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ORIGINAL ARTICLE

Occurrence of *Angiostrongylus cantonensis* in rodents from the rice granary of the Philippines and associated risk factors for zoonotic transmission

Diane Shiela C. Castillo[1] · **Vachel Gay V. Paller**[2]

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Abstract *Rattus tanezumi* and *Rattus norvegicus* are the most common rodent species in the Philippines, with the former mainly inhabiting agricultural land and the latter in urban areas. Generating information on the *Angiostrongylus cantonensis* harbored by these rodents would be useful for public health. A total of 600 rodents were collected from traps placed each month from July to December 2012 in selected villages of Muñoz, Nueva Ecija; the rice granary of the Philippines. The prevalence of *A. cantonensis* in *R. tanezumi* and *R. norvegicus* was, 100%; all the rodents from the five villages were infected. The study shows that regardless of stages, body weight and length, rodents are vulnerable to infection by *Angiostrongylus cantonensis* present in the environment. However in terms of sex, results revelead that the male and female in *R. tanezumi* has no statistically significance while *R. norvegicus* revealed that male and female has statistically significance. This study also show that, rodents that were caught in the agricultural area has higher mean intensity compared to rodents that were trapped in the residential areas but found to be insignificant. Also through survey interviews the various risk factors with zoonotic implication of *A. cantonensis* in the area was also discussed.

Keywords Zoonotic · *Rattus tanezumi* · *Rattus norvegicus* · *Angiostrongylus cantonensis*

✉ Diane Shiela C. Castillo
dianeshiela20@gmail.com

[1] Department of Environmental Science, College of Arts and Sciences, Central Luzon State University, Nueva Ecija, Philippines

[2] Division of Animal Biology, Institute of Biological Sciences, University of the Philippines Los Baños, Los Baños, Philippines

Introduction

Rodents are a vital mammalian group which can be found in various environments throughout the globe, consisting of more than 42% of the known mammalian species (Singleton et al. 2003). Rodents belonging to the family Muridae are vastly successful and dominant species in most regions of the world this is due to the ability of rodents to adapt and utilize this changing environment at a great rate (Singleton et al. 2003). The genus *Rattus* consist of various sub- species that can be further grouped into numerous species. Most rodents that are members of this genus are found in forest or island, urban/rural and agricultural areas. *Rattus tanezumi* and *Rattus norvegicus* are the two most successful species adapting to all kinds of environment and found widespread in distribution (Roberts 1977).

Rodents serve as definitive host of various parasites, whereas humans become infected due to close association of rodents to human. The *Angiostrongylus cantonensis* is one of the nematode parasites that inhabiting the lungs and heart of the rodents. Rodents that are infected by this parasite can pass larvae of the worm through their feces to the soils, water or even in vegetables. Moreover, invertebrates such as snails, slugs, freshwater shrimp, land crabs as well as the amphibian such as frogs can become infected by ingesting the larvae of the parasite; the animals mentioned are usually considered to be the intermediate host of *A. cantonensis* (Kwon et al. 2013). People will become infected after ingesting the third stage larvae (L3) which is the infective stage can be harbor by humans through eating raw or inadequately cooked intermediate hosts. Humans can also be infected by eating such as leafy green vegetables contaminated by the invertebrates or even rodents (Rosen et al. 1967; Ash 1976).

In the case of human infection, the neurotropic larvae migrate to the central nervous system, but are unable to complete their lifecycle and subsequently die. The death of these larvae is assumed to result in diffuse meningeal inflammation characterized by an eosinophilic infiltration (Slom and Johnson 2003). Most cases of Angiostrogyliasis have been reported in Southeast Asia (Uchi-kawa et al. 1984; Kliks and Palumbo 1992). There were only few data about the *A. cantonensis* in the Philippines. Therefore, this study aimed to determine the prevalence, mean intensity, and abundance of *A. cantonensis* in two *Rattus* species inhabiting agricultural and residential areas in the rice granary of the Philippines which is Muñoz, Nueva Ecija. Also this study aimed to determine some associated risk factors for zoonotic transmission.

