



High prevalence of cattle fascioliasis in coastal areas of Thua Thien Hue province, Vietnam

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ABSTRACT. In Vietnam, especially central Vietnam, patients with fascioliasis are increasingly being reported. Since the fascioliasis is zoonotic, survey on the cattle fascioliasis should be informative for the control of human fascioliasis. In this study, the prevalence of cattle fascioliasis as well as the density of the intermediate host snails, *Lymnaea swinhoei* and *L. viridis*, were studied in Thua Thien Hue (TTH) province during 2014–2015. A total of 572 cattle feces were examined from 27 communes in 9 districts. *Fasciola* eggs were detected in cattle from 24 communes with an average prevalence of 23.4% (134/ 572). The highest prevalence was detected in cattle in the coastal plain terrain (31.0%) followed by plain (25.5%), mountain (21.7%), and low hilly (16.2%) terrains. The highest proportion of heavy infection (>200 EPG) was observed in the coastal plain terrain (36.1%), followed by mountains (20.0%), low hills (13.0%), and plains (8.9%). Low number of heavy infection, as well as relatively low prevalence in low hills and plains were associated with the extensive use of anti-fluke treatments. High number of intermediate host snails in low hilly and plain terrains also indicate high risk of fascioliasis. In this study, the density of *Lymnaea* snails in the coastal plain terrain was found to be very high (17.3 snails/m²) compared to that in previous studies. This is the first report indicating the recent expansion of cattle fascioliasis in the coastal region in Vietnam.

KEY WORDS: cattle, fascioliasis, *Lymnaea*, Thua Thien Hue province, Vietnam

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Human fascioliasis is emerging or re-emerging in many countries [58]. In Vietnam, the status of human fascioliasis has changed significantly in recent years; only 2 cases of human fascioliasis occurred in 1978 and 500 cases were reported from 1997 to 2000 [51]. However, the number of reported cases drastically increased recently: 3,838 (2006), 2,196 (2007), 2,000 (2008), 4,300 (2009) and 9,985 (2011) [52–54]. Importantly, 84.8–92.3% of cases of human fascioliasis occurred in central Vietnam [52]. Humans may be infected with *Fasciola* parasites by eating aquatic plants, by drinking fresh untreated water contaminated with metacercariae of the *Fasciola* spp. [1, 2, 6], or by consuming dishes with raw liver from cattle infected with the juvenile flukes [48].

Cattle infected with *Fasciola* spp. may be responsible for human infection since the cattle-raising area is often in close proximity to the aquatic plants grown for human consumption [6, 13, 17, 26, 34, 47]. The prevalence of cattle fascioliasis has been reported to be 22.0–23.5% in northern Vietnam [21] and 1.4–51.9% in southern Vietnam [20, 34]. In central Vietnam, where the highest number of human cases has been reported, survey on cattle fascioliasis was restricted in south–central Vietnam [33, 35], and no recent survey focusing on central and north–central Vietnam, including the Thua Thien Hue (TTH) province, has been reported. Thus, in the present study, an investigation into the infection of liver flukes (*Fasciola* spp.) in cattle of the TTH province was conducted, and the distribution of snails, which are the intermediate hosts, was examined. The knowledge of fascioliasis in this province may aid in the improved understanding of the disease in central Vietnam.

