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Effect of Calcium Chloride on Growth and Survival of *Biomphalaria pfeifferi*-Intermediate Host of *Schistosoma mansoni*

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Abstract: *Biomphalaria pfeifferi*, the snail intermediate host of *Schistosoma mansoni* was cultured in the laboratory to show the effects of calcium chloride ($CaCl_2$) on its growth rate and survival. Ten snails of approximately the same size and age were cultured in each of 4 tanks set up. In 3 of the tanks, $CaCl_2$ was dissolved in 1000 mL of water in various concentrations. The 4th tank served as control experiment where snails were bred without any $CaCl_2$ added. Highest growth rate was recorded in tank with highest concentration but percentage rate of survival was lowest here. Lowest growth was recorded in the control tank. Highest survival rate was recorded in tank 1 where calcium concentration was lowest. Significant increase in growth ($p<0.05$) was recorded in tank 1 with optimum concentration of calcium. Calcium chloride in optimal concentration was found to improve the growth and survival rates of *B. pfeifferi*.

Key words: *Biomphalaria pfeifferi*, *Shistosoma mansoni*, $CaCl_2$, growth rate, rate of survival

INTRODUCTION

If schistosomiasis must be controlled, adequate knowledge of the snail intermediate host, which is an essential link in the schistosome life cycle is required. Blood flukes, also referred to as Schistosomes are the only flukes which are dioecious and whose circarial larvae have bifid tails. They inhabit the blood stream of warm-blooded vertebrates. Schistosomiasis (Bilherziasis) originated from the Nile valley and from there spread to other parts of Africa and the entire world through migration and slave trade (Bandoni *et al*., 2000). Snails of the class Gastropoda and sub-class Pulmonata are the intermediate hosts of *Schistosoma* (Okere and Odaibo, 2005). *Biomphalaria pfeifferi* is the most important species in endemic areas of West Africa. It is involved in the propagation of *Schistosoma mansoni* (Bandoni *et al*., 2000).

B. Pfeifferi prefers fresh water habitats with sandy substrates. They are widely distributed in Africa, south of Sahara. Also present in Egypt, Israel, parts of Arabia, South Africa, Southern USA etc. (de Kock *et al*., 2004).

Biomphalaria specie, the snail intermediate host of *Schistosoma mansoni* belongs to the phylum Mollusca, class Gastropoda, subclass Pulmonata, order Basommatophora, family Planorbidae, subfamily Planarians and genus *Biomphalaria* (Jordan and Webbe, 1982; de Kock *et al*., 2004).

The following Physicochemical factors influence the distribution and occurrence of snail intermediate hosts in their habitats:

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In natural waters, hardness is caused by ions of Bicarbonate (HCO_3), Sulphate (SO_4) Chloride (Cl), Nitrates (NO_3), Magnesium (Mg) and Calcium (Ca) (Nduku and Harrison 1976). The snail intermediate hosts show more tolerance to higher alkalinity than low. When they are found in water with low alkalinity, the number is reduced and they have relatively thin shells (Okwuosa and Ukoli, 1980; de Kock *et al*., 2004).

Broderson and Madsen (1991) found that *B. Pfeifferi* was absent in water bodies with high concentration of Mg and present where calcium ion were abundant. Okwuosa (1982) had also observed an upper limit of 5.0 mg L^{-1} for Ca in the intermediate snails' habitat.

This is one of the maintenance challenges encountered in breeding snail intermediate hosts in the laboratory. Generally, low temperature (winter) reduces snail breeding in tropical and subtropical zones (Jordan and Webb, 1982). Temperature limits the distribution of the snails and they are found at lower altitudes (Pfluger, 1978). Southgate *et al*. (2001) found that the optimal temperature for the rapid expansion of this species was close to 25°C. The maximum temperature tolerated by *B. Pfeifferi* is 32°C (Appleton *et al*., 1996).

To study the quality of water bodies, which serve as habitats for the snail intermediate hosts, various factors have been considered. Such factors are Hydrogen ion concentration (pH), Dissolved Oxygen (DO), aeration, water level, dissolved solids etc.

The intermediate hosts of *Schistosoma* are pulmonates, which possess both lung and pseudobranch. When the snails are sub-merged in water, the pseudobranch serves as respiratory organ and uses Dissolved Oxygen (DO) (Susan *et al*., 1990).

The snail intermediate hosts depend more on DO than atmospheric oxygen (Mead, 1979). pH slightly less than 7.0 is optimal for the snail intermediate hosts (Okwuosa and Ukoli, 1980; de Kock *et al*., 2004).

Biomphalaria pfeifferi remains an essential link in the schistosome life cycle, its ecology, bionomics and population dynamics is required for a proper understanding of schistosome transmission. This should also form a basis for planning and control measures against the snail intermediate hosts in the control of schistosomiasis. *B. pfeifferi* is small in size (diameter = 10-12 mm) and very difficult to maintain under laboratory conditions. There is therefore the need to research into the different cultural conditions required for maximum yield under laboratory conditions.

This study is therefore designed to assess the effect of Calcium on the rates of growth and survival of *B. pfeifferi*.

MATERIALS AND METHODS

This work was carried out between February and May, 2005 at the Schistosomiasis Research Laboratory, Lagos University Teaching Hospital, (LUTH), Lagos.

The snail intermediate hosts were collected from the snails' pond in the zoological garden of the university of Ibadan. The snails were carefully taken to the laboratory and allowed to acclimatize for 72 h before setting up the experiment. Four Breeding tanks were set up using sand as the substrate.1000 mL of dechlorinated water was measured and poured in to each of the tanks.

