# Article

# Zoonotic organisms in selected species of freshwater gastropods in Lanao del Norte, Mindanao, the Philippines

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#### Abstract

Urgency and worldwide attention are now focused on parasites causing zoonotic diseases. An inventory of parasites inhabiting the previously identified freshwater gastropods in Lanao del Norte is the next step necessary to elucidate and determine the etiology of zoonoses. Four sampling sites, composed of thirty areas within the Municipalities of Lala, Kapatagan, and Salvador from the island of Mindanao, the Philippines, were thoroughly searched in this study from February to August 2018 using an explorative-investigative study design. Out of the 2,460 sampled individuals, eight species of gastropods were observed and collected, namely: Melanoides tuberculata, Melanoides turriculus, Tarebia granifera, Pomacea canaliculata, Oncomelania quadrasi, Gyraulus crista Linn., Vitta virginea and Radix. These snails were then cleaned and rinsed with water and were then brought to the laboratory for examination. Small Sizes of snails were crushed, while the larger sizes were subjected to shedding. The parasites sampled were then identified using key manuals. After the examination, three morphotypes of cercariae were sampled and recorded in this study, namely: Vivax cercariae, schistosome cercariae, and Pigmentata amphisome cercariae. Two parasitic protozoans (Paramecium and Entamoeba coli) and one parasitic microscopic animal (rotifer) were also sampled. Paramecium and E. coli were found out to be the most prevalent in all the parasites. Although these species were reported to be of no ecological and medical significance, they were also reported to cause physiological effects to the host snails. Schistosome cercariae and Pigmentata amphisome cercariae observed were of significant medical importance; thus, since these snails were found near in areas where there are human dwellings, the transmission of diseases caused by parasites vectored by these snails is possible. It is, therefore crucial that there is a need to implement proper sanitation practices in the communities and adequate management of these snails to prevent, manage, and control the transmission of diseases caused by the parasites.

Keywords Amphistome; Cercariae; Schistosome; Vivax; zoonosis.

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# **1** Introduction

Increased attention and urgency worldwide had been focused on the prevalence of parasites-caused zoonoses, which contributed to an increasing number of human mortality and morbidity. In the prevention of a zoonotic disease, it is very relevant to study and understand the manner of its transmission. This basic understanding could further improve its surveillance and develop an intervention system that can be effective for its prevention and control.

However, before strategies can be crafted and preventive measures can be implemented, it is also very relevant to set baseline information for what are the vectors, and parasitic species are present in the area, where they are strategically found, how transmissions can happen to humans and animals and what are the specific hosts. Studies conducted on the incidence of zoonosis have documented in the different parts of the Philippines (Sosa et al., 2014; Farahnak et al., 2005) and other areas of the world (Pandey et al., 2002; Caron et al., 2008; Mohammed et al., 2016; Chontananarth et al., 2017). The prevalence of parasites caused zoonoses have gained increased attention and urgency worldwide because it contributed to increased human mortality and morbidity (PAHO, 2003). It had been of great concern since social and environmental factors also influenced its distribution, prevalence, and transmission patterns making its management and control a very tedious effort (Komba, 2017). Since the prevention of zoonoses is highly dependent on the identification of the animals and foodstuffs that can be the primary source of infection (EFSA, 2010) including information on the vectors that facilitated the spread of the disease, this study was therefore conducted. In the Philippines and other parts of the world, snails had been looked into as one of the vectors for zoonoses since they are used as source of food Venugopal et al., 2017) and medicine (Bonnemain, 2005) and they may be intermediate hosts of several zoonotic organisms that cause diseases in human and animals (Mohammed et al., 2016; Rae, 2017; WHO, 2010). The presence of these freshwater snails to areas wherein human and animal activities are centered can be one of the significant points of transmission (Jones et al., 2008). This study can be very vital because the data that can be generated from this study can trace the etiology of zoonotic diseases and can be very important, especially in crafting strategies for disease prevention, management, and control.

### 2 Methodology

#### 2.1 Study area

The study was conducted in the Municipalities of Lala, Kapatagan, and Salvador, all in the Province of Lanao del Norte (Fig. 1). All of the identified sites are endemic areas of schistosomiasis. Different ecosystems were purposely selected, including rice fields, streams/rivers, irrigation canals, and waterfalls/seepages.