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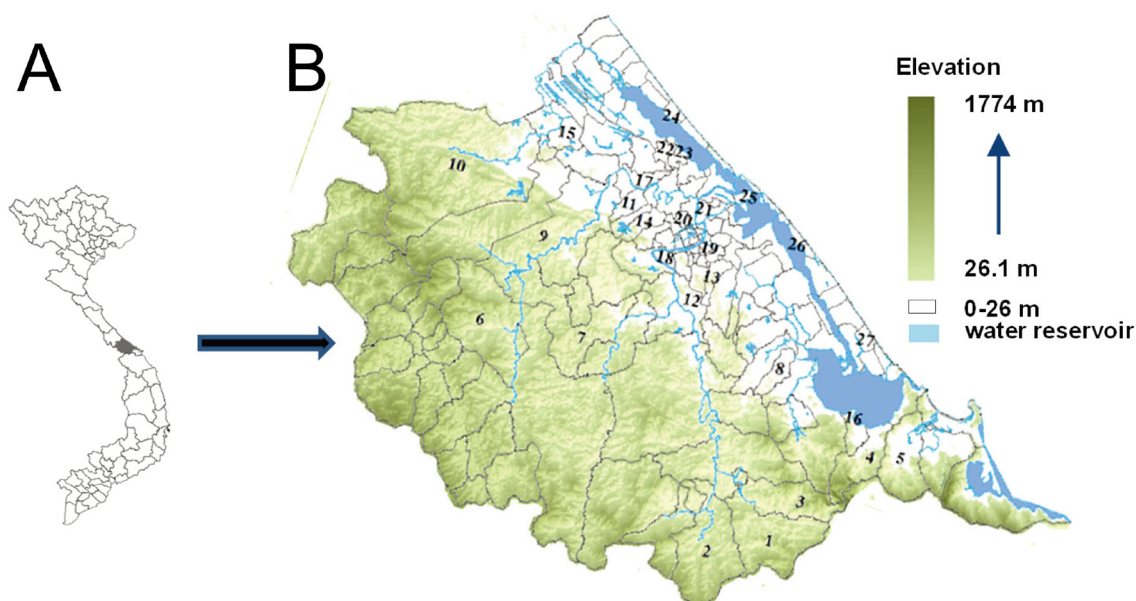


Fig. 1. A: Map of Vietnam. Thua Thien Hue (TTH) province is filled. B: Enlarged map of TTH province. Light blue color illustrated for water reservoir. Gradient green color represent altitude. Numbers indicated are communes where cattle feces and snails were sampled, which are corresponding to those indicated in Table 1.

Although fascioliasis is recognized in Vietnam, no national campaign for fascioliasis prevention has been established. In TTH province, cattle are treated for fascioliasis independently by owner's will or veterinarian's suggestion. Most of the fascioliasis surveillance were conducted by fecal examination for *Fasciola* eggs [20, 21, 33–35]. In order to file epidemiological data in TTH province, fecal egg counting was also applied in this study. Since individual medical treatment might affect the results of fecal examination, interview to cattle owners and veterinarians were conducted.

The species of the Lymnaeidae family are known as intermediate hosts for *F. gigantica* or *F. hepatica* [3, 27, 35, 43, 46]. In Vietnam, it has been reported that the most frequent intermediate hosts of *F. gigantica* are *Lymnaea swinhoei* and *L. viridis* [20, 35, 43, 57]. The habitat preference of snails is lowland plains with slow moving streams; generally, a high number of snails reside in this environment [8], resulting in the high prevalence of cattle fascioliasis [23, 24, 50]. Highlands and low hills with fast currents or coastal areas with higher salinity are considered unsuitable habitats for the snails [8, 23, 57], resulting in a low prevalence of the disease [20, 50, 57]. Thus, areas in TTH were categorized as mountain, low hilly, plain, or coastal plain terrains and were analyzed separately.

MATERIALS AND METHODS

Study area

TTH is located in north-central Vietnam (16.0 to 16.5° latitude and 107.0 to 108.8° longitude), with an area of 5,053,990 m², long and narrow land with an average width of 60 km and length of 127 km (Fig. 1A). TTH lies in the monsoon tropical area, which has a dry season that lasts from March to August and a rainy season from September to February.

TTH province involves 9 districts and 152 communes. There were 27 communes in 9 districts (Table 1) located near main rivers that were chosen as study areas and categorized into 4 topographic terrains by altitude; mountains (250–1,774 m), low hills (30–250 m), plains, and coastal plains (0–30 m) (Table 1). Coastal plain terrains were defined as plains areas facing the sea or lagoon (Fig. 1B).

Cattle-fecal sample collection

Fecal samples were collected from domestic yellow cattle and yellow crossbred with zebu raised in the study areas between April and October, 2014. Animals were selected randomly regardless of age or gender. In most of the communes, multiple smallholders shared pastures. In each commune, cattle were followed and feces were collected immediately after they dropped. Samples were collected one time in each commune to avoid double counting. More than 10 samples per commune were collected, except in Loc Tri and Binh Dien, where only 9 samples were collected due to low cattle numbers. For the sampled cattle, age was estimated by the owners' information and the number of horn growth rings (HGRs) [16, 29]. The sex of cattle was recorded but not separately analyzed. On each farm, about 10 g of fecal samples from each animal were collected using a polythene bag worn over the fingers. All samples were labeled and transported to the Parasitology Laboratory, Hue University for examination.

