Asian Journal of Research in Medical and Pharmaceutical Sciences

1(4): 1-6, 2017; Article no.AJRIMPS.36070

In vitro Studies on the Effects of Cercariae Shedding (Schistosoma Parasitosis) on Fecundity, Hatchability and Longevity of *Bulinus globosus*

V. U. Obisike[1*], G. N. Imandeh[1], E. U. Amuta[1] and T. F. Ikpa[1]

[1]Department of Biological Sciences, University of Agriculture Makurdi, P.M.B. 2373, Makurdi, Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors GNI and EUA designed the study. Author VUO carried out the research, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VUO and TFI managed the analyses of the study. Author VUO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2017/36070
Editor(s):
(1) Aurora Martinez Romero, Clinical Biochemistry, Juarez University, Durango.
(2) Ashalata Gannepalli, Department of Oral & maxillofacial pathology, Panineeya Institute of Dental sciences & Research centre, Hyderabad, India.
(3) Mario Ciampolini, (Rtd) Department of Pediatrics, University of Florence, Italy.
Reviewers:
(1) Abdoualye Dabo, University of Sciences, Mali.
(2) Ali Mahmud Umar, Kano University of Science and Technology, Nigeria.
(3) Calvin Bisong Ebai, University of Buea, Cameroon.
Complete Peer review History: http://www.sciencedomain.org/review-history/21493

Original Research Article

Received 11th August 2017
Accepted 16th October 2017
Published 20th October 2017

ABSTRACT

Schistosomiasis is considered the second most prevalent worldwide parasitic disease ranked next to malaria. *Bulinus globosus* is one of the intermediate hosts for *Schistosoma haematobium*.
Aim: This research work investigated the effects of cercariae shedding (schistosoma parasitosis) on fecundity, hatchability, and longevity of *Bulinus globosus in vitro*.
Study Design/Methodology: One hundred snail eggs were hatched and grown *in vitro*. Fifty (50) were infected with miracidia while fifty (50) were treated as control. Observations were made on cercariae shedding, fecundity, hatchability and longevity of the snails.
Results: The result shows significance difference in the longevity rate between infected and control snails in their offspring's $P<0.05$. There was also high fecundity rate in control snails (84%).

**Corresponding author: E-mail: talk2vu@gmail.com;*

Hatchability rate was recorded higher in infected snails (97.2%).
Conclusion: The mortality rate of infected snails was observed to increase with shedding of cercariae. It is therefore recommended that prospective control programmes should aim at incorporating the control of snails and associated cercariae shedding to help sustain the gains made through chemotherapy. Greater efforts should also be placed on reducing environmental contamination by improvement of local water sanitation and hygiene.

Keywords: Freshwater; snail; vector; Trematode; parasites; fecundity; hatchability; longevity; cercariae shedding.

1. INTRODUCTION

Schistosomiasis is considered the second most prevalent worldwide parasitic disease ranked next to malaria, [1]. It has significant economic and health consequences in many developing countries [1].Various human pathogenic species of schistosomes are known. *Schistosoma hoematobium* causes urinary Schistosomiasis and is the most prevalent and widespread species in Africa, Eastern Mediterranean and the Middle East, [2].

The other four species causes intestinal shistosomiasis; *Schistosoma intercalatum* occurs in ten countries in the rain forest belt of Africa, *Schistosoma mansoni* is found in over 52 countries mainly in of Africa; carribbean, Eastern mediterancean, Latin America; *Schistosoma japonicum* and *Schistosoma mekongi* are prevalent in Africa and the Pacific region [2].

Bulinus globosus snails as specific intermediate host for *Schistosoma haematobium* as well as *B. truncatus and B. senegalensis* is prevalent in many parts of Africa forming a main favourable factor for schistosomiasis transmission. This snail species is found in the irrigation areas and drainage system and also water sources in reclaimed areas leading to infection in previously uninfected populations. This may eventually leads to increase schistosomiasis transmission [3].

A key determination of schistosomiasis transmission success is compatibility of the local snail population to Schistosome infection [3]. Compatibility here is defined as the likelihood that exposure to a miracidium or miracidia leads to a cercariae-producing infection, affects the survival rate (longevity) of the snail, egg production (fecoundity) and hatchability of the snail eggs. High compatibility will lead to more snail infections. This is because more Schistosome eggs will be shedded into the habitat, which may lead to increase in the number of cercariae that are produced, resulting in increased transmission [3]. *Bulinius globosus* is widely distributed in major irrigation schemes, lake, rivers and other water bodies. It is also found in small impoundments and both seasonal and perennial streams. Artificial light has been observed to induce cercariae shedding, it is often used in the laboratories [1,3].

