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Prevalence and risks of fascioliasis among adult cohorts in Binh Dinh and Quang Ngai provinces-central Viet Nam

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Abstract
Fascioliasis (liver fluke disease) has raised significant public health concerns in the 15 regional provinces of Central Vietnam, accounting for 93% of the national incidence of the disease. No control measures to date have proven effective. Annual reports show increasing incidence of fascioliasis but they are incomplete. This cross-sectional study was conducted to identify the prevalence of fascioliasis and to describe its associated risks in three communes in Central Vietnam. 500 human blood samples were examined (ELISA); and a survey of knowledge, attitude and practice (KAP) was conducted for 600 randomly selected adults per commune. The findings suggest that overall seroprevalence was 7.75% (95% CI 6.54-9.16%). Among the infected cases, people aged from 18-59 years (85.6%) and farmers (68.0%) accounted for majority of infection. Less than half of participants in all three communes (24.6% - 46.0%) knew the causes of fascioliasis; and considerable proportions ate improperly boiled vegetables (28.2-33.8%), drank unboiled water (23.5-42.5%), and did not own a household toilet (14.2-20.5%). Relatively high prevalence and risks of fascioliasis were found in Central Vietnam, supporting the need for comprehensive intervention measures including selective treatment, health education, and multisectoral approaches to reduce the morbidity associated with fascioliasis and thus improve the health status of the people.

Keywords
provinces, ngai, quang, dinh, binh, cohorts, adult, among, fascioliasis, risks, prevalence, viet, nam, central

Disciplines
Education | Social and Behavioral Sciences

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Prevalence and risks of fascioliasis among adult cohorts in Binh Dinh and Quang Ngai provinces-central Viet Nam

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ABSTRACT

Fascioliasis (liver fluke disease) has raised significant public health concerns in the[15] regional provinces of Central Vietnam, accounting for 93% of the national incidence of the disease. No control measures to date have proven effective. Annual reports show increasing incidence of fascioliasis but they are incomplete. This cross-sectional study was conducted to identify the prevalence of fascioliasis and to describe its associated risks in three communes in Central Vietnam. 500 human blood samples were examined (ELISA); and a survey of knowledge, attitude and practice (KAP) was conducted for 600 randomly selected adults per commune. The findings suggest that overall seroprevalence was 7.75% (95%CI 6.54-9.16%). Among the infected cases, people aged from 18-59 years (85.6%) and farmers (68.0%) accounted for majority of infection. Less than half of participants in all three communes (24.6% - 46.0%) knew the causes of fascioliasis; and considerable proportions ate improperly boiled vegetables (28.2-33.8%), drank unboiled water (23.5-42.5%), and did not own a household toilet (14.2-20.5%). Relatively high prevalence and risks of fascioliasis were found in Central Vietnam, supporting the need for comprehensive intervention measures including selective treatment, health education, and multisectoral approaches to reduce the morbidity associated with fascioliasis and thus improve the health status of the people.

Keywords: *Blood survey, Central Vietnam fascioliasis, F. hepatica, F. gigantica, KAP, prevalence, risk factors,*

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INTRODUCTION

Fascioliasis (liver fluke disease) is the parasitic disease caused by two food-borne species. Fasciola hepatica (F. hepatica) and Fasciola gigantica (F. gigantica) are classified as liver flukes (trematodes) and belong to the genus Fasciola[1]. Among the digenerean species, F. hepatica has a wide distribution in all continents[2,3], whereas F. gigantica is restricted to the low altitudes in tropical regions of Africa, Asia and the Middle East[4,5]. In addition, the two fasciolids can infect a wide range of final hosts, including water buffaloes, bovines, goats, sheep and humans[6,7]. Snails belonging to the Lymnaeid species are known for their intermediate hosts of Fasciola[8]. The spread of fascioliasis to new environments and geographical areas reflects the marked adaptation of the parasites to the Lymnaeid snails and expansion of the existing snail populations[9]. Increasingly, recent molecular tools have reported the prevalence of the two fasciolids to be overlapping[10], possibly because they are very adaptive with Lymnaeid snails as their intermediate hosts[11]. It is the distribution and adaptability of the aquatic Lymnaeid snails to a wide range of environmental niches that have resulted in the recent expansion of fascioliasis transmission in Europe, South America, Africa, Asia and the Middle East[12-15].

Since the mid-1990s fascioliasis has been considered a secondary emerging/re-emerging zoonotic disease[16] and it is posing negative impacts on public health systems and livestock industries worldwide[17, 6]. With the annual prediction of 35,000 DALYs lost[18], human fascioliasis is reportedly affecting about 180 million people and infecting from 2.4 to 17 million others in 51 countries worldwide[1, 19, 20], Recent outbreaks of human fascioliasis have made the disease a major public health problem in developing parts of the world and it is listed by WHO as a priority of neglected tropical diseases (NTDs)[8, 21, 22]. In addition, various studies[23-27] report significant reduction or losses in production of milk and meat products attributed to fascioliasis.

In Vietnam, fascioliasis has raised significant public health concerns.The National Institute of Malariology-Parasitology and Entomology (NIMPE) reported a total incidence of 15,761 cases between 2006 and mid-2010 in 47 of 63 provinces nationwide[28]. Central Vietnam, with favourable geographical and weather conditions for parasitic diseases, is the area most affected by fascioliasis[29]. Recent studies[30, 31] reported the emergence of the disease in all 15 regional provinces, accounting for 93% of the total national incidence. Other studies[32, 33] have indicated human fascioliasis infection rates ranging from 5.6% to 11.1% in coastal provinces of Binh Dinh, Phu Yen, and Khanh Hoa. However, studies conducted previously have not provided detailed understanding of fascioliasis as the potential public health problem in Central Vietnam, and annual reports documenting increasing incidence are incomplete.

In the absence of a more comprehensive exploration of the prevalence of fascioliasis and the detailed description of the associated risks of the disease, this study was conducted to determine the prevalence of fascioliasis by laboratory methods and to describe the risk factors associated with fascioliasis infections in three adult cohorts.

METHODS

The study was undertaken in Nhon Hau and Nhon Thanh communes (An Nhon town-Binh

Dinh province) and Tinh Giang commune (Son Tinh district-Quang Ngai province) in Central Vietnam between March and May 2013 (Figure 1). The selection of the actual communes in the two different provinces was based on the convenience of accessibility (transports, health system facilities, and engagement of local authority), and site distance (control commune away from the influences of the intervention communes). The study duration was at beginning of the dry season in two provinces, with the measured meteorological data at the Binh Dinh station for three months of temperaturesat 26.9^{0}C, 28.3^{0}C, and 28.9^{0}C; rainfalls of 22.1, 38.9, and 255.6 millimetres; and sunshine duration of 269.6, 242.9, and 307.2 hours, respectively[34]. A cross-sectional descriptive design comprising human blood survey and knowledge, attitudes and practices (KAP) survey was applied in this study. In each commune, randomly-selected 500 adults (aged from 18 years old) had their blood samples examined (ELISA); and 600 randomly selected adults (household representatives) were involved in the KAP survey on fascioliasis. In addition, snail surveys were undertaken to evaluate the prevalence of fascioliasis in the intermediate hosts in the aquatic biotopes of the three communes.

