

IDENTIFICATION OF THE SNAIL SECOND INTERMEDIATE HOSTS OF *Echinostoma* species IN BARANGAY NEW SIBONGA, NABUNTURAN, COMPOSTELA VALLEY

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Echinostomiasis is a foodborne, intestinal, zoonotic, snail-mediated parasitosis by the Genus *Echinostoma*. It is endemic in Southeast Asia and the Far East. In the Philippines, two echinostome species, *Echinostoma ilocanum* and *Echinostoma malayanum* infect humans with high prevalence. The foci of pathogen transmission and natural reservoirs are widespread and are linked to fresh or brackish water habitats. Metacercariae, the infective stage of the parasite, are ingested by humans in raw or undercooked fresh or brackish water mollusks, fishes, crustaceans, and amphibians (tadpoles or frogs), which constitute a substantial portion of the diet in endemic areas. The parasite became endemic because of ingestion of the raw second intermediate hosts infected with metacercariae. The reported snail second intermediate hosts are *Pila luzonica* ("kuhol") and *Vivipara angularis* for *Echinostoma ilocanum* and *Lymnaea (Bullastra) cumingiana* for *Echinostoma malayanum*. This study aimed to identify the snail second intermediate hosts of *Echinostoma* species as well as the *Echinostoma* species found in Barangay New Sibonga, Nabunturan, Compostela Valley Province. A total of 2,100 freshwater snails were collected and identified from the research locale. Snail species identified were *Pila* species, *Pomacea canaliculata*, and *Melanoides juncea*. The shells of the snails were cracked and the muscle parts were removed and digested using artificial gastric juice (0.1% pepsin and 0.7% hydrochloric acid) for 1 to 2 hours at 370 Celsius. After incubation, the digested muscle parts were washed with physiologic saline solution (0.9% sodium chloride) and sieved and filtered to separate the sediments from the suspension. The metacercariae recovered were fed to the albino rats by oral gavage. Feces of albino rats were examined 7, 14, and 21 days post-infection for the identification of eggs of *Echinostoma* using Direct Fecal Smear Technique. Operculated ova measuring 80 to 140 µm by 58 to 90 µm were recovered 21 days post-infection from albino rats inoculated with metacercariae from *Pomacea canaliculata* snails. The characteristics of the recovered egg were compatible with the characteristics of *Echinostoma* species eggs. Therefore, the snail second intermediate hosts of *Echinostoma* species in Barangay New Sibonga, Nabunturan, Compostela Valley Province is possibly *Pomacea canaliculata*.

However, after 21 days post-infection, no adult flukes were recovered from the infected albino rats because of some technical difficulties in the recovery of the minute organism. For this reason, this study was unable to identify the species of *Echinostoma*, which produces the abovementioned eggs.

Keywords: *Echinostomiasis*, snail second intermediate hosts, *Pomacea canaliculata*, Compostela Valley Province, *Echinostoma* species

Introduction

Foodborne trematodes infections are major public health problems with over 40 million persons affected throughout the world, mainly in eastern and southern Asia (Abdussalam, Kaferstein, & Mott, 1995). In all cases, infection took place through the ingestion of raw plants, freshwater fishes, freshwater snails, freshwater shellfishes or amphibians (tadpoles or frogs) containing the infective stages of these parasites (metacercariae) especially those parasites belong to Family Echinostomatidae.

Members of the Family Echinostomatidae, a group of intestinal trematodes of birds and mammals, are morphologically characterized by the presence of head crown with collar spines. Several of these are known to infect man and cause gastrointestinal troubles. Echinostomiasis is a foodborne, intestinal, zoonotic, snail-mediated parasitosis by the Genus *Echinostoma* (Graczyk & Fried, 1998). Adult trematodes infect variety of mammals and aquatic birds. Two separate life cycles of echinostomes, the human life cycle and the sylvatic life cycle (which have common zoonotic potential), have been identified and demonstrated to efficiently operate in endemic areas (Carney, 1991). In endemic areas, the disease occurs focally and was associated with common socio-economic practices (Tangtrongchitr & Monzon, 1991). The foci of pathogen transmission and natural reservoirs are widespread and linked to fresh or brackish water habitats. Metacercariae, the infective stage of trematodes, are ingested by humans in raw or undercooked fresh or brackish water mollusks (pulmonate and opisthobranch snails or bivalves), fishes, crustaceans, and amphibians (tadpoles or frogs), which constitute a substantial portion of the diet in endemic areas. Adult flukes are also found in wide range of domesticated mammals and birds in these areas. The parasite is then endemic because of ingestion of the raw second intermediate hosts infected with metacercariae.

