Original Article

Prevalence, risk factors, and spatial distribution of *Fasciola* in carabao and intermediate host in Baybay, Leyte, Philippines

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ABSTRACT

Fasciolosis remains an important parasitic disease of ruminants in Southeast Asia. We investigated the prevalence and spatial distribution of *Fasciola* spp. in carabao (*Bubalus bubalis*) and snail intermediate hosts (IHs) in Baybay, Leyte, Philippines. Using one-stage cluster sampling, we examined 335 fecal samples of carabaos from 138 herds for *Fasciola* spp. eggs, and in parallel collected IH snail data in the area. Risk factor analyses were performed and GIS maps were created for *Fasciola* spp. prevalence and IH snail distribution. The prevalence of *Fasciola* spp. in animal and herd level was 63.58% and 86.96%, respectively. Risk factor analyses showed that increasing age and drinking from irrigation water were highly associated with *Fasciola* infection in carabaos. Inversely, access to wallowing ponds seemed to be a significant protective factor. Lymnaeid snail presence was higher in areas with intensive networks of irrigated water system and rivers, land elevation above sea level, and moderate vegetation. Our epidemiologic data suggest a high endemicity of *Fasciola* spp. in Baybay, Leyte and present essential factors to consider in implementing control measures.

1. Introduction

Fasciolosis or liver fluke infection is an important parasitic disease of ruminants caused mainly by *Fasciola hepatica* and *F. gigantica*. Both species complete their life cycle by infecting the intermediate snail host and the definitive host such as sheep, goat, cattle, and buffalo. These parasite species inhabit and damage the liver of the mammalian host leading to biliary cirrhosis, anorexia, general intoxication, and death (Belina et al., 2015). In the Philippines, *F. gigantica* is the most common liver fluke in ruminants (Molina et al., 2005).

The Philippine ruminant industry considers *Fasciola* infection as a major setback in attaining the full potential of carabao production (Domingo, 2014). The economic impact brought by the parasite comprises the cost of interventions, diminished production of all ruminant products, reduced animal reproductive capacity, reduced draught power, and increased mortality. Therefore, concerned authorities should prioritize the formulation of effective measures to stop fasciolosis in livestock (Molina-Hernández et al., 2015).

The strategies to control fasciolosis often involve anthelmintic treatment in infected animals and molluscicide application in the field. Among the biggest challenges, however, are the increasing reports of anthelmintic resistance and the environmental risks of molluscicides. These concerns necessitate the call for a holistic strategy in controlling fasciolosis (Gray et al., 2008; Calumpang et al., 1995). Sound epidemiology proves to be an effective approach to improve the timing and efficiency of intervention. Previous studies highlighted the importance of epidemiologic data in addressing *Fasciola* infection and fasciolosis, specifically. For example, Pedersen et al. (2014) used the geospatial distribution of intermediate snail host and parasite transmission to anticipate the potential spread and predict outbreaks of the disease. In addition, various epidemiologic studies incorporated the Geographic Information System (GIS) to visualize spatial prevalence and high-risk areas for a rapid formulation of preventive and control measures against *Fasciola* infection (Stensgaard et al., 2006; Tum et al., 2004; Malone et al., 1998).

In the Philippines, studies on the epidemiology of *Fasciola* infection and its geospatial distribution remain limited, if not unreported. A few studies on *Fasciola* spp. prevalence in ruminants are available from Samar (Gordon et al., 2015), Nueva Ecija (Domingo, 2014), and selected areas in Mindanao (Molina et al., 2011). In this study, we present epidemiologic data that suggest high endemicity of *Fasciola* infection in carabaos and the risk factors independently associated with the parasite prevalence in Baybay, Leyte. We further created a GIS map to visualize easily the geographic pattern of the intermediate host and *Fasciola* infection. Our goal is to provide valuable information for development of future intervention strategies in Baybay, Leyte and its neighboring

Fig. 1. Study location. The dots are the sites where carabaos are pastured and sampled. The red and blue dots represent carabaos that are either positive or negative for *Fasciola* infection, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Multivariate logistic regression analysis of the risk factors associated with *Fasciola* infection in carabao.

Variable	Adjusted odds ratio	95% CI	Coef.	P-value
Age of animal	1.13	1.05–1.24	0.13	0.001
Wallowing pond	0.13	0.08–0.23	−2.00	0.001
Irrigation water	10.40	1.33–81.65	2.34	0.026

95% CI = 95% Confidence Interval; Coef = Coefficient.

localities.

2. Materials and methods

2.1. Description of the study area

Baybay, Leyte is located in the eastern part of the Visayan Region, the central part of the Philippines (Fig. 1). Baybay covers 92 small administrative units called barangays, 68 of which are rural. The eastern portion is mountainous, while the east side slopes down towards the shoreline. The common means of livelihood are farming, fishing, and forest-related activities. The region has no seasonal demarcation. During the study period, the average humidity and rainfall was 78.43%, and 87.28 mm, respectively. The ambient temperature was between 29 and 32.1 °C.

2.2. Study design and sampling

A cross-sectional study was conducted between December 2015 and June 2016. For sample size determination, we initially collected records of the carabao population from the Department of Agriculture-Baybay and the Philippine Carabao Center-VSU. After the determination of carabao distribution per barangay (i.e., 17 rural barangays with carabaos), we performed a one-stage cluster sampling using the StatCalc of the Epi-Info™ 7 (CDC, Atlanta) software. At the level of 95% confidence interval, sampling of at least 23 carabaos from each barangay, for a
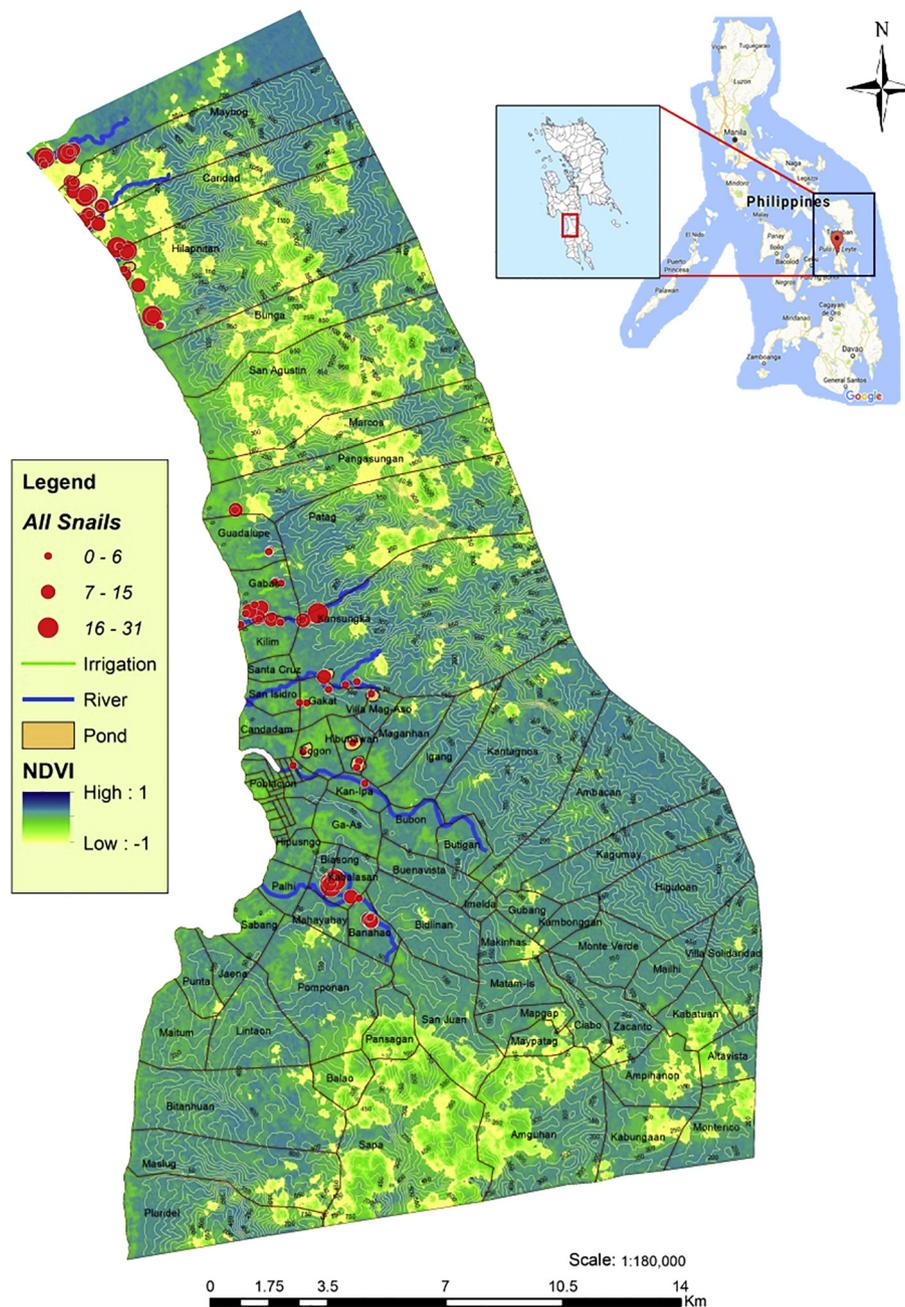
Fig. 2. Spatial distribution of *Fasciola* spp. intermediate snail host in Baybay, Leyte. The diameter of red circle represents the number of IH snail collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

total of ≥ 391 animals, was required. However, due to on-site sampling limitations (e.g., insurgent areas), we sampled 335 animals in 138 herds. Each herd had at least 1 carabao.

Snail sampling and georeferencing the site using a handheld Global Positioning System (GPS) device (Gecko 101 Garmin®) were conducted in rural barangays, specifically in areas where farmers use their carabaos for field work and pasture and wallowing areas. Riverbanks, ponds, and irrigation streams were also included for sampling. Two to nine collection spots were selected randomly for every sampling site. The entire 10-m radius for each spot was surveyed for the presence of snails. A total of 1189 snails from 92 GPS spots were collected and morphologically classified into either *Fasciola* spp. intermediate host (IH) or non-IH.

2.3. Fecal sampling and analysis

The collection of a fecal sample from carabao was performed either through direct rectal collection or free catch method. The collected samples were placed in a labeled container and stored on ice to stop the development and hatching of *Fasciola* eggs. Examination of *Fasciola* eggs was carried out using the modified Danish Bilharziasis Laboratory (DBL) technique (Carabin et al., 2005). *Fasciola* eggs were identified following the descriptions and measurements described by Valero et al. (2009).

2.4. Snail identification

Morphological identification of snails under the family Lymnaeidae was conducted according to the visual identification key of lymnaeid

Table 2

Multivariate logistic regression analysis of the environmental factors associated with the distribution of lymnaeid snail in 92 sampling areas.

Variable	Adjusted odds ratio	95% CI	Coef.	p–value
Bodies of water				
Pond	1	-	-	-
River	6.18	1.24–30.95	1.82	0.027
Irrigation	20.22	3.66–111.71	3.01	0.001
Land elevation				
Below sea level (− 21 to 0 m)	1	-	-	-
Above sea level (3 to 8 m)	4.75	1.57–12.24	1.56	0.030
NDVI				
Low vegetation (< 0)	1	-	-	-
Moderate vegetation (0 to 1)	4.33	1.34–14.00	1.46	0.015

95% CI = 95% Confidence Interval; Coef = Coefficient; NDVI = Normalized Difference Vegetation Index.

snails found in the Philippines (Young and Fontanilla, 2014). In addition, snail samples were morphologically compared to the lymnaeid specimens at the Parasitology Laboratory of the College of Veterinary Medicine, Visayas State University. Snails were identified to the genus level and lymnaeid snail genera, i.e., *Lymnaea, Radix,* and *Myxas,* were considered potential IH of *Fasciola spp.* (Correa et al., 2010).

2.5. Trematode larvae identification

Sampled snails were crushed using mortar and pestle or between two glass slides. The crushed flesh and fluid were examined under high power objective for *Fasciola*-like larval morphology. The identification of *Fasciola* larval stages was made according to published works elsewhere (Phalee et al., 2015; Nguyen et al., 2012; Soulsby, 1982).

2.6. Survey questionnaire

A structured survey questionnaire was designed to capture the information of the owner, animal, and management practices. The questions were made in the English language and translated into local dialect during the face-to-face interview. The gathered data were organized in Microsoft Excel.

2.7. Geographic information system (GIS) mapping

GIS maps were created using the ArcGIS software 10.3 (Esri, CA, USA). The map was formed by adding the coordinates of the samples on the layers of geographic and administrative shapefiles. Shapefiles of the administrative boundaries and detailed bodies of water were derived from the Philippine GIS Data Clearinghouse (philgis.org). Elevation shapefile was derived from SRTM 90 m Digital Elevation database. The Normalized Difference Vegetation Index (NDVI) was computed using the Landsat Enhanced Thematic Mapper (ETM) image.

