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Infection rate of *Schistosoma japonicum* in the snail *Oncomelania hupensis quadrasi* in endemic villages in the Philippines: Need for snail surveillance technique

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Abstract. Schistosomiasis japonica is one of seven NTDs endemic in the Philippines that continues to threaten public health in the country. The causative agent, the blood fluke *Schistosoma japonicum*, uses an amphibious snail *Oncomelania hupensis quadrasi* which can harbor larval stages that multiply asexually, eventually producing the infective cercariae which are shed into the water. Contamination of freshwater bodies inhabited by the snail intermediate host occurs through release of human and animal feces containing *S. japonicum* eggs. Miracidia hatching from these eggs subsequently infect the snails that inhabit these water bodies. The degree of fecal contamination can vary across snail sites and influences snail infection rates in these sites. In this study, conventional malacological surveys using intensive manual search for snails were conducted from 2015 to 2016 in seven selected endemic provinces, namely Leyte and Bohol in the Visayas and Surigao del Norte, Agusan del Sur, Bukidnon, Lanao del Norte and Compostela Valley in Mindanao. A total of 6,279 *O. hupensis quadrasi* snails were collected from 38 snail sites. The municipality of Trento in Agusan del Sur recorded the highest number of snail sites (7) that yielded *O. hupensis quadrasi* snails while only one snail site was found positive for *O. hupensis quadrasi* snails in Kapatagan in Lanao del Norte and Talibon in Bohol. Alegria in Surigao del Norte yielded the highest number of snail sites (5) that were found to harbor snails positive for *S. japonicum* infection. The snail infection rates in this municipality ranged from 0.43% to 14.71%. None of the snails collected from Talibon in Bohol was infected. Bohol is the only province among the 28 schistosomiasis-endemic provinces which has reached near elimination status. Snail infection rates were found to vary considerably across snail sites, which could be due to the degree of fecal contamination of the snail sites and their connectivity to water that can serve as contamination source.

INTRODUCTION

Schistosomiasis japonica which was discovered in the Philippines in 1906 continues to threaten the health of agricultural communities especially the farmers, fishermen and children who remain constantly exposed to the disease through their work and habits (Blas *et al.*, 2004). The disease is endemic in 12 regions of the country covering 28 provinces, 14 cities, 189 municipalities and 2,221 barangays (Leonardo *et al.*, 2016). *O. hupensis quadrasi* was first described by Mollendörff from specimens collected in Surigao (McMullen *et al.*, 1947) (Figure 1). In 1932, Dr. Marcos Tubangui confirmed the epidemiological role of *O. hupensis quadrasi* as intermediate host to *S. japonicum* from samples collected at Barrio Gacao, Palo, Leyte (Blas 1988-89; Leonardo *et al.*, 2016).

To date, approximately 12 million Filipinos are living in endemic areas, and 2.5 million individuals are directly exposed to infection (Blas *et al.*, 2004; Leonardo *et al.*, 2012, 2015, 2016). *O. hupensis quadrasi* thrives in a wide array of freshwater bodies such as rice fields, cemented waterways, canals, irrigation systems, swamps, lakeshores, riverbanks, and other shaded and waterlogged areas (Blas, 1988-89; Tanaka *et al.*, 1978; Ohmae *et al.*, 2003). Some snail sites are located in close proximity to households in endemic areas. Since snail colonies populate rice fields or irrigation canals, *S. japonicum* is considered an occupational hazard among farmers and inland fishermen (Blas, 1988-89).

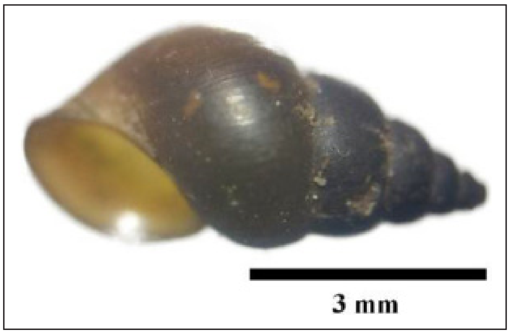

Figure 1. *Oncomelania hupensis quadrasi* (Mollendörff, 1895) snail collected from Alegria, Surigao del Norte, Philippines.