Equation 1. Formula 1 for sample size calculation used in blood survey

$$n = \frac{Z^2_{(1-\alpha/2)} P(1-P)}{d^2}$$

n: minimum sample size needed
p: referential infection rate of 6.0% (P= 0.06) from the study conducted previously[35].
d: absolute percentage point of precision, 2.3% (d= 0.023)
Z(1-α/2) = 1.96 at 95% CI

Equation 2. Formulas for sample size calculations used in KAP survey

$$n = Z^2(\alpha,\beta)\frac{[p1(1-p1)+p2(1-p2)]}{(p1-p2)^2} \qquad n = Z^2(\alpha,\beta)\frac{[p1(1-p1)+p3(1-p3)]}{(p1-p3)^2}$$

$$n = Z^2(\alpha,\beta)\frac{[p2(1-p2)+p3(1-p3)]}{(p2-p3)^2}$$

n: minimum sample size needed
Z(1-α/2) = 2.576 at the confident interval (CI) of 99%
Z(1- β) = 0.84 when 1-β = 80%; $Z^2(\alpha,\beta) = 11,669$
- p1: proportion of population in intervention commune 1 owning knowledge, attitude and practice against fascioliasis after the intervention, expected at 80% (p1=0.84);
- p2: proportion of population in intervention commune 2 owning knowledge, attitude and practice against fascioliasis after the intervention, expected at 69% (p2=0.69);
- p3: proportion of population in control commune owning knowledge, attitude and practice against fascioliasis after the intervention, expected at 56% (p3=0.59).

The seroprevalence of fascioliasis in three communities was determined by laboratory-based blood ELISA and comparing the eosinophilia counts with the guidelines of diagnosis and treatment of fascioliasis by the Ministry of Health, Vietnam[36]. Determining the prevalence of fascioliasis in the community settings can be based on either of two main diagnostic methods: the classical coprology and serology[37]. The coprological examination provides a direct measure of the infection but has low sensitivity and is not routinely conducted in Vietnam[38, 39]. In this study, serological ELISA (Enzyme-Linked Immunosorbent Assay) was used to indirectly detect the antibodies specific to *Fasciola spp*. (IgG) in human sera. This study used the test kits (FASCELISA), specific for *F.gigantica* infection, which were produced and distributed by the Viet Sinh Chemical Producing & Trading Co., Ltd (formerly the Faculty of Pharmacy-Ho Chi Minh City University of Medicine and Pharmacy-Vietnam). The test kits were previously proven to have high sensitivity and specificity[40], and the protocol had been approved for use by the Ministry of Health.

The sample for cross-sectional study was calculated (formula 1) for statistical methods for sample size determination by Lemeshow et al.[41], with known referential infection rate from previous study conducted in a neighbouring province of Khanh Hoa[42]. The total sample population in each selected commune was 428 people. To assure the desired number of participants an additional 20% or 85 more people were added in case of loss to follow-up or inaccessibility, resulting in a total sample population in each commune of 504 (rounded to 500 people). As the prevalence of fascioliasis among children in the area had been found in recent studies to be low[43-45], only adults aged from 18 were selected for this study. Households were randomly selected from the records available at the communal registrar's office, applying a 5-household interval, until the required number of households was reached. One adult member of each household was invited to participate.

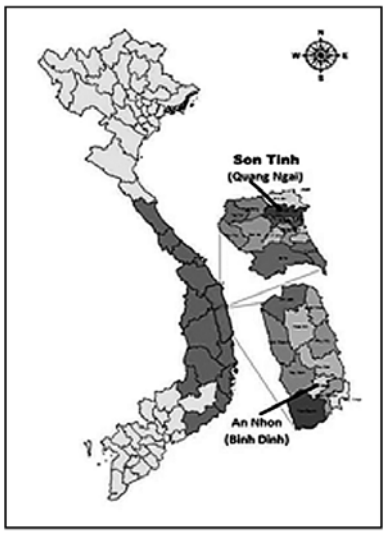

Fig1. Map of Vietnam showing the studied sites in Binh Dinh and Quang Ngai provinces

The blood samples were used for ELISA to identify if a person was infected with fascioliasis and for eosinophilia evaluation as a supplementary indicator of positive case[36]. The results of the assays were calculated by dividing the optical density (OD) reading of each sample well of the plate by the cut-off value (determined by the mean OD negative controls plus 3 standard deviations). Any result from 1.0 was considered positive, and results less than 1.0 were recorded as negative[40].

As the KAP survey involved adult cohorts in three communes, the manual Bonferroni adjustment was used to allow for multiple comparisons to be made while assuring the overall confidence coefficient[46]. The sample size for the study was calculated for estimating the difference between two population proportions with specified absolute precision (Two-sample situations) by Chadha[47], with equal sample size in each pair of three comparisons (formula 2). Previous proportions of knowledge were used as referential indicators[48] and the average samples were calculated for each pair of comparisons (p1 and p2, p1 and p3, and p2 and p3). Accordingly, the total sample population in each commune (plus 10 of sample in case of attrition) was 600 people. All adults (aged from 18 and above) in every second household participating in the blood survey were invited to undertake the KAP survey.

As a result, 1,800 representatives (usually householders) aged from 18 years old were invited to participate in the KAP survey. The KAP surveys were administered by face-to-face interviews at the householder's residence, taking approximately 20 minutes.

Participation of individuals for blood and KAP surveys was on voluntary basis. Participants had the choice of participating or not in the study and could discontinue the interviews at any time without bearing any consequences.

Equation 3. Formula 3 for sample size calculation used in Lymnaied snail surveys

$$n = Z^2_{1-\alpha/2}\frac{(1-p)}{p.\varepsilon^2}$$

n: minimum sample size needed
p: referential infection rate of 1.7% (p= 0.017) from previous study conducted in BinhDinh49.
ε: relative precision at 25% or 0.25, Z(1-α/2) = 1.96 at 95% CI

Surveys of *Lymnaeid* snails as intermediate hosts of fascioliasis were conducted at all rice fields, lakes, ponds, agricultural canals or small streams of three study sites to identify the prevalence of fascioliasis infection in the snail (Equation 3). Estimated total of 3,554 snails plus 10% to account for sampling attrition or 3,909 snails were collected in each of the three study sites. The snails were collected from the aquatic habitats using paddles with 1.5 metre-long wooden handle and 15 by 15 cm net size. The collected snails were then put into plastic, screw capped containers with an amount of water and transferred to the laboratory of IMPE-QN. Before examination, the *Lymnaeid* snails were identified by snail taxonomy[50]. Each of the snails was crushed and smeared on microscopic slides for the presence of *Fasciola* larvae (radiae and cercariae) using microscope (40X) and with the taxonomy of *F.gigantica* cercariae[51]. The infection rate was calculated in percentage by the amount of *Lymnaeid snails* infected by the larvae.

