

# Population Abundance and Disease Transmission Potential of Snail intermediate hosts of Human Schistosomiasis in Fishing Communities of Mwanza Region, North-western, Tanzania

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**Abstract:** *Schistosomiasis remains a major public health problem with estimated 240 million cases worldwide whereby more than 90% of these occur in Sub-Saharan Africa. Tanzania is ranked second after Nigeria in terms of disease burden with prevalence of infection as high as 100% in some communities. We conducted a cross-sectional malacological survey to examine for abundance, identity and disease transmission potential of snails in fishing communities of Mwanza region, Tanzania. Snails were collected from selected sites with high human water contact using standard methods. The most abundant snails species were *Biomphalaria sudanica* which accounted for 81.25% (n=1470) of snails collected. *Bulinus nasutus* was the second abundant snail accounting for 13.87% (n=251) while *Bulinus globosus* was the least abundant snail accounting for 4.86% (n=88). Only 0.57% (n=11) of collected *Biomphalaria* snails were shedding *S. mansoni* cercariae while none of the *Bulinus* snail species were shedding cercariae. Vegetation cover was significantly associated with snail abundance ( $P<0.001$ ). A positive correlation between water temperature and snail abundance was observed. No correlation was found between pH and snail abundance. The low numbers of snails shedding schistosome cercariae could be as a result of many factors including timing of the study in relation to transmission season.*

**Keyword:** Schistosomiasis, *Biomphalaria*, *Bulinus*, Cercariae, Mwanza region.

## 1. Introduction

Schistosomiasis remains a major public health problem with almost 240 million cases worldwide, and more than 90% of these are concentrated in Sub-Saharan Africa [1]. Schistosomiasis in humans results from infection with parasitic trematodes (blood flukes) of the genus *Schistosoma* [2]. There are five species of schistosomes causing infection in humans namely *S. haematobium*, *S. japonicum*, *S. mansoni*, *S. intercalatum* and *S. mekongi*. Schistosomiasis in Tanzania is caused by *Schistosoma mansoni* and *Schistosoma haematobium* and transmitted by snails of the genus *Biomphalaria* (*B. sudanica*, *B. pfeifferi*, *B. choanomphala*) and *Bulinus* (*B. nasutus*, *B. globosus* and *B. africanus*) respectively [3], [4]. While the disease is now predominantly in Africa, it also occurs in the America, the Eastern Mediterranean region, the Southeast Asian region and the Western Pacific [5]. Tanzania is the second country after Nigeria in terms of disease burden with high prevalence of infection of up to 100% in some communities particularly in the Lake Victoria basin [6]. While infection with *S. haematobium* causes urogenital schistosomiasis in humans,

infections with *S. mansoni* causes intestinal schistosomiasis and when chronic, may have severe consequences such as severe hepatomegaly, splenomegaly, hepatosplenomegaly, esophageal varices, bleeding and death [7]. In school children the disease causes poor growth performance, haematuria, chronic pain and hepatosplenomegaly all of which contribute to poor academic achievement [8]. In women of childbearing age, infection with *S. haematobium* in the genital organs causes granulomas in uterus, fallopian tube and ovaries. It leads to uterine enlargement, menstrual disorders, cervicitis and infertility [9]. The disease also causes anaemia and poor birth outcomes among others [10],[11],[12]. Infection with schistosomes at young age leads to serious complications in adulthood such as severe damage to liver, spleen, urinary tract and bladder. It has been observed that infection with urogenital schistosomiasis increases the susceptibility to other infections such as malaria, tuberculosis and HIV/AIDS [10],[13].

Based on WHO 2013, control and eventual elimination of schistosomiasis in endemic countries depend on chemotherapy using the anthelmintic drug praziquantel to

interrupt transmission and improvements have been realized. In 2010, 34.8 million people were treated for schistosomiasis in 30 countries [14]. Most of the studies conducted on schistosomiasis in Tanzania have focused on epidemiology and disease control with few data available on the role played by snails in the transmission and maintenance of infection. This cross-sectional malacological study therefore examined the identity, abundance, and disease transmission potential of snail intermediate hosts of schistosomes in fishing communities of Mwanza region, North-western Tanzania.

## 2. Literature Survey

In Tanzania, schistosomiasis occurs throughout the country but high transmission is concentrated along the Indian Ocean coast and the Lake Victoria basin and its vicinity and is caused by *Schistosoma mansoni* and *Schistosoma haematobium* via their respective intermediate host snails [2]. The main snail species responsible for transmission of urinary schistosomiasis in Tanzania are *Bulinus globosus*, *Bulinus africanus* and *Bulinus nasutus* [15],[3]. *Bulinus nasutus* is the major intermediate host for the transmission of urinary schistosomiasis in the North-western Tanzania [16],[17],[18] while *Bulinus globosus* occurs in permanent water bodies and is largely important vector in coastal areas [3], [19],[2]. *Bulinus africanus* occurs in temporary streams and is important host for *S.haematobium* transmission in temporary habitat areas [3]. The *Bulinus* species in the area are sporadically distributed occupying various sources of water ranging from small and temporary water pools to large and permanent water sources throughout the country [20].

Intestinal schistosomiasis in Tanzania is transmitted by snails of the genus *Biomphalaria*. Of these, *Biomphalaria sudanica* occurs close to the lake shore while *Biomphalaria choanomphala* prefers the bottom of the lake and *Biomphalaria pfeifferi* occurs in streams and are the most important intermediate hosts for the transmission of *S.mansoni* in the area [22],[16]. Numerous epidemiological studies have been undertaken focusing primarily on distribution and burden of the disease [23],[21],[24], hence very few data are available on snail intermediate hosts in the study area. This study presents insights on population abundance and disease transmission potential of snail intermediate hosts of schistosomiasis in Northwestern Tanzania. The findings may be useful in design of schistosomiasis control strategies based on identification of disease transmission hotspots and guiding selections and targeting of disease control interventions.

### PROBLEM DEFINITION

Schistosomiasis is a major public health problem in Tanzania with prevalence of infection as high as 100% in some communities [6]. The disease causes poor growth performance, haematuria, chronic pain and hepatosplenomegaly in school children all contributing to poor academic achievement. In women of childbearing age, the disease causes anemia and poor birth outcomes among others. Infection with schistosomes at young age leads to complications in adulthood such as severe damage of liver, spleen, urinary tract and bladder. There is increasing evidence suggesting that the disease increases the

vulnerability to other infections such as malaria, tuberculosis and HIV/AIDS [10]. In Tanzania Schistosomiasis occurs in all regions and consistently co-exists with malaria and hookworm. The group mostly at risk is school children, adolescents, fisherman, farmers and women of child bearing age [21]. Realizing the public health burden of schistosomiasis, the government of the United Republic of Tanzania established the National Schistosomiasis and Soil-Transmitted Helminthiasis (NSSCP) in the year 2005 with efforts to reduce the public health burden associated with the disease.

## 3. Methodology

### 3.1 Study area and population

The study was conducted in two villages namely Fogofogo (Busega district) and Lutale (Magu district) in Mwanza region, Northwestern Tanzania. The two districts are contiguous to each other and located along the Mwanza-Nairobi road. They lie between 2° 10' and 2° 50' South of the equator and 33° to 34° East. The area is mainly covered by Lake Victoria waters by 56.1% the remaining part consists of low lying land with undulating hills. The area has two zones of rainfall ranging between 700mm to 1000mm between October to December and between March and May. The major ethnic groups in the area are Sukuma, Jita, Kerewe, and Kurya. Of these, the Sukuma ethnic group is the most predominant group in the area practicing subsistence mode of farming which creates conducive environment for snails breeding sites along their farms. Fogofogo village is among the villages within Kabita ward with a total population of 22,777, of these 10,754 (47.2%) are males. In Lutale village the total number of inhabitants are 12,307 of these 6,026 (49.0%) are males (NBS 2012). Major income generating activities conducted within the area are mainly subsistence farming and fishing. The hygienic condition for both villages was found to be poor with few toilets available for use by community members. Plenty of human excreta were found in places where people perform various day to day activities including the sites where *Biomphalaria* species and *Bulinus* species were collected, which exposes people to higher risk of infection.

### 3.2 Site selection, snail collection and identification of transmission hotspots.

The study sites were selected along the Lake Victoria basin, where people have direct contact with water as a result of routine activities such as swimming, collecting water, bathing or occupational work like farming and fishing. Identified sites were mapped using hand held GPS. Snail collection was done using hand held scoops and dredges. Scooping was done by two people collecting snails for 15 minutes per site. Eight schistosomiasis transmission sites were visited, four sites for each village. Snails collected from these sites were screened for cercariae shedding by exposure to 12 well ELISA plate filled with distilled water and placed under artificial light for two hours. Snails were checked for cercariae shedding using a dissecting microscope. Identification of transmission hotspots was thus done by assessing cercariae shedding capacity of infected snails in respective sites.

### 3.3 Snail identification

In the field, collected snails were identified based on shell morphology using the WHO snail identification guide (WHO, 1973)\*. Snails were placed in individual well plates and placed under natural light for two hours to allow cercariae shedding. Emerging cercariae were caught individually using a p20 pipette and stored onto the Whatman FTA cards for further genetic analysis of schistosomes species. All infected snails were preserved in absolute ethanol and a subset of non-shedding snails from each site was preserved in 70% percent ethanol.

### 3.4 Cercariae DNA extraction

Cercariae samples stored into the Whatman FTA cards was punched out using Harris 2mm, the discs were placed in an individual eppendorf tube followed by 90µl of ATL buffer solution to lyse the tissue and 20µl of proteinase K enzyme. Vortexing was done to mix the mixture thoroughly, and then incubated at 56°C for one hour while vortexing in every 15 minutes. About 100µl of lysis buffer (AL) was added then vortexed and later incubated for 10 minutes at 70°C. About 100µL of ethanol was added and vortexing, the mixture was then pipetted to DNeasy column and spun for 1 minute at 8000rpm. The column was then placed in a new collection tube where 500µl of wash buffer 1(AW1) was added and spun for 1 minute at 8000rpm. The column was transferred to a new collection tube and 500µl of washing buffer 2 (AW2) was added and spun for 3 minutes at 14000rpm. The column was again placed in an eppendorf tube where 20µl of eluting buffer (AE) was added and spun for 1 minute at 8000rpm. This step was repeated two times and finally the column was discarded and the gDNA was already extracted ready for polymerase chain reaction (PCR).

### 3.5 Polymerase chain reaction (PCR)

In preparation of the PCR master mix, 25µl was made by 7.5µl of nuclease free water, 1µl of each ITTS1 TGCTTAAGTTCAGCGGGT and ITTS2 AACAAGGTTTCCGTAGGTGAA Primers, 12.5µl of master mix and 3µl of gDNA was added to make PCR master mix based on the manufacturer's protocol.

### 3.6 PCR Cycles and gel electrophoresis

The Polymerase chain reaction was set at 95°C for 5 minutes, 40 cycles of 30 seconds at 95°C, 30 seconds at 40°C and 1:30 minutes at 72°C. The final extension period was 7 minutes at 72°C running both positive and negative PCRs. The PCR product was allowed to run in an agarose gel where 2.25 g of agarose powder was measured and poured into 150 ml of 1×TBE buffer then warmed in a microwave for 3 minutes to allow the agarose powder dissolve and make a clear solution, 7.5µl of Red safe™ nucleic acid staining solution was added. The solution was poured in an electrophoresis tract, the comb was placed and then the solution was allowed to cool and solidify for about 30 minutes then placed in an electrophoresis tank. About 8µl of PCR product was pipetted and mixed with 2µl of DNA loading dye and then loaded in the agarose gel lane, DNA ladder and negative control was also placed.

### 3.7 Data handling and statistical analysis

All data collected was entered into the Census and Survey Processing System (CSPro) software (U.S. Census Bureau, USA) and analyzed using STATA version 12 software (STATA Cooperation, Texas, USA). Mean values of snail abundance were calculated and presented as means  $\pm$  standard deviation (SD). The normality and homogeneity of variance were checked as well as log transformed ( $\log_x+1$ ) where applicable. The difference in snail abundance, temperature and P<sup>H</sup> values between and among sites was compared using the student's t-test or one way analysis of variance (ANOVA), as appropriate. The association between snail abundance and environmental variables were determined using Spearman correlation (r). A p-value of <0.05 was considered to be statistically significant.