Materials and methods

Ethical considerations

Prior to the conduct of the study, the protocol was approved by the research ethical panel of the Institute with accordance with Administrative Order No. 40 series of 1999 otherwise known as "Rules and Regulations on the Conduct of Scientific Procedures Using Animals" pursuant to Republic Act no. 8485 otherwise known as the "Animal Welfare Act of 1998".

Study area

Five villages were selected out of 37 total villages in Muñoz, Nueva Ecija where population was 75,462 as of 2011. The site is globally situated at 15. 71N latitude and 120.90° longitude E and has a total land area of 16,305 hectares which is mainly for agricultural utilization (9819 ha) followed by residential zone (2847 ha) (retrieved at Science City of Muñoz, July 2012). The collection of two rodent species was done every day during one cropping season from July to December, 2012.

Collections of *Angiostrongylus cantonensis* in rodents

R. tanezumi and *R. norvegicus* were collected using single capture live traps and baited with roasted coconut and soaked in peanut butter. Morphometrics such as sex, weight, body measurements, species, and the place of collection were also recorded. The determination of the age classes of *Rattus* sp. was based on MacDonald and Barrett (1993). Captured rodents were euthanized by ether and subjected to cervical dislocation technique before dissecting to recover *A. cantonensis*. The lungs and heart of the rodents were examined for adult worms. The worms that were collected were preserved in small vials with 70% ethanol. Identification of adult *A. cantonensis* was identified through their distinct characteristics wherein male have a copulatory bursa and female having a barber pole (Vitta et al. 2011). The heart and liver organs were also artificially digested with pepsin-HCL solution using a hot plate with magnetic stirrer at 37 °C for 1 h. The digested organs were filtered and placed in petri dish for microscopic examination of the larvae using dissecting microscope.

Survey on awareness and local practices of people on rodents

Written informed consent was prepared prior to the conduct of interviews. One hundred (100) selected farm owners and local people were interviewed to record some knowledge and practices regarding rodents in their areas and to know the level of awareness on the zoonotic potential that is linked to rodents.

Data analysis

The prevalence and mean intensity were determined for both rodent species. Moreover, all data was tested for normality of distribution using SPSS software at 95% confidence level. The relationship of the mean intensity of parasite to the length and weight were analyzed using Pearson's Correlation Analyses. Chi square test of independence was used to analyze the differences of mean intensity in residential and agricultural area. Meanwhile, age and sex were analyzed using Fisher's exact test. Statistical computations were done using Quantitative Parasitology version 3.0 (QP3), Predictive Analytics Software (PASW) version 18.0.

Results

A total of 600 rodents belonging to two species, the common field rat, *R. tanezumi* (n = 334) and the common house rat, *R. norvegicus* (n = 266), were trapped and examined for *A. cantonensis*. Samples consisted of 151 females and 149 males for *R. tanezumi* and 142 females and 158 males for *R. norvegicus*. Samples were collected from five selected villages in Muñoz, Nueva Ecija within a period of six months (July–August, 2012). Many of the rodent samples included in the study were collected through the help and effort of local people and local government through their program "Rat Management Plan". A. *cantonensis* were harvested from the lungs and heart of the rodents.

Table 1 Over- all prevalence and mean intensity of *A. cantonensis* in *Rattus tanezumi* and *Rattus norvegicus* in Science City of Muñoz, Nueva Ecija

	Prevalence (%)	Mean intensity (MI)
Rattus tanezumi	100	22.8
Rattus norvegicus	100	23.13

The result of this study shows that the prevalence of *A. cantonensis* in *R. tanezumi* and *R. norvegicus* was, 100%; all the rodents from the five villages were infected. Meanwhile, the mean intensity of *A. cantonensis* in *R. tanezumi* and *R. norvegicus* was 22.8 MI and 23.13 MI, respectively. However, mean intensity in both *Rattus* species was found to be insignificant ($p > 0.05$) (Table 1).

Meanwhile, the mean intensity of *A. cantonensis* in agricultural area shows that *R. tanezumi* has the highest mean intensity with 22 (MI) compared to *R. norvegicus* with 9 MI. For residential area, *R. tanezumi* also has the highest mean intensity with 15 MI and 10 MI for *R. norvegicus* (Table 2).However, the total mean intensity of *A. cantonensis* between agricultural land and residential areas was found to be insignificant ($p > 0.05$).