In the Philippines, the most common species of *Echinostoma* are *Echinostoma ilocanum* and *Echinostoma malayanum*. The second intermediate hosts are freshwater snails (Belding, 1964). In the Philippines, the reported second intermediate hosts were *Pila luzonica* ("kuhol") and *Vivipara angularis* for *E. ilocanum* and *Lymnaea (Bullastra) cumingiana* for *E. malayanum*. *Pila luzonica* infected with metacercariae is eaten uncooked by some people (Cross & Basaca-Sevilla, 1986). A study on eating habits associated with *Echinostoma malayanum* infections was conducted in the Philippines by Tangtrongchitr and Monzon (1991). The said parasite is endemic in Isabela, Northern Luzon because of ingestion of raw second intermediate hosts, the *Lymnaea (Bullastra) cumingiana*. Since these snails are common to any localities of the country, cases of *echinostomiasis* may increase but may remain unreported. As such, prevention and control programs are not yet established in their localities maybe because of lack of knowledge regarding the parasitic infection.

Morbidity and mortality due to *echinostomiasis* are difficult to assess in endemic areas because of a prolonged latent phase, a short acute phase, asymptomatic presentations, and also because of a similarity of clinical symptoms to other intestinal *helminthiasis* (Shekhar, 1991). Clinical symptoms are related to parasite load. In light-to-moderate infections, anemia, headache, dizziness, slight anemia, stomachache, gastric pain, and loose stools have most often been reported (Belding, 1964). Heavy infections are associated with *eosinophilia*, abdominal pain, profuse watery diarrhea, anemia, edema, and anorexia (Carney, 1991). Pathologically, echinostomes damage the

intestinal mucosa and cause extensive intestinal and duodenal erosions (Chai et al., 1994) and catarrhal inflammation (Belding, 1964).

Human *echinostomiasis* is endemic to the Southeast Asia and the Far East, i.e., mainland China, Taiwan, India, Korea, Malaysia, Philippines, and Indonesia (Huffman & Fried, 1990). In the Philippines, two echinostome species, *Echinostoma ilocanum* and *Echinostoma malayanum* infect human with an overall prevalence of 44 percent (Huffman & Fried, 1990; Graczyk et al., 1998). Garrison in Manila and later in Northern Luzon first noted *Echinostoma ilocanum* in 1907 with an average prevalence of 11 percent among Ilocanos (Cross, & Basaca-Sevilla, 1986). It was also noted that the parasite is also endemic in some areas of Southern Mindanao.

In the year 2000, Compostela Valley Province has been the subject of epidemiologic study due to the outbreak of what was called a “mysterious disease” which was eventually identified as *capillariasis*. Several people from this locality died from common symptoms of diarrhea, *borborygmi*, abdominal pain, loss of weight, and dehydration. The first reported case of *capillariasis* was in Ilocos Region where echinostomiasis was thought to originate. These epidemiologic phenomena sparked interest to conduct a pilot study on the reported cases of *echinostomiasis* in Barangay New Sibonga, Nabunturan, Compostela Valley. The initial interview of the residents of the said locality revealed that due to economic reasons, eating snails and fresh water fishes was inevitable. Lakes, rivers, rice fields, and brackish water were the primary source of these snails and fresh water fishes, which were the possible second intermediate hosts of *Echinostoma species*.

The University of the Philippines-College of Public Health and Department of Health conducted stool examination among 12 to 71 month-old children in Barangay New Sibonga, Nabunturan, Compostela Valley last October 2004 and trematode ova were found, which was identified as *Echinostoma*. This was the first reported case of *echinostomiasis* in Nabunturan, Compostela Valley. The known first intermediate hosts of the parasite are the snails and the known second intermediate hosts could be another species of snails, amphibians (tadpoles or frogs), freshwater fishes, and crustaceans. Ingestion of raw second intermediate hosts infected with *metacercariae* of the parasite is the possible source of infection but there was no further study conducted in this locality to identify the snail second intermediate hosts of the parasite. The echinostomes display extremely broad specificity for the second intermediate hosts. Life cycle may be completed without involvement of humans because of the presence of animal reservoir hosts. But it is evident that in Barangay New Sibonga, Nabunturan, Compostela Valley, humans are also infected and the acquisition of the infection is not yet elucidated in the area.

This study is formulated to identify the snail second intermediate hosts carrying the infective stage of *Echinostoma* in Barangay New Sibonga, Nabunturan, Compostela Valley. The result of this study shall raise the awareness of the community about the parasite which may aid in the formulation of prevention and control measures for echinostomiasis.