Tanks 1, 2 and 3 contained 1.0 g (0.36 g of Ca), 2.0 g (0.72 g of Ca), 2.50 g (0.90 g of Ca) $CaCl_2$ in 1000 mL of water, respectively. The fourth tank designated as Tank 4 had no calcium in it and therefore served as the control. These tanks were aerated for 24 h after which 10 snails of approximately the same size and age were put in each tank. The snails were fed regularly using fresh lettuce. In order to forestall contamination by excreted nitrogenous waste and maintain the levels of $CaCl_2$ in the tanks, aged tap water and $CaCl_2$ were introduced weekly. Two pieces of 4×4 cm transparent cellophanes were dropped in each tank for the snails to lay their eggs on.

Growth Measurement and Survivorship

The size of the snails in each of the four tanks were monitored and recorded weekly. The diameters of the snails were measured using a pair of vernier callipers. The shells were handled carefully to prevent cracking. Changes in the size, shape, colour and strength of the shells of the living snails were observed and noted. The surviving snails were counted and a weekly log on their survivorship was kept. The percentage rate of survival was calculated using:

$$\text{Rate of survival (\%)} = \frac{\text{No. of surviving snail}}{\text{Initial No. of snails}} \times 100$$

The tank and laboratory ambient temperatures were measured daily and the weekly mean temperatures were calculated. The laboratory ambient and water tanks temperatures for the period of this study was between 27.6 -29.4°C and 26.1- 28.2°C, respectively.

RESULTS AND DISCUSSION

Table 1 shows the mean of weekly growth rate of snails cultured in various concentrations of calcium chloride. The results generally indicate a steady increase in shell diameter with the highest mean diameter recorded in the culture medium containing 2.5 g L^{-1} $CaCl_2$ while the lowest mean was recorded for biospecimens cultured in the control medium. Notably, Tank 1 (1.0 g L^{-1} $CaCl_2$) showed a steady increase but the growth rate was not as fast as it was in Tank 3 with the highest concentration of $CaCl_2$ (2.5 g L^{-1} $CaCl_2$) where maximum growth (1.6 mm) was attained in three weeks. However, in the reference tank (Tank 4), the growth rate was very slow and the maximum growth was recorded only in the 9th week.

It was observed that after 10 weeks, 50% of the snails that were cultured in Tank 1 survived. Similar observations were made in Tanks 2 and 3 with percentage survival rate of 41.7 and 25%, respectively. However, about 41.7% survival rate was recorded for the control medium. In general, the survival rates decreased as the age of the snails increased but there was stability in the survival rate after the 6th week. The highest mortality of biospecimens was recorded in the culture with the highest concentration of calcium chloride (Table 2).

The results of this study showed that calcium ion in different concentrations have significant effect ($p<0.05$) on growth and survivorship of *B. pfeifferi*. At enhanced concentrations, $CaCl_2$ was found to be lethal to the snail intermediate host. However, it was observed that the biospecimens survived in 2.5 g of $CaCl_2$ (0.90 g L^{-1} Ca) concentrations but at concentration higher than this, the mortality rate was 100% within one week. This finding corroborates similar reports by Okwuosa (1982).

Table 1: Mean growth rates (Shell diameter in mm) of *B. pfeifferi* in the cultured and reference tanks

Weeks	Tank 1	Tank 2	Tank 3	Tank 4
0	0.60±0.017	0.64±0.024	0.60±0.165	0.72±0.014
1	0.64±0.010*	0.68±0.010*	0.65±0.009	0.74±0.029
2	0.69±0.017	0.72±0.012	0.85±0.008	0.79±0.019
3	0.92±0.016	0.78±0.022	1.00±0.018	0.84±0.011
4	0.86±0.022	0.79±0.021	0.96±0.017	0.90±0.014
5	0.91±0.012	0.84±0.014	1.01±0.020	0.94±0.014
6	0.94±0.022	0.86±0.007	1.06±0.010	1.06±0.012
7	0.95±0.009	0.88±0.016	0.73±0.569	1.04±0.013
8	1.06±0.009	0.90±0.009	1.13±0.017	1.10±0.012
9	1.05±0.009	1.01±0.008	1.15±0.010	1.10±0.012
10	1.13±0.009*	1.06±0.014	1.16±0.010*	1.10±0.012

Tabulated values are mean±SD of 10 determinations. *: $p<0.05$ vs control

Table 2: Percentage survival rate of *B. pfeifferi* in reference and cultured tanks

Weeks	Tank 1 (1.0 g $L^{-1}CaCl_2$)	Tank 2 (2.0 g $L^{-1}CaCl_2$)	Tank 3 (2.5 g $L^{-1}CaCl_2$)	Control (0.0 g $L^{-1}CaCl_2$)
0	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)
1	100.0 (12)	91.7 (11)	50.0 (6)	91.7 (11)
2	91.7 (11)	91.7 (11)	33.3 (4)	91.1 (11)
3	91.7 (11)	91.7 (11)	33.3 (4)	75.00 (9)
4	75.0 (9)	91.7 (11)	33.3 (4)	75.0 (9)
5	75.0 (9)	58.3 (7)	25.0 (3)	66.7 (8)
6	66.7 (8)	41.7 (5)	25.0 (3)	66.7 (8)
7	50.0 (6)	41.7 (5)	25.0 (3)	58.3 (7)
8	50.0 (6)	41.7 (5)	25.0 (3)	41.7 (5)
9	50.0 (6)	41.7 (5)	25.0 (3)	41.7 (5)
10	50.0 (6)	41.7 (5)	25.0 (3)	41.7 (5)

Figures shown are in % and the numbers of surviving snails in parenthesis

In Tