Cercarial Diversity in Freshwater Snails from Selected Freshwater Bodies and Its Implication for Veterinary and Public Health in Kaduna State, Nigeria

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Abstract—A study conducted to determine cercariae diversity and prevalence of trematode infection in freshwater snails from six freshwater bodies selected by systematic random sampling in Kaduna State was carried from January 2013 to December 2013. Freshwater snails and cercariae harvested from the study sites were morphologically identified. A total of 23,823 freshwater snails were collected from the six freshwater bodies: Bagoma dam, Gimbawa dam, Kangimi dam, Kubacha dam, Manchok water intake and Saminaka water intake. The observed freshwater snail species were: Melanoides tuberculata, Biomphalaria pfeifferi, Bulinus globosus, Lymnaea natalensis, Physa sp., Cleopatra bulimoides, Bellamya unicolor and Lanistes varius. The freshwater snails were exposed to artificial bright light from a 100 Watt electric bulb in the laboratory to induce cercarial shedding. Of the total freshwater snails collected, 10.55% released one or more types of cercariae. Seven morphological types of cercariae were shed by six freshwater snail species namely: Brevifurcate-apharyngeate distome, Amphistome, Gymnocephalus, Longifurcate-ephyngeate monostome, Longifurcate-ephyngeate distome, Echinostome and Xiphidid cercariae. Infection was monotye in most of the freshwater snails collected; however, Physa species presented a mixed infection with Gymnocephalus and Longifurcate-ephyngeate distome cercariae. B. globosus and B. pfeifferi were the most preferred intermediate hosts with the prevalence of 13.48% and 13.46%, respectively. The diversity and prevalence of cercariae varied among the six freshwater bodies with Manchok water intake having the highest infestation (14.3%) and the least recorded in Kangimi dam (3.9%). There was a correlation trend between the number of freshwater snails and trematode infection with Manchok exhibiting the highest and Bagoma none. The highest cercarial diversity was observed in B. pfeifferi and B. globosus with four morphotypes each, and the lowest was in M. tuberculata with one morphotype. The general distribution of freshwater snails and the trematode cercariae they shed suggests the risk of human and animals to trematodiases in Manchok community. Public health education to raise awareness on individual and communal action that may control snail breeding sites, prevent transmission and provide access to treatment should be intensified.

Keywords—Cercariae, diversity, freshwater snails, prevalence, trematodiases.

I. INTRODUCTION

TREMATODES (flukes) are a group of parasitic helminthes that infect humans and animals worldwide [1]. They cause parasitic diseases of man and animals of economic and public health importance, especially in tropics and developing countries like Nigeria [2]. Trematodes exhibit a strong and persistent association with aquatic organisms for transmission. Many species of freshwater snails serve as intermediate hosts for the trematodes larvae (cercariae) [3]. Species of trematodes such as Fasciola, Schistosoma, Clonorchis and Echinostoma are responsible for a number of diseases such as Fascioliasis and Schistosomiasis [4] which today remain the most important cause of global health problem to man and his domestic animal [5]. In most cases, cercaria is the infective form in trematodes, but in some, it is the metacercaria [1]. Cercariae are usually described based on the position and number of suckers [6], which includes Monostome, Amphistome, Gastrostome and Distome or described based on the shape and relative size of tail as Pleurolorhophocercous, Cystocercous, Furcocercous, Microcercous, Gymnophallus, Macrocerous, Cercariae, Leptocercous, Rat-king, Trichocercous and Cotylocercous [7]. The presence of other body structures such as collar spine (Echinostome), anterior stylet (Xiphidio cercaria), and eye spot (Ophthalmo cercaria) are often used [7]. In any geographical area, the presence of freshwater snail species could serve to indicate the presence of particular trematodiases [8]. Several countries forecast the spread of trematodiases on the bases of freshwater snail species present in a particular area, so that preventive measures could be taken in advance [9], [10]. The prevalence and intensity of trematode infection is dependent on the number of snails in the environment and the number infected in the freshwater body [11]. This study was therefore aimed at determining cercarial diversity and prevalence of trematode infection in freshwater snails of veterinary and public health significance in selected water bodies of Kaduna state, Nigeria.

II. MATERIALS AND METHODS

A. Study Area

Kaduna State, with its capital Kaduna, is geographically located on latitude 10°21'23"N and longitude 7°26'21"E. It covers an area of 46053 km² and has a population of 6,066,562 [12]. The state is endowed with two large rivers (Gurara and Kaduna) that have been dammed at various locations to provide water for drinking, fishing, agriculture and other human needs. In addition to this water impoundment along the
two rivers, the state government has constructed several earthed dams and water intakes as feeders for treatment plants to provide potable drinking water to communities hosting them. The two climatic conditions (rainy and dry seasons) of the state greatly influences the lives of the people. Thus, the predominantly agricultural inhabitant is occupied with farming in the rainy season which is supplemented with irrigation in the dry season.

B. Study Sites
Six freshwater bodies were selected by systematic random sampling technique to cover the three senatorial district of the State. The freshwater bodies selected are: Kangimi dam, Gimbawa dam, Kubacha dam, Saminaka water intake, Bagoma dam and Manchok water intake.

C. Malacological Studies

1. Snail Collection
Freshwater snails were collected with the aid of a scooping net or manually collected with forceps when seen. Freshwater snails were collected from the six selected freshwater bodies monthly for a period of one year (January to December, 2013) from sites where there were obvious human activities [13]. Freshwater snail sample collection was carried out between 8.30 a.m. and 11.00 a.m., within an area of approximately 5 m², at each of the selected freshwater bodies [13]. Freshwater snails collected were transferred to labelled perforated plastic containers and transported to the Department of Biological Sciences laboratory, Nigerian Defence Academy, Kaduna, for identification. In the laboratory, the freshwater snails were washed to remove dirt and sorted out according to species using morphological characteristics keys [14] and counted.

D. Cercarial Collection

1. Shedding and Harvesting of Cercaria from Freshwater Snails
Freshwater snails collected from each of the freshwater bodies were placed separately in Petri dishes containing water filtered from their sites of collection. The snails were exposed to artificial bright light from a 100 Watt electric bulb for one to four hours to induce shedding of cercariae. Each snail was
carefully observed under dissecting microscope and examined for possible emergence of cercariae at regular intervals [15]. The snails were crushed after shedding and examined for Sporocyst or Rediae. Images of the different larval forms were captured using a Lumix Vario digital camera (12 megapixels, Panasonic model DMC FS10).

2. Morphological Identification of Cercaria

Live cercariae shed by each separated freshwater snail species in a Petri dish were transferred in water solution onto a microscopic slide, covered with a cover slip and observed under X40 objective of the light microscope. The observed cercariae were then stained with neutral red (0.1%). A cover slip was carefully placed on the stained slide and observed under the X40 objective of the light microscope. The movement and the morphological details of the live and stained cercariae observed under the microscope, respectively, were recorded for identification purposes using standard key [15].

E. Data Analysis

Correlation coefficient was used to find the relationship between freshwater snail abundance and cercariae infestation.

III. RESULTS

A. Prevalence of Trematode Infection in Freshwater Snails

1. Overall Prevalence of Trematode Infection in Freshwater Snails

The overall prevalence of trematode infection in freshwater snail species collected from the six freshwater bodies surveyed in Kaduna State is shown in Table I.

The overall prevalence of trematode in freshwater snails collected in the six freshwater bodies surveyed was 10.55%. Bulinus globosus, Biomphalaria pfeifferi, Cleopatra bulimoides, Physa spp., Lymnaea natalensis and Melanoides tuberculata were found to harbour larval stage (cercariae) of different trematodes, while none of the Bellamya unicolor and Lanistes varicus showed any sign of infection.

Prevalence of trematode infection was highest in B. globosus (13.48%). This was closely followed by B. pfeifferi (13.46%), L. natalensis (10.95%), M. tuberculata (10.71%) and Physa spp. (8.33%).

The distribution of trematode infected freshwater snails from the freshwater bodies sampled is presented in Table II.

The highest prevalence (14.30%) of infected freshwater snails was recorded at Manchok water intake. Next were Kubacha dam, Gimbawa dam and Saminaka water intake with prevalence of 13.68%, 7.15% and 5.96%, respectively. The least number of infected freshwater snails was recorded at Kangimi dam (3.90%). However, none of the freshwater snails recorded at Bagoma dam was found to harbour trematode infection. Correlation analysis showed a significant association (P < 0.05) between freshwater snails at the various freshwater bodies and trematode infection (r = 0.979; P = 0.000).