The results from ELISA for blood samples were entered in the EpiData 3.1 and transferred into SPSS (Statistical Package for Social Sciences) version 19.0. Descriptive statistical analyses were undertaken, such as overall prevalence of fascioliasis in the population and distribution of the disease according to gender, age, education background and occupation. With the statistical χ^2 and Fisher's exact tests being used, statistical significances were reached if the p-values were less than 0.05. The associated risks of fascioliasis among three adult cohorts were evaluated using the data obtained from the knowledge, attitude and practice surveys in three communes. Data entry and statistical analyses of the KAP data were similar to procedures applied to blood survey data. Descriptive analyses were undertaken: ethnographical characteristics were compared between variables to explore the risks among the communities in terms of their knowledge, attitude and practice of fascioliasis. As the Bonferroni adjustment was used to allow multiple comparisons in the two-sample situations[47] in this survey, significant differences from χ^2 and Fisher's exact tests were reached if the p-values were less than 0.017.

The research protocol was approved by the Bio-medical Ethics Committee at the Institute of Malariology-Parasitology and Entomology, Quy Nhon (approval number 364/VSR-CV) and by the Human Research Ethics Committee at the University of Wollongong (Approval number HE 12/405).

RESULTS

Prevalence of fascioliasis among adult cohorts in two Central provinces of Viet Nam

A total of 1,612 participants aged from 18 years old and above were involved in the cross-sectional study by ELISA-based blood surveys. The seroprevalence of fascioliasis in three communes of Nhon Hau, Tinh Giang and Nhon Thanh was 8.8%, 8.4% and 6.1%, respectively; and the overall infection rate was 7.8% (Table 1).The descriptions of clinical and

para-clinical characteristics of infected cases, as recommended in the guidelines as supplementary indicators of a fascioliasis case confirmation[36], are presented in Table 1. At each location the majority of the people with infection reported abdominal pains in the right upper quadrant (ranging from 66.0% to 82.4%), followed by fatigue (68.4%-79.4%), urticarial (skin rash) (40.4%-76.5%), and fever (49.0%-52.9%). Digestive system disturbance (36.2%-47.1%) and other symptoms such as nausea and anorexia (36.2%-41.2%) were less reported. In Nhon Hau commune, 57.4% of the infected cases had eosinophilia; followed by those in Tinh Giang (56.8%) and Nhon Thanh communes (52.9%). With the 95% CI of the mean eosinophils in three cohorts of 7.0-8.4, 7.5-9.8, and 7.3-10.3, these were not significantly different.

No significant differences were indicated in fascioliasis infection rate by gender. Infection rates of different age groups were not significantly different in Nhon Hau and Nhon Thanh communes. In Tinh Giang commune, the infection rate of fascioliasis among people aged from 40-49 years was significantly higher ($p<0.05$) than in those within the age ranges of 18-29, 50-59 and 60+ years. Participants with lower education background (secondary level and under) in Nhon Hau commune accounted for high infection rate (10.1%), whereas lower infection (4.2%) was found among those with higher education background; and the difference was significant ($p<0.05$). In the other two communes, no difference was found in infection rates across education levels. Across the occupations, farmers in our study represented the highest incidence of infection in the three communes, (72.3% in Nhon Hau, 70.5% in Tinh Giang, and 58.8% in Nhon Thanh communes, respectively) (data not shown). Other occupational groups had relatively low infection rates (typical infection proportions of other occupations not shown); but no significant differences was found among the groups ($p>0.05$).

Table 1. Prevalence of fascioliasis in three cohorts under study

Characteristics	Nhon Hau (n=535)		Tinh Giang (n=522)		Nhon Thanh (n=555)	
	Number (%)	p	Number (%)	p	Number (%)	p
Infected cases (7.8%)	**47(8.8)**		**44(8.4)**		**34(6.1)**	
Clinical symptoms						
Pain in RUQ[1]	34 (72.3)	NS	31 (66.0)	NS	28/(82.4)	NS
Fever	24 (51.1)		23 (48.9)		18 (52.9)	
Digestive disturbance	22 (46.8)		17 (36.2)		16 (47.1)	
Urticaria	31 (66.0)		19 (40.4)		26 (76.5)	
Fatigue	35 (74.5)		30 (63.8)		27 (79.4)	
Vomiting, anorexia	26 (55.3)		17 (36.2)		14 (41.2)	
Eosinophilia						
No. ≥ 8.0%[2]	27 (57.4)	NS	25 (56.8)	NS	18 (52.9)	NS
Min-max	2-14		2-17		2-22	
% mean ±SD	7.7±2.3		8.6±3.8		10.5±4.3	
95% CI	7.0-8.4		7.5-9.8		7.3-10.3	
Gender						
Males	15/180 (8.3)	NS	21/216 (9.7)	NS	16/221 (7.2)	NS
Females	32/357 (9.0)		23/306 (7.5)		18/334 (5.4)	
Age (mean±SD)	**(47.7±12.1)**		**(44.8±12.5)**		**(46.2±13.2)**	
18-29	0/39	NS	2/65 (3.1)	<0.05	3/62 (4.8)	NS
30-39	12/98 (12.2)		10/118 (8.5)		9/113(8.0)	
40-49	13/152 (8.6)		20/120(16.7)	<0.05	9/141 (6.4)	
50-59	18/148 (12.2)		9/157 (5.7)	<0.05	11/145 (7.6)	
60+	4/94 (4.1)		3/62 (6.8)		2/94 (2.1)	
Education level						
Secondary & under	42/417 (10.1)	<0.05	34/365 (9.4)	NS	25/402 (6.2)	NS
High school & above	5/118 (4.2)		10/157 (17.5)		9/153 (5.9)	
Occupation						
Farmers	34/342 (9.9)	NS	31/313 (9.9)	NS	20/300 (6.7)	NS
Others	13/193 (6.7)		13/209 (6.2)		14/255 (5.5)	

[1] *RUQ: right upper quadrant of the abdomen*

[2] *Recommended by the MOH (2006) as a supplementary indicator of a positive case confirmation*

Perception and practice in relation to risks of fascioliasis infection in studied cohorts

General perceptions of fascioliasis wereexplored in the three cohorts under study. The overall findings indicated low awareness (less than 50%) of the participants in the three communes in terms of their knowledge of fascioliasis (Table 2). Participants in Nhon Hau commune had significantly higher awareness of the disease than those in Tinh Giang and Nhon Thanh communes ($p<0.001$). Among participants who knew about the disease in the three communes, more than 50% stated the correct answers of the transmission

routes such as eating improperly treated* vegetables and drinking unboiled water; however, less than 50% of them knew about the signs and symptoms of fascioliasis infection. Disparities were found across the communes in the participants' knowledge of whether fascioliasis was curable: more participants in Nhon Hau commune than those in Tinh Giang commune agreed the disease was curable ($\chi 2=7.2$, $p<0.01$) but no significant difference was indicated in comparison with participants in Nhon Thanh commune ($p>0.017$).