Prior to the conduct of the study, the Local Chief Executives of the three municipalities were consulted and informed on the objectives of the study. Ethical permission was sought, and affirmative support was granted.

The explorative-investigative study design was employed in the conduct of this study. Before the collection of the sample, the snail habitats were determined and located visually on the field. The area was mapped, and

geographic references were recorded. An indefinite number of snail species present in the area were collected using forceps and an improvised long-handled kitchen sieve within a 15-minute period per site. Collected snails were kept in a sealed container, labeled appropriately, and transported to the laboratory for examination. Snails were then categorized and grouped according to the site where they were sampled, species, and sizes.



Fig. 1 Geographical location of the study sites.

All samples that were collected per site were placed in a polyethylene bag and were correctly labeled. The samples were then washed, counted, and photographed. Identification was done using identification manuals (Harold and Guralnick, 2010; Frest et al., 1999; Thompson, 2004). The snails were then grouped according to their species and sizes.

### 2.2 Examination of parasite infection

### 2.2.1 Isolation method / monitoring of cercariae shedding

Larger sizes of snails were placed in a glass beaker and were added with 20 ml of dechlorinated tap water per 20 individuals (Frandsen et al., 1984). The beakers were then subjected under intense simulated lighting at  $20-20^{0}$ C for 4-6 hours to facilitate the release of the cercariae. Once cercariae were observed, snails were transferred individually to small beakers. The same procedure was followed in each snail, and details were recorded. Identification of cercariae-producing snails was made and was vital in the identification of the cercariae.

# 2.2.2 Squash technique

Methods used by Caron et al. in 2008 were used in this study by placing small-sized snails in a glass slide and crushing it with a forceps. The crushed snails were then be added with a drop or two of water and minced with a pair of dissecting needles. It was then examined using a microscope. All possible parasite found in a single snail were recorded and were later on identified.

A record was then derived, including time and place of the collection, the time of examination, the number of snails harboring each species of cercariae encountered, and the presence of other species of parasites.

#### 2.2.3 Identification of cercariae

A field guide on the identification of cercariae from Frandsen and Christensen (1984) was used for proper and precise identification of the cercariae. A similar publication like Farahnak et al. (2006), Mohammed et al. (2015), and Chontananarth et al. (2017) had also been used in the identification.

Collected data were analyzed using descriptive statistics.

#### **3** Results and Discussion

A total of 2,460 individual samples of snails were collected, examined, and identified from different sampling sites (Tables 1 and 2). The collected samples belonged to eight different species (Fig. 2) and were grouped into six families, namely: Thiariidae, Ampullariidae, Pomatiopsidae, Planorbidae, Lymnacidae, and Meritidae. It was observed that *Melanoides tuberculata* was the most widely distributed species, which was present in 20 areas out of the 30 that composed the four sampling sites. In Site 1, *Tarebia granifera* was the most dominant, Site 2 had *Pomacea canaliculata* and Sites 3 and 4 had *Oncomelania quadrasi*.

The rate of infection also varies per site. It was observed that the irrigation canal had the highest percentage (69.40%) of the entire four sites. It was closely followed by the ricefield (61.73%), bodies of freshwater (14.42%) and waterfalls (1.34%). It was observed from the conduct of the study that the irrigational canals had been favorable sites for the parasites to increase because of the nutrient availability that was attributed by the combination of nutrient enrichment from the ricefields and the organic waste discharges by the surrounding community, these findings were contrary to the conclusions of the study conducted by Devkota et al. (2011) wherein accordingly contained water bodies like temporary ponds and lakes had higher prevalence of parasite infection. The areas under this site had waters that were generally turbid which were usually fast flowing. Characteristic vegetation in the area consisted of *Paspalum conjagatum* Brgius, *Colocasia esculenia* and *Musa acuminata*. The irrigation canals that were sampled during the study were generally exposed to sunlight. There were draft animals that were pasturing in the area like *Bubalus bubalis*, *Anas luzonica, Bos taurus, Equus feruscaballus*, and *Sus scrofadomesticus* that excreted waste in the water adding to the organic waste that was commonly carried away by the water. Houses were also observed along the irrigation canal, and their waste water was directly discharged to the water flowing in the irrigation canal.