Relationship of sex, age and body index in the parasite intensity of *A. cantonensis* in rodents

In this study, results revealed that male and female in *R. tanezumi* ($x^2 = 0.387$; $0.172 > 0.05$) has no statistically significance while *R. norvegicus* ($x^2 = 0.539$; $0.000 < 0.05$) revealed that male and female has statistically significance; the prevalence was 100% to both male and female of *R. tanezumi* and *R. norvegicus* (Fig. 1).

Based from the rodent weight, age class was classified as juvenile, sub- adult and adult. The prevalence of *A. cantonensis* in both rodents was 100%. In terms of mean intensity, the differences between the two species was not statistically significant among all stages in *R. tanezumi* ($x^2 = 0.351$, $0.482 > 0.05$), as well as in *R. norvegicus* ($x^2 = 0.399$, $0.063 > 0.05$) (Table 3).

Measurement shows that the body length of *R. tanezumi* was 169.58 mm ± 19.74 while *R. norvegicus* was 199.08 mm ± 29. 86. Statistical analysis showed that the body length of *R.tanezumi* (r = 0.40, $p > .492$) has no significant relationship with parasite intensity of *A. cantonensis*. The same trend was also observed with *R. norvegicus* (r = 0.102, $p > 0.077$). In addition, *R. tanezumi* mean weight was 158.16 g ± 30.30 while *R. norvegicus* was 227.31 g ± 52.37. The statistical analysis showed that there was weak correlation between *A. cantonensis* parasite intensity with the body weight of *R. tanezumi* (r = 0.047, $p > 0.420$). For *R. norvegicus* the body weight had a moderate positive correlation with parasite intensity. (r = .102, $p > 0.78$). However, both correlations are not significant (Fig. 2).

Survey and risk associated with *A. cantonensis* in local farmers

The survey was conducted in five (5) selected villages in Muñoz, Nueva Ecija. There were 100 respondents consisting of adult males (n = 83) and females (n = 17) were interviewed for the study (Table 4). According to the survey, all male respondents and few females eat rat. The adult males eat cooked rat while they drink alcohol in drinking sessions. Also, most of the respondents said that rodent meat was their alternative viand because some of them cannot afford to buy pork or chicken meat for their family. Meanwhile, most of the interviewed households also cook and eat freshwater snails, vegetable such as water spinach (kangkong), snakes and fish. Locals are not aware though rodents may serve as vectors to many diseases aside from leptospirosis. In addition, local communities were not aware that eating raw snails and other invertebrates or vegetables that are contaminated by *A. cantonensis* larvae can cause eosinophilic meningitis to humans.

Discussions

Agriculture activity is the key industry in Muñoz, Nueva Ecija, of which it became the rice bowl or granary of the Philippines. And since the land area of Muñoz is primarily agricultural area, rodents are abundant. Rodents are associated to serious damage to the rice crops in the area which can inflict considerable economic loss. Local farmers cooperate with the local government units in controlling the rodent pest. Farmers conduct community campaigns before the ricefield rodent breeding season using local methods, such as burrow hunting. However, concerning the knowledge of the majority, rodents are also an important public health issue. Rodents transmit many diseases causing pathogens such as parasites to humans. Among all parasitic nematode, the rat lungworm (*Angiostrongylus cantonensis*), is the most common cause of human eosinophilic meningitis in Southeast Asia and it has been slowly spreading around the world. Rodents are the definitive host and the main reservoir of *A. cantonensis* although other species of rodents that are found in rural area and forested areas are also reported to be natural hosts (Wu 2006; Cross and Chen 2007). Rodents are necessary for the establishment of *A. cantonensis* foci in an area as they act as a continuous source of infection to maintain its life cycle in its area. *A. cantonensis* has become an important emerging pathogen worldwide especially in Southeast Asia due to frequent outbreaks and increased

Table 2 Prevalence (%) and mean intensity of *A. cantonensis* in *Rattus* species collected from agricultural and residential areas in Muñoz, Nueva Ecija

	Agricultural area		Residential Area	
	R.tanezumi	*R. norvegicus*	*R. tanezumi*	*R. norvegicus*
Prevalence (%)	100	100	100	100
Mean Intensity (MI)	22	9	15	10

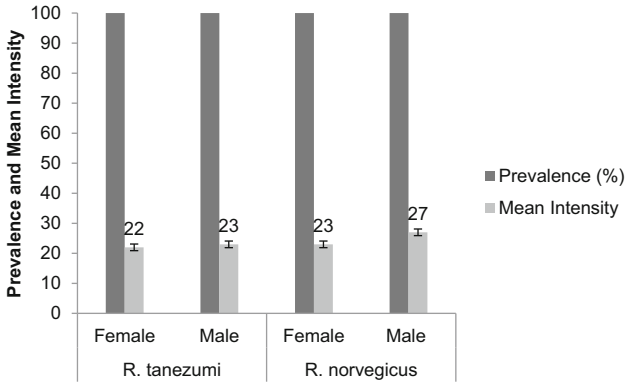

Fig. 1 *A. cantonensis* prevalence and mean intensity between male and female of *R. tanezumi* and *R. norvegicus* from Muñoz, Nueva Ecija

Table 3 *A. cantonensis* prevalence and mean intensity in various stages of *R. tanezumi* and *R. norvegicus* from Muñoz, Nueva Ecija

	R. tanezumi			*R. norvegicus*		
	Juvenile	Sub-adult	Adult	Juvenile	Sub-adult	Adult
Prevalence (%)	100	100	100	100	100	100
Mean Intensity (MI)	23	24	22	22	23	24

reports of sporadic cases in new areas. However, case reports of Angiostrongyliasis in the Philippines are not yet fully established. The study of the transmission and life cycle of *A. cantonensis* in the Philippines must be established first in order to understand how to control and prevent Angiostrongyliasis. Knowledge on the prevalence of *A. cantonensis* in rodents can provide relevant information for future epidemiological studies of *A. cantonensis* that can cause eosonophilic meningitis.

In this study, the sampling sites were comprised of residential and agricultural land areas where *R. norvegicus* and *R. tanezumi* exists. The result of the study shows *A. cantonensi* was 100% prevalent in both rodents; this may be attributed to the abundance of snails in the area, which is known as *A. cantonensis'* intermediate hosts. The abundance of snail could be attributed to the characteristics of the sampling sites, since the sites were located in rice fields or small swamps, the perfect habitats for snails. Moreover, the study was conducted during wet season (July- December) where the intermediate host is more abundant because of its favorable environmental conditions. The over- all mean intensity of *A. cantonensis* in both rodents revealed that *R. tanezumi* was found to have higher mean intensity than *R. norvegicus.* This could be due to the territorial behavior of two rodent species present in the area (Barnett 1997). *R. norvegicus* is usually found in towns and cities and less in agricultural areas. In this study, *R. norvegicus* was also found in the agricultural area this can be attributed to the abundance of invertebrates in the field. This species commonly consumes other animals as a regular part of their diet such as crabs, snails, and other invertebrates (Shiels and Pitt 2014), which may explains why *R. norvegicus* is also infected by *A. cantonensis* despite of its preferred habitat. Meanwhile, *R. tanezumi's* main diet consists of 75–80% of plant material (Kami 1996, Norman 1970, Clark 1981, Cole et al. 2000, Shiels et al. 2013). Furthermore, de Guia and Quibod (2014) revealed that *R. tanezumi* does not only consume plant material but also animal matter and invertebrates. Quick (1990), also stated that *R. tanezumi* consumes' insects, snails and slugs that can be found in their preferred habitat. Based on prior studies, it is obvious that the two *Rattus* sp. consumes snails, slugs and other invertebrates despite of their preferred habitat and territorial behavior; which strongly supports the result of this study wherein *A. cantonensis* was 100% prevalent in both rodents.

