SCHISTOSOMA JAPONICUM IN THE BLACK RAT, *RATTUS RATTUS MINDANENSIS*, FROM LEYTE, PHILIPPINES IN RELATION TO *ONCOMELANIA* SNAIL COLONIES WITH REFERENCE TO OTHER ENDOPARASITES

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Abstract. This study examined the prevalence of *Schistosoma japonicum* infections in field rats, *Rattus rattus mindanensis*, according to different trapping locations. Between October 1995 and January 1996, traps were set in the municipality of Palo, Leyte, Philippines to determine the correlation of rats infected with schistosomiasis to the proximity of the intermediate snail host, *Oncomelania hupensis quadrasi*, colonies. Of the 22 rats that were caught within a snail colony, 21 (95.5%) were positive for schistosomiasis. Of the 23 rats that were caught 100 meters from a snail colony, 13 (56.5%) were positive for schistosomiasis. Of the 17 rats that were caught approximately 1 km from a snail colony, zero (0%) were positive for schistosomiasis. Infection rates were highest within the habitat of the intermediate host and lowest in rats captured far from snail colonies. Captured rats were also examined for the presence of other endoparasites. Infections of the following were found: *Angiostrongylus cantonensis, Gonylonema neoplasticum, Hymenolepis diminuta, Nippostrongylus muris, Strongyloides ratti, Syphacia obvelata, Taenia crassicollis* and *Trichuris muris*, but there was no correlation between trapping location and prevalence. None of the rats were infected with *Moniliformis moniliformis, Trichinella spiralis, Trypanosoma lewisi* or *Vampirolepis nana.*

INTRODUCTION

It is well known that the Philippine Island of Leyte is endemic to *Schistosoma japonicum* (Pesigan *et al*, 1958). Several investigations have been carried out on the prevalence of *S. japonicum* infection in field rats, *Rattus rattus mindanensis*, of Leyte (Cabrera, 1976; Oshima *et al*, 1978; Kamiya *et al*, 1980), and one study was done in Manila to sample the helminthic fauna of wild rats in Manila (Tabangui, 1931). However, this study was designed to examine the prevalence of rat infection depending on the location of the captured rats to the proximity of the habitat of the intermediate snail host, *Oncomelania hupensis quadrasi*. Therefore, traps were set within snail colony habitat, bordering snail colony habitat, and removed from snail colony habitat. Observations were also made on the number of adult flukes residing within each host, identification of the number of egg capsules passed with the feces per animal per day, calculation of the average amount of feces deposited per rat per day, and a survey of other co-inhabiting parasites. With this information, the importance of field rats as a vector of *S. japonicum* can be evaluated and proper means of control can be determined. Also, future epidemiological studies may profit from the surveillance data gathered on the co-inhabiting parasites residing within the rat population.

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MATERIALS AND METHODS

Fifteen different sites located in selected barangays (villages) in the municipality of Palo, Leyte, Philippines were chosen as areas to set rat traps (Blas *et al*, 1990). These sites included environments such as uncultivated rice fields, plots adjacent to rice fields, bogs, swamps, streamside, coconut groves, and suburban areas.

Traps set within snail colonies were:

I. Barangay Cavite: Upper Hubang in an uncultivated rice field
II. Barangay Gacao: Along both sides of the Gacao stream
III. Barangay Gacao: Adjacent to a recently harvested rice field
IV. Barangay Cogon: Road side ditch and bogy area
V. Barangay Libertad (Mailrong): Along the border of a small pond
VI. Barangay Caibaan: Within a swampy area between two cement homes

Traps set bordering snail colonies (approximately 100 meters) were:

I. Barangay Cangumbang: Floodplain on the Hibaca-an Tributary-B

II. Barangay Cangumbang: Sparsely vegetated area between two nipa huts
III. Barangay San Antonio: Grassy area near a rice field
IV. Barangay Caibaan: Small garden and grassy area
V. Barangay Cabarasan Guti: Coconut grove with vegetation
VI. Barangay Cabarasan Guti: Coconut grove with vegetation

Traps set removed from snail colonies (approximately one kilometer) were:

I. Barangay Salvacion: Coconut grove including a trash dump
II. Barangay Salvacion: Dry, vegetated area encompassing a trash burning pit
III. Barangay Fatima Village: Swampy snail free area with tall grass.