Site 2 or the Ricefields, on the other hand, were the next more favorable to the parasites. The areas under Site 2 were generally exposed to sunlight, with a sparse amount of trees that partially shade some of the exposed areas. Solid waste materials were evident in the ricefields, including food wrappers, empty bottles of pesticides, and fertilizers. Generally, the water in the ricefieldswas turbid. Waterflow was nearly stagnant. Aside from *Oryza sativa*, *P. conjagatum Brgius*, *C. esculenia*, and *M. acuminata* were also present in the spaces in between the ricefields and these vegetations provided shade to the area.

Site 3, or the bodies of freshwater was the third area where parasites favored. Common characteristics of the areas under this site are partially exposed to sunlight. There were farm animals observed to be grazing around which contributed to the organic waste. Solid waste materials were also present. Human activities are active in the area like quarrying, which was one of the primary livelihood of the people. Other areas were observed to be near houses with no clean comfort rooms making the creeks place where human increment was deposited. Generally, water in the sampled freshwater bodies was turbid during the time of the conduct of the study attributed to the rain during the time of sampling. Water flow was slow to moderate, and vegetational cover includes *Bambuseae*, Site 4 or the waterfalls, and seepages were the least favored by the parasites. This can be attributed to the constant disturbance of the area by the tourist visiting the waterfalls. There was the less vegetational cover, which can only be found in the seepage area; the rocky substrate of the waterfalls made a

low productive ecosystem for parasites to thrive. The water was turbid with the slow-moderate flow. The details of the collection areas are presented in Table 2.

Site	Tarebia granifera	Melanoides tuberculata	Melanoide sturriculus	Oncomelania quadrasi	Pomacea canaliculata	Gyraulus crista	Radix	Vittavirginea				
Irrigation Canal (Site 1)												
Pinuyak 1												
Tenazas IC	+	+	+	-	-	-	-	-				
Sto. Thomas 1	-	-	-	-	+	-	-	-				
Sto. Thomas 2	+	+	+	-	-	-	+	-				
Curva-Miagao	+	+	-	-	-	- 1	+	-				
Inagasan IC	-	-	+	+	+	- 1	+	-				
Cumpra IC	+	+	-	-	-	- 1	-	-				
Cumpra IC 2	+	+	-	-	-	- 1	-	-				
			Rice	field(Site 2)		1						
Lala Proper												
Schoolside RF	-	+	-	+	-	-	-	-				
Butadon RF	-	+	-	+	+	-	+	-				
CumpraMiagao		_	_	_	+	+	+	_				
RF	-	-	-	-	т	т	т	-				
Flowing Waterbodies (Site 3)												
Lala Proper												
Schoolside	-	+	-	+	-	-	-	-				
Creek												
Suba 5,	+	+	+	+	-	+	+	-				
Tenazas		'	·	'		·						
Abaga Bridge	+	-	-	+	+	+	+	-				
Simpak Creek	-	+	-	-	+	-	+	-				
Tunaan Creek	-	-	-	+	-	-	-	+				
Rebe 1	-	-	-	-	-	-	+	-				
Rebe 2	+	+	+	-	-	+	+	-				
Cabasagan	+	_	_	+	-			-				
Stream				'								
Cabasagan	+	+	+	+	-	-		-				
Creek												
Maguindanao	+	+	+	+	-	-	+	-				
Creek								ļ				
Cabasagan	+	+	+	+	-	+	+	-				
Tributary 2												
Cabasagan	+	+	+	+	+	-	-	-				
A discont to												
Aujacent to Municipal Hall	+	+	+	+	+	+	+	-				
Noar												
Lanipaobray	+	+	+	+	+	_	+	_				
Hall		1		1		_		-				
CumpraMiagao												
River	-	-	-	-	+	-	+	-				
Pinuvak 2	+	-	+	+	+		+	-				
San Vicente												
Creek	+	+	+	+	-	-	-	-				
	•	•	Waterfalls	Sepages(Site 4)	•			•				
Cathedral Hills												
sepages	-	+	+	+	-	-		-				
Waterfalls	-	-	-	+	-	-	-	-				

Table 1 Snails that were present in area sites of collections in various villages.