In the three communes, 28.2% to 33.8% of the participants reportedly ate improperly treated vegetables, with no significant differences found between the communes. Regarding the practice of drinking water, significance differences were found as participants in Nhon Hau commune reported better practice of drinking properly treated water than the two other communes ($p<0.01$). In addition, although a majority of households in the three surveyed communes owned toilets, significantly lower household toilet ownership was reported in Tinh Giang commune in comparison to Nhon Thanh commune ($p<0.01$). Without a toilet, more than half of the participants reported defecating outdoors such as on sand banks, in rice fields or into surface water sources such as canals, streams or rivers. The contaminated water sources were then used for watering vegetables or cooking, hence posing high risks of fascioliasis transmission, especially in the rainy seasons.

Table 2. Description of cohorts' knowledge, attitude and practice of fascioliasis

Item	NhonHau[a]	TinhGiang[b]	NhonThanh[c]	χ^2, p
	Number /total (%)	Number /total (%)	Number /total (%)	
Know about fascioliasis	276/600 (46.0)	148/600 (24.6)	224/600 (37.3)	a-b: 59.8;<0.01 a-c: 22.5;<0.01 b-c: 9.3; NS
Know transmission routes	n=276	n=148	n=224	
Eat improperly treated vegetables	173 (62.7)	91 (61.5)	141 (62.9)	a-b: 0.2; NS
Drink unboiledwater	165 (59.8)	95 (64.2)	129 (57.6)	a-c: 0.1; NS b-c: 0.5; NS
Know signs & symptoms	121 (43.8)	56 (37.8)	102 (45.5)	a-b: 1.4; NS a-c: 0.2; NS b-c: 2.2; NS
Know it is controllable	144 (52.2)	81 (54.7)	111 (49.6)	a-b: 0.3; NS a-c: 0.4; NS b-c: 1.0; NS
Know it is curable	145 (52.5)	59 (40.0)	97 (43.3)	a-b:6.2; <0.01 a-c: 4.2; NS b-c: 0.4; NS
Behaviours:				
Eat improperly treated vegetables	169/600 (28.2)	197/600 (32.8)	203/600 (33.8)	a-b: 3.1; NS a-c:4.5; NS b-c: 0.1; NS
Drink unboiled water	141/600 (23.5)	255/600 (42.5)	220/600 (36.7)	a-b:49.0; <0.01 a-c:24.7; <0.01 b-c: 4.3; NS
Don't own household toilets	104/600 (17.3)	85/600 (14.2)	123/600 (20.5)	a-b: 2.3; NS a-c: 2.0; NS b-c: 8.4; <0.01
Outdoor defecation[1]	65/104 (62.5)	66/85 (77.6)	90/123 (73.2)	a-b: 5.1; NS a-c: 3.0; NS b-c: 0.5; NS

[4] *Sand banks, rice fields, hilly areas and surface water such as streams, canals or river banks.*

Snail surveys

During April-May, 2013, a total number of 2,669 *Lymnaeid* spp. snails were collected at various aquatic habitats such as rice fields, canals and small streams; considered as appropriate habitats of the snails. More snails were caught from rice fields than in streams and canals, but the number of fascioliasis infected snails collected from the latter was higher than the former. Laboratory examination for species identification was performed at IMPE. The major species found

Table 3. Fascioliasis infection in collected Lymnaeid spp. snails (April-May, 2013)

Aquatic habitats	Nhon Hau	Tinh Giang	Nhon Thanh
	No./total (%)	No./total (%)	No./total (%)
Rice fields	4/849 (0.47)	4/656 (0.61)	3/684 (0.44)
Streams, canals	1/164 (0.61)	1/154 (0.65)	1/162 (0.62)
Total	5/1,013 (0.49)	5/810 (0.62)	4/846 (0.47)

* *treated – dipped in potassium permanganate, acetic acid (vinegar), or sodium chloride before being washed under running water*

was *L. viridis* (2,189 snails, 82.1%), followed by *L. swinhoei* (480 snails, 17.9%). These *Lymnaeid* snails were then further examined to identify the infection of Fasciola larva. The snail infection rates of Fasciola larva in Nhon Hau, Tinh Giang and Nhon Thanh communes were 0.49%, 0.62% and 0.47%, respectively.

DISCUSSION

The overall human seroprevalence as determined by ELISA was 7.75%, which categorised Central Vietnam as the mesoendemic area of fascioliasis. Although a variety of infection was indicated in the ethnographic characteristics, no differences were significant within the variables and across the communes.

The KAP survey revealed important gaps in knowledge and practices, resulting in considerable risks of fascioliasis transmission among the cohorts under the study. Low proportions of participants in the three communes were aware of fascioliasis. In addition, although more than half of those in three communes who were aware of fascioliasis stated the correct transmission routes of the disease, less than half of them did not know the signs and symptoms of fascioliasis. Considerable proportions of participants reported they ate improperly treated and raw vegetables, except for the cohort in Nhon Hau who performed good practice of drinking boiled water.

Considerable numbers of participants lived in households without a toilet and went to defecate outdoors, presenting further potential risks of fascioliasis transmission. The overall prevalence of fascioliasis in three cohorts found in this study (7.75%); was higher than some studies previously conducted in some Central provinces of Quang Nam (3.2%) Quang Ngai and Gia Lai (3.4%), Khanh Hoa (3.7%), Binh Dinh (6.0%), and Gia Lai (7.1%), and Phu Yen (7.1%)[35, 48, 52, 53], and lower than in others such as Quang Ngai (8.7%) and Gia Lai (10.2%)[54-56]. The differences might be attributed to the different time frames of surveys and or the use of different ELISA kits.

