

## *Schistosoma japonicum* (Katsurada 1904) in Selected Rice Fields Surrounding Lake Mainit, Philippines

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### ABSTRACT

The occurrence of schistosomiasis is still highly endemic and prevailing in the Caraga region. The presence of an intermediate snail host is a good indicator of the endemicity of schistosomiasis in an area. This study was conducted to assess the distribution of snail infection in selected rice fields surrounding Lake Mainit. Snail sampling was done by handpicking using fine forceps. Isolation of cercaria and other larval stages of trematode was done using cercarial emergence and crushing technique, respectively. *Oncomelania quadrasi* snails exhibited relative abundance (%) and high prevalence (%) in Poblacion (40.1%; 82%), followed by Roxas (13.2%; 68%), San Isidro (13.9%; 64%), Magpayang (16.8%; 62%) and Matin-ao (15.9%; 62%). The physico-chemical properties of water revealed that only total dissolved solids ( $r_s = -0.900$ ) showed a significant negative relationship to the snail abundance. The over-all trematode mean infection was also noted to be significant ( $p = 0.000$ ) across sampling areas. Also, another possible trematode was considered to be harboring *O. quadrasi* snails as an intermediate snail host was recorded. The results showed that there is a significant relationship between the vector snail and the larval stages of *Schistosoma japonicum*. Known risk factors to the zoonotic transmission of *S. japonicum* were considered and observed across sampling areas, thus creating a convincing explanation of the rate of snail infection in the area. A high level of awareness about the presence of a snail host is recommended to help impede and prevent the zoonotic transmission of schistosomiasis in an area.

**Keywords:** Lake Mainit, Schistosomiasis, Zoonotic parasites, Blood fluke

### 1 Introduction

The host specificity of a parasite causes endemism of diseases (Morand, & Guegan, 2000). A host is needed to attain, arrest, and harbors either sexual or asexual development during their maturation (Viney & Cable, 2011). It provides an environment for the parasite to exploit, sustain, and complete their considerable growth and reproduction (Lockyer et al., 2004). *Oncomelania hupensis quadrasi* (Möllendorf 1895) is a gastropod species that is widespread and most abundant in areas of Southeast Asian countries, including the Philippines (Rintelen, 2011). Distribution and transmission of *S. japonicum* depend on the presence of intermediate snail host *O. quadrasi* (Legaspino et al., 2014). The snail acts as an intermediate host for the schistosome parasite poses a significant threat to humans in terms of their health, leading to chronic health conditions (Leonardo et al., 2008).

In the Philippines, the prevalence of schistosomiasis infection has declined up to less than 3% and but the disease is still endemic to 12 out of 17 regions in the country (Leonardo et al., 2013). Snail abundance of known snail vector (*O. hupensis quadrasi*) is influenced by environmental factors, the composition of the water present that is sometimes altered by human activities, and food supply (Ayanda, 2009; Amoah et al., 2017). Pathology of schistosomiasis is exhibited mostly by farmers who are directly exposed to the natural

habitats of the intermediate snail host (Omonijo et al., 2015).

Lake Mainit is known to be the fourth-largest lake in the Philippines. The lake is considered to be one of the key biodiversity areas in the country (Demetillo et al. 2016). Lake Mainit has potential tourist spots, abundant resources such as rare species of fishes, and also houses rare, threatened wildlife that gives the lake its high economic value. Schistosomiasis ranks third after malaria and intestinal helminthiasis on the Neglected Tropical Diseases (NTDs) database. Up-to-date research should be done to note the prevalence accurately and mean intensity of the diseases that harbor the known vectors that may pose a significant threat to the health system of the community (WHO, 2002). Baseline data is already available regarding the distribution of *Oncomelania* snails in areas surrounding Lake Mainit (Jumawan et al., 2016). This study was conducted to determine the abundance of snail vector, the prevalence and mean intensity of *S. japonicum* among vector snails, and also to generate a map showing the spatial patterns of *Schistosoma*-harboring snails along ricefields surrounding Lake Mainit.