F. Cercarial Diversity in Infected Freshwater Snails

The cercarial diversity in freshwater snail species in sampled freshwater bodies is presented in Table III.

Seven morphologically distinguishable cercariae types were recorded from infected freshwater snail species collected at the various freshwater bodies sampled. The cercariae recorded were Fig. 2 (Amphistome cercariae), Fig. 3 (Echinostome cercariae), Fig. 4 (Gymnocephalus cercariae), Fig. 5 (Xiphidio cercariae), Figs. 6 (a) and (b), (Brevifurcate-apharyngeate distome cercariae), Fig. 7 (Longifurcate–apharyngeate monostome cercariae), Fig. 8 (Longifurcate-apharyngeate distome cercariae) and Fig. 9 (mixed infection with Longifurcate-apharyngeate distome and Gymnocephalus cercariae).

Freshwater snail species found to harbour different trematode cercariae recorded were as follows: C. bulimoides (Longifurcate-apharyngeate monostome cercariae and Xiphidio cercariae), B. pfeifferi (Amphistome cercariae, Brevifurcate-apharyngeate distome cercariae Xiphidio cercariae and Longifurcate-apharyngeate distome cercariae), B. globosus (Echinostome cercariae, Gymnocephalus cercariae, Brevifurcate-apharyngeate distome cercariae and Longifurcate – pharyngeate distome cercariae), L. natalensis (Amphistome cercariae and Gymnocephalus cercariae), M. tuberculata (Amphistome cercariae), Physa spp. (Longifurcate-apharyngeate distome cercariae with Gymnocephalus cercariae).

TABLE I

<table>
<thead>
<tr>
<th>Snails species</th>
<th>Number of freshwater snails examined</th>
<th>Number of trematode infected freshwater snails</th>
<th>Prevalence of trematode Infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulinus globosus</td>
<td>0816</td>
<td>0110</td>
<td>13.48</td>
</tr>
<tr>
<td>Biomphalaria pfeiffer</td>
<td>9030</td>
<td>1216</td>
<td>13.46</td>
</tr>
<tr>
<td>Lymnaea natalensis</td>
<td>0630</td>
<td>0069</td>
<td>10.95</td>
</tr>
<tr>
<td>Melanoides tuberculata</td>
<td>9562</td>
<td>1024</td>
<td>10.71</td>
</tr>
<tr>
<td>Cleopatra bulimoides</td>
<td>1003</td>
<td>0094</td>
<td>09.37</td>
</tr>
<tr>
<td>Physa species</td>
<td>0024</td>
<td>0002</td>
<td>08.33</td>
</tr>
<tr>
<td>Bellamya unicolor</td>
<td>2743</td>
<td>0000</td>
<td>00.00</td>
</tr>
<tr>
<td>Lanistes varicus</td>
<td>15</td>
<td>0000</td>
<td>00.00</td>
</tr>
<tr>
<td>Total</td>
<td>23823</td>
<td>2515</td>
<td>10.55</td>
</tr>
</tbody>
</table>
### TABLE II