## 4. Results

### 4.1 Snail abundance, species distribution and transmission hotspots

A total of 1,809 snails were collected from 8 sites in the two villages of Fogofogo and Lutale. Of these 1,276 (70.5%) were collected from Fogofogo and 533 (29.5%) from Lutale. The mean number of snails collected from Fogofogo and Lutale was 284  $\pm$  95 and 133  $\pm$  52, respectively. Based on shell morphology, 81.26% (n=1,470) of all snail collected were identified as *Biomphalaria* species while 18.73% (n=339) were identified as *Bulinus* species. Of all the snails identified as *Biomphalaria* species belonged to *Biomphalaria sudanica* while *Bulinus* species belonged to *Bulinus globosus* and *Bulinus nasutus* (see table 1).

**Table 1:** Distribution of snails by village and site

Village	Number of snails	snail species			Mean snail abundance
		<i>Biomphalaria sudanica</i>	<i>Bulinus globosus</i>	<i>Bulinus nasutus</i>	
<b>Fogofogo</b>					
A	504	504	0	0	
B	251	0	0	251	284 $\pm$ 95
C	251	163	88	0	
D	270	270	0	0	
<b>Lutale</b>					
A	174	174	0	0	
B	58	58	0	0	
C	158	158	0	0	133 $\pm$ 52
D	143	143	0	0	
<b>Total</b>	<b>1809</b>	<b>1470</b>	<b>88</b>	<b>251</b>	

In terms of snail abundance and distribution, for Fogofogo village, snails were clustered in 4 collection sites (Table 1). Site A, C and D were found in marshy environments along the lake shore where *Biomphalaria* species were collected. Interestingly, site C was found to be inhabited by both *Biomphalaria sudanica* and *Bulinus globosus* while site B, an inland temporary pool was inhabited by *Bulinus nasutus* only. Based on sites of collection, site A and D recorded the highest number of snails as compared to other sites and the majority of snails collected in the two sites belonged to *Biomphalaria* species. *Biomphalaria sudanica* and *Bulinus globosus* were mainly dominant in the lake shore collection points whereas *Bulinus nasutus* were commonly found in inland collection sites with temporary water bodies. For



Lutale village all sites A, B, C and D were marshy sites along the lake shore where a total of 533 (29.5%) were collected all being *Biomphalaria sudanica*. No *Bulinus snail* species were collected from this village. Overall, majority (81.26% (n=1470) of snails collected from lake shore sites were *Biomphalaria* species. Only 13.87% (n=251) belonged to *Bulinus nasutus* and 4.86% (n=88) belonged to *Bulinus globosus*. *Biomphalaria sudanica* dominated all the sites along the lake shore while *Bulinus nasutus* dominated inland collection sites. There was a significant difference in number of snails collected from the two villages ( $p < 0.001$ ).

#### 4.2 Snail infectivity testing

Out of the 1,809 snails collected, only 11 (0.6%) were found to be shedding human schistosome cercariae. All snails found to be shedding cercariae belonged to the genus *Biomphalaria* and all were collected from the lake shore sites. In relation to sites of collection, snails which were found shedding cercariae were mainly collected from Fogofogo village on site A (n=5, 45.4%) and site D (n=5, 45.4%). Only one infected snail (9.1%) was collected from site C at Lutale village. None of the collected *Bulinus* species were shedding cercariae.

#### 4.3 Environmental factors

The common vegetation cover found in the collection sites were a combination of water hyacinth and grasses. Vegetation cover was associated with snail abundance ( $P < 0.001$ ) with *Biomphalaria* species found abundantly inhabiting water hyacinth compared to *Bulinus* species ( $P < 0.001$ ).

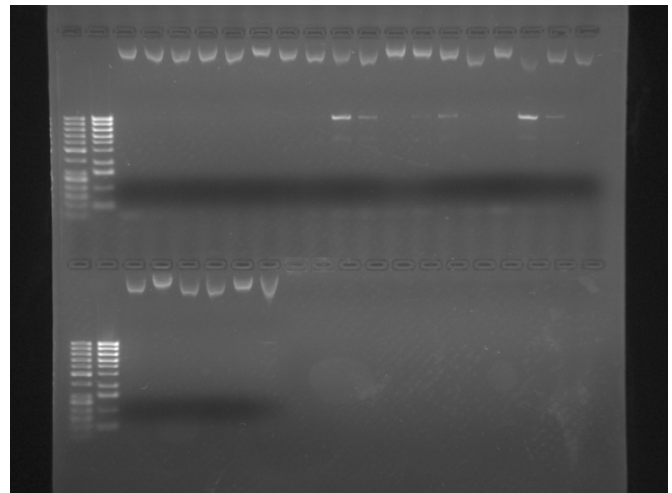
The overall mean temperature was  $28 \pm 0.75^{\circ}\text{C}$  (range  $26.35$ - $29.64^{\circ}\text{C}$ ). In relation to sites of collection, the inland collection sites which were mainly dominated by *Bulinus* species and had significantly higher mean temperature ( $31.45 \pm 0.95^{\circ}\text{C}$ ) as compared to the lake shore sites ( $27.31 \pm 0.69^{\circ}\text{C}$ ) ( $p = 0.03$ ).