# Table 2 Vegetations present in the sampling sites.

Site	Coo	Vegetation				
Irrigation Canal (Site 1)						
Pinuyak 1, Lala, LDN	7°55.580"N	123°43.583"E	Oryza sativa, Ipomoea aquatic, Paspalum conjagatum Brgius, Musa acuminata, Bambuseae, Cocos nucifera			
Tenazas, Lala, LDN	7°56.100"N	123°46.783"E	Musa acuminata, Paspalum conjagatum Brgius, Colocasia esculenta			
Sto. Thomas 1, Kapatagan, LDN	7°54.770"N	123°47.384"E	Musa acuminata, Colocasia esculenta, Paspalum conjagatum Brgius, Cocos nucifera, Terminalia catappa			
Sto. Thomas 2, Kapatagan, LDN	7°54.758"N	123°47.377"E	Paspalum conjagatum Brgius			
Curva-Miagao, Salvador, LDN	7°54.052"N	123°49.271"E	Colocasia esculenta, Paspalum conjagatum Brgius			
Inagasan IC, Salvador, LDN	7°55.025"N	123°50.827"E	Paspalum conjagatum Brgius			
Cumpra IC, Salvador, LDN	7°54.106"N	123°49.583"E	Musa acuminata, Colocasia esculenta			
Cumpra IC 2, Salvador, LDN	7°54.108"N	123°49.581"E	Musa acuminata, Colocasia esculenta			
Ricefield (Site 2)						
Lala Proper School side RF, Lala, LDN	7°57.855"N	123°44.508"E	Oryza sativa, Paspalum conjagatum Brgius, Colocasia esculenta			
Butadon, Kapatagan, LDN	7°53.220"N	123°44.701"E	Colocasia esculenta, Paspalum conjagatum Brgius, Cocos nucifera			
CumpraMiagao RF, Salvador, LDN	7°53.572"N	123°49.561"E	Paspalum conjagatum Brgius, Musa acuminata			
Flowing Waterbodies (Site 3)						
Lala Proper School side Creek	7°57.343"N	123°48.082"E	Bambuseae, Oryza sativa, Colocasia esculenta, Citrus, Cocos nucifera, Mangifera indica, Musa acuminata, Pandanus amaryllifolius			
Suba 5, Tenazas, Lala, LDN	7°56.395"N	123°47.535"E	Gliridiasepium, Chrysophyllum cainito, Cocos nucifera, Durio, Sandoricum koetjapa, Leucaena leucocephala, Terminalia catappa, Colocasia esculenta, Mangifera indica			
Abaga Bridge, Lala, LDN	7°56.232"N	123°46.038"E	Colocasia esculenta, Pteridophytes, Bambuseae, Paspalum conjagatum Brgius, Musa acuminata, Cocos nucifera			
Simpak Creek, Lala, LDN	7°56.170"N	123°44.918"E	Colocasia esculenta, Cocos nucifera, Terminalia catappa, Musa acuminata, Bambuseae, Pteridophytes			
Tunaan Creek, Lala, LDN	7°58.742"N	123°45.806"E	Paspalum conjagatumB rgius, Gliricidia sepium, Musa acuminata, Cocos nucifera			
Rebe 1, Lala, LDN	7°54.822"N	123°47.818"E	Nymphaea spp, Bambuseae, Ipomoea aquatica, lemon, Swietenia macrophylla, Annona squamosa, Gliricidia sepium			
Rebe 2, Lala, LDN	7°54.262"N	123°47.840"E	Bambuseae, Musa acuminata, Artocarpus odoratissimus, Colocasia esculenta			
Cabasagan Stream, Lala, LDN	7°58.007"N	123°48.068"E	Musa acuminata, Mangiferaindica, Colocasia esculenta, Bambuseae, Cocos nucifera, Imperata cylindrica, Tamarindus indica, Saccharum, Gliricidiasepium, kaimito, Terminalia catappa, Artocarpu s odoratissimus, citrus			
Cabasagan Creek, Lala, LDN	7°57.343"N	123°48.082"E	Bambuseae, Oryza sativa, Colocasia esculenta, citrus, Cocos nucifera, Mangifera indica, Musa acuminata, Pandanus amaryllifolius			
Maguindanao Creek, Lala, LDN	7°56.805"N	123°48.084"E	Gliricidia sepium, Cocos nucifera, Bambuseae, Leucaena leucocephala, Moringa oleifera, Paspalum conjagatumB rgius, Colocasia esculenta			
Cabasagan Tributary 2, Lala, LDN	7°57.602"N	123°47.054"E	Paspalum conjagatum Brgius, Colocasia esculenta, Moringa oleifera, Cocos nucifera			
Cabasagan Tributary 1	7°57.701"N	123°46.984"E	Paspalum conjagatum Brgius, Colocasia esculenta, Moringa oleifera, Cocos nucifera			
Adjacent to Municipal Hall, Lala, LDN	7°57.896"N	123°46.640"E	Paspalum conjagatum Brgius, Leucaena leucocephala			
Near Lanipaobrgy. Hall, Lala, LDN	7°57.613"N	123°46.603"E	Paspalum conjagatum Brgius, Colocasia esculenta,			
CumpraMiagao River, Salvador, LDN	7°53.552"N	123°49.625"E	Colocasia esculenta, Paspalum conjagatum Brgius, Bambuseae			
Pinuyak 2, Lala, LDN	7°55.588"N	123°43.689"E	Leucaena leucocephala, Musa acuminata, Paspalum conjagatum Brgius, Colocasia esculenta, Ipomoea aquatica, Musa acuminata, Cocos nucifera, Swietenia macrophylla, Nymphaea spp, Crescentia cujete			
San Vicente Creek, Kapatagan, LDN	7°53.141"N	123°46.724"E	Paspalum conjagatum Brgius, Colocasia esculenta, Cocos nucifera, Terminalia catappa			
Waterfalls/Seepages (Site 4)						
Cathedral Hills seepages,	7°52.266"N	123°46.315"E				
Waterfalls, Kapatagan, LDN	7°52.233"N	123°46.639"E	Oryza sativa, Colocasia esculenta, Cocos nucifera, Leucaena			



Fig. 2 Freshwater snail species sampled in the area and studied for parasite infections.

Three morphotypes of cercariae were sampled and recorded in this study namely: *Vivax* cercariae (sampled from *Radix* spp and *P. canaliculata*), schistosome cercariae (from *O. quadrasi*) and Pigmentata Amphistome cercariae (from *Radix* spp). The identification of these cercariae was only up to the morphotypes because the determination of their species is only possible after conducting experimental infection in the lab (Fig. 3).

Parasites other than cercariae like Protozoans were also recorded namely, Schistosoma and Pigmentata Amphistome cercariae both had significant medical and veterinary relevance, while the Vivax cercariae had no economic importance. Schistosoma cercariae were reported to: Paramecium (on all gastropods sampled) and *Entamoeba coli* (on all samples except on *V. virginea*). In addition, microscopic aquatic animals like Rotifers were also prevalent on *P. canaliculata, T. granifera, V. virginea* and *G. crista.* cause the prevalence of the debilitating schistosomiasis, and the pigmentate Amphistome cercariae were reported as intestinal parasites of mammals, especially ruminants.

Consequently, the remaining parasitic protozoans were of no high medical relevance; however, their presence can also disrupt the natural physiology of the host snails, which may cause lowered survival rate, growth, access to mates, or fertility of the host (Ruiz, 1991). The distribution of these parasitic protozoans is presented in Table 3 and Fig. 4.



Fig. 3 Parasite species from the sampled freshwater gastropods (a. *Entamoeba coli*, b. Paramecium, c. Pigmentata aphistome cercariae, d. rotifer, e. Schistosoma Cercariae, f. Vivax Cercariae).

Snails	dividuals	Number of positive cases infected with parasites										wunou t Parasite	Total number of	Infection	
	Number of in	Paramecium	Entamoeba coli	Rotifer	Vivax	Amphistome	Schistosome	Paramecium + Entamoeba	Rotifer + Para	Rotifer + Entameoba	Para + Roti + Enta	Vivax + Para		infected snails	Rate:
T. granifera	639	258	43	0	0	0	0	56	10	0	0	0	272	367	57.43
M. tuberculata	387	43	2	0	0	0	0	2	0	0	0	0	347	47	11.93
M. turriculus	273	15	0	0	0	0	0	1	0	0	0	0	257	16	5.86
O. quadrasi	778	10	0	0	0	0	7	2	0	0	0	0	752	19	2.46
P. canaliculata	177	112	2	2 0	0	0	0	22	0	0	2	2	17	160	90.40
G. crista	63	25	8	8	0	0	0	0	0	2	0	0	20	43	68.25
Radix	111	55	2	0	5	5	0	3	0	0	0	0	55	72	56.69
V. virginea	32	31	0	0	0	0	0	0	1	0	0	0	0	32	100.00
	2460												1720	756	30.53

# Table 3 Freshwater snail species with parasite infection rates.



Fig. 4 A graphical presentation of the snails and the parasites present.

#### **4** Discussion

It can be seen from the results that the different species of snails vary with the kind of species and number of individuals infected with the parasites. The number of species infecting the snails varies from 1 to 5. The majority of the total parasites found from the samples were Paramecium. Some were a combination of 2 or more parasites (Table 3 and Fig. 4). The most vulnerable species sampled and examined in this study were the V. virginea, where the majority of the snails harbored paramecium (99%), and only 1% harbored both rotifer and paramecium. Second species where a majority of the infection was in the invasive P. canaliculta snail by Paramecium. Other individual snails have a combination of Paramecium and E. coli, a Rotifer and E. coli, Paramecium, Rotifer, and E. coli and Vivax and Paramecium. There was also an aggregate that was not infected with any parasites. For G. crista, a majority of individual snails infected were caused by paramecium, followed by E. coli and Rotifer, and a combination of Rotifer and E. coli. There were sampled snails that were also not infected by any parasites. T. granifera recorded an almost similar infected number of infected and uninfected samples. The majority of the infected snails were also caused by Paramecium. Others were caused by the combination of Paramecium and E. coli, by E. coli alone and a combination of Rotifer and Paramecium, although these were in only for a few individual snails. Selected *Radix* spp. Individuals were found to be infected with either Paramecium, Vivax cercariea or Amphistome, or both. Most individuals of M. tuberculata snails were mostlyaffected with Paramecium and a few with only E. coli, or a combination of Paramecium and E. coli.

Very few individuals of *M. turriculus* species were infected by the Paramecium, by *E. coli* and a combination of both Paramecium and *E. coli*. The lowest infection rate was recorded on *O.* species by Paramecium, Schistosome cercariae, and a combination of Paramecium and *E. coli*. Although there was no reported significant medical and economic importance in the presence of these species as parasites to the snails, they can potentially cause any alteration in the physiological features of snails, which may affect their survival, growth, and reproduction rates (Ruiz, 1991). However, the cercarial infection from schistosome cercariae and Pigmentata amphistome cercariae observed on Radix, *O. quadrasi*, and *P. canaliculata* had significant medical

value since they were known to have caused debilitating diseases for many human individuals in the area. While the infection rates of the cercariae had been in low percentage, their presence can result in the transmission of diseases carried by these parasites as shown by some studies conducted from the Philippines (Sosa et al., 2012; Farahnak et al., 2005) and other areas of the world (Pandey et al. 2002; Caron et al., 2008; Mohammed et al., 2016; Chontananarth et al., 2017). The prevalence of parasitic zoonoses is known to have contributed to increased human mortality and morbidity in human populations (PAN, 2003) since some species of these snails are used as a source of food (Venugopal et al., 2017) and medicine (Bonnemain, 2005). As intermediate hosts of several zoonotic organisms that cause diseases in humans and animals (Mohammed et al., 2016; Rae, 2017; WHO, 2010), the presence of these freshwater snails can be considered as points of transmission (Jones et al., 2008) Thus; control measures should be in place for the prevention, management, and control of diseases which may be accomplished by proper sanitation and possibly the management of these parasite hosts in the area.

# **5** Conclusion

This study has shown that disease-causing parasites were observed in selected species of freshwater gastropods. Since these species were found near in areas where there are human dwellings, the transmission of diseases caused by parasites vectored by these snails is not only possible. It is, therefore, crucial that proper sanitation practices and control of the presence of these snails be done to prevent, manage, and control the transmission of diseases caused by the parasites.

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