The serological surveys of this study were conducted in March 2013, which is at the end of the rainy season in Central Vietnam, and hence after the development season of *Lymnaied* snails as intermediate hosts of *Fasciola*. In other studies conducted in Central Vietnam, higher prevalence of human fascioliasis was found in summer-autumn months[45,48,56]; and lower morbidity of the disease was reported in other months of the year[33,52,53]. In addition, the seasonal transmission of fascioliasis is dependent on geographical characteristics, the adaptability of the intermediate host snails and the parasite itself[57].

In some previous studies researchers[32, 56] have used different test kits, produced by the National Institute of Veterinary Research-Ministry of Agriculture and Rural Development. Neither of these test kits have been assessed for validity for use in Vietnam, especially in the context of reported cross reactivityand suspected overlapped *Fasciola* species[15, 58-61]. In addition, studies on cross reactivity between fascioliasis and other trematodiasis in the region have not been reported.

Although this studyused the same test kits (FASCELISA) as other studies[40], the results may have differed as the interpretations of the test results were based on different criteria. In this study the results were based on the quantified OD readings, while other researchers read the results on the basis of the

antibody titres at 1/1600 and 1/3200 (positive results range from 1/3200-1/12800)[52-54, 62].

Most of the studies conducted in other countries use the ELISA-based serological rather than the manual coprological (stool) tests to identity the infection of fascioliasis in the human antibody because of its higher specificity and sensitivity. Particularly in the field-based settings the ELISA serological blood test provides conclusive immuno diagnosis as it possesses low cut-off sensitivity at all stages of the liver fluke life cycle, especially during the invasive or acute phases[63-65]. However, serological results may detect past infections, as the fluke antibodies may remain in the human body for a long period post-treatment and even after the elimination the flukes from the recipient's body[66]. In addition, it should be noted that limited commercial supplies of test kits, issues of specific antigens, test procedures, and lack of a validated optimal test system may challenge the serological diagnosis in many areas in which the disease is endemic, including Vietnam[67].

Clinical examinations reported abdominal pains in the right upper quadrant was the most complaint symptom among infected patients, followed by fatigue, urticarial (skin rash), and fever as among the signs recommended by the MoH as the guidelines on diagnosis and treatment of fascioliasis[36]. Similar results were also reported in previous studies conducted in some provinces of Central region[35, 56, 68], with colic pain in the right upper quadrant being the predominant symptoms of fascioliasis. However, the fluctuated proportions of reported symptoms in these studies might come from different number of reported cases, patients' recalls, and interpersonal skills of medical staff during clinical examinations[52]. Considered an important sign of fascioliasis, eosinophilia occurs in infected patients during the acute stage, with high concentrations of the white blood cells in peripheral vessels and liver granulomas as a result of the host defences against the infection[21, 69-71]. Subsequently, the MoH[36] recommended the use of elevated eosinophils as good indicators of suspected fascioliasis at the peripheral health facilities.

The findings in this survey of no difference in rates of fascioliasis infection between genders is consistent with other studies conducted in Central Vietnam[30, 43, 45, 52, 55, 72]. An explanation for this may be that in study areas, women were more often to contact with Fasciola larvae than men in daily household activities such as housekeeping, washing vegetables, preparing meals, washing clothes and grazing cattle[52, 73,76].

With respect to infection of fascioliasis by education level, the study reported that participants with education background up to secondary level presented much higher infection proportion than those with high school education and above; however, the only significant difference was found in Nhon Hau commune ($p<0.05$). The results in this study are consistent with reported results in previous studies, yet the differences might come from the different samples sizes and study localities[45, 52].

By occupation, farmers represented a higher proportion of infection in comparison with other occupations; however, no significant differences were indicated. Similar findings were reported in other studies, which were probably conducted in the rural areas with farmers as major attendants, whose typical career is related to exposing risks of fascioliasis infection[45, 52, 54, 77].

Parasitological diagnosis of fascioliasis was not conducted in this study due to pragmatic considerations. However, it has been recognized that animal reservoirs may play an important role in the transmission of fascioliasis[49,78,79]. In this study an assumption was made that the relationship between human and animal fascioliasis would correlate at a basic level[10]. Thus the study included consideration of those factors thought to contribute to the high prevalence in both human and animal fascioliasis, including low awareness of fascioliasis, unsafe living habits and high-risk daily practices.

In this study, less than 50% of the participants in the three communes had knowledge of fascioliasis. Among participants having the awareness of fascioliasis, although more than 50% knew the transmission routes of the disease, less than 50% did not know the symptoms and signs. Previous studies conducted in Central Vietnam indicated similar results[48, 56].

Noticeable proportions of participants in the communes reported eating raw and improperly treated vegetables, a common practice of Vietnamese people. Vegetables improperly treated prior to consumption or previously fertilized with cattle manure can lead to fascioliasis transmission in humans[80]. In Central Vietnam, studies[35, 45] have reported that some aquatic plants were contaminated with fasciola larvae, with rates varying from 0.40 to 1.34 metacercaria per kilogram of vegetable. Another study reported significant relationship between human fascioliasis and eating raw lettuce, a popular plant in mixed vegetables[54]. In Cuba, China, Japan, Thailand and elsewhere in the world, various studies reported similar connection between fascioliasis and raw consumption of vegetables such as watercress, houttuynia, and lettuce[22, 80-83]. Water-borne parasites including *Fasciola* larvae utilise water bodies as direct habitats for their intermediate and final hosts and for transmission of diseases[84]. Although some authors[54,55] reported no relationship between drinking unboiled water and fascioliasis transmission, others indicated the proportional infections of fascioliasis among unboiled water drinkers in Central provinces[52, 54, 55].

Humans also can facilitate fascioliasis transmission through their living habits. For example, outdoor and indiscriminate defecation, a common practice in developing countries, facilitates egg shedding[19, 85-87]. In this study, a high percentage of participants lived in households without toilets and went to defecate outdoors (on the hills, sand banks, rice field or into the river banks or other water sources), which spread the *Fasciola* eggs into the environments, facilitating the transmission risks of fascioliasis in the community.

Snails belonging to the *Lymnaeid* species are known for their intermediate hosts of *Fasciola*[8]. Increasingly, wide adaptation of the parasites to the existing *Lymnaeid* snail population in a new geographical areas contributes to the spread of fascioliasis[9]. In endemic areas of Europe, South America, Africa, Asia and the Middle East, the expansion of fascioliasis transmission in recent years has been reported to be the result of the distribution and ability of the aquatic *Lymnaeid* snails to adapt to a wide range of environmental niches[12-15]. In Central Vietnam there aret wo *Lymnaeid* snails (*L. viridis* and *L. swinhoei*)[88, 89]. In this study, the collection of *Lymnaeid* snails was conducted in May, the end of rice harvest and beginning of summer. This might explain a majority of *L.viridis* (prefer to live in rice field) caught in comparison with *L. swinhoei* (prefer

large water body habitats). In addition, as a result of the dry season, not many snails were caught in the aquatic biotopes. However, the appearance of *Fasciola* larvae in two snail species proves the capacity of larval transmission of fascioliasis even in the least favourable season of the year.