Mean pH was  $7.44 \pm 0.1$  (range  $7.24$ - $7.64$ ). Inland sites had significantly higher mean pH ( $8.06 \pm 0.24^{\circ}\text{C}$ ) as compared to  $7.32 \pm 0.28^{\circ}\text{C}$  for shoreline sites ( $p < 0.001$ ). Confined water conductivity across sampling sites varied from  $94.4\mu\text{S}$  to  $1001\mu\text{S}$  indicating that total dissolved salt was high in the sampling sites.

There was a significant association between snail abundance and water pH ( $P < 0.001$ ). A large number of snails were found in a pH range of  $7.2$ - $7.5$ . Among sampling sites no snail were collected in areas with  $\text{pH} > 7.6$ . Likewise, there was a significant association between snail abundance and water temperature ( $P < 0.001$ ). Majority of the snails were collected in water temperature ranging from  $21.9^{\circ}\text{C}$  to  $30.4^{\circ}\text{C}$ .

#### 4.4 Identification of *S. mansoni* cercariae by PCR

Genomic DNA was successfully extracted from 6 FTA® card samples and the positive bands confirmed in the electrophoretic report revealed that the parasite was *S. mansoni* a human schistosome (Figure 2).



**Figure 2:** Shows image of agarose gel electrophoretic profile of *S. mansoni* cercariae

#### 5. Discussion

Results of the current study demonstrated higher abundance of snail intermediate hosts of schistosomiasis. The major snail species identified were *Biomphalaria sudanica*, *Bulinus nasutus* and *Bulinus globosus* corresponding to findings of previous studies in the area [3], [25], [26]. Snail population abundance seem to be primarily influenced by environmental factors that vary significantly within diminutive distances in the water bodies from site to site in snail sampling areas. In this study the physico-chemical factors examined in snail sampling sites include vegetation cover which was a major determinant factor for abundance of fresh water snails in sampled sites particularly *Biomphalaria sudanica* species. This observation suggests that snail population is mainly enhanced by availability of abundant emerging microflora from which snails gets food and deposit their egg masses. The observed association between vegetation cover and snail abundance was similar to observations made by Kariuki and his colleagues where vegetation cover had significant association with various snail species [27]. A comparable observation was made by Opisa and others [28] where vegetation cover was associated with snail abundance.

In this study, water temperature was significantly associated with snail abundance. Water temperature showed fluctuation across sampling sites with a range from  $21.9$  to  $30.4^{\circ}\text{C}$  suggesting suitable condition for the *Biomphalaria sudanica* to reproduce leading to increase in snails population density. Elsewhere, the association between water temperature and snail abundance was reported [28], [16], [29]. Comparable findings were reported in Uganda with significant correlation between water temperature and *B. sudanica* abundance [30]. However, in contrast to association between water temperature and snail abundance, findings from Kenya observed no association between water temperature and snail abundance [27]. In addition, pH range in this study was negatively correlated with snail abundance and ranged from  $7.2$  to  $7.5$ . This observation was similarly reported elsewhere [30], [16], [31], [32] indicating that snail abundance is not influenced by PH only rather by a combination of other environmental factors. Low pH in snail habitats could be detrimental to fresh water snails by causing coagulation of

mucus on bare skin surface and enhancing fragility of snail shells.

The negative correlation between water conductivity and snail abundance suggest that water conductivity did not influence snail abundance in the study area. However, this observation was centrally to what was reported by Kazibwe and others in Lake Albert, western Uganda [30].