The rats were caught using live traps that were set in the late afternoon and recovered the next morning. Bait was switched every other day, alternating between cooked coconut and dried fish. Ten traps were placed on 25 m^2 plots.

Feces collection

Captured rats were kept in the traps, which served as their cages. Debris was removed, and the cages were cleaned with water. The cage was then suspended over a collecting tray for 24 hours to catch the rat's feces. The rats were not fed during this time. After 24 hours, the stools were collected, weighed, and placed in 9.4 ml of merthiolate-formaldehyde (MF) stock solution and stirred. The egg capsule count using the Merthiolate, Iodine, Formaldehyde Concentration (MIFC) Technic (Franciscio and Blas, 1978) was employed to recover the egg capsules from the feces.

Rat dissection

The rats were sacrificed and then dissected. The heart, lungs, liver, spleen, stomach, intestines, and all associated blood vessels were removed and placed in a petri dish containing 0.85% NaCl. Animals were quickly tested for schistosomiasis by excising a small piece of liver tissue, crushing it between two glass slides, and then looking for egg capsules of *S. japonicum* lodged within the tissue. Whether eggs were detected or not, all organs and blood vessels were searched for egg capsules or adult flukes. Male and female flukes were observed using a hand lens.

Co-inhabiting parasites

During the dissection of the rat and the collection of the feces, the presence of other endoparasites was recorded. For the detection of *Angiostrongylus cantonensis*, lung tissue of the rat was crushed between two glass slides and examined for juveniles. The nematode stomach worm, *Gonylonema neoplasticum*, was located by cutting open the stomach, and looking for adult worms. A *Nippostrongylus muris* infection was indicated when adults were seen in the small intestine or egg capsules in the feces. *Strongyloides ratti* infections were detected by the presence of egg capsules or rhabditiform larvae in the feces. *Taenia crassicollis* infections were visualized by observing the liver for pearly, white cysts. A *Trichinella spiralis* infection was indicated by the presence of juveniles in the left diaphragm muscle, in a section of intercostal muscle, or in a section of the tongue. A *Trichuris muris* infection was suggested by the presence of eggs in the feces. *Syphacia obvelata* infections were determined by the presence of adults in the cecum or large intestine. *Moniliformis moniliformis* infection was determined by the presence of adults in the small intestine. *Hymenolepis diminuta* and *Vampirolepis nana* infections were determined by the examination of the feces for egg capsules and by the collection of adult worms. Two thin blood smears were prepared to check for the presence of *Trypanosoma lewisi.*

RESULTS

Sixty-two rats were caught and 31 or 50% were positive for schistosomiasis. There were 22 rats caught within the intermediate host, *Oncomelania hupensis quadrasi*, snail colonies and 21 (95.5%) were positive for schistosomiasis. Twenty-three rats were caught approximately 100 m from a snail colony, and 13 (56.5%) were infected with schistosomiasis. Seventeen rats were caught approximately 1 km away from a snail colony and zero (0%) were positive for schistosomiasis. There was a significant difference in the prevalence rate of schistosomiasis based on location (Table 1). Those rats caught in *O. quadrasi* snail colonies showed a much higher prevalence than those caught near, but not within, snail colonies. The prevalence was lowest in rats caught far from snail colonies. Each infected rat carried an average of 20.8 adult male flukes and 21.8 adult female flukes. There was no significant difference in infection with schistosomes between the sexes (Table 2). There was a strong correlation between rats with high worm burdens and increased egg capsule output, correlation = 0.908, $p < 0.001$. There was no correlation between the mass of stool produced and the number of eggs passed correlation = 0.366, $0.20 < p < 0.1$. Different sexes passed an equal amount of fecal material per day, by Two-tailed Variance Ratio Test, F 0.05 [1,48] = 5.35. The

Table 1
Comparison of site location versus prevalence.