Table 1. Study area, prevalence of cattle *Fasciola* spp. infection, and treatment

Terrain	Commune	No. examined	No. infected	Prevalence (%)	Treatment ^{a)}
Mountain terrain	Thuong Lo (1)	25	0	0.0	Ra
	Thuong Nhat (2)	32	10	31.3	-
	Huong Loc (3)	23	12	52.2	-
	Loc Tri (4)	9	2	22.2	Ni
	Loc Thuy (5)	22	4	18.2	Ra
	Hong Ha (6)	27	2	7.4	Ni
Subtotal		138	30	21.7	
Low hilly terrain	Binh Dien (7)	9	1	11.1	Ra
	Loc Son (8)	19	1	5.3	Ni
	Phong Son (9)	29	3	10.3	Tr, Al
	Phong My (10)	20	7	35.0	-
	Huong Van (11)	15	9	60.0	- ^{b)}
	Thuy Bang (12)	23	1	4.3	Ni
Subtotal		142	23	16.2	
Plain terrain	Huong Chu (14)	21	14	66.7	Ni/ ^{c)}
	Phong Dien town (15)	20	1	5.0	Ni
	Phu Loc town (16)	28	1	3.6	Tr
	Quang Phu (17)	14	3	21.4	Bi
	Thuy Bieu (18)	25	17	68.0	-
	Xuan Phu (19)	21	4	19.0	Ni
Coastal plain terrain	Huong So (20)	18	5	27.8	Ni
	Phu Mau (21)	29	0	0.0	Tr
Subtotal		176	45	25.6	
Coastal plain terrain	Sia town (22)	10	5	50.0	-
	Quang Phuoc (23)	13	5	38.5	-
	Quang Ngan (24)	20	10	50.0	-
	Thuan An (25)	26	4	15.4	Ni
	Phu Dien (26)	24	0	0.0	Ni
	Vinh My (27)	23	12	52.2	-
Subtotal		116	36	31.0	
Total		572	134	23.4	

a) Abreviation of anti-fluke treatments are; Ra: Rafozanide, Ni: Nitroxylin, Tr: Triclabendazol, Al: Albendazol, Bi: Bithionol. b) Cattle were treated with Levamisol. c) Among 9 farms involved, cattle in 3 farms were treated with Nitroxylin and 6 farms with Levamisol.

Fasciola spp. egg detection by fecal sedimentation

Fasciola eggs were detected by the fecal sedimentation method [36]. Briefly, 5 g of cattle feces were re-suspended in 500 ml water and passed through a 250- μ m pore sieve colander. The retained material was washed thoroughly; the filtrate was transferred to a 500 ml conical beaker that was allowed to stand for 30 min; the supernatant was discarded. This step was repeated 3–5 times, depending on the sample. After the supernatant was discarded, the remaining 12–15 ml was transferred to a 10 cm petri dish and a few drops of methylene blue were added. The sediment was applied to a slide, covered with a coverslip, and examined under a microscope at 100 \times magnification to view the eggs. The procedure was repeated until all sediment was examined. Identification of *Fasciola* eggs was performed using the standard parasitological keys as described by Hussein [22], Phalee [42] and Valero [55].

Determination of eggs per gram (EPG) by the McMaster method

The positive feces for *Fasciola* eggs obtained through the fecal sedimentation method were processed to count the EPG using the McMaster method [19, 49]. Two grams of feces were placed into a flask containing 60 ml of ZnSO₄ saturated solution. While mixing vigorously a sample of the mixture was obtained with a pipette and transferred into one of the chambers of the McMaster slide. The procedure was repeated and the sample was put into another chamber. After 30 sec, the total number of eggs in the designated area of six chambers (900 μ l) were counted. The EPG was calculated by multiplying the number by 100/3. The intensity of infection with *Fasciola* parasites was classified as light (EPG \leq 100), moderate (100<EPG \leq 200) and heavy (200<EPG).

Chemotherapy for cattle fascioliasis

In each commune where cattle feces were sampled, farm owners and veterinarians working on their farms were interviewed for the use of parasitocidal medicines.