## 2 Materials and Methods

### *Locale and duration of the study*

The study was conducted in five selected rice fields surrounding Lake Mainit in Surigao del Norte (Figure 1) from July to August 2018. Lake Mainit is surrounded by four municipalities that include the Municipalities of Mainit and Alegria. Surrounding the lake are different barangays that include barangays of San Isidro, Magpayang, Matin-ao, Alegria, and Roxas. These barangays were selected based on schistosomiasis cases reported from stool data from rural health centers/ units and a baseline data of the occurrence of snail vector in the Lake Mainit (Jumawan et al., 2016).

### *Collection of snail samples*

Before the collection of snail samples, the physicochemical properties of water (dissolved oxygen, water pH, water conductivity, total dissolved solids, and surface water temperature) were obtained on-site between 6:00-7:00 in the morning, using a Multimeter Tester (Hanna) with triplicate readings in every 100m transect. For snail collection, a triplicate 100 m transect line was set along the rice field with 1 m perpendicular to the central transect where all possible *Oncomelania* snails were collected. Snail sampling was performed by handpicking using fine-forceps from 7:00- 10:00 pm for 1 hour per 100m transect line to avoid bias (Leonardo et al. 2013). The collected snails were then immediately transferred to the laboratory for cercarial/sporocyst examination.

The identification of *O. quadrasi* was distinguished based on its shell morphology/size (Legaspino et al., 2014; Ponder et al., 2016). The sampling sites, which were positive for *O. quadrasi* was tracked down and mapped out using GPS (Global Positioning System) device and GIS (Geographic Information System). A checklist of the identified risk factors, as suggested and presented by several studies (Madsen et al., 2008; Opisa et al., 2011; Tujan et al., 2016), was also recorded. The identification of risk factors was recorded simultaneously upon the collection of snail samples.

### *Isolation of Schistosoma japonicum from Oncomelania quadrasi*

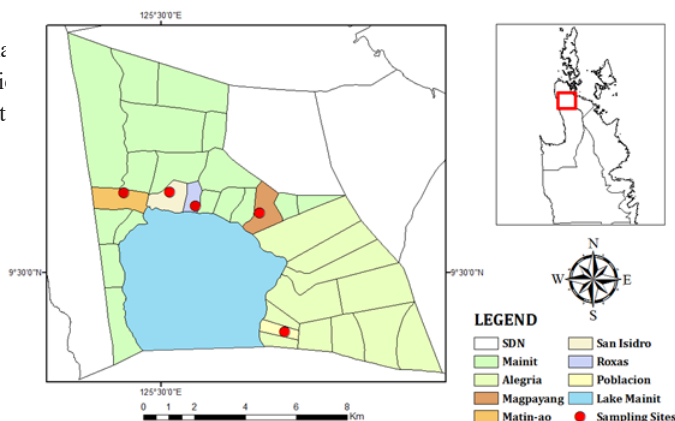


Figure 1. Map showing the five sampling areas in selected rice fields surrounding Lake Mainit, Philippines.

A total of 250 snails were dissected, as per stated in the DENR Gratuitous Permit Grant. The isolation method was adapted from the classical cercarial shedding or emergence technique (Frandsen and Christensen, 1984; Legaspino et al., 2014). Briefly, the collected snails were washed with distilled water and exposed to sunlight for two to three hours to induce shedding of cercaria larvae. Snails were carefully crushed between two glass slides, and the internal tissues were wet-mounted then viewed with a compound microscope for a thorough examination of cercarial infection and other larval stages (miracidium and sporocyst). The identification of the examined trematode was based on its diagnostic features and parasite life cycle.