Poor sanitation practices in endemic areas perpetuate environmental contamination with schistosome eggs while lack of access to clean water increases risk of exposure to the disease when people go to possibly contaminated water bodies to launder their clothes, wash their dishes and bathe (Blas, 1991). A human infection survey by Leonardo *et al.* (2008) in 13 provinces in Mindanao and five provinces in the Visayas reported a prevalence rate ranging from 0.08% (Agusan del Norte) to 3.95% (Agusan del Sur). New endemic foci for schistosomiasis were further discovered in Gonzaga, Cagayan Valley and Calatrava, Negros Occidental in 2002 and 2005, respectively. Various diagnostic procedures from stool examination (Kato-Katz), serodiagnosis (COPT and ELISA), and ultrasound were used to confirm cases of schistosomiasis in these new foci (Leonardo *et al.*, 2015). Based from the report of the Philippine Department of Health in 2018, 1611 villages (barangays) are still endemic to schistosomiasis with around 188 are low endemic (11.67%), 382 are moderately endemic, and 453 are highly endemic (28.12%). For zero prevalence barangays, 242 (15.02%) have been recorded, but 346 remain to be determined due to lack of focal surveys done in these areas. Stratification of endemicity of villages were based on the prevalence recorded during focal surveys with $\geq$5% in high endemic areas, $\geq$1% but <5% in moderately endemic and <1% in low endemic areas (DOH, 2018).

A multifaceted schistosomiasis program is required for the successful control and eventual elimination of the disease, and this necessitates sustained and regular data from human, animal, and snail surveys. Malacological surveys yield data such as snail density, distribution and infection rate, which can be used as indicators of the possible extent of distribution and rate of transmission of the disease (UP CPH Foundation, 2012). This study therefore aimed to provide baseline data of the snail infection rate in seven selected endemic provinces and provide information for potential areas for transmission.

MATERIALS AND METHODS

Malacological surveys were conducted in seven municipalities representing seven schistosomiasis-endemic provinces that were selected based on the national prevalence survey conducted in 2005-2008 (Leonardo *et al.*, 2008, 2012). These provinces were Bohol and Leyte in the Visayas and Surigao del Norte, Agusan del Sur, Bukidnon, Lanao del Norte, and Compostela Valley in Mindanao (Table 1, Figure 2A). Surveys were done from April 2015 to January 2016 (Table 1) through intensive and purposive search in sites known to support *O. hupensis quadrasi* snails. There were common ecological characteristics in the snail sites visited, which include being waterlogged and with thick vegetation shading swamps, riverbanks, rice fields, irrigation canals, ponds, and streams (Figure 2B-E). Survey was done for one day from 8 am to 12 noon and 1 pm to 3 pm. The number of snail collectors per site varied depending on the availability of personnel at the time of survey (Table 1). The snails were collected using forceps and placed in properly labeled containers, after which they were air dried in a conventional filter paper away from direct exposure to sunlight or heat. Air dried snails were transferred and packed carefully in clean sheets of filter paper and transported to the Department of Parasitology, University of the Philippines Manila for further processing.

Identity of snails was confirmed by examination of shell morphology. *O. hupensis quadrasi* snails have a distinct ovately conic shell shape with sharp apex. Shell color may vary from brown to black. Juvenile snails are 3 milimeters (mm) in

Table 1. List of selected barangays surveyed in municipalities across seven provinces endemic to schistosomiasis japonica in the Philippines

Island	Province	Endemic Municipality/ City	Villages/ Barangays	Date Surveyed	No. of Collectors	No. of sites surveyed
Leyte	Northern Leyte	Alang-alang	Bugho, San Antonio Farm, San Vicente	Apr 13-17, 2015	5	8
Bohol	Bohol	Talibon	San Roque	Jul 28-31, 2015	4	1
Mindanao	Agusan del Sur	Trento	Manat, Tudela, San Isidro	Jul 13-17, 2015	5	11
	Surigao del Norte	Alegria	Poblacion, Alipao, San Pedro	Aug 4-7, 2015	5	8
	Lanao del Norte	Kapatagan	Bagong Silang Curvada, De Asis	Oct 27-30, 2015	4	3
	Bukidnon	Valencia	Kahaponan, Vintar, San Isidro	Nov 30- Dec 4, 2015	3	3
	Compostela Valley	Maragusan	Tigbao, Mapawa, New Albay	Jan 24-27, 2016	6	4