**PREVALENCE OF TREMATODE INFECTION IN FRESHWATER SNAILS IN THE SIX FRESHWATER BODIES STUDIED**

<table>
<thead>
<tr>
<th>Species</th>
<th>Kangimi dam</th>
<th>Gimbawa dam</th>
<th>Kubacha dam</th>
<th>Saminaka water intake</th>
<th>Manchok water intake</th>
<th>Bagoma dam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number examined</td>
<td>Number Infected</td>
<td>Number examined</td>
<td>Number Infected</td>
<td>Number examined</td>
<td>Number Infected</td>
</tr>
<tr>
<td><em>Bulinus globosus</em></td>
<td>10</td>
<td>1(10)</td>
<td>232</td>
<td>46(19.82)</td>
<td>574</td>
<td>63(10.97)</td>
</tr>
<tr>
<td><em>Biomphalaria pfeifferi</em></td>
<td>49</td>
<td>(20.4)</td>
<td>495</td>
<td>0(0)</td>
<td>190</td>
<td>24(12.65)</td>
</tr>
<tr>
<td><em>Physa species</em></td>
<td>0</td>
<td>0(0)</td>
<td>2</td>
<td>1(50)</td>
<td>0</td>
<td>0(0)</td>
</tr>
<tr>
<td><em>Cleopatra bulimoides</em></td>
<td>984</td>
<td>90(9.14)</td>
<td>19</td>
<td>4(21.05)</td>
<td>0</td>
<td>0(0)</td>
</tr>
<tr>
<td><em>Bellamya anicolar</em></td>
<td>329</td>
<td>0(0)</td>
<td>488</td>
<td>0(0)</td>
<td>1364</td>
<td>0(0)</td>
</tr>
<tr>
<td><em>Melanoides tuberculata</em></td>
<td>1101</td>
<td>0(0)</td>
<td>2793</td>
<td>219(7.8)</td>
<td>4515</td>
<td>805(17.85)</td>
</tr>
<tr>
<td><em>Lymnaea natalensis</em></td>
<td>306</td>
<td>8(2.6)</td>
<td>177</td>
<td>31(17.51)</td>
<td>84</td>
<td>13(15.47)</td>
</tr>
<tr>
<td><em>Lanistes varicus</em></td>
<td>15</td>
<td>0(0)</td>
<td>0</td>
<td>0(0)</td>
<td>0</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

**Total**

|                       | 2794        | 109(3.90)   | 4206        | 301(7.15)            | 6153                 | 842(13.60)    | 1174         | 70(5.96)        | 8341         | 1193(14.30)    | 1155         | 0(0)        |

**Total**

|                       | 2794        | 109(3.90)   | 4206        | 301(7.15)            | 6153                 | 842(13.60)    | 1174         | 70(5.96)        | 8341         | 1193(14.30)    | 1155         | 0(0)        |

### TABLE III

**CERCARIAL DIVERSITY IN FRESHWATER SNAIL SPECIES OF SAMPLED FRESHWATER BODIES IN KADUNA STATE**

<table>
<thead>
<tr>
<th>Cercaria type</th>
<th>Snail Intermediate host</th>
<th>Water body</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Longifurcate-pharyngeate monostome</em> Cercariae</td>
<td><em>Cleopatra bulimoides</em></td>
<td>Kangimi dam, Gimbawa dam</td>
</tr>
<tr>
<td><em>Amphistome cercaria, Gymnocephalus cercariae</em></td>
<td><em>Biomphalaria pfeifferi</em></td>
<td>Gimbawa dam, Manchok water intake, Gimbawa dam, Kubacha dam</td>
</tr>
<tr>
<td><em>Echinostome cercariae</em></td>
<td><em>Lymnaea natalensis</em></td>
<td>Saminaka water intake</td>
</tr>
<tr>
<td><em>Brevifurcate-apharyngeate distome</em> Cercariae</td>
<td><em>Melanoides tuberculata</em></td>
<td>Kubacha dam, Manchok water intake, Gimbawa dam, Saminaka water intake</td>
</tr>
<tr>
<td><em>Xiphidio cercariae</em></td>
<td><em>Cleopatra bulimoides</em></td>
<td>Gimbawa dam</td>
</tr>
<tr>
<td><em>Longifurcate-pharyngeate distome</em> Cercariae</td>
<td><em>Biomphalaria pfeifferi</em></td>
<td>Gimbawa dam, Kubacha dam</td>
</tr>
<tr>
<td>Mixed infection with <em>Longifurcate-Pharyngeate distome cercariae</em> and <em>Gymnocephalus cercariae</em></td>
<td><em>Bulinus globosus</em></td>
<td>Gimbawa dam, Saminaka water intake</td>
</tr>
</tbody>
</table>

**Cercariae Types Identified in the Sampled Water Bodies in Kaduna State**

![Fig. 2 Amphistome cercaria](image2.png)

![Fig. 3 Echinostome cercaria](image3.png)

![Fig. 4 Gymnocephalus cercaria](image4.png)

![Fig. 5 Xiphidio cercaria](image5.png)
The overall trematode prevalence of 10.55% recorded in the present study corroborates with the works of [16]-[20], [3], [13] that reported prevalence within the range of 1.8% to 18% in various freshwater snail hosts. The low infestation rate in the freshwater snails is viewed as a characteristic of parasites in short lived host [21]. Another study attributed it to host-parasite co-evolution [22].