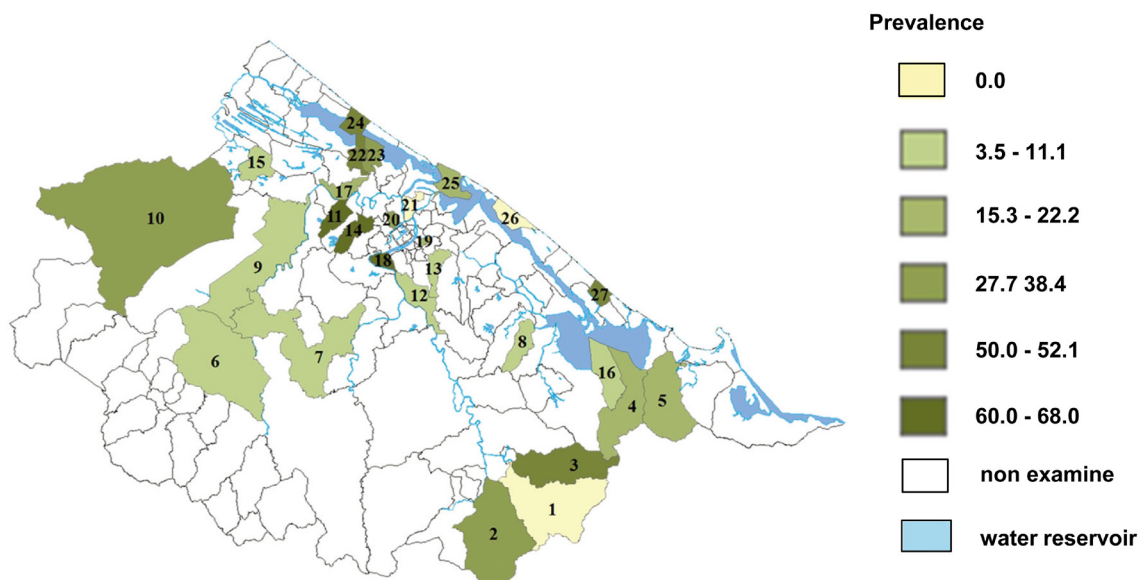


Fig. 2. Prevalence of *Fasciola* spp. of cattle in TTH province. Light blue color illustrated for water reservoir. Light yellow color indicate the commune where all cattle were negative for *Fasciola* spp. eggs. Gradient green color represent communes with cattle positive for *Fasciola* spp. eggs; intensity of color corresponding to the infectivity as indicated. White color indicates communes not examined. Numbers indicated are communes where cattle feces and snails were sampled, which are corresponding to those indicated in Table 1.

Collection and identification of *Lymnaea* snails

Lymnaea snails were collected and examined from November 2014 to October 2015. Snails less than 2 mm were not collected because species identification was difficult. Snail collection sites included irrigation canals, ponds, and paddy fields, which were located near cattle grazing areas. Snails were collected from at least 3 sites including each of above mentioned different habitats per commune. At each collection site, snails were collected from 5 locations. The average snail density (snails/m²) of each site was calculated from snail counts in 5 locations where at least one snail was found. A monthly average of snail density was calculated from sites in each terrain. At each sampling location, a 1-m² frame was established; all snails on the surface and those attached to water plants were picked up by hand. Snails that were in deep area were collected using a 50 cm diameter mesh bag. The contents of each mesh bag were transferred to a 70 cm bamboo sieve. *Lymnaea* snails, including *L. swinhoei* and *L. viridis*, known intermediate hosts of *F. gigantica*, were collected. Samples were transported to the laboratory where *Lymnaea* snails were identified morphologically according to the standard protocol of Dang *et al.* [7], and the snails identified as *L. swinhoei* and *L. viridis* were counted. The snail were identified as juveniles and adults size base on the shell length, 2–4 mm and above 4 mm for *Lymnaea viridis* [28], 2–5 mm and above 5 mm for *Lymnaea swinhoei* [43], respectively.

Topographic profile

To assess the topographic profile, ArcGis software 10.3 (Eri, Redlands, CA, U.S.A.), and a Digital Elevation Model from ASTGM2-N16E107 dataset to ASTGM2-N16E108 data aggregated to seconds, produced by the United States Geological Survey (USGS).

Data management and statistical analysis

The statistical analysis of data collected was reduced to contingency tables. Statistical Package for Social Science (SPSS), version 22.0 (SPSS Chicago Inc., Chicago, IL, U.S.A.) was used to determine Chi-square or Fisher's exact test where appropriate *P* values were less than 0.05 (*P*<0.05).