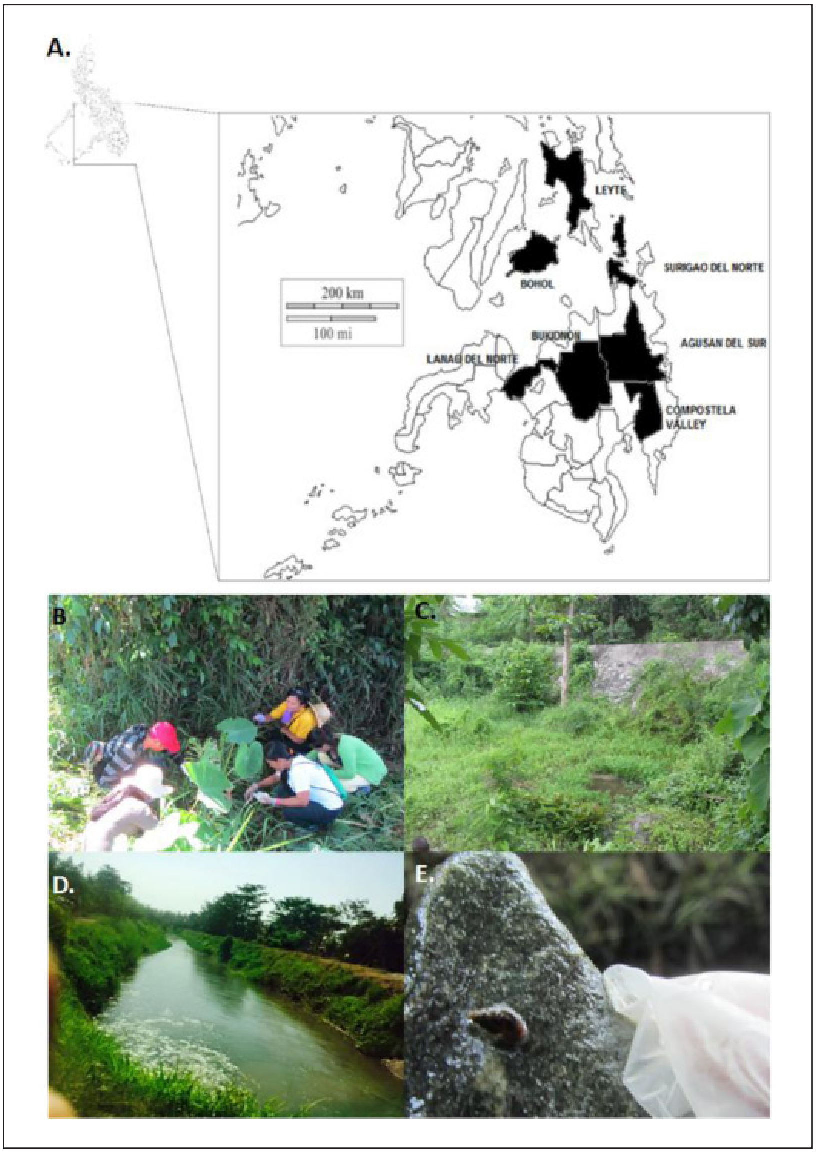

Figure 2. (A) Seven provinces in the Philippines surveyed for *S. japonicum* infection to *O. hupensis quadrasi* snails. Visayas: Leyte, Bohol; Mindanao: Surigao del Norte, Agusan del Sur, Bukidnon, Lanao del Norte, Compostela Valley. (B) Malacological survey conducted in a rice field in Trento, Agusan del Sur with *O. h. quadrasi* snails, (C) Sarong boggy, a snail site in a swampy area in Talibon, Bohol, (D) wide and deep irrigation canals supplying water in many rice fields in Agusan del Sur, (E) an *O. hupensis quadrasi* attached to a stone collected from a riverside in Valencia, Bukidnon.

length or less and adult snails are 3 mm with maximum of 6 mm in length (Garcia, 1988; Leonardo & Solon, 1996).