This study aimed to focus on identifying the snail second intermediate hosts of *Echinostoma species*, particularly those found in Barangay New Sibonga, Nabunturan, Compostela Valley Province.

Materials and Methods

This study was conducted in Barangay New Sibonga, Nabunturan, Compostela Valley with 8 *puroks*. Freshwater snails were collected by handpicking technique from the riverbanks, fishponds, and rice fields of the said locality. A total of 2,000 snails were collected and identified as *Pila species*, *Pomacea caniculata*, and *Melanoides juncea*. The shells of the snails were cracked and the muscle parts were removed and digested using artificial gastric juice (0.1 percent pepsin and 0.7 percent hydrochloric acid) for 1 to 2 hours at 37° Celsius. After incubation, the digested muscle parts were washed with physiologic saline solution (0.9 percent sodium chloride) and sieved to separate the sediments from the suspension. The recovered *metacercariae* were fed to albino rats by oral gavage. Rat feces were examined for 7, 14, and 21 days post-infection for the identification of eggs of *Echinostoma* using Direct Fecal Smear Technique. Immediately after finding the eggs, the infected albino rats were dissected to remove the whole length of intestines to possibly recover adult flukes.

The apparently recovered adult flukes were fixed in 10 percent formalin and stained with Carmine Red and Malachite Green. The stained adult fluke were mounted in Canada balsam. The final phase of the study will be the identification of the species of the recovered parasites based on the number of spines and testicular lobes.



Figure 1. *Pila species* snail



Figure 2. *Pomacea caniculata* snail

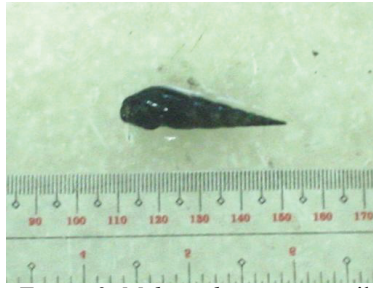


Figure 3. *Melanoides juncea* snail

Results and Discussions

Infection Status of Possible Echinostoma Metacercariae

Possible metacercariae of *Echinostoma* were detected in *Pila species*, *Pomacea caniculata*, and *Melanoides juncea* snails. Despite the apparently recovered metacercariae were not specific for a certain parasite, these were still inoculated to the test animal to prove whether it is a metacercariae of a parasitic fluke. Possible larval stages of parasites were also identified from *Pila species* and *Pomacea juncea* snails. Cystic stages of free-living protozoan parasites were identified *Pila species* and *Pomacea caniculata*. Specifications of such larvae and cysts were not yet done.

Table 1

Percentage of Snails Infected with Possible Metacercariae

Snail Species	No. of snails collected	% of snails infected with possible metacercariae	Average number of metacercariae detected per snail
<i>Pila species</i>	700	32 (4.5%)	6
<i>Pomacea caniculata</i>	700	167 (23.9%)	21
<i>Melanoides juncea</i>	600	95 (15.8%)	15



Figure 4. Possible Metacercaria Recovered from *Pila species* Snails



Figure 5. Possible Metacercaria Recovered from Pomacea caniculata Snails

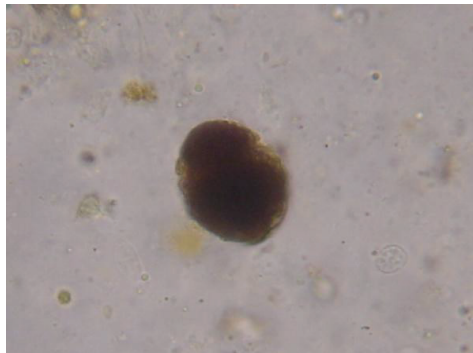


Figure 6. Possible Metacercaria Recovered from Melanoides junccea Snails



Figure 7. Possible Larval Stages of Parasite from Pila species Snails



Figure 8. Possible Larval Stages of Parasite from *Pomacea caniculata* Snails



Figure 9. Possible Cystic Stage of Protozoa from *Pila species* Snails

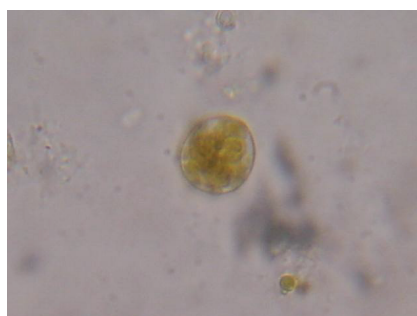


Figure 10. Possible Cystic Stage of Protozoa from *Pomacea caniculata* Snails

Possible Second Intermediate Hosts and Parasite Stages

The recovered possible *metacercariae* were fed to albino rats by oral gavage. The rats' feces were examined 7, 14, and 21 days post-infection for the identification of eggs. No fluke eggs were recovered on the 7th and 14th day post-infection. However, operculated ova measuring 80 to 140 μm by 58 to 90 μm were recovered 21 days post-infection inoculated with *metacercariae* from *Pomacea canaliculata* snails. The characteristics of the recovered egg were compatible with the characteristics of *Echinostoma species* eggs. Therefore, the snail second intermediate hosts of *Echinostoma*

