 2008 [25]. Epidemiological and risk factors investigations





Factors relationship		Positive	Negative		
Factors rela	ationsnip	n (%)	n (%)	p-value	
Trichinella infection		225 (5.1)	4,138 (94.9)	<0.05	
0	Male	56 (24.8)	1,427 (34.5)	-0.0F	
Sex	Female	169 (75.1)	2,711 (65.5)	<0.05	
	6-15	19 (8.4)	226 (5.5)		
Age	16-59	181 (80.4)	3,610 (87.2)	<0.05	
	60-92	25 (11.2)	302 (7.3)		
	Kinh	7 (3.1)	288 (7.0)		
Ethnic group	Thai	114 (51.4)	2,125 (51.4)	0.05	
	Mong	98 (44.1)	1,525 (36.8)	<0.05	
	Muong	3 (1.4)	200 (4.8)		

in these two provinces after the epidemic determined that animals (wild boars, synanthropic rats and local-roaming pigs) had Trichinella infection [11,12,26]. The results of this study are also lower than the local epidemiological surveys after the epidemics such as Thanh Hoa 1.7% compared to the survey data in 2012 was 7.4% [27]. Dien Bien 10.5% compared to the survey data in 2013 was 28.5% [28].

In this study, most infected Trichinalle person were age of labor from 16-59 (80.4%), and females was two times higher than males. Other studies reported, the infected patients were adults of the 41- to 50-year-old age group (35.1%). Only one 6-year-old child acquired the infection in the 2012 outbreak. Males were more infected (84.2%) than females (15.8%) [27]. This difference may be due to the fact that this report is available to patients at the outbreak rather than a large-scale regional survey as this study. The study in Thailand, It is not uncommon to see patients in the 10-14 and 65+ age groups, but most patients are in the age 35-44 groups, and the disease occurred more frequently in men than women during 1962-2003, with no significant sex difference during 2004-2006 [7]. In Lao People's Democratic Republic, the patients were primarily adults in the range of 28-33 years of age and about equally affected men and women [29].

This result is similar to other reports (from 1967 to 2013) belonged to the ethnic minorities Thai, Tay, and H'mong [30], most of the infected people Thai ethnic group (51.4%) and living in poverty (67.6%. Lap is a traditional food of Thai ethnic group, is made from raw pork mixed with roasted rice flour and wild herbs. Lap food is high risk factor for Trichinella infection. This has also been verified in six outbreak Trichinella reports at study sites [17,22-24,27]. As ThaiLand and Lao PR, epidemiological investigations reveal that the outbreaks have mostly occurred in rural areas where villagers often celebrate traditional festivals such as northern Thai New Year, wedding ceremonies, etc. Typically, the source of infection is domestic hilltribe pigs raised like wild animals or from wild boar. Infection is usually acquired from consuming the ethnic dish called "Lahb" served during the festivities in Thailand [7]. Several outbreaks and sporadic cases of trichinellosis have occurred in Laos, Thailand and Vietnam over the past five years with the majority of the reported cases being associated with consumption of raw pork [6,7,10,24,27-29]. Trichinella spiralis is thought to be endemic in the pig population in mountain province in Vietnam where

Factors relationship		Positive		Negative		-			
Factors rela	itionsnip	n 86	% 38.2	n 666	% 16.1	Total 752	p-value < 0.05	RR 2.37	95% CI 2.07-2.95
Eating wild meet	Yes								
- Kind of meet (n=752)	Wild pig	28	31.5	101	15.2	129	< 0.05	2.11	1.56-2.85
	Wild rat	24	27	294	44.3	318			
	others	37	41.6	268	40.4	305			
Raw pork	Yes	53	23.6	345	8.3	398	<0.05	2.84	2.19-3.65
-Kind of meet (n=398)	In maket	42	79.2	218	63.2	260	<0.05	1.25	1.07-1.47
	House pig	11	20.8	127	36.8	138			
How to make food (n=398)	Lap traditional food	45	84.9	132	38.3	177	<0.05	2.22	1.86-2.65
	Blood pudding	8	13.2	213	61.7	221			

share boder with Lao PDR and infection in humans occurs via the ingestion of raw or undercooked meat containing the larvae of *T. spiralis* nematodes [31–41]. Suspected human cases occur regularly in Vietnam, however, diagnostic facilities and outbreak investigation are lacking [10]. Today in Vietnam, people buy pigs in the mountains transported to the delta and the city slaughtered meat, especially processed foods of this Lap food of ethnicity are also widely disseminated. This does mean the Kinh people, people of the main Vietnamese ethnic group, also acquired the Trichinella infection.

Conclusion

The risk of *Trichinella* antibody detection in the ES ELISA increased significantly with increasing age and Lao-Tai people were at significantly greater risk Anti-positive *Trichinella* IgG ELISA rate is high 5.1% (0.0%-10.5%) in the communities

The risk of *Trichinella* antibody detection was associated with sex, age, and certain ethnic groups, suggesting that prevention measures could be targeted toward specific populations. Higher prevalence was recorded in the labor age group than other age groups. Trichinosis female ratio two times higher than men. Prevalence among Thai ethnic group was higher than other ethnic groups.

Risk of *Trichinella* antibody detection was also associated with specific food practices, such as eating raw pork. This suggests that prevention efforts could be targeted toward specific food preparation and consumption practices. Lap traditional food is risk factor for *Trichinella* infection.

Recommendation

Prevention efforts, such as education around safe food handling and preparation practices, should be developed and introduced into the communities identified in this investigation. Further efforts should be made to understand the interactions among human health, animal health and the environment using a One Health approach.

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