RESULTS

A total of 572 cattle fecal samples were collected. The prevalence of cattle fascioliasis varied within each terrain, as well as between terrains (Fig. 2 and Table 1). Cattle in 24 of 27 communes in the four terrains were found to be positive for *Fasciola* eggs. The highest and second highest prevalence rates were observed in the communes belonging to plain terrain—68.0% in Thuy Bieu and 66.6% in Huong Chu—followed by 60.0% in Huong Van, a commune in low hilly terrain. Interestingly, the highest average prevalence of *Fasciola* spp. in the TTH province was found in the coastal plain (31.0%), followed by plain (25.5%), mountain (21.7%), and low hilly (16.2%) terrains.

The distribution of the severity of infection in the 572 cattle from all terrains examined was as follows: light, 10.5% (60/572);

Table 2. *Fasciola* spp. infection intensity in cattle of TTH province in association to the age and topography

Age ^a	Mountain terrain			Low hilly terrain			Plain terrain			Coastal plain terrain			Total ^d (%)
	Light ^b (%)	Moderate (%)	Heavy (%)	Light (%)	Moderate (%)	Heavy (%)	Light (%)	Moderate (%)	Heavy (%)	Light (%)	Moderate (%)	Heavy (%)	
<1	0/0 ^c (–)	0/0 (–)	0/0 (–)	1/1 (100.0)	0/1 (0.0)	0/1 (0.0)	2/2 (100.0)	0/2 (0.0)	0/2 (0.0)	2/2 (100.0)	0/2 (0.0)	0/2 (0.0)	5/30 (16.7)
1–3	6/12 (50.0)	4/12 (33.3)	2/12 (16.7)	7/8 (87.5)	1/8 (12.5)	0/8 (0.0)	18/29 (62.1)	10/29 (34.5)	1/29 (3.5)	5/15 (33.3)	5/15 (33.3)	5/15 (33.3)	64/267 (24.1)
>3–5	5/9 (55.6)	3/9 (33.3)	1/9 (11.1)	2/6 (33.3)	3/6 (50.0)	1/6 (16.7)	1/9 (11.1)	6/9 (66.7)	2/9 (22.2)	3/13 (23.0)	6/13 (46.2)	4/13 (30.8)	37/150 (24.7)
>5	5/9 (55.6)	1/9 (11.1)	3/9 (33.3)	2/8 (25.0)	4/8 (50.0)	2/8 (25.0)	1/5 (20.0)	3/5 (60.0)	1/5 (20.0)	3/6 (50.0)	1/6 (16.7)	2/6 (33.3)	28/125 (22.4)
Total	16/30 (53.3)	8/30 (26.7)	6/30 (20.0)	12/23 (52.2)	8/23 (34.8)	3/23 (13.0)	22/45 (48.9)	19/45 (42.2)	4/45 (8.9)	13/36 (36.1)	12/36 (33.3)	11/36 (30.6)	134/572 (23.4)

a) Ages were estimated by owners' information and the number of horn growth rings (HGRs) [16, 29]. b) The intensity of infection with *Fasciola* parasites was classified as light (EPG<100), moderate (100<EPG<200) and heavy (200<EPG). c) Infection intensity presented as numbers of infected cattle in each category /total number of cattle infected in each terrain (percentage). d) Total infection intensity presented as numbers of infected cattle in each age category /total number of cattle in each age category (percentage).

moderate, 8.4% (24/572); and high, 4.5% (26/572). Cattle with the fluke eggs in each terrain were listed according to the severity of infection and age group (Table 2). The highest proportion of high severity of infection was detected in the coastal plain (36.1%), followed by mountain (20.0%), low hilly (13.0%), and plain (8.9%) terrains. Although fecal examinations for cattle less than 1 year were limited, the fluke eggs were found in 16.3% (5/30) of cattle less than 1 year, which was slightly lower than cattle in other categories, and all of them were light infections (Table 2). No significant difference of infectivity was observed between cattle aged 1 year and older.