2x2 Contingence test.
Ho: There is no significant difference between infection and site.
Ha: There is a significant difference between infection and site
alpha =0.05

Site	No. positive	No. negative
Within a snail colony	21	1
Near a snail colony	10	13
Removed from a snail colony	0	17

Chi-squared = 33.35
DF= 2
Chi-squared at (0.05) (2) = 5.991
Therefore, reject Ho.
$p < 0.001$

Table 2
Infection of host relative to sex.

2x2 Contingence test.
Ho: There is no significant difference of infection relative to sex.
Ha: There is a significant difference of infection relative to sex.
alpha =0.05

Sex	Positive	Negative
Male	23	20
Female	8	11

Chi-squared = 0.419
DF= 1
Chi-squared at (0.05) (1) = 3.814
Therefore, do not reject Ho.
$0.25 < p < 0.50$

average mass of feces deposited was 1.633 g/day. Sixty-one percent of the rats were mildly infected with less than ten paired adults. Rats hosting 11 to 20 paired adult flukes comprised 17.8% of the population. Those rats hosting 21 to 30 paired adult flukes comprised 14.2% of the population, while no rats hosted 41 to 50 paired flukes. Rats superinfected with over 50 paired adult flukes comprised 7% of the population (Fig 1).

Co-inhabiting parasites within *Rattus rattus mindanensis*

Other parasites identified within the rats were recorded, and their prevalences were calculated. *Nippostrongylus muris* and *Strongyloides ratti* were the most common intestinal parasites encountered, with prevalences of 79.2% and 77.1% respectively. About half the rats, 54.2% were infected with juveniles of *Angiostrongylus cantonensis*. Exactly 18.75% rats were infected with the tapeworm *Hymenolepis diminuta* and the whipworm *Trichuris muris*. Both *Gongylonema neoplasticum* and *Taenia crassicollis* were found about 13% of the time after dissection, while *Syphacia obvelata* was found to have a prevalence of 8.3%. No rats were infected with the following: *Moniliformis moniliformis, Trichinella spiralis, Trypanosoma lewisi*, or *Vampirolepis nana* (Table 3).

DISCUSSION

In this study, of 62 rats caught, 31, or 50% were positive for *S. japonicum*. Twenty-five of the infected rats were examined for egg capsules in the feces. Nine, or 36%, were passing egg capsules, and the average number of egg capsules deposited was 2.4 per per day. Similar studies done on the prevalence of *S. japonicum* in field rats on the island of Leyte vary. Pesigan *et al* (1961), Cabrara (1976), Oshima *et al* (1978), and Kamiya *et al* (1980), individually discovered prevalences of 22.7%, 72.7%, 86%, and 81.9%, respectively.

In this study, the prevalence was highest when

Table 3
Percent prevalence of common endoparasites of wild rats. (Luttermoser, 1936; Price and Chitwood, 1931; Tubangui, 1931).

Parasite	Locality and Year			
	Washington, DC 1931	Manila, Philippines 1931	Baltimore, MD 1935	Palo, Leyte, Philippines 1995
G. neoplasticum	5	44		13
H. diminuta	31	64	16.6	18.75
M. moniliformis	4			0
N. muris		58		79.2
S. ratti		74	20.2	77.1
S. obvelata			0.48	8.3
T. crassicollis	54		19.2	12.9
T. lewisi	7			0
T. muris				18.75
V. nana	41	1.7	14.4	0