Of all snail intermediate hosts collected from this study, only 11 *Biomphalaria sudanica* were shedding cercariae. Given the known association of snails to humans, cercariae emergence time corresponds to availability of their definitive host, which enhances the disease transmission cycle among human populations [26],[31].

It may seem surprising that very few snails shed cercariae from a high schistosomiasis transmission area. This findings are similar to other studies conducted in endemic areas with high transmission and snail abundance but with very few or no snails shedding cercariae [33],[34]. In contrast to high human prevalence of *S.mansoni* infection among school children and adults in Mwanza region [35],[36],[3], few percentages of collected snails were capable of shedding cercariae. In Lutale site C one of the sampling site in this study it was observed that cercarial shedding was very low (only one snail shed) while the prevalence of *S.mansoni* infection in the village was 49.9% in adults (results of a parallel study not shown here). In a recent study in Sesse island of Lake Victoria, Uganda, findings reported none of the snails collected from a high transmission areas shed any cercariae [34].

Considering this situation, different explanations may be put forward for the absence or low numbers of snails shedding cercariae. First, fluctuations of snail population abundance, infectivity rates and cercarial productivity could inhibit continuous flow of miracidia for snails infection and subsequent shedding of cercariae [27],[37],[38],[39]. Second, stages of parasite development within the snails intermediate host, the collected snails may be in prepatent infection (the period between penetration of miracidia to the development of infective cercaria) that can last for several weeks with a very few percentages of snails reaching stages of cercariae shedding. This suggests that when snails are in prepatent infection, identification of infection cannot be performed by cercarial shedding observation due to limitation in cercarial release [27] [40],[41]. The timing of the study in relation to the season of transmission may also have contributed to low number of snails shedding cercariae.

Ongoing mass drug administration (MDA) for control of schistosomiasis currently being implemented in the area may have reduced the transmission cycle by killing the parasite within the definitive host as a result less or no continuation of miracidia flow in the environment is occurring in the environment for snail infection. It may also be possible that the number of infected snails is very low or the cercariae are shed in phases. This may be enhanced by the focal nature of schistosomiasis and complication in snail sampling leading to difficulties in recognizing sites with high number of infected snails. Cercarial shedding may be inhibited by various contaminants harbored by snail which may prevent cercariae release. Although it is accepted that verification of

schistosomiasis transmission is by identification of infected snails, the findings of the present study suggest that molecular techniques may be useful in determination of infected snails by examining both prepatent and patent snail stages of infection. Repetitions of cercarial shedding several times in the laboratory and crushing of snails in search for larvae may give better estimates of the prevalence of infected snails in the area.

## 6. Implication of the snail data in designing cost effective control interventions

The insights on snail abundance and infectivity have implications in designing cost effective measures for control and elimination of schistosomiasis. From the current findings, snail abundance was high in the sampling sites but very few snails were infected compared to the infection rates observed in the human population. This suggests that the schistosome parasite is more prevalent in the human host than in the snail intermediate host. This in turn suggests that application of molluscicides may have no impact on schistosomiasis control unless combined with population based mass drug administration (MDA) strategies targeting both school based children and adults. This will allow killing of mature worms in the definitive host and interfering with continuation of the schistosome life cycle. Thus to ensure successful and sustainable control and eventual elimination of schistosomiasis, a combination of control methods is necessary targeting both the snail intermediate hosts and treatment of the definitive host in addition to health education and sanitation.

## 7. Conclusion

The high abundance of *Biomphalaria* and *Bulinus* species collected in the study site suggest that schistosomiasis could be transmitted easily in this area. However, only few snails were shedding schistosome cercaria which could be caused by many factors including timing of the study in relation to transmission season.

## 8. Recommendations

The current study was limited by several factors. First, weather condition, majority of the inland transmission sites were dry during sampling period making it difficult to obtain the transmission sources for *Schistosoma haematobium* where *Bulinus* species could be found. Given the known *Bulinus* species distribution throughout the country [42], further studies are required to determine snail abundance and their infection rates in the study area. Second, snails were sampled on a single day at each site. Based on seasonal variation in snail abundance and dynamics of infection, further studies with close follow up may improve snail sampling to determine snail abundance and infectivity rates. Third, the pre-patent stage of infection in snails was not examined, given that infection could be in the early stage in the intermediate host [27]. Further, molecular techniques such as PCR are required in order to improve determination of snail infectivity.

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## Author Profile



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