As for the control of fascioliasis, rafozanide, nitroxylin, triclavendazol, alvendarol coupled with triclavendazol, and bithionol were used (Table 1). In this study, 60.1, 75.4, 75.6 and 43.1% of examined cattle in mountain, low hilly, plain and coastal plain terrains, respectively, were from farms using anti-fluke chemicals (Table 3). Cattle in farms located in the same commune were treated as the same way, except for Huong Chu, where 3 out of 9 farms used nitroxylin. All infected cattle in Huong Chu were from farms without anti-liver fluke treatment. In some farm, levamisole was treated. The effect of anti-fluke treatment on cattle fascioliasis prevalence was shown in Table 3. In all terrains, anti-fluke-treated cattle showed lower prevalence (6.5–10.5%) compared to non-treated cattle (40.0–72.1%). The fascioliasis prevalence of non-used cattle in the plain terrain (72.1%) was higher than those in other terrains (40.0–48.5%) (Table 3). Average EPG of positive cattle were not different between different terrains, as well as between anti-fluke-treated and non-treated cattle (Table 3).

The monthly density of *L. swinhoi* and *L. viridis* was studied in four topographies from September 2014 to October 2015 in Thua Thien Hue province (Fig. 3). A total of 43,507 snails were collected during this period. Snails were collected from each terrain; mountain terrain, 170; low hilly terrain, 14,128; plain terrain, 24,053; and coastal plain terrain, 5,156. In mountain terrains, where the velocity of streams is high, snails were found only in 2 months; April and September. They were most commonly observed in paddy fields and small canals of irrigation systems year-round in low hilly, plain, and coastal plain terrains, exceptions included the month of October in low hilly terrain and December, May, and July in coastal plain terrain. Seasonal changes of the snail density in the low hilly, plain, and coastal plain terrains were similar. The snail decline in December may have been due to floods after the peak of rain in November. In January, small size snails increased. The snail density again declined in March. At the middle and end of the dry season, in August in low hilly terrain and October in plain terrain, snail density increased (Fig. 3).

DISCUSSION

In Vietnam, fascioliasis is most endemic in low hilly or plain terrains, respectively [13, 14, 32, 44, 57]. In this study, the prevalence of *Fasciola* parasite in low hilly and plain terrains were 16.2 and 25.6%, respectively, which were relatively lower compared with previous researches in which those in low hilly and plain terrains were between 28.0 and 76.0% [32]. In addition, EPG of *Fasciola* eggs-positive feces was also relatively lower in low hilly and plain terrains (Table 2). One of the possible

Table 3. Effect of anti-flukes on cattle prevalence and EPG

Terrain	Treatment ^a	Prevalence ^b	Average EPG ^c
Mountain	yes	9.6 (8/83)	171.0 ± 72.3
	no	40.0 (22/55)	118.1 ± 89.6
Low hilly	yes	6.5 (7/107)	138.1 ± 80.5
	no	45.7 (16/35)	120.9 ± 65.4
Plain	yes	10.5 (14/133)	130.9 ± 59.3
	no	72.1 (31/43)	118.2 ± 70.5
Coastal plain	yes	8.0 (4/50)	75.3 ± 16.5
	no	48.5 (32/66)	182.4 ± 93.2
Total		23.4 (134/572)	138.1 ± 81.6

a) Data on each anti-fluke treatment described in Table 1 were combined. b) Data are described as % prevalence followed by (infected/ total cattle examined). c) Data are described as mean ± s.d.