### Data Analyses

Prevalence (%) was computed based on the total number of snails harboring *S. japonicum* over the total number of snails dissected and examined. For the Mean Intensity (larvae per snail), the total count of parasite was determined over the total number of snail samples harboring *S. japonicum*. Statistical analyses were also performed using IBM SPSS, and the normality test and homogeneity of variances were obtained before the statistical tests. The difference in intensity of trematode larval infection in snails across sampling areas was determined using Kruskal-Wallis test and Mann Whitney U test. Spearman correlation analysis was also used to determine the correlation of trematode infection rate to the water physicochemical properties and abundance of *Oncomelania* in the area. The prevalence and frequency of the infected snail samples across the sampling area were presented in a map using ArcGIS.

## 3 Results and Discussion

### Physical and chemical properties of water

The physical and chemical parameters of water are shown in Table 1. The water pH ranges from 4.98 – 5.70, which means that the water pH in the rice fields was acidic. It was observed that Roxas had the highest pH, while San Isidro had the lowest pH. Roxas is situated openly near the lake and adjacent to Quezon, where the wet market of the Municipality of Mainit is located. According to farmers, mollusciciding activity was already performed before the collection of samples in Alegria since cropping season had already started. This observation was opposite in Roxas, where the mollusciciding schedule was yet to be performed. According to the National Statistics Office (2000), the municipality of Mainit is much more populated than in Alegria, which best explains that high human activities (open-field activities) of draining household chemicals could alter water pH from irrigation canals. Alegria has more agricultural lands than in Mainit since most of the areas are converted to residential areas, and only a few hectares are used for agricultural purposes.

Table 1. Mean and standard error mean (Mean  $\pm$  SEM) for the physico-chemical properties of water across selected rice fields surrounding Lake Mainit, Mindanao, Philippines, July-August, 2018.

Study Area	Water temperature (°C)	Total Dissolved Solids (mg/L)	Dissolved Oxygen (mg/L)	Water Conductivity ( $\mu$ S/cm)	Water Resistivity (k $\Omega$ .cm)	Water pH
1	28.88 $\pm$ 0.283	291.4 $\pm$ 11.95	4.687 $\pm$ 0.058	327.9 $\pm$ 18.06	1.757 $\pm$ 0.3571	5.703 $\pm$ 0.0033
2	28.65 $\pm$ 0.224	237.4 $\pm$ 36.86	4.397 $\pm$ 0.207	384.7 $\pm$ 6.735	2.303 $\pm$ 0.3756	4.983 $\pm$ 0.0133
3	27.4 $\pm$ 0.086	108.8 $\pm$ 17.69	3.113 $\pm$ 0.132	199 $\pm$ 18.07	5.077 $\pm$ 0.6276	4.997 $\pm$ 0.0088
4	30.96 $\pm$ 0.062	122.7 $\pm$ 15.42	3.103 $\pm$ 0.012	282.4 $\pm$ 3.964	4.21 $\pm$ 0.6951	5.253 $\pm$ 0.0722
5	27.61 $\pm$ 0.563	159.9 $\pm$ 7.278	3.187 $\pm$ 0.119	246.3 $\pm$ 4.012	1.757 $\pm$ 0.0809	5.247 $\pm$ 0.0857

1- Roxas; 2- San Isidro; 3- Magpayang; 4- Poblacion; 5- Matin-ao

### The abundance of *Oncomelania hupensis quadrasi*

A total of 416 snail samples were collected throughout the study period. As shown in Figure 2, Barangay Poblacion, Alegria had the highest number/percent abundance (%) of snail samples collected (n = 167; 40.1%). During snail collection, snails were observed to be found slightly submerged on the soil