Meanwhile, the mean intensity of *A. cantonensis* within a host species can be influenced by different reproductive stages, sex, body weight and length. In this study, it was revealed that the parasite mean intensity of *R. norvegicus* is significantly correlated with the host sex but not significant in *R. tanezumi*. This could attribute to the behavior of *R. norvegicus* wherein male rodents are more mobile than female rodents (Tew and Macdonald 1994). *R. norvegicus* had fewer intake of food but longer feeding visits while female have many short visits (Ingles et al. 1996). Since *R. norvegicus* is more active and can travel significantly farther than females; making them more likely to infect by parasites, their broad diet makes them adaptive to travel longer distances (Sanchez et al. 1985; Heaney et al. 1999; Soliman et al. 2001) and also making them more susceptible to parasitic infection (Tew and Macdonald 1994). In the case of *R. tanezumi*, findings were not similar with *R. norvegicus* with regards with parasite intensity in relation with sex. The study suggests that sex will not matter with parasite intensity harboredby *R. tanezumi* which could be due to the preferred habitat of *R. tanezumi.* This species is the principal rodent pest of rice and other crops in the

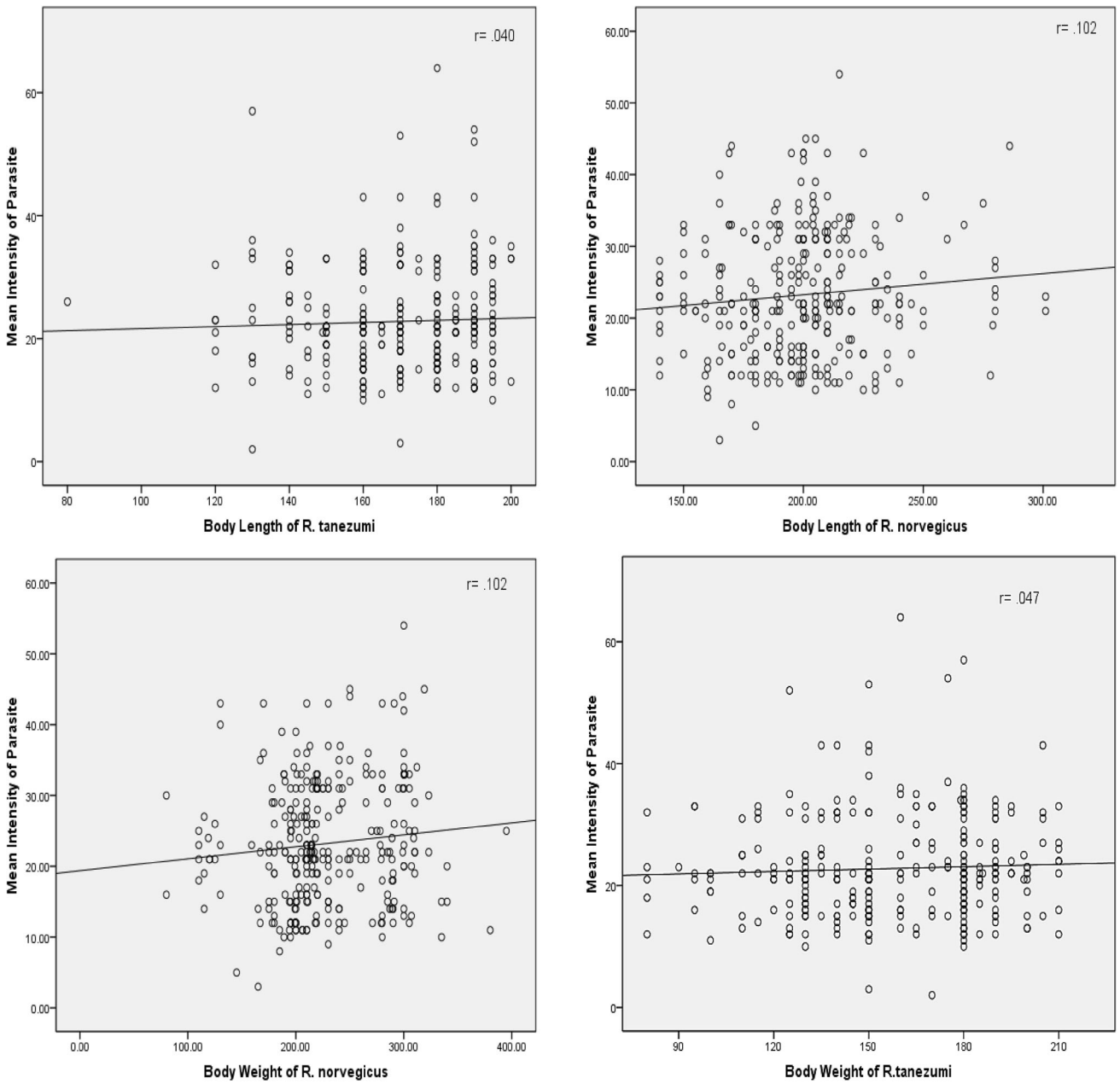

Fig. 2 Relationship between *R. tanezumi* and *R. norvegicus* body length and weight of *A. cantonensis* parasite intensity