species in Barangay New Sibonga, Nabunturan, Compostela Valley Province is possible *Pomacea canaliculata*. No parasitic ova were identified from the rest of the albino rats.

Table 2

Stool Examination Results of Inoculated Rats

Snail Species	7 days post-infection	14 days post-infection	21 days post-infection
<i>Pila species</i>	No ova found	No ova found	No ova found
<i>Pomacea canaliculata</i>	No ova found	No ova found	Operculated Ova found
<i>Melanoides juncea</i>	No ova found	No ova found	No ova found

The whole length of the intestines of the albino rat inoculated with *metacercariae* from *Pomacea caniculata* was removed to possibly recover an adult stage of parasite. However, no adult flukes were recovered from the infected albino rats because of some technical difficulties in the recovery of the minute organism. For this reason, this study was unable to identify the species of *Echinostoma*, which produces the abovementioned eggs.

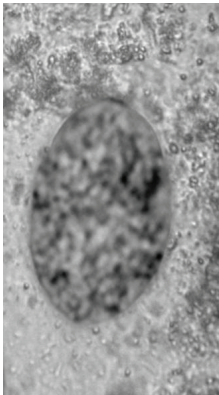


Figure 11. Ovum Recovered from Feces of Albino Rat Inoculated with Metacercariae from *Pomacea caniculata* Snails

Conclusions and Recommendations

Investigations of the infection rates of *metacercariae* in snail second intermediate hosts, in combination with identification on adult flukes, can usefully analyze the epidemiology of fluke infections in a particular area. In the case of *echinostomiasis*, the second intermediate hosts are not well investigated. Therefore, in order to provide epidemiologic information on *echinostomiasis*, the snail second intermediate hosts of *Echinostoma* species were identified as well as the identification of *Echinostoma* species

in Barangay New Sibonga, Nabunturan, Compostela Valley. Several researches have reported on *Echinostoma* fluke *metacercariae* collected from fresh water snails, tadpoles and frogs. The reported snail second intermediate hosts of *Echinostoma ilocanum* were *Pila luzonica* and *Vivipara angularis* and *Lymnaea (Bullastra) cumingiana* for *Echinostoma malayanum*. The identified fresh water snails from the research locale were *Pila species*, *Pomacea caniculata*, and *Melanoides juncea*, which were possible second intermediate hosts of *Echinostoma* and other flukes. Stool samples were taken from the residents and it was found that *Echinostoma* infection was present in all age groups. The possibility of exposure through the usual food consumed is likely. Higher infection rates of *echinostomiasis* may suggest higher transmission in these groups of people in the said locality. Higher transmission may mean higher consumption of infected snail second intermediate hosts. This study recovered different larval stages of flukes in *Pila species* and *Pomacea caniculata* snails and would indicate that these snails may serve as both the first and second intermediate hosts. Furthermore, cystic stages of free-living protozoans were recovered from *Pila species* and *Pomacea caniculata* snails indicate transmission of protozoan infections from snails to reservoir hosts is most likely. Specifications of these stages of parasites were not yet done, thus, further investigation is recommended. As for the possible intermediate hosts, the recovery of different larval stages of trematodal parasites in freshwater snail species is alarming to consider that snails were major source of food in the area. This may have large implication to health conditions of the area, thus, it is necessary that this occurrence be well understood by the local residents and health advocates to lessen this problem. Recommendations regarding possible intermediate hosts include the need to inform the local residents to avoid eating of freshwater snails or cook it properly to prevent acquisition of fluke and protozoan infections. Edible field frogs also present in the locality could be an intermediate host, thus, must be cooked properly as majority of parasites were observed in its gut (Belizario, et. al, 2000). There is a possibility freshwater fishes were also infected with flukes' *metacercariae*, thus, survey of all fish food in the area is necessary for identification of parasites. Other possible intermediate hosts, such as tadpoles, frogs, freshwater fishes, and ducks, are recommended for further studies.

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