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study sites are five water bodies within Makurdi metropolis and includes Bank of River Benue a pond at Demekpe, Ichwa dam rivers Ityomu and Fete. Makurdi is a town that lies between latitudes 7^o 15'N - 7^o 45'N and longitude 8^o 15' E - 8^o 40'E covering an area of 16 km^2 [4]. The main drainage system is Benue River with other smaller tributaries traversing the town. Makurdi is located within Guinea savannah of vegetation belt with annual rainfall between 150-180 mm and temperatures of 26°C -29^{o}C. The wet season spans from November to March.

.

2.2 Study Population/ Study Design

The study population comprises selected water bodies (Ichwa dam, rivers Ityomu, Bank of River Benue, Demekpe and Fete) and selected schools closer to water bodies. The population comprises students and snail species from selected schools, giving a total of one hundred and fifty (150) pupils were sampled and one hundred snails of *Bulinus globosus* (50 control and 50 infected) were used in the study. Three primary schools found close to freshwater bodies in Makurdi Local Government area of Benue State were randomly selected, 50 pupils from each each of the selected school were selected randomly for the study.

2.3 Snail Sampling

Sampling of snails was carried out from June to September 2016 in five water bodies (three river,

one dam and a ponds). Sampling at each site was done in the morning period from 8 – 11 am Two sampling techniques were employed, mainly the hand-net method [5] and hand picking techniques were used [6]. The operators were protected from direct contact with water by wearing knee-length rubber boots, and latex hand gloves Snails obtained were put in specimen bottles bearing labels showing the location of collection; reference number and dates of collection. The samples were taken to the Biological Sciences Laboratory of University of Agriculture, Makurdi Nigeria for identification.

2.4 Snail Identification

The snail samples were taken to the Biological Sciences Laboratory of University of Agriculture, Makurdi Nigeria for identification. Morphological identification of the collected snails was performed using a handbook of Danish BilhaziasisLaboratory [7].

2.5 Ethical Clearance

Prior to collection of samples, all the school headmasters were given a copy of an introductory letter from University of Agriculture, Makurdi for approval, cooperation and necessary briefing regarding the purpose, relevance and personal involvements of the exercise. In each of the schools, health education lectures were carried out for enlightenment about the disease.

2.6 Urine Samples

Sterile specimen bottles were given to selected pupils based on those that reported haematuria in questionnaire to collect the urine samples. This was supervised by their teachers. The samples were collected between 9:00 am and 2:00 pm this time coincides with the time of cercariae shedding [8,9]. The samples were properly labeled in a clean and sterile specimen bottle containing 0.1g of boric acid [10] to serve as buffer, with the help of their teachers. The specimen bottle preserves and prevents the eggs shistosomes from hatching during transportation to the laboratory [8]. The sampled population included Primary school children selected randomly. The specimen bottles were labeled with same identification codes to match with the one on the questionnaire. Microscopic examination of the samples was performed at the Laboratory of Biological Sciences Department, University of Agriculture, Makurdi using direct microscopy [9].

2.7 Detection of Eggs of Schistosome

Detection of the eggs of *S. haematobium* was done by microscopic examination of urine samples [11]. Concentration method was done where 10 mls of urine was centrifuged at 2000rpm for 3 minutes in order to concentrate eggs of *Schistosoma*. The supernatant was decanted and deposit tabbed gently and a drop was placed on a clean slide which was carefully covered with cover slip and viewed under the microscope first with x10 and then x40 objective lens. Eggs were preserved in refrigerator [9,12].

2.8 Snail Rearing

The snail samples were reared in transparent plastic containers which served as aquaria to mimic the natural environment [11,13] for a period of 56 days (14 weeks). The snails were reared based on the measures of successful snail rearing/breeding [12,13]. The snails were fed with lettuce and cabbage [13], water was changed every two days with water from their natural environment [12, 13]. This was to make sure they were not exposed to a toxic environment because chlorinated water makes the environment toxic for the snails [12,13] and to mimic the natural environment Five snails were kept in each container to avoid overcrowding and the aquaria were constantly cleaned to avoid other organisms [12,13,14].

2.9 Hatching the Eggs and Infecting the Snail

Juvenile snails were kept one snail per petridish to enhance miracidum penetration and competion among the snails and samples containing the eggs of *Schistosoma haematobium* were introduced into the petri dish containing dechlorinated water. Each Petri dish was exposed to artificial light for a period of 2-6 hours (200watts of electric bulb) to induce hatching of the eggs. Miracidia swarm out of eggs that hatched successfully and penetrate the snail where they are known to have biochemical attraction [14]. Fourteen weeks after the introduction of the eggs of the *Schistosoma haematobium*, when the eggs should have undergone several developments [15], the snails were exposed to artificial light to induce cercaria shedding. All the above processes were monitored using a magnifying lens. The offspring of exposed (infected snail) and offspring of control snails were monitored [16] for longevity.