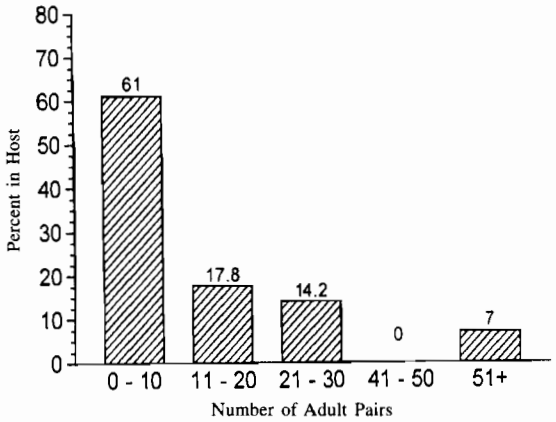

Fig 1–Frequency distribution of *S. japonicum* in *R. r. mindanensis.*

rats were caught in areas within *Oncomelania* snail colonies such as the 100% infection rates seen in Barangays Cogon, Cavite, Gacao, and Libertad. In areas removed from snail colonies, the prevalence dropped dramatically. No infected rats were caught in Barangays Fatima Village, and Salvacion. Areas bordering snail colonies had mixed percentages. In Barangay Cangumbang 80% of the rats were infected, while rats from Barangay San Antonio, and an area in Caibaan showed a 50% infection rate. Table 1 illustrates that site and infection rates are directly related. This means that trapping further away from snail colonies diminishes the probability of catching an infected rat. It can further be deduced that the rats were unlikely to spread the disease to new areas that were originally non-endemic or where the disease had been eradicated. Humans and their behavior have a greater potential to spread and maintain schistosomiasis (Gillett, 1984).

Pesigan and Hairston (1961) captured all their rats in the municipality of Palo in 1953 and 1954. A low prevalence of 22.7% in their study may be attributed the method of detecting rats positive for schistosomes. During the 1950's, efficient techniques for detecting egg capsules in the stool had not been developed. Therefore, if rats were passing few, if any, egg capsules, and if the technic were less effective, then false negatives would be recorded. The technic used by Pesigan and Hairston (1961) were the direct fecal smear, acid-ether sedimentation, and the Faust-Meleney egg-hatching technic. It can also be argued that an increase in the prevalence of rats of 22.7% in the 1950s to higher rates today could be attributed to an increase in human population, but in an indirect way. The human population of Palo in the 1950s was about 25,000 with a prevalence of about 30%. This means that about 7,500 people were infected. In 1990, the human population increased to 37,777, but with a prevalence of only 2.12% (National Statistic Office, Manila, 1992). This means that only about 800 people were infected. Therefore, despite the increase in human population, the actual number of infected individuals that could contribute to the spread of the disease had decreased. The low prevalence in Palo today is attributed to the use of the safe and effective antischistosomal drug, praziquantel, and the convenient location of the Schistosomiasis Research Hospital to the population for treatment. However, the increase in the prevalence in rats could be indirectly

due to the increase in human population. As the human population increases, a concomitant increase in the domestic animal population should occur. Since Fernandez *et al* (1983) determined that domestic animals play a more important role in the epidemiology than previously thought, the increase in the prevalence in the rat population could be rationally explained.

Cabrera (1976) performed his study in 1975 on Leyte in the inland municipalities of Jaro, Santa Fe, and Pastrana, and recorded an overall prevalence of 73%. A 73% prevalence can be explained by examining the location of the trapping sites. In Santa Fe and Pastrana snail colonies are practically ubiquitous, unlike Jaro which has large tracts of land that are *Oncomelania*-free (Blas *et al*, 1990). The prevalence should be high, near 100%, in the rats taken from Santa Fe and Pastrana, while those rats taken from the non-endemic areas of Jaro will have few, if any rats infected with schistosomiasis. If equal numbers of rats were taken from the three municipalities, the overall average prevalence should be 66.7%. Therefore, Cabrera's 72.7% infection rate is approximate to the findings in this study. Oshima *et al* (1978) recorded a considerably high prevalence of 86% in field rats. This may be attributed to their study areas. Oshima *et al* (1978) studied in the following locations: Santa Fe, Libertad, Anahauay, Gacao, Palo, and Caibaan. All of these areas with the exception of Palo, are cursed with large tracts of snail-infested land (Blas *et al*, 1990). Kamiya *et al* (1980) did their study in the inland, endemic barangay of Dagami. The trapping sites were all adjacent to snail colonies and an average yearly prevalence in rats of 81% was recorded. The selection of trapping sites remains the most important factor in prevalence amongst *Rattus rattus mindanensis.*