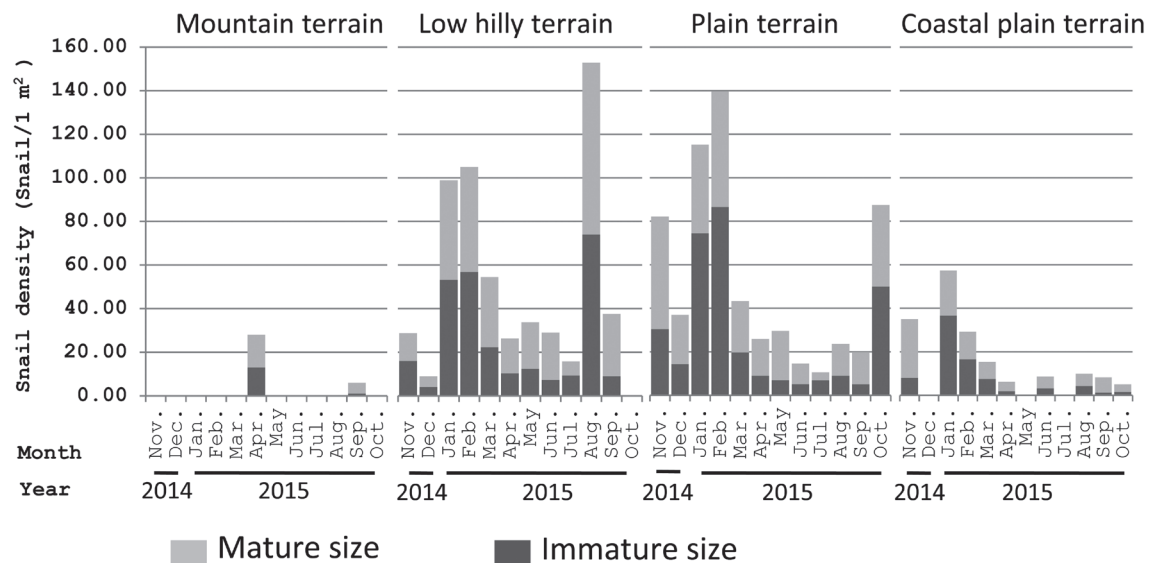


Fig. 3. Monthly density of the intermediate hosts of *Fasciola* spp. Only *Lymnaea swinhoei* and *L. viridis*, known intermediate hosts of *F. gigantica*, were identified morphologically according to the standard protocol of Dang *et al.* [7] and collected. Snails were separately counted for mature and immature sizes as indicated. Average density of snails in each terrain are calculated and presented as snail per m².

reason for the decrease in number of heavy infection in low hilly and plain terrains is deworming treatments for cattle, since the prevalence of fascioliasis in untreated cattle in low hilly and plain terrains was comparable or rather higher (72.1% in plain terrain) than other terrains (Table 3). The number of the intermediate host snails collected in this study was also high in plain terrain, followed by low hilly terrain, indicating the high risk of *Fasciola* infection in these areas. It was also notable to find high prevalence of fascioliasis in non-treated cattle from mountain terrain (Table 3), though snail density was very low.

There might be possible reasons for the seasonal change in snail density; the declination of snail density in December may have been due to the increasing water surface in rainy season plus with floods after the peak of rain in November. An increasing number of juvenile size snails observed indicated the hatching of new generations in January and February in low hilly, plain and coastal plain terrain. The snail density again declined in March to April, which may be due to the pesticides, such as dichlorvos, dinotefuran, and isoprocarb for preventing brown plant hopper (*Nilaparvata lugens*) and rice stem borers and herbicides, such as fenoxaprop-P-ethyl, diquat, paraquat, and ethoxysulfuron, for preventing weed in paddy field, since some of these pesticides and herbicides were reported to affect snail productivity and mortality [5, 15, 56]. The importance of these environmental pesticides used for paddy field on intermediate host *Lymnaea* snails should be studied in the future. Small floods in April may also contribute to the decrease of the snail density. At the middle and end of the dry season, in August in low hilly terrain and October in plain terrain, snail density increased possibly due to the reduction of water and enrichment of snails (Fig. 3).

The high rate of heavy infection in coastal plain terrain (36.1%, Table 2) may indicate the recent expansion of infection in coastal plain terrain. Several risk factors may explain the high prevalence of fascioliasis in the coastal plains of TTH province, Vietnam; the expansion of dams for blocking brackish water entry to the field for agricultural production and irrigation systems for supplying fresh water [9–12, 18, 30, 31, 37–41], leading to the enlargement of field for cultivation and cattle raising, may enlarge the habitable area for intermediate host snails [4, 45], as is shown in bilharziasis [25], and enhance snail migration and/or the presence of *Fasciola* parasite eggs. Floods during the rainy season may also contribute to the dissemination of snails and *Fasciola* eggs to the coastal terrain. The increase of number of cattle introduced, both infected and uninfected with *Fasciola* spp., may also contribute to the high prevalence of fascioliasis. Overall, these factors lead to an increased distribution of the intermediate host to coastal areas. In this study, the density of the intermediate host snail of *Fasciola* spp. in coastal plains was high (17.3 snails/m²), varying from 0–57.4 snails/m². This contrasts with results of previous studies, in which the density of the intermediate host in coastal areas was low [57]. For the control of fascioliasis in the coastal terrain, further surveys and studies on intermediate host snails including their infectivity of *Fasciola* larvae may be required.

In the present study, the use of anti-flukes were also surveyed in addition to the general fecal egg examination. It is true that EPG counting for all cattle is important for the comparison to the regional data from other province in Vietnam or from other countries. Knowing the use of medical treatment would provide additional information to control the disease.

CONFLICT OF INTEREST. None declared.

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