The high prevalence observed in *B. globosus* and *B. pfeifferi* (Table I) probably suggest that these freshwater snails are more preferred intermediate hosts for these trematodes than all the others. These are freshwater snails that serve as an intermediate host to *Schistosomes* that causes Schistosomiasis, which is one of the most widespread and prevalent parasitic water borne disease and second only to malaria in terms of its socio-economic and public health significance [23]. The prevalence of 10.31% observed in *L. natalensis* that serves as an intermediate host to *Fasciola* (liver fluke) which causes Fascioliasis in line with the work of [20]. An infection with *Fasciola* is associated with liver damage in animals [2].

The absence of infection in *B. unicolor* and *L. varicus* in the present study may be due to the presence of operculum in these freshwater snails that serve as a cover against predation as well as helping the resistance of the freshwater snail to invasion by trematode larvae. Similar observation was made which considered them as non pathogenic [24].

Generally, the freshwater bodies studied with the exception of Bagoma dam, harboured infective freshwater snails. The high level of cercarial infestation (14.30%) observed in Manchok water intake informed the intense activity of definitive host in the site (man, livestock, and birds perching on the forest trees) which probably led to faecal contamination of the water body. Thus, the diversity of trematode fauna depends on conditions conducive for the transmission and the presence of the definitive host and intermediate host snail [25], [26]. Therefore, humans and animals utilizing this water body for washing, bathing, drinking and irrigation stand the risk of trematode infection. Kubacha dam with an infestation rate of 13.68%, mostly from *B. pfeifferi* and *L. natalensis* results from the presence of definitive hosts that are always utilizing the water body for domestic use. This makes the water body unsafe. Similarly, Gibbawa dam and Saminaka water intake were found to be infested with trematodes in *B. globosus*, *L. natalensis* and *M. tuberculata* with varying degrees of infestation. These water bodies therefore pose a threat to their respective communities. The least infected water body with cercariae was Kangimi dam (3.9%). The low prevalence might be as a result of the fast flowing water current that characterizes the dam and which displaces the snails and make the possible snail-miracidium contact a rare occurrence. Infestation of freshwater snail was not recorded from Bagoma dam during the period of study. This could be due to variation in local habitat and non availability of freshwater snail host for the infective stage of parasite to develop. Here, the only freshwater snail species that occurred in the freshwater body was *M. tuberculata*. The absence of other snail species might be attributed to the fact that *M. tuberculata* has been reported to be a competitor snail and has the potential to eliminate other species [27]. It was further reported that instability of habitat has the potential to influence the distribution of a particular species [16]. And since this dam over flooded during the period of study, it is eminent that the flood might have washed away other snail species, hence their absence.

The diversity of the freshwater snails is reflected in the diversity observed in cercariae from the six freshwater bodies surveyed in Kaduna state. This may be attributed to the conducive environment provided by the freshwater bodies for the freshwater snails to proliferate. The result agreed with that
of [18] who reported the occurrence of digenean trematode cercariae namely: Furcocercous cercariae, Gymnocephalus cercariae Echinostome cercariae and Xiphidio cercariae in some freshwater snail species. The presence of these cercariae types in the freshwater bodies studied implied that there is the likelihood of the spread of trematodiasis amongst their respective definitive hosts (man and other animals). Reference [13] further stated that these cercariae types are of great economic importance to humans and animals in the transmission of digenetic trematode freshwater snail borne diseases.

The present study revealed the occurrence of additional cercariae species namely; Longifurcate-pharyngeate distome, Longifurcate-pharyngeate monostome and Amphistome cercariae. [3] identified six morphological types of cercariae in nine species of freshwater snail hosts which had high potentials in spreading freshwater snail borne diseases, [28] identified nine species of trematode cercariae in freshwater snails with four human Schistosome transmitting snails and the presence of 20 morphologically distinguishable cercariae in 12 species of freshwater snails was reported in another study [30]. The occurrence of five morphological types of cercariae (Xiphidio, Echinostome, Gymnocephalus, Longifurcate and Cystophorous monostome cercariae) in some freshwater bodies in Zaria, Kaduna state was also reported [31]. These cercariae types were found in the current study except Cystophorous monostome cercariae. These cercariae have potentials of transmitting trematode infection.

It was also found in this study that some freshwater snails did not shed any cercaria. Reference [32] observed non-shedding of cercariae by three freshwater snail species in their studies (Lymanea natalensis, Pila ovata and Anisus stagnicola). This is consistent with other studies from endemic areas with high transmission where few or none of the freshwater snails collected shed any cercariae [13]. Furthermore, some of the freshwater snail species recorded in the current study shed up to four different species of trematode cercariae. The shedding of Amphistome, Gymnocephalus, Brevifurcate-apharyngeate distome cercariae by Biomphalaria, Bulinus and Lymnaea harvested in Gimmbawa dam, Saminaka water intake, Manchok water intake and Kubacha dam may suggest that humans and livestock utilizing these freshwater bodies are at higher risk of contracting infections carried by these freshwater snails than any other site surveyed during this study. This buttressed the previous findings of [26] who observed that cercarial diversity may be influenced by distribution and abundance of definitive host.

The present study reported single individual of Physa species with double infection (Longifurcate-pharyngeate distome and Gymnocephalus cercariae). Similar observations were made [33], [3] with triple and double infections in M. tuberculata, respectively. Double infection could mean more worm load resulting in higher mortality that may lead to under representation of freshwater snails [34]. This could be the reason why this was the least freshwater snail species collected during the study. The current study also observed that Longifurcate-pharyngeate monostome cercariae were harboured by C. bulimoides only. This showed that this type of cercaria is host specific. Longifurcate-pharyngeate monostome cercariae has been reported in the family Cystocephalidae which are intestinal parasites of reptiles, birds and mammals in Africa, however, with no economic importance [15].

Amphistome cercariae in this study was not host specific, as it was found to be harboured by three species of freshwater snails (B. pfeifferi, L. natelansis and M. tuberculata) (Table III). This cercaria occurs in the family Paramphistomatidae and those that belong to the Pigmentata group which are intestinal parasites of mammals (ruminant) which are of great veterinary importance [15].

It was also noted that Brevifurcate-apharyngeate distome cercariae were harboured by B. globosus and B. pfeifferi (Table III) Fig. 6 (a) is an avian cercaria with the adult mostly found in ducks and geese. It has been reported that this type of cercaria is able to penetrate human skin and cause Cercarial dermatitis as a result of allergic response [35]. However, this type of cercaria was observed in L. natelansis among others [36]. Fig. 6 (b) is the cercaria that causes urinary and intestinal Schistosomiasis which to date, many causes of natural human infection had been confirmed [37], [38].

Longifurcate-pharyngeate distome cercaria has no economic importance. It parasitizes amphibians, reptiles and fish with not much harm to them. Therefore, its presence in the water bodies does not pose any threat to the communities around.

Echinostome cercariae is the least observed cercariae type in the water bodies studied. They are intestinal parasites of birds, reptiles and mammals of the family Echinostomidae. This also has no economic importance [15].

Gymnocephalus cercariae is the cercarial form of liver flukes of domestic stock animals. They are of great veterinary importance, as many cases of Fascioliasis had been reported in Nigeria [20], [39] with established infestation in the four studied freshwater bodies. Xipido cercariae produced by trematodes of the family Plagiorchioidea are of no great economic importance. It is obvious from this study that the prevalence of freshwater snail species infected with larval trematodes varied from one water body to another. The distribution of trematodes in freshwater snails in the study areas indicated great variation, which was similarly observed in earlier research [3], who observed that, the degree of infection may vary in freshwater snails of the same species from one location to another.

V. CONCLUSION AND RECOMMENDATION

The presence of Brevifurcate-apharyngeate distome and Gymnocephalus cercariae is of serious concern considering the fact that they are the causative agents of Schistosomiasis and Fascioliasis in humans and animals, respectively. Of more serious concerns is the presence of these cercariae and the intermediate host snails in more than 50% of freshwater
bodies surveyed in the present study. It is therefore recommended that relevant government agencies and other stake holders should intensify awareness and education campaigns among communities that are at high risk of contracting these infections. Also, efforts should be intensified at controlling the freshwater snails (intermediate host) through the use of cheaper technologies such as the use of biological control and or other integrated control method.

REFERENCES