Snail infection was determined microscopically by demonstrating schistosome cercariae and sporocysts in crushed snails. Snails were placed individually in three drops of distilled water in a glass slide. The snails were crushed by pressing another clean slide on top of the slide with the snails. Snail tissues were further teased and shell debris removed to better expose the schistosome cercariae and sporocysts. The crushed snails were examined under a stereomicroscope (Bausch & Lomb OPT. Co. U.S.A.). Snails were considered infected if the characteristic furcocercous cercariae and sac-like sporocysts were seen. The rate of infection for the municipality surveyed was computed as follows: [(number of infected snails)/ (total number of collected snails examined)] x100.

RESULTS

A total of 6,279 *O. hupensis quadrasi* snails were collected from the seven municipalities (Table 2). Agusan del Sur yielded the highest number of sites surveyed at 11 sites while the least number was in Talibon, Bohol with only one site (Tables 1 & 2). The highest number of collected snails was recorded in Agusan del Sur with a total of 1,683 snails from Barangays Manat, Tudela, and San Isidro (Table 2). The least number was recorded in Valencia, Bukidnon with 133 snails from Barangays Vintar, Kahaponan, and San Isidro (Table 2). It was observed during the survey that not much is known about snail sites in Valencia, Bukidnon. In the light of this paucity of information, the survey was extended to all possible snail habitats such as tributaries of rivers, rice fields and water-logged areas, which revealed few interspersed clumps of snails. In Agusan del Sur where prevalence of the disease has been known to be high, sanitary inspectors and malacologists previously conducted malacological surveys to monitor the snail intermediate hosts. Thus, records of snail sites and their location provided crucial *a priori* information on the municipality, leading to a more focused survey and subsequently resulting to a very high number of collected snails.

The highest number of snail sites with infected snails was recorded in Alegria, Surigao del Norte (SDN) with five sites (Table 1, Figure 3). Table 2 shows that snails positive for schistosome infection were found in snail sites surveyed in the 6 of the 7 provinces covered. The snails found from the single site in Talibon, Bohol were negative for *S. japonicum* cercariae or sporocysts, the only province in this study to yield such result (Tables 2 & 3). It should be noted that among the 28 provinces which are presently endemic for schistosomiasis, only Bohol has reached near elimination status on the basis of the absence of human cases for the past many years according to health authorities (Leonardo personal communication). Widest range of snail infection rate (IR) was noted in Alegria, Surigao del Norte (0.43%–14.71%) while the narrowest IR range was recorded in Valencia, Bukidnon (0%-1.72%) (Table 3).

DISCUSSION

In the Philippines, the cornerstone of schistosomiasis control has been mass drug administration (MDA) while snail control has not been given that much attention. Malacological surveys are not regularly

Table 2. Summary of results for the different municipalities based on the malacological surveys conducted from April 2015 to January 2016

Municipality	Sites with Ohq	Sites with infected Ohq (%)	Number of collected snails	Number of infected snails (%)
Talibon	1	0 (0.00)	388	0 (0.00)
Alang-alang	5	3 (60.0)	1588	9 (0.57)
Trento	7	3 (42.9)	1683	6 (0.36)
Alegria	6	5 (83.3)	1229	12 (0.98)
Kapatagan	1	1 (100.0)	461	4 (0.87)
Valencia	3	1 (33.3)	133	1 (0.75)
Maragusan	4	3 (75.0)	797	12 (1.51)

Note: Ohq = *Oncomelania hupensis quadrasi.*

Table 3. Summary of snail infection rates (IR) of snail sites from the endemic municipalities