2.10 Data Analysis

The data obtained from the study was subjected to t-test analysis using SPSS version 20.with the level of significance determined at P<0.05.

3. RESULTS

It was observed that 37 (24.7%) of the pupils were positive to *S. haematobium*, questionnaire reviewed that 68 (45.3%) pupils use the dams as their major source of water. People were seen doing some water activities in the dams especially bathing, washing etc. The results obtained showed that both the control snail and infected snail had appreciable longevity though the control was significantly higher than the infected snails t= 17.740 as shown in Table 1. Fecundity was higher in control snails than in infected snail while hatchability was higher in infected snails as shown in Table 2. The longevity of second generation offspring as shown in Fig. 1 was slightly infected as the offspring of the control group had higher longevity.

Table 1. Longevity rate of infected and control snails

Weeks	Control (%) N=(50)	Infected (%) N=(50)
1	48 (96)	48 (96)
2	47(94)	47 (94)
3	47 (94)	47 (94)
4	46 (92)	46 (92)
5	44 (88)	44 (88)
6	43 (86)	43 (86)
7	43 (86)	43 (86)
8	41 (82)	41 (82)
9	40 (80)	33(66)
10	40 (80)	32 (64)
11	40 (80)	31 (62)
12	39 (78)	31(62)
13	37 (74)	30 (60)
14	35 (70)	25 (50)

t= 17.740

4. DISCUSSION

Both control and exposed snails in this studies had a longer life span than those observed in related studies carried out in 2000 in southern part of Nigeria [13] This may be due to the procedure used to rear the snails in this experiment (one individual per dish), avoiding the negative effects of high population density and the nutritional supply and general maintenance employed in this study.

The above result shows higher longevity rate in control snails than in exposed snails, this is in agreement with the work conducted in 1990 and 1996 [17,18], which state that at least for the genus *Lymnea* as well as *Bulinus,* parasitism decreases the longevity and fecundity of the hosts.

Mortality was high in exposed snails possibly as a result of the cercarial emission of infected snails.

The fecundity rate was higher in control snails than exposed snails. However, the hatchability rate was more pronounced in exposed snails as 97.2% off the eggs hatched than control snails which 92.9% eggs hatched.

Table 2. Fecundity and hatchability rate of the first generation of infected and control snails

	Infected number (%)	Control number (%)
No. of eggs laid	37 (74)	42 (84)
No. of eggs hatched	36 (97.2)	39 (92.9)
No. of eggs unhatched	1 (2.7)	3 (7.1)

This is in agreement with the work carried out previously in 2000 [16] which states that the infected snails increase the hatching probability of their eggs as a compensation for their lowered fecundity compared to non-exposed snails. Also, the offspring of the snails in control group had higher longevity than that of offspring of infected parents.

This study revealed the effect of cercariae shedding on the reproductive parameters of *Bulinus globosus*. Although snails which shed high doses of cercariae ensure high infection rates but tend to die earlier. Longevity was observed to be higher in snails that had low cercariae shedding compared with the snails that had heavy shedding. Snails that had at least one cercaria shedding could transmit the parasite longer as they tend to have higher longevity. If such snails that despite infection survives longer than others “super-survivor snails” occur in the field, they could pose problems for Schistosomiasis control initiative which is mostly on mass drug administration, and pays little or no attention to the control of the snails which are the intermediate host of schistosomes. Even if drug treatment were timed more frequently than currently practiced (say every 4 or 6 months)

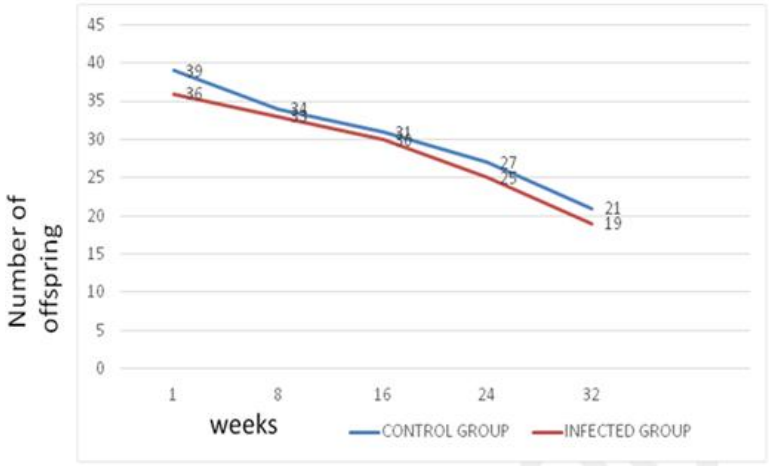

Fig. 1. Longevity rate of offspring's of second generation of infected and control snails
t = 9.502

as a means to minimize new snail infection more efficiently and lower the eventual rate of reinfections in people, super-survivor snails could potentially continue to persist through multiple rounds of treatment and still be there to initiate reinfections. As the proportion of super-surviving snails would probably be low, this effect may prove to be trivial, but along with contributions of schistosome egges by reservoir hosts, may favour persistence of schistosomes even in the face of more frequent treatments.

5. CONCLUSION

Longevity, hatchability and fecundity are affected by cercariae shedding in snail hosts of *Schistosoma* as observed in the study. The higher survival rate in snails that recorded low cercariae is an indication that the life cycle of *Schistosoma* could continue even in low cercariae load.

We therefore recommend an all inclusive environmental sanitation and use of environmental friendly mulluscicide to reduce the snail population.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENT

We wish to acknowledge the entire staff of the Biological Sciences Department of University of Agriculture Makurdi for their encouragements. we say thank you to Mr. Angula, A.M. on his efforts and encouragement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Baraket R, Morshedy H, Farghaly A. Human schistosomiasis in middle east and north Africa region. Neglected Tropical Diseases. Spinger Varley Wein. McDowell, Rafati (Eds). High Institute of Public Health, Alexandra University, Egypt; 2014.
2. Utzinger G, N'Govan E. Schistosomiasis and negleted tropical diseases: Towards integrated and sustainable control and a word of caution. Parasitology. 2002;136: 1859-1874.
3. World Health Organization. Prevention and control of schistosomiasis and soil transmitted helminthiasis: Report of who expert committee. WHO Technical Report Series. 2002;912:1-57.
4. Terwase S, Terese E. Tyonum. Residents coping measures in flood prone areas of Makurdi, Benue State. Applied Ecology and Environmental; 2013.
DOI: 10.12691/aees-1-6-4

5. Okafor F, Okolie D. Schistosoma transmission patterns in freshwater systems of Anambra State of Nigeria. Angewandte Parasitologie. 1990;37:159-166.
6. Ofoezie IE. Distribution of freshwater snails in the man-made Oyan Reservoir, Ogun State, Nigeria. Hydrobiology. 1999;416: 181-191.
7. World Health Organization. A Field Guide to African Freshwater Snails. Danish Bilharziasis Laboratory. Charlottenlund, Denmark: WHO collaborating Centre for Alied Malacology. 1998;65-76.
8. Lucas A, Gilles H. A new short textbook of preventive medecine for the tropics. Edward Arnold: Educational Low Priced Scheme. 1990;147-150.
9. Cheesbrough M. Medical laboratory manual for tropical countries. 2nd edition. ELBS, Cambridge. 2002;1:323-341.
10. Appannavavar SB, Biswal M, Rajkumari N, Mohan B, Tanega N. Evaluation of commercial boric acid containing vials for urine culture: Low risk of contamination ans cost effectivenes and considerations. Indian Journal of Pathol Microbial. 2013; 56:261-264.
11. Okon O, Udoutun M, Oku E, Nta A, Etim S, Abraham J, Apan P. The prevalence of urinary schistorsomiasis in Abini community, biase local government area, Cross River State, Nigeria. Nigeria Journal of Parasitology. 2007;28:29-31.
12. Evelyn L, Haseeb M, Laboratory rearing of *Biomphalaria glabarata* snails and maintenance of larval schistosomes *in vitro* and vivo in *Biomphalaria* snails and larval trematodes. Springer New York. 2011;33-55.
13. Okete JA, Edu EA, OKU EE, Sadig IA. Efficacy of the ethanolic extracts of *Talinum triangulare* (JACQ) for control of the freshwater snail, *Bulinus globosus*, the vector of urinary schistosomiasis. International Journal of Pure and Applied Zoology. 2015;3:76-86.
14. World Health Organization, Prevention and Control of Schistosomiasis and soil transmitted helminthiasis: Report of WHO expert committee. WHO Technical Report Series. 2002;912:1-57.
15. Center for disease control parasitic diseases. New England Journal of Medicine. 2016;17:43-67.
16. Gultierrez A, Pereta G, Yong M, Sanchez J, Wong L. Life history traits of *Fossaria cubensis* under experimental exposure to *Fasciola hepatica*. SciELO Analytics. 2000; 95:747-752.
17. Jong-Brink M. How *Trematode* parasites interfere with reproduction of their intermediate host, freshwater snails. Journal of Medical and Appllied Malacology. 1990;2:101-133.
18. Jarne P, Njioku F, Bellec C, Delay B. Population genetics structure and mating systems in hermaphrodites freshwater snails the example of *Bulinus globosus* and *B. truncates.* Malacol. 1996;6:115-120.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21493