2.8. Data management and analysis

Risk factor analyses were performed using Epi-Info™ 7 (CDC, Atlanta, GA, USA). Firstly, the associations between *Fasciola* infection and potential risk factors in carabao were evaluated using Yates' corrected chi-square test. The variables with *p*-value < .20 were retained. Following the Erb (2006) approach of handling complex data, the final multivariate model was selected when all the independent variables entered and the regression model had a p-value of < 0.05.

In the analyses of snail, we assessed the associations between the IH snail distribution(i.e., GPS sampling sites) and the environmental factors. Also, we assessed the association between *Fasciola* larvae infection in snail and the environmental factors. The environmental variables collected were land elevation, bodies of water, and NDVI. Daily rainfall and temperature did not vary throughout the study so were excluded from the analysis. Univariate logistic regression analysis was initially performed to screen out environmental variables that were unlikely to be associated with the dependent variable. The variables that passed the screening (p < 0.20) were included in the multivariate logistic regression model. The multivariate analysis was conducted through backward elimination and the *p*-value of < 0.05 was the basis for the final model.

3. Results

3.1. Prevalence of Fasciola spp. in carabaos

A total of 335 animals and 138 herds were examined for the presence of *Fasciola* spp. eggs using modified DBL technique. At the animal level, 63.58% (95% CI 58.15% - 68.70%) of the carabaos showed *Fasciola* infection. The herd-level prevalence of *Fasciola* infection was 86.96% (95% CI 80.17% - 92.08%). Eight out of 17 barangays showed 100% herd prevalence. These barangays were Cogon, Maybog, Gacat, San Isidro, Pangasugan, Ciabo, Mapgap, and Maganhan. The remaining barangays showed *Fasciola* spp. herd prevalence between 60 and 92%.

3.2. Risk factors of Fasciola infection in carabaos

Of the putative risk factors analyzed for *Fasciola* infection in carabaos (Supplement 1), three were identified as the most significant variables. The most significant risk factors were the animal's age, irrigation water, and wallowing pond (Table 1). The average and median age of carabaos in the study area were 4.7 and 3 years, respectively. *Fasciola* infection was higher in older animals. For every year of increased age of the carabao, the odds ratio (OR) of *Fasciola* infection significantly increased by 1.13 (95% CI 1.05–1.24; $p = .001$). Moreover, animals that drank water from the irrigation system of rice fields were 10.40 times (95% CI 1.33–81.65) more likely to be infected with *Fasciola spp.* ($p = .026$). On the contrary, use of wallowing ponds appeared to be a protective variable (OR 0.13 [0.08–0.23]) against *Fasciola* infection (p < 0.001). We also created an interaction model for the three risk factors to have an idea of the current field scenario by using the formula log y = x (yrs) + x2 (irrigation) – x3 (pond). The model suggested an almost double (OR 1.6) the odds of acquiring *Fasciola* infection when a 10-year-old carabao wallows in the pond and drinks from irrigation water.

3.3. Intermediate host distribution

From the total of 92 GPS sampling sites, 1189 snails were collected and classified into either IH or non-IH. The proportion of the IH was 62.91% (95% CI 60.16%- 65.66%), of which 58.03% (95% CI 55.22%- 60.84%) were *Lymnaea*, 4.46% (95% CI 3.29%–5.63%) were *Radix*, and 0.42% (95% CI: 0.05–0.79) were *Myxas*.

3.4. Environmental factors associated with IH snail distribution

Fig. 2 shows the spatial distribution of IH snails in Baybay, Leyte. Waterbody, land elevation, and vegetation were significantly associated with IH snails' proliferation (p < 0.05; Table 2). The odds of finding lymnaeid snails were significantly higher in areas within irrigation water (OR 20.22; p = 0.001) and rivers (OR 6.18; p = 0.027). On the other hand, wallowing ponds were unlikely to contain IH snails (p < 0.05). We observed an increase in IH for every meter increase in land elevation (p = 0.04). The odds of finding lymnaeid snail was significantly higher in areas above sea level (3 to 8 m) than those areas below sea level (p = 0.030). Based on the NDVI values, moderately
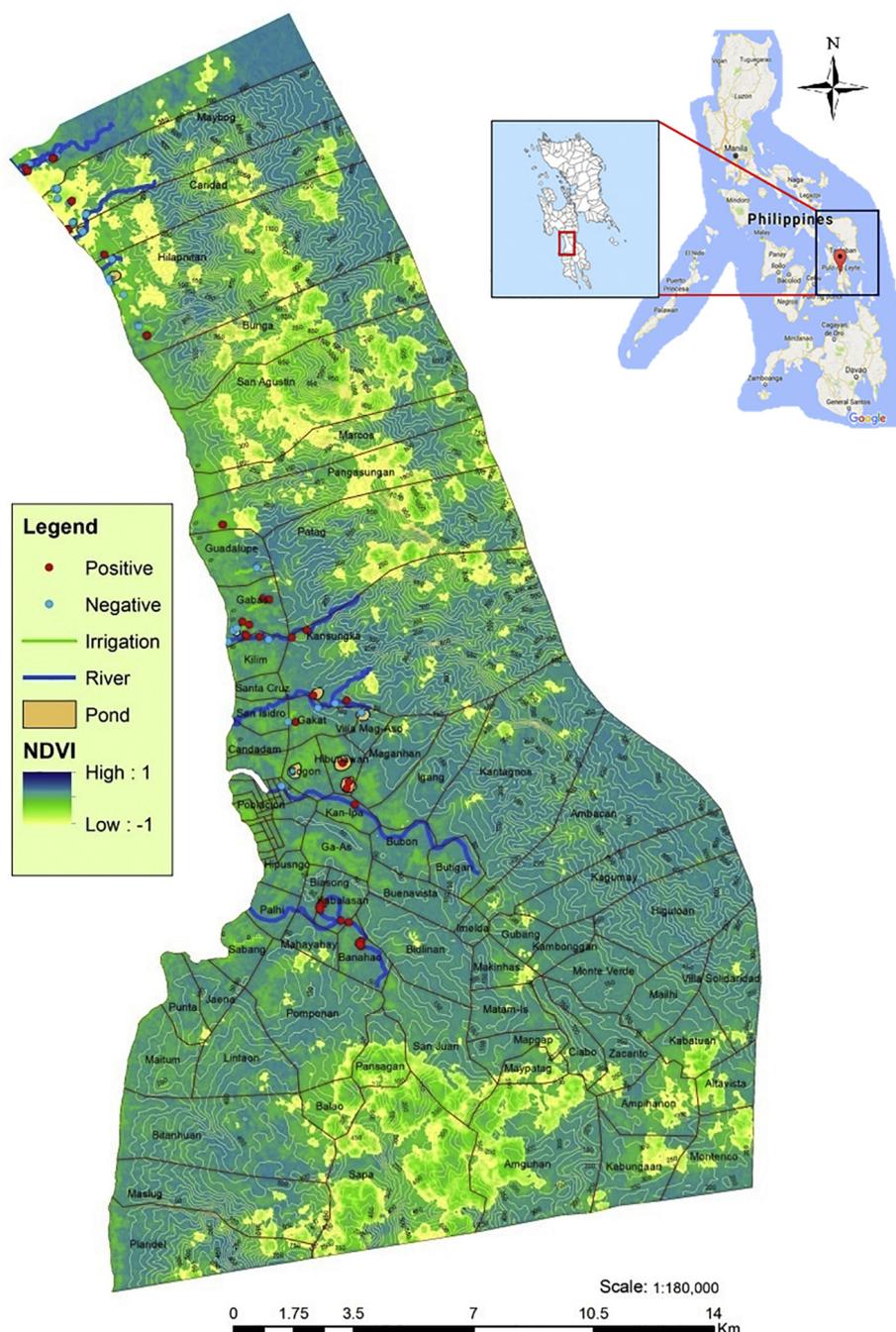
Fig. 3. Spatial distribution of *Fasciola* larvae in intermediate snail host in Baybay, Leyte. The red dots represent the 10-m radius where IH snails are collected and positive for *Fasciola* larvae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

vegetated areas (NDVI of 0 to 1) have more IH snails than in lowly vegetated areas (NDVI < 0; p = 0.015).

3.5. Fasciola larvae in IH snail

Out of 748 intermediate host snails collected, 495 were positive (66.18% [62.64%–69.54%]) for *Fasciola* larvae. Fig. 3 presents the map of focal areas where *Fasciola* larvae were positive or negative. Moderate vegetation appeared to be associated with *Fasciola* larvae positivity in IH snail. We observed three-times more *Fasciola*-positive IH snails in moderately vegetated areas than those in lowly vegetated (p < 0.001). On the

4. Discussion

We investigated the prevalence and risk factors of *Fasciola* spp. both in the definitive carabao host and intermediate snail host in Baybay, Leyte, Philippines. Our results showed that 86.96% of the herd and 63.58% of the individual animals examined were positive for *Fasciola* spp. eggs. A study in Samar, the neighboring island of Leyte, reported a very high prevalence between 95 and 96% of *F. gigantica* eggs in cattle and carabao (Gordon et al., 2015). These findings indicate high endemicity of *Fasciola* in many areas of Leyte and Samar. *Fasciola* hyperendemicity in the area may cause a possible spill-over transmission between livestock and wildlife (Portugaliza et al., 2015). On the other hand, studies in other Philippine municipalities showed relatively lower prevalence when compared to our results. The prevalence of *Fasciola*

infection in carabao is 16.25% in Gabaldon Nueva Ecija (Domingo, 2014), and 51.11% in selected municipalities of Cotabato (Molina et al., 2011).

In our study, *Fasciola* infection in carabao showed positive association with the increasing age of animal and drinking water from irrigation areas and rivers. Older carabaos are more likely to be infected with *Fasciola* spp. because of longer exposure in rice paddies as a draft animal (Molina et al., 2005). Use of irrigation areas and nearby rivers as drinking water for carabaos increase the odds of *Fasciola* infection because both environments sustain the habitat of the IH snails (Gray et al., 2008). Contrariwise, we observed that a wallowing pond is a protective factor against *Fasciola* infection. Most of the ponds in the area are man-made and belong to an animal center that distributes regular animal health services. These factors may act as potential confounders of *Fasciola* infection in the area. Unlike the wallowing ponds, irrigation waters in the rice paddy are often clear, shallow, and slow-moving which greatly favors the reproduction of the IH snails (Young and Fontanilla, 2014).

The use of dewormer showed no significant effect in decreasing *Fasciola* infection at the herd and animal levels in the final multivariate model. Albendazole is widely used by farmers as an anthelmintic in the study location. However, a previous report suggested drug underdosing and incorrect treatment regimen of albendazole in ruminants, which may explain the poor efficacy against helminths (Rupa and Portugaliza, 2016). Also, albendazole resistant parasites are becoming common due to indiscriminate anthelmintic use (Cabardo Jr and Portugaliza, 2017; Venturina et al., 2015).

Fasciola species utilize snails under the family Lymnaeidae as IH, which are reportedly present in Leyte Island (Young and Fontanilla, 2014). Of the snail population sampled, *Lymnaea* accounted for the most number of IH. We investigated how lymnaeid spatial distribution is affected by land elevation, the presence of waterbody, and vegetation. Daily rainfall and temperature did not vary throughout the study so were excluded from the analysis. We observed that the IH snail distribution appeared higher in areas with land elevation above sea level possibly because most of the rice paddies are within this altitude. In other countries, the distribution of lymnaeid snails occurred in a wide range of altitude (Mas-Coma et al., 2001). Furthermore, IH snails seemed to be concentrated in areas with intensive networks of irrigated water systems and rivers, essentially in rice fields. These features appeared as well in some Southeast Asian countries such as in Central Vietnam and Cambodia (Nguyen et al., 2012; Tum et al., 2004). In reference to vegetation, moderate NDVI (i.e., suggestive of rice fields and grassland areas) favored the proliferation of IH snails and positivity of *Fasciola* larvae. Indeed, lymnaeid snails prefer swampy and green environments where food resources are abundant (Young and Fontanilla, 2014; De Kock et al., 1989).

In conclusion, our epidemiologic data suggest a high endemicity of *Fasciola* spp. in Baybay, Leyte. The corroborating distributions of parasite between carabao and snail hosts indicate a sustained and successful life cycle in the area. While suitable interventions need careful investigation, we suggest to include in the strategy how farmers prevent ruminants from consuming metacercaria-contaminated plants, rice straws, and water from rice fields or rivers. Our GIS maps could serve as a rapid guide to examine which areas are high risk and in need for more efforts to control fasciolosis. Deworming programs should be implemented wisely and re-strategized accordingly. The high *Fasciola* infection prevalence warrants further investigation, particularly regarding its impact on livestock economy and public health with the data presented herein providing a baseline for assessing the impact of changes in management and factors such as climate change.

Conflict of interest

The authors declare no conflict of interest.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Ethical statement

No animal experimentation was conducted in the study titled "Prevalence, risk factors, and spatial distribution of *Fasciola* in carabao and intermediate host in Baybay, Leyte, Philippines", authored by Harvie P. Portugaliza, Ivy Mae C. Balaso, June Clyde B. Descallar, and Eugene B. Lañada. In addition, handling of animals for fecal samples were conducted following the guide for the care and use of agricultural animals in research and teaching (FASS, 3rd Ed., 2010).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2018.100261.

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