CONCLUSIONS

The high rate of fascioliasis infection, poor knowledge of the disease and frequency of high-risk behaviours supports the need for intervention measures to increase disease awareness and appropriate behaviours and practices in the community. Comprehensive fascioliasis control strategies are required. This study provides strong support that a broadly-based control model, including chemotherapy, vector control, health education, surveillance and management and evaluation, and involvement of the range of concerned agencies such as education, agriculture and community organizations, can be effective in the control and management of fascioliasis.

CONFLICT OF INTERESTS

The authors declared no conflicts of interest with respect to the research, authorship and/or publication of this article.

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REFERENCES

1. Keiser, J. and I. Utzinger, Food-borne trematodiasis. Clinical Miriobiological Review, 2009. 22: p. 466-483.
2. Ozturhan, H., et al., Seroepidemiology of Fasciola Hepatica in Mersin province and surrounding towns and the role of family history of the Fascioliasis in the transmission of the parasite. The Turkish journal of gastroenterology : the official journal of Turkish Society of Gastroenterology, 2009. 20(3): p. 198-203.
3. Sakru, N., et al., Fasciola hepatica Infection in echinococcosis suspected cases. Türkiye parazitolojii dergisi / Türkiye Parazitoloji Derneği = Acta parasitologica Turcica / Turkish Society for Parasitology, 2011. 35(2): p. 77.
4. Walker, S.M., et al., The distribution of Fasciola hepatica and Fasciola gigantica within southern Tanzania - constraints associated with the intermediate host. Parasitology, 2008. 135(4): p. 495-503.
5. Sothoeun, S., H. Davun, and B. Copeman, Abattoir study on Fasciola gigantica in Cambodian cattle. Tropical Animal Health and Production, 2006. 38(2): p. 113-5.
6. Mas-Coma, S., Epidemiology of fascioliasis in human endemic areas. Journal of Helminthology, 2005a. 79(3): p. 207-216.
7. A Marcos, L., et al., Hepatic fibrosis and Fasciola hepatica infection in cattle. Journal of Helminthology, 2007. 81(4): p. 381-6.
8. Maha, F.M.S., Epidemiological review of human and animal fascioliasis in Egypt. Journal of Infection in Developing Countries, 2008. 2(3): p. 182-189.
9. Mas-Coma, S., Human fascioliasis: Epidemiological patterns in human endemic areas of South America, Africa and Asia. Southeast Asian Journal of Tropical Medicine and Public Health, 2004b. 35 (Suppl 1): p. 1-11.
10. Mas-Coma, S., Epidemiology of fascioliasis in human endemic areas. Journal of Helminthology, 2005. 79(3): p. 207-216.
11. Taraschewski, H., Hosts and parasites as aliens. Journal of Helminthology, 2006. 80(2): p. 99-128.
12. Kenyon, F., et al., Sheep helminth parasitic disease in south eastern Scotland arising as a possible consequence of climate change. Veterinary parasitology, 2009. 163(4): p. 293-297.
13. Bargues, M.D., et al., Molecular characterisation of Galba truncatula, Lymnaea neotropica and L. schirazensis from Cajamarca, Peru and their potential role in transmission of human and animal fascioliasis. Parasites & vectors, 2012. 5(1): p. 174-174.
14. Mas-Coma, S., M.D. Bargues, and M.A. Valero, Fascioliasis and other plant-borne trematode zoonoses. International journal for parasitology, 2005b. 35: p. 1255-1278.
15. Mas-Coma, S., M.A. Valero, and M.D. Bargues, Fasciola, Lymnaeids and Human Fascioliasis, with a Global Overview on Disease Transmission, Epidemiology, Evolutionary Genetics, Molecular Epidemiology and Control, in Advances in Parasitology Vol 69, D. Rollinson and S.I. Hay, Editors. 2009b, Elsevier Academic Press Inc: San Diego. p. 41-+.
16. Molyneux, D.H., Control of human parasitic diseases: Context and overview, 2006, ELSEVIER ACADEMIC PRESS INC: SAN DIEGO. p. 1-45.
17. Mas-Coma, S., The Importance of Emerging and Re-emerging Zoonotic Diseases: Recognition, Monitoring and Control, in FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health, N.E.G. Odongo, M.;Viljoen, G. J. , Editor 2010, Food and Agriculture Organization of the United Nations, Rome.: Vienna, Austria. p. 277-284.
18. Fürst, T., J. Keiser, and J. Utzinger, Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. The Lancet infectious diseases, 2012. 12(3): p. 210-221.
19. Mas-Coma, S., M.A. Valero, and M.D. Bargues, Fasciola, Lymnaeids and Human Fascioliasis, with a Global Overview on Disease Transmission, Epidemiology, Evolutionary Genetics, Molecular Epidemiology and Control, in Advances in Parasitology Vol 69, D. Rollinson and S.I. Hay, Editors. 2009, Elsevier Academic Press Inc: San Diego. p. 41-+.

20. WHO, Triclabendazole and fascioliasis - A new drug to combat an age-old disease. . Fact sheet No. 191, 1998: p. 3 pages.

21. WHO, The "Neglected" Worms. Action Against Worms. 2007a.

22. Chen, J.-X., et al., An Outbreak of Human Fascioliasis gigantica in Southwest China. . PLoS ONE, 2013. 8(8).

23. Alawa, C.B.I., et al., In vivo efficacy of Vernonia amygdalina (compositae) against natural helminth infection in Bunaji (Bos indicus) calves. Pakistan Vet. J., 2010. 30: p. 215-218.

24. Charlier, J., et al., Associations between anti-fasciola hepatica antibody levels in bulk tank milk samples and production parameters in dairy heards. Preventive Veterinary Medicine, 2007. 78: p. 57-66.

25. Schweitzer, G., et al., Estimating the financial losses due to bovine fascioliasis in Switzerland. Veterinary Record, 2005. 157: p. 188-193.

26. Rioux, M.C., et al., Discovery and validation of serum biomarkers expressed over the first twelve weeks of Fasciola hepatica infection in sheep. International journal for parasitology, 2008. 38(1): p. 123-136.

27. Gray, G.D., R.S. Copland, and D.B. Copeman, Overcoming liver fluke as a constraint to ruminant production in South-East Asia, A.C.f.I.A. Research, Editor 2008, Australian Government: Canberra.