Philippines (Stuart et al. 2014). According to Barnett (1997) *R. tanezumi* is more territorial than *R. norvegicus*; the latter only defend their immediate surroundings. The main diet of *R. tanezumi* regardless of sex is found mainly in agricultural areas which may explain why the mean intensity of *A. cantonensis* in male and female of *R. tanezumi* does not differ. Agricultural land is quite suitable for parasites life cycle due to availability of the intermediate hosts. The study shows that regardless of stages, body weight and length, rodents are vulnerable to infection by *Angiostrongylus cantonensis* present in the environment because this species is a voracious eater regardless of their body composition.

The study shows that *R. tanezumi* and *R. norvegicus* is highly infected by *A. cantonensis*, high infection rate in both rodent species is due to the season. Through this, it is possible that the local people living in close association with the hosts can possibly be infected by this parasite. It was reported that there is rapid increasing frequency of *A. cantonensis* in Southeast Asia including Philippines (Alto 2001). There was only one study conducted by Hochberg et al. (2007) that *A. cantonensis* infection in humans was reported in three (3) Filipino. In this study, local practice of

Table 4 Response of the local people in Munoz, Nueva Ecija regarding awareness on associated risk factors on zoonotic transmission

Survey	Respondents (%)
Cook and eat field rodents	
Male	100
Female	20
Food prepared by locals other than field rodents	
Snails	70
Water spinach (kangkong)	20
Snakes	5
Fish	5
Food preference other than meat	
Rodents	20
Snails	60
Fish	20
Zoonotic disease known to be associated with rodents	
Leptospirosis	100
Parasitic	0
Knowledge about *A. cantonensis*	
With knowledge	0
None	100

the community will have great possibility of being infected by *A. cantonensis*. People can pick up the infection by handling or eating any infected critter such as rodent eating produce that has been contaminated by roaming infected snails and slugs. Infection might also occur from ingestion of contaminated water or unwashed vegetable that may contain small snail and slugs that is infected by *A. cantonensis.*

Summary and conclusion

This study revealed for the first time the occurrence of *A. cantonensis* in both *R. tanezumi* and *R. norvegicus* in Muñoz Nueva Ecija, the rice granary of the Philippines. *R. tanezumi* was found to have higher mean intensity and prevalence than *R. norvegicus* but found to be insignificant. Rodents feed on a variety of food in their environment; the presence of the potential intermediate hosts such as snails, slugs, contaminated water and vegetables in agricultural lands could increase the prevalence in rodents. Since the parasite was found to be prevalent in rodents in the area, the local people should be educated about the occurrence of this parasite and the potential risk of infection to humans. Various practices that could reduce risk of infection should be also being part of the information for risk mitigation. Interviews among locals and medical personnel mentioned rare reports of meningitis in the area. Albeit rare, the main cause of meningitis is not usually properly diagnosed. Health government officials also disseminate information about leptospirosis caused by rodents, but none of them had knowledge on rodents as vehicle of parasitic infection to humans. Finally, this study revealed that the people in the community are lack of knowledge on the possible role of rodents as agent of parasitic infection.

Recommendation

This study suggested analyzing the effect of changing seasons on the occurrence of *A. cantonensis* in rodents and intermediate hosts is recommended. Since this study revealed that the infection rate of *A. cantonenisis* in both rodent species is 100% in all sampling areas, it is also suggested to conduct molecular study on parasites for further species identification. This study also recommends conducting preliminary study on the infection *A. cantonensis* in human for future study since Angiostrongyliasis in the Philippines has not been further established yet.

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Authors Contribution Conceived and designed the experiments: DSCC, VGV; Performed the experiment, Data analysis: DSCC; Both authors participated in writing the final paper.

Compliance with ethical standards

Conflict of interest All authors declares that they have no conflict of interest.

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