and sometimes can be found attached to dead leaves. Similar results are found in the study of Legaspino et al., (2014), where *Oncomelania* is mostly found on submerged materials rather than immersed in the soil. This observation was also noted to be similar to Leonardo et al., (2013) in Cagayan Valley with the extensive manual search for *Oncomelania* using five different sampling techniques, namely bamboo poles, filter paper, ring, tube, and banana leaf that was left overnight. *Oncomelania* prefer shaded areas. The presence of trees that gives shade on rice fields promotes growth and reproduction of *Oncomelania* where it is most likely to be found. Similar results were reported by Yirenya-Tawiah et al., (2011) and Madsen et al. (2008) in Samar, Philippines, that trees had a slightly positive correlation to the snail abundance. Sudomo and Pretty (2007) and Garjito et al., (2008) also noted that *Oncomelania* are found in muddy areas and are under the shadow of direct sunlight exposure. It is also similarly reported that *Oncomelania* snail's habitat is scattered and improved in areas such as irrigation canal, rice fields, and ditch-like pools (Rosmini et al., 2014). Moreover, several studies also revealed that the presence of macrophytes contributes to the abundance of snails in the area (Abe et al., 2017; Owojori et al., 2006; Salawu & Odaibo 2014).

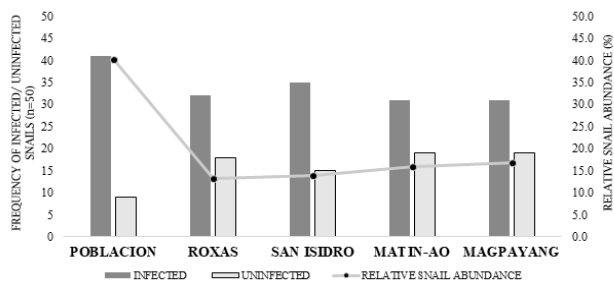


Figure 2. Relative abundance and frequency of snails infected/uninfected between sampling stations in selected rice fields surrounding Lake Mainit, Mindanao, Philippines

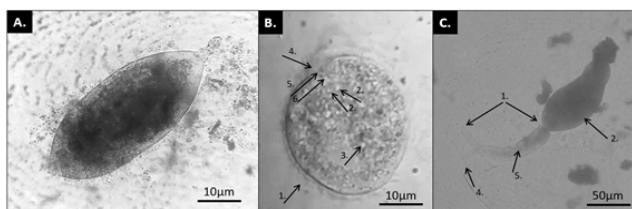


Figure 3. Observed early developmental stages of *Schistosoma japonicum*. A: 1st generation Sporocyst. B: Miracidium arrows pointing 1. Cilia 2. Eyespot 3. Rudimentary Germ Cells 4. Papilla 5. Apical gland 6. Brain. C: *Schistosoma japonicum* cercaria arrows pointing 1. Tail region 2. Head region 3. Excretory tubule 4. Bifurcocercous tail stained with safranin. (under LPO 100x; Scale Bar = 10 µm)

### Parasitological analyses of trematode infection

Out of 250 dissected snails, total prevalence of 67.71% was obtained for *Oncomelania* with water-borne blood fluke trematode, *S. japonicum* (Figure 3). A summary of schistosome infection in *O. quadrasi* across sampling sites was presented in Table 2. A total of 109 *Oncomelania* snails were infected with miracidium (n= 300 miracidia). Intensity for miracidia infection across sampling areas was considered significant (Table 3). Of all the larval-trematode, miracidia had the highest total count in terms of infection in snails. It was stated that an increasing infection rate in snails is due to the growing number of miracidia harboring the snail's body (Leonardo et al., 2016). According to Legaspino et al., (2014), the presence of intermediate snail host in an area is an excellent indicator for active transmission of schistosomiasis. Its role as an intermediate snail host is needed to attain the sexual maturation of *S. japonicum* (Viney & Cable, 2011). The presence of infected host in an area causes a release of schistosome eggs in areas where water and snail are present. Within the intestine of an infected host, mostly of mammals, adult worm male and female of *S. japonicum* reproduce sexually producing thousands of eggs. Contamination of eggs in potable and agricultural water system resulted in hatching from eggs to miracidia, free-living larvae of *S. japonicum*, infecting snail through skin penetration (Payne et al., 2013). Miracidia is defined as a free-swimming larval stage of a trematode that hatches from an egg and infects its intermediate host, which is a snail (Amoah et al., 2017).