28. Nguyen, M.H., Workshop on reviewing malaria and helminthiasis control activities during 2006-2010 and planning for 2012, 2011, National Institute of Malariology-Parasitology and Entomology. p. 109-111.

29. Trieu, N.T., Annual workshop reviewing control activities of malaria, parasitic and vector-borne diseases in Central Vietnam in 2010, 2011, Institute of Malariology-Parasitology and Entomology, Quy Nhon. p. 7.

30. Huynh, H.Q., N.T. Trieu, and V.C. Nguyen, Effectiveness of the therapeutic regiment with triclabendazole on Fasciola gigantica infected patients in Central Vietnam from 2004-2006. Monograph of Scientific research studies, Instiute of Malariology-Parasitology and Entomology, Quy Nhon, 2007: p. 433-443.

31. Huynh, H.Q., N.T. Trieu, and V.C. Nguyen, Effectiveness and absortion of triclabendazole on Fasciola gigantica and preliminary use of metronidazole for treatment failure cases with triclabendazol in Central Vietnam from 2004-2006. Monograph of Scientific research studies, Instiute of Malariology-Parasitology and Entomology, Quy Nhon, 2007a: p. 444-456.

32. Nguyen, V.D., et al., Preliminary surveys of fascioliasis in Khanh Hoa province. Journal of Practical Medicine, 2003. 447: p. 125-128.

33. Nguyen, V.C., V.K. Nguyen, and V.T. Bui, Study on some epidemiological characteristics of fascioliasis in some areas of two provinces in Central Vietnam. Monograph of Scientific research studies (2001-2006). Institute of Malariology-Parasitology and Entomology, Quy Nhon, 2008.

34. GSO. Statistical data: 1. Administrative Unit and Climate. 2013 [cited 2013 June 22, 2015]; Available from: http://www.gso.gov.vn/default_en.aspx?tabid=773.

35. Nguyen, V.C., et al., Study on some epidemiological characteristics of fasioliasis in some study sites of Central Vietnam. Monograph of Scientific research studies, 2007: p. 410-416.

36. MOH, Guidelines on diagnosis and treatment of fascioliasis in humans, Departmen of Treatment, Editor 2006, Ministry of Health: Hanoi.

37. WHO, Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control. 2007b.

38. De, N.V., et al., Evaluation of triclabendazole in fascioliasis treatment in Vietnam in National Institute of Malariology-Parasitology and Entomology. National workshop on Malariology-Parasitology and Entomology during 2001-2005, NIMPE, Editor 2006, NIMPE: Ha Noi. p. 45-53.

39. Trieu, N.T., Annual workshop on malaria and helminthiasis control in 2005, planning for activities in 2006, 2006, Institute of Malariology-Parasitology and Entomology, Quy Nhon: Quy Nhon.

40. Tran, V.H. and T.K.D. Tran, Test kits for diagnoses of parasitological diseases. Journal of Practical Medicine, MOH, Viet Nam, 2004. 447: p. 112-116.

41. Lemeshow, S., et al., Adequacy of sample size in health studies1990, Chichester [England]: Published on behalf of the World Health Organization by Wiley.

42. Nguyen, V.D. and T.H. Le, Human fascioliasis and Fasciola species in Vietnam. Monograph of Scientific research studies, Instiute of Malariology-Parasitology and Entomology, Quy Nhon, 2007: p. 474-482.

43. Dang, T.C.T., Fascioliasis in Vietnam and suggested control measures. Journal of Malaria and Parasitology, 2007. 781: p. 129-131.

44. Dang, T.C.T. and M. To, Clinical, para-clinical characteristics and effectiveness of triclabendazole for the treatment of fascioliasis in patients admitted at North Quang Nam regional general hospital, 2006-2009 Journal of Malaria and Parasitology, 2010. 4: p. 45-55.

45. Nguyen, K.L., Study on some characteristics of fascioliasis (Fasciola spp.) and effectiveness of intervention measures in Dai Loc district, Quang Nam province, 2010, Vietnam Military Medical University: Ha Noi.

46. Bland, J.M. and D.G. Altman, Multiple Significance Tests - The Bonferroni Method.10. British Medical Journal, 1995. 310(6973): p. 170-170.

47. Chadha, V.K., Sample size determination in health studies. NTI Bulletin, 2006. 42(3-4): p. 55-62.

48. Nguyen, V.C., et al., Trial model of fascioliasis control in two communes of Phu Cat district, Binh Dinh province, Vietnam. Journal of Practical Medicine, 2011. 796: p. 152-156.

49. Nguyen, V.Q., et al., Animal infection of fasioliasis in Binh Dinh province and preventive and control measures Journal of Science and Technology, Department of Science and Technology, 2010.

50. Dang, N.T., Morphological identification of fress water invertebrates in Northern Vietnam. Journal of Science, Hanoi-Vietnam, 1980: p. 440-490.

51. Pham, N.D. and T.L. Nguyen, Characteristics of identifying cercaria of trematoda and differentiation of cercaria in Fasciola gigantica Cobbold 1885 in Lymnaid snails in Vietnam Journal of Biology-Vietnam National Centre for Natural Science and Technology, 2005. 27(3A): p. 31-36.

52. Nguyen, V.V., Study on human fascioliasis and effectiveness of intervention measures in a community of Quang Nam during 2009-2011., in Parasitology Department2012, National Institute of Malariology-Parasitology and Entomology: Ha Noi.

53. Nguyen, V.K., Prevalence of fascioliasis in some study sites of two central provinces of Quang Ngai and Gia Lai. Monograph of Scientific and Technological Research 2001-2006, 2007(Institute of Malariology-Parasitology and Entomology, Quy Nhon): p. 462-465.

54. Nguyen, X.M., Study on prevalence and related factors of human infection in some plain areas of Quang Ngai province, 2009, Department of Science and Technology-Quang Ngai province: Quang Ngai.

55. Tran, V.H., et al., Infection rates of Fasciola sp. in two communes Chu Pa and H'Bong of Gia Lai province Journal of Military Pharmaco-Medicine - Vietnam Military Medical University, 2008. 2: p. 75-78.

56. Nguyen, T.H., Study on some characteristics and treatment effectiveness of fascioliasis with triclabendazole in Tinh Ky and Nghia Son communes, Quang Ngai province, 2012, National Institute of Malariology-Parasitology and Entomology: Ha Noi. p. 163.

57. Mas-Coma, S., J.G. Esteban, and M.D. Bargues, Epidemiology of human fascioliasis: a review and proposed new classification. Bulletin of the World Health Organization, 1999a. 77: p. 340-346.

58. Ashrafi, K., et al., Plant- borne human contamination by fascioliasis. Am. J. Trop. Med. Hyg., 2006. 75(2): p. 295-302.

59. Le, T.H., et al., Human fascioliasis and the presence of hybrid/introgressed forms of Fasciola hepatica and Fasciola gigantica in Vietnam. International journal for parasitology, 2008. 38(6): p. 725-730.

60. Das, K., et al., Non-resolving liver abscess with Echinococcus cross-reactivity in a non-endemic

region. Indian Journal of Gastroenterology, 2007. 26(2): p. 92-93.

61. Ramzy, R.M.R., et al., An enzyme-linked immunosorbent assay for detection of IgG1 antibodies specific to human cystic echinococcosis in Egypt. Tropical Medicine & International Health, 1999. 4(9): p. 616-620.

62. Nguyen, K.L., N.A. Do, and N.S. Nguyen, Identification of fascioliasis infection in cattle and risk factors of human transmission in Dai Loc district-Quang Nam province. Jounal of Malaria, Parasitic and Entomological control-National Institute of Malariology-Parasitology and Entomology, 2011. 3: p. 76-82.

63. Kumar, N., S. Ghosh, and S.C. Gupta, Early detection of Fasciola gigantica infection in buffaloes by enzyme-linked immunosorbent assay and dot enzyme-linked immunosorbent assay. Parasitology research, 2008. 103(1): p. 141-150.

64. Rokni, M.B., J. Massoud, and A. Hanilo, Comparison of adult somatic and cysteine proteinase antigens of Fasciola gigantica in enzyme linked immunosorbent assay for serodiagnosis of human fasciolosis. Acta Tropica, 2003. 88(1): p. 69-75.

65. Reichel, M.P., Performance characteristics of an enzyme-linked immunosorbent assay for the detection of liver fluke (Fasciola hepatica) infection in sheep and cattle. Veterinary parasitology, 2002. 107(1): p. 65-72.

66. Apt, W., X. Aguilera, and F. Vega, Treatment of human chronic fascioliasis with triclabendazole: drug efficacy and serologic response. Am. J. Trop. Med. Hyg., 1995. 52: p. 532-535.

67. Espinoza, J.R., et al., EVALUATION OF FAS2-ELISA FOR THE SEROLOGICAL DETECTION OF FASCIOLA HEPATICA INFECTION IN HUMANS. American Journal of Tropical Medicine and Hygiene, 2007. 76(5): p. 977-982.

68. Huynh, H.Q., et al., Reviews on animal husbandry and awareness of community on fascioliasis in two Central provinces of Quang Nam and Quang Ngai. Monograph of Scientific research studies, Instiute of Malariology-Parasitology and Entomology, Quy Nhon, 2011.

69. David, A., et al., Immunoglobulin E and Eosinophil-Dependent Protective Immunity to Larval Onchocerca volvulus in Mice Immunized with Irradiated Larvae. Infection and Immunity, 2004. 72(2): p. 810-817.

70. Meeusen, E.N.T. and A. Balic, Do Eosinophils have a Role in the Killing of Helminth Parasites?, 2000, Elsevier B.V: ENGLAND. p. 95-101.

71. Aly, I.R., et al., Fasciola gigantica Fatty Acid Binding Protein (FABP) as a Prophylactic Agent against Schistosoma mansoni Infection in CD1 Mice. The Korean Journal of Parasitology, 2012. 50(1): p. 37-43.

72. Nguyen, K.L., T.C.T. Dang, and M. To, Fascioliasis infection in Dai Loc district, Quang Nam province and effectiveness of community-based control measreas. Journal of Military Pharmaco-Medicine - Vietnam Military Medical University, 2008. 33(2): p. 71-74.

73. Curtale, F., et al., Human fascioliasis infection: gender differences within school-age children from endemic areas of the Nile Delta, Egypt. Transactions of the Royal Society of Tropical Medicine and Hygiene, 2007. 101(2): p. 155-160.

74. Curtale, F., et al., Clinical signs and household characteristics associated with human fascioliasis among rural population in Egypt: A case-control study. Parassitologia, 2003. 45(1): p. 5-11.

75. Esteban, J.G., et al., High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1999. 93: p. 151-156.

76. Esteban, J.G., et al., High fascioliasis infection in children linked to a man-made irrigation zone in Peru. Tropical Medicine & International Health, 2002. 93: p. 151-156.

77. Phan, G.C., K.S. Pham, and D. Nguyen, Some reviews on fascioliasis in the first 9 months of 2008 in Gia Lai province. Monograph of Scientific research studies, National Instiute of Malariology-Parasitology and Entomology, Hanoi, 2008. 6: p. 82-87.

78. Nguyen, T.G., et al., Bovine fasciolosis in the human fasciolosis hyperendemic Binh Dinh

province in Central Vietnam. Acta tropica, 2011. 117(1): p. 19-22.

79. Nguyen, S.T., et al., Prevalence of Fasciola in cattle and of its intermediate host Lymnaea snails in central Vietnam. Tropical Animal Health and Production, 2012. 44(8): p. 1847-1853.

80. Marcos, L., et al., Risk factors for Fasciola hepatica infection in children: a case-control study. Transactions of the Royal Society of Tropical Medicine and Hygiene, 2006. 100(2): p. 158-166.

81. Rojas, L., et al., Fascioliasis: can Cuba conquer this emerging parasitosis? Trends in parasitology, 2010. 26(1): p. 26-34.

82. Muhsin Kaya Remzi Bestas Sedat, C., Clinical presentation and management of Fasciola hepatica infection：Single-center experience.世界胃肠病学杂志：英文版, 2011. 17(44): p. 4899-4904.

83. Aroonroch, R., et al., Hepatic fascioliasis due to Fasciola hepatica: a two-case report. Journal of the Medical Association of Thailand = Chotmaihet thangphaet, 2006. 89(10): p. 1770.

84. Zhou, X.-N., Prioritizing research for "One health - One world". Infectious diseases of poverty, 2012. 1(1): p. 1-1.

85. Agnamey, P., et al., Cross-sectional serological survey of human fascioliasis in haiti. Journal of parasitology research, 2012. 2012(Journal Article): p. 751951-3.

86. Tolan, R.W., Fascioliasis Due to Fasciola hepatica and Fasciola gigantica Infection: An Update on This 'Neglected' Neglected Tropical Disease. LABMEDICINE, 2011. 42(2): p. 107-116.

87. Crompton, D.W.T. and L. Savioli, Handbook of Helminthiasis for Public Health2006, Boca Raton, FL. USA: Taylor & Francis Group.

88. Dang, T.C.T., et al., Epidemiological charateristics and control measures of fascioliasis in Vietnam N.P. Works, Editor 2011, Ministry of Health: Hanoi.

89. Dang, T.T. and Y. Nawa, Fasciola and fascioliasis in Vietnam. Asia Parasitology, 2005. 1: p. 57-60.