Island	Province	Municipality	Site	IR (%)
Mindanao	Compostela Valley	Maragusan	Maragusan 1	0
			Maragusan 2	1.89
			Maragusan 3	1.03
			Maragusan 4	2.15
	Surigao del Norte	Alegria	SDN 1	14.71
			SDN 2	0.65
			SDN 3	1.19
			SDN 4	0.43
			SDN 5	0.65
	Bukidnon	Valencia	BKN 1	1.72
			BKN 2	0
			BKN 3	0
	Agusan del Norte	Trento	Agusan 1	0
			Agusan 2	0
			Agusan 3	2.22
			Agusan 4	0.32
			Agusan 5	0
			Agusan 6	2.38
			Agusan 7	0
Bohol	Bohol	Talibon	Bohol	0
Leyte	Leyte	Alang-alang	Leyte 1	1
			Leyte 2	0
			Leyte 3	0.36
			Leyte 4	0

conducted so that snail sites are not updated and transmission sites are not identified. The situation in Valencia, Bukidnon illustrates this problem clearly where paucity of information is further perpetuated by the lack of manpower to conduct snail surveys. In contrast, control efforts in Agusan del Sur have included snails to bring down the consistently high prevalence of schistosomiasis in the province. While the data for Bohol may be promising, i.e. no infected snails and human cases, further surveillance using more sensitive serologic tests on humans and other animal reservoir hosts and regular snail surveys should be conducted to confirm the near elimination status of schistosomiasis in the province and potentially towards full elimination status. In China, infected snails were still observed even in areas where transmission was declared to be controlled. Few areas showed a decreasing trend in snail infection rate while other areas that were monitored for snail surveillance worsened, indicating that snail control should be focused and reinforced most especially in areas where transmission were under control (Gen-Ming *et al.*, 2005).

In Alegria, Surigao del Norte, one snail site (SDN1) was observed to have the highest infection with 14.71% (Table 3). This snail site was observed to be near human settlement and even a pig farm and was therefore a recipient of domestic wastes.

High snail infection rates are indicative of intense fecal contamination either from infected humans or infected animals caused by poor environmental sanitation or failure to manage waste disposal from livestock.

While snail population density and snail distribution are important indicators of possible presence and spatial distribution of schistosomiasis, a better parameter that indicates disease transmission is snail infection rate. The lone snail site in Kapatagan, Lanao del Norte (1/1 = 100%) was found to harbor infected snails (Table 2). This result should be a red flag for the municipality, and efforts should be intensified to locate more snail sites and examine these for the presence of snails and determine if they are infected. The presence of infected snails means that their habitat is being contaminated with feces coming from infected vertebrates. Unless access by potential hosts are restricted or removed to disrupt the life cycle of the parasite, this site will remain a transmission site. Moreover, snail infection rate can be an ideal monitoring tool to assess progress in intervention programs such as MDA and environmental sanitation. If these two main key strategies are neglected and efforts not sustained, infected cases without access to clean and safe water for domestic purposes will certainly contaminate freshwater bodies where *O. hupensis quadrasi* colonies thrive, infecting these snails.

Previous studies reporting snail infection rates were conducted in other endemic areas in the Philippines such as Gonzaga in Cagayan Valley, Calatrava in Negros Occidental, and Samar (Madsen *et al.*, 2008; Leonardo *et al.*, 2015). For instance, Leonardo *et al.* (2015) noted higher snail infection rates in Gonzaga than in Calatrava, particularly in Barangay Magrafil that can be attributed to the closer proximity of snails to human habitation and the presence of infected animals that may continuously contaminate the water and subsequently infect the snails. Other barangays showed comparatively lower snail infection rates, and variability of snail infection rates across barangays was high. In another study, Madsen *et al.* (2008) compared naturally rain-fed and artificially irrigated villages and noted no significant difference in snail density but with significantly higher infection rate in artificially irrigated villages, which they attributed to the steady and consistent supply of water in these areas that allowed for longer stay of snail colonies and therefore greater exposure time to the parasites.

The current study shows the importance of snail data in elucidating the status of schistosomiasis in endemic areas. Snail population density, spatial distribution and snail infection rates are critical indicators of the possible presence and distribution of the disease as well as occurrence of transmission. The number of snails collected and snail infection rate vary even if the snail sites demonstrate striking similarity in ecological characteristics such as being waterlogged with thick vegetation. Therefore, snail surveys must be regularly conducted in schistosomiasis endemic provinces.

The importance of identifying sites positive not only for snails but for snails infected with *S. japonicum* could not be overemphasized. Whilst municipalities like Talibon, Kapatagan and Valencia have only few snail sites or sites with infected snails, this information should spur them to intensify efforts in expanding their surveys to include other unvisited sites to determine potential transmission sites.

For other endemic municipalities such as Alang-alang, Trento, Alegria and Maragusan, having identified the snail sites and those with infected snails puts them at first base, but further steps should be undertaken such as restricting access of humans and domesticated animals to these sites in order to disrupt the life cycle of *S. japonicum*.

Identification and mapping of snail sites and continuous monitoring for snail infection are therefore important components of snail surveillance, which should be standard for schistosomiasis intervention programs in endemic areas. Snail control methods can be done through environmental modification measures such as cement lining, dredging, and burying in order to destroy the snail habitats. Japan was able to eliminate schistosomiasis in the 1990's using mostly by mostly transforming waterlogged areas into fruit plantations, burying snail habitats, and converting them into residences and even golf courses. To date, however, *O. hupensis nosophora*, the snail intermediate host of schistosomiasis in Japan, remains in large number in previously endemic areas in Japan such as Yamanashi-Kofu River Basin, though no human and animal cases have been noted since 1977 after continuous control efforts (Tanaka & Tsuji, 1997). This just goes to show that the disease can be eliminated without eliminating the snail intermediate host. What is essential is to prevent transmission by keeping the snails free from *S. japonicum* infection.

In light of the call for elimination of schistosomiasis by 2030, endemic countries are encouraged to intensify control and elimination efforts starting with development not only of highly sensitive diagnostic methods to detect the disease in humans and animal reservoirs but identify snail sites and determine snail infection rate. The Philippines can place its hopes on Bohol to be the first province to eliminate schistosomiasis given its present status of no infected snails and no infected humans (using Kato-Katz for diagnosis).

However, 0% infection rate status must be confidently ascertained through extensive snail survey coupled with the use of sensitive diagnostic tools. Malacological surveys could be very laborious, time-consuming, and unreliable especially since many of those involved may not have the necessary expertise in identifying snails let alone determine snail infection rate. Another downside of intensive manual search is the physical hazard in accessing possible snail sites that can only be reached after crossing steep and slippery slopes or boggy marsh with unstable substrate.

A possible complement currently being explored is detection of environmental DNA (eDNA) from the parasite or the snail intermediate host. This provides a rapid and more accurate method that is also safer to use. Several molecular markers have already been used in detecting helminth parasites. These markers include transposons and retrotransposons for *S. japonicum* (Driscoll *et al.*, 2005; Hung & Remais, 2008), and cytochrome *c* oxidase subunit 1 gene (*cox1*) for *O. viverrini* and *O. lobatus* (Hashizume *et al.*, 2017). Presence of environmental DNA (eDNA) is indicative of the target organism's presence and can be used to estimate range of endemicity of the parasite in a sampled area.

CONCLUSION

Malacological surveys using intensive search were used to confirm the presence of snails in previously located snail sites in selected villages in endemic municipalities in seven schistosomiasis endemic provinces. The extent of collection in terms of how many snail sites surveyed and the number of snails collected was influenced by available information on these snail sites. Snail infection rates varied across snail sites even if conditions seem to be similar like heavily shaded water logged areas. Variation was attributed to degree of fecal contamination of the sites and/or their degree of connectivity to sources of fecal contamination.

RECOMMENDATIONS

The present conventional malacological surveys through intensive search can yield fruitful results if there is enough manpower and enough expertise in identifying snails and the infective cercariae and sporocysts. In the light of intensifying surveillance to support efforts for elimination of the disease, an alternative technique through eDNA detection in water samples collected from existing snail sites and possible snail sites can be explored.

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