The presence of egg capsules in the feces presents an interesting enigma. Pesigan *et al* (1958) determined that rats pass a mean of 21 egg capsules per day. This was a staggering number compared to the 2.4 egg capsules per day in the author's study. However, an explanation that might be considered was dependent upon the fact that Pesigan *et al* (1958) used only three rats that had high worm burdens. In the author's study, only one rat had an egg capsule output (27 egg capsules/day) close to the Pesigan *et al* (1958) average, and this was due to a superinfection with 211 adult flukes. Also in the author' s study, there was a direct correlation between increasing numbers of adult flukes within the rats and a high egg capsule count.

Hairston's study on the population ecology of *S. japonicum* in Leyte hinted that rats were significantly contributing to the maintenance and transmission of the disease (Hairston, 1962; 1965). His data collected from an inland village crudely estimated that there were 60 rats per hectare with a prevalence of 64%. He then estimated that the individual rats were passing an average of 222 hatchable eggs per day. Hairston used these arguably high estimates of the rat population (60 rats/hectare) and included information gleaned from laboratory rat studies on egg capsule hatchability to make his population ecology equation for *S. japonicum* in Leyte more workable. In 1975, the study done by Cabrera (Cabrera, 1976) would later help to support Hairston's estimates on egg capsule deposition. Cabrera selected 19 rats from his study and found that 11 of these rats (58%) were passing viable *S. japonicum* egg capsules with a mean of 5 miracidia per rat, taking just two to three pellets from the distal colon immediately after autopsy for his egg capsule hatchability tests. It was determined in this investigation that the average amount of fecal matter deposited per rat was 1.633 g and one pellet weighed roughly 0.025 g. Therefore, about 150 miracidia per rat per day should be liberated. Also, Hairston argued that large numbers of rats superseded the impact of large mammals, like water buffalos, which have a lower prevalence and smaller populations. He used the following rational to support his estimates. Snail infection rates were shown to be higher (7.8%) within 70 m of houses than elsewhere, in comparison to areas more distant from homes (2.66%). Hairston argued that rats could only contribute the rate of infection of the snails in the areas away from homes. Second, the emergence of cercariae from snails showed a daily cycle, with a high emergence in the early evening (Hariston, 1962; 1965; Hariston and Santos, 1961). Hairston believed the emergence was initiated by natural selection because field rats are nocturnal animals and therefore, were the most important definitive host. There are four major flaws to Hairston's rationale. First, he misread the behavior of humans and their defecation habits. Remote areas that seem to be an unlikely spot for a person to defecate are, in reality, prime locations for this personal function. Also, Filipinos living in these endemic areas use neither bathrooms (Blas and Rona, 1998) nor toilet paper on a regular basis. These bathrooms have no outdoor plumbing and are generally not maintained, except when company is expected. Filipinos usually defecate near a water source so that they are able to wash immediately afterwards. Once the feces are deposited in water, they are easily

washed to remote areas by heavy rains, which are common, causing rivulets to disseminate infected snails or contaminated feces into remote regions (Makiya *et al*, 1981; 1982; Pesigan and Hairston 1961; Pesigan *et al*, 1958). This could explain the high number of infected snails in remote areas. Secondly, rats may not represent the most important definitive host as supported by Loker's argument (Locker, 1983). Locker detected a correlation between the pre-patent period of development of the adult flukes, and the longevity of its definitive mammalian host. He stated that the population stability of the known mammalian schistosomes was reliant of the role of relatively large mammals as reservoirs. *Schistosoma japonicum* has a pre-patent period of 33.5 to 37.5 days, while the life expectancy of a wild rat is 60.6 days (Watson, 1950). This means that unless an infection occurred early in the rat's life, the rat would contribute very little to the spread of the disease. Loker reasons that rodents, despite their great abundance and likelihood to come in contact with freshwater habitats indigenous to schistosomes, do not make good definitive hosts. He states that their limited longevity, periodic population fluctuations, and extremely high turnover rates of their populations make them less than adequate epidemiological factors. Only *S. douthitti* is exclusively a rodent parasite and has the shortest prepatent period, just 28 days (Loker, 1983). This is about 20% to 34% shorter than *S. japonicum*. A third fault in Hairston's presentation ragards his interpretation of cercarial emergence. Cercarial emergence is not restricted to only the early evening hours, as Hairston believed, but occurs at various times of the day (Kawashima *et al*, 1985; Nojima, 1980). Snails are more likely to come in contact with water in the evening when they lay their eggs at or near the water surface. However, this does not necessarily indicate that the most important definitive host must be nocturnal, especially since cercariae can remain infective for up to 36 hours (Kawashima *et al*, 1985; Nojima *et al*, 1980). The fourth flaw involved Hairston's incorrect data regarding the importance of domestic animals as reservoir hosts. Fernandez *et al* (1983) indicated that dogs, pigs and water buffalos were passing larger amounts of egg capsules with higher hatchability rates than was previously recorded. Perhaps, if Hairston had the updated figures on the contribution of domestic animals, then he would not have had discrepancies in the population ecology equation.

Magath and Mathieson in 1946 and Oshima *et al* in 1978 correlated a high worm burden with a visibly diseased liver. Therefore, Cabrera's results had a built-in bias, since he only chose rats for hatchability studies that exhibited damaged livers, thereby suggesting a heavy worm burden and probably a high egg capsule count in the feces. Also, since these egg capsules were taken directly from the distal portion of the gut, there may be a greater likelihood of hatching. Table 2 shows that one sex in not more prone to infection than the other does. There is no correlation between fecal mass and egg capsule output, while the mass of male and female rat fecal deposits per day are equal. Therefore, worm burden remains the sole variable determining egg capsule output.

Fig 1 indicates that the frequency distribution of *S. japonicum* in *R. r. mindanensis* matches those predicted by the mathematical model known as the negative binomial. The negative binomial distribution predicts that a small portion of the host population will contain a majority of the parasites. In this study, a relatively small portion (7%) of the rat population is superinfected with *S. japonicum*, so 7% of the rat population has the potential to significantly contribute to the epidemiology. If there are only 30 rats per hectare (Salamat, 1995), then only approximately two rats are potentially transmitting the disease. If one considers that some of these rats are not passing hatchable egg capsules due to flukes located in areas of the body not conducive to egress such as the lungs, as shown by Oshima *et al* (1978), then their importance as reservoir hosts becomes even less significant.

In this study, no rats were infected with *Moniliformis moniliformis, Trichinella spiralis, Trypanosoma lewsi,* and *Vampirolepis nana.* Tubangui (1931) did a similar survey in Manila, Philippines and found no cases of *Trichinella spiralis* and low prevalence of *Moniliformis moniliformis* (4.2%) and *Vampirolepis nana* (1.7%). No reported cases of *Trypansoma lewsi* were found, and this is not unusual, since older rats develop an adequate defense (Duca, 1939). Rats infected with *Strongyloides ratti* (77.1%) compared nearly equally with Tubangui's study done over sixty years earlier, which found a prevalence of 74% in the rat population. Infections with *Gonylema neoplasticum, Taenia crassicollis,* and *Trichuris muris* were similar to prevalence of rats in Baltimore during the 1930s (Luttermoser, 1936). *Nippostrongylus muris* was the most common intestinal parasite found in this study, with a prevalence of 79.2%, which is over 20% higher than rats caught in Manila during the 1930s (Tubangui, 1931). This increase may be due to the rural environment of the trapping sites in Palo, Leyte.

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