The result of the study also observed that the prevalence of Poblacion is higher compared to a study conducted in Lanao del Norte (Legaspino et al., 2014) where Kapatagan had the highest prevalence of 64% (18% smaller to Poblacion). Also, comparing the prevalence of Roxas, San Isidro and Magpayang (68%, 64%, 18

62%) respectively to Lala, Lanao del Norte which had a prevalence of 60%, that is 2%-8% smaller than the three abovementioned areas.

Table 2. Summary of schistosome (miracidia, sporocysts, and cercaria) infection in *Oncomelania hupensis quadrasi* from selected rice fields surrounding Lake Mainit, Mindanao, Philippines.

Study Area	No. of snails infected with bifurcocercous cercaria	MI of infection with cercaria	No. of non-fork-tailed cercaria	No. of snails infected with sporocysts	MI of sporocysts	No. of snails infected with miracidium	MI of miracidia	No. of snails infected with metacercaria	Prevalence (%)
1	1/1	1	1	37/21	2	36/22	2	0	68
2	2/1	2	1	65/27	3	42/18	2	0	64
3	0	0	1	35/19	2	40/22	2	3	62
4	187/5	37	3	85/25	4	157/31	5	1	82
5	2/1	2	1	42/21	4	25/16	2	2	62

1- Roxas; 2- San Isidro; 3- Magpayang; 4- Poblacion; 5- Matin-ao; MI – Mean Intensity (LPS – Larvae per snail host); n = 50

Table 3. Comparison of trematode larval infection across sampling areas in selected rice fields surrounding Lake Mainit, Mindanao, Philippines.

Larval Stages	Intensity of parasitic load in snails (P-value)	Remarks
Miracidia	0.044*	Significant
Sporocysts	0.063	Not Significant
Cercaria	0.001*	Significant
Total Trematode Infection per Barangay	0.000*	Significant

\*significant at  $\alpha=0.05$  level (two-tailed) Tested in Kruskal-Wallis Test.

In terms of sporocyst infection, a total of 113 *Oncomelania* were infected (n=264 sporocysts). Sporocyst is an initial stage of larval infection that hatches from miracidium and later on become a cercaria. Accordingly, a decreasing number in sporocysts infections may be due to a large number of miracidium harboring the snail, as this finding is arguably in contrast to Payne et al. (2013). Moreover, eight *Oncomelania* snails were infected with cercaria. The intensity of cercaria among sampling areas was found to be statistically significant. Cercaria isolated in this study were bifurcocercous which is considered to be of mammalian origin (Opisa et al., 2011).

Prevalence of trematode including cercaria, sporocyst, and miracidium in areas favorable for snail infection is high however prevalence in terms of cercarial infection is low with a prevalence of only 3.2%. This result is similarly reported elsewhere (Ayanda 2009; Opisa et al., 2011; Leonardo et al., 2013; Legaspino et al., 2014; Omonijo et al., 2015). Cercarial infection in snails indicates that zoonotic transmission involving definitive mammalian hosts is active and very alarming.

Aggressive behavior of emerged cercaria during the examination was observed, and this observation was supplemented by the study of Cheng et al. (2013) which presented that abnormal behavior of cercaria indicate problems in the area where light intensity is high, and host's morphology is being altered. It was observed that cercarial emergence occurred after 3 hours of direct exposure to sunlight.

Notably, infection rate vary across sampling areas. As shown in the map (Figure 4), mean intensity for miracidium and cercaria and prevalence was highest in Poblacion. The lowest intensity and prevalence of sporocyst and miracidium was in Magpayang.

Furthermore, the result of the study revealed no significant correlation between the water physicochemical parameters and the mean intensity of trematode infection in selected rice fields surrounding Lake Mainit. The results could imply that the endoparasites are indirectly affected by the changes of the external environment



(Ross et al., 2002; Muth et al., 2010).

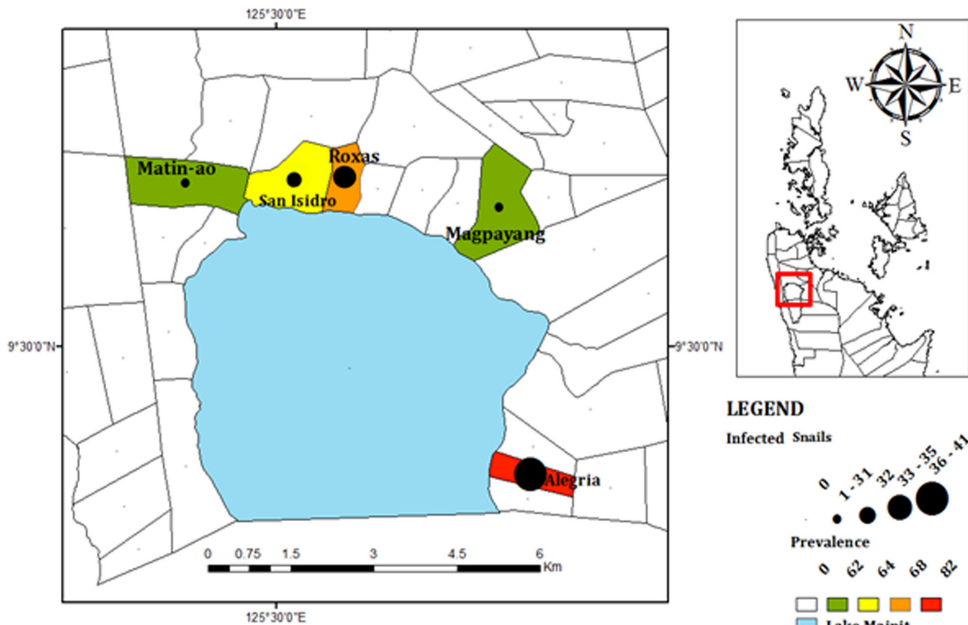


Figure 4. Map of prevalence (%) and frequency of infected snails in selected rice fields surrounding Lake Mainit, Mindanao, Philippines

***Oncomelania as an intermediate host of another parasitic trematode***

It was observed that there are metacercaria and cercaria of unidentified trematode species, probably *Paragonimus* sp (Figure 5) in the cavity of *Oncomelania*. Such findings lead to an assumption that *Paragonimus* sp., a species of lung flukes, also uses *Oncomelania* snails as their first intermediate snail host. Paragonimiasis is a food-borne trematode infection that is acquired by eating undercooked or raw crustaceans (McNulthy et al., 2014).

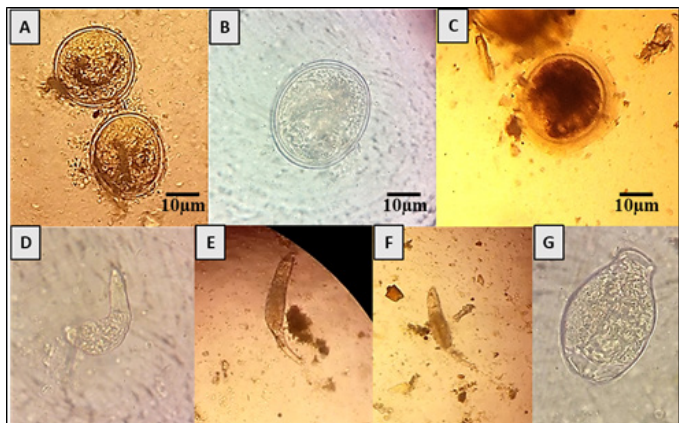


Figure 5. Other trematode larval stages recovered from *Oncomelania hupensis quadrasi* in Lake Mainit, Philippines. (A-C) Metacercaria; (D-G) Cercaria

In some cases, the symptoms and diagnosis of paragonimiasis can be similar to tuberculosis. Tuberculosis is one of the leading diseases in the country, especially in the Caraga Region. This parasite is often misdiagnosed as tuberculosis when, in fact, it is a lung fluke parasite that takes the same signs and symptoms with tuberculosis (WHO, 2002). Such findings of *O. quadrasi* harboring the possible *Paragonimus* sp. contributes to the real cases of paragonimiasis in the country.

***Risk factors to snail infection and parasite transmission***

During the sampling period, it was observed that animals such as carabaos, pigs, dogs, cats, and rats were

present in the area. Rats are considered to be agricultural and household pests, according to the interviewed residents in each sampling area. Pigs were observed to be domesticated in a pig cage within a 1-kilometer distance from the rice fields. Potential reservoir hosts in areas where intermediate snail hosts are present indicate an active transmission of the parasite. All the animals observed in this study are considered to be a potential reservoir host to zoonotic parasites, including *Schistosoma japonicum* (Leonardo et al., 2013; Abao and Paller, 2019).

Human activities such as laundry near the rice fields and irrigation canals were recorded in Magpayang and San Isidro. Households were near irrigation canals that water the rice fields. Walking barefooted near rice fields, especially during mollusciciding and plowing the rice fields using carabaos, were observed. Usage of irrigation water as a source for consumable water was also rampant in the areas as they believed that the water is clean and safe to use and consume. Comfort rooms adjacent to rice fields (open defecation) were also observed among areas. Human activities enable the transmission of schistosomiasis if snail host is found to be positive in the area (Ayanda, 2009).

The livelihood of residents was of farming and fishing. The majority of people exposed to such risk areas were males. Accordingly, farmers were included and made a top priority in the medical plan of treating residents exposed to endemic areas surround the Lake. Awareness of people about the snail as an intermediate host for the disease is deficient. Residents are aware of schistosomiasis being endemic in each area; however, knowledge of its zoonotic nature is deficient. According to Leonardo et al. (2016), these abovementioned factors are some of the reliable indicators of schistosomiasis infection in the area.

Aside from those cercariae that emerge from the snails, animal excreta of those abovementioned reservoir hosts can transmit schistosome eggs. Schistosome eggs hatch to become miracidium, which will swim to its intermediate snail host that later on mature to become cercaria. This scenario is best highlighted in the study of Opisa et al. (2011) that even if a single exposure to water infested with cercariae is enough to affect transmission.

Low awareness of the snail vector is also observed. Risk infection in definitive and reservoir hosts was found to be occurring across sampling areas. According to Leonardo et al. (2013), risk factors play a crucial role in *S. japonicum* transmission. Infection to cercaria, for example, mammals, is due to the development of cercaria that uses skin penetration as its route of infection (CDC, 2012).

The results implied that the active transmission of *S. japonicum* in the area was mainly influenced by agricultural practices (rice farming and small scale/ backyard livestock farming) where poor sanitation and low level of awareness of parasite transmission dynamics are observed. Given the zoonotic nature of schistosomiasis, it is evident that the prevalence among snail vectors will not be controlled by molluscicides alone (Cassion et al., 2013). There is a need for innovative and cost-effective strategies to control schistosomiasis in the long term with the efforts and initiatives from the Department of Health (DOH), Local Government Units (LGUs), and academic Institutions. There is a need to augment mitigating measures such as non-toxic snail control, environmental sanitation, and health education that could bring more enduring outcomes (Blas et al., 2004).

#### 4 Conclusion

The prevalence of *Schistosoma* infection among barangays were considered high and varies among sampling areas. Transmission of *S. japonicum* is influenced by many factors. Some key factors would include the abundance of snail vector, *Oncomelania hupensis quadrasi* and environmental contamination with animal excreta, in particular, from stray cats and dogs, swine and cattle and sanitation and proper hygiene is low. As a result, *S. japonicum* exhibits marked spatial heterogeneity.

#### 5 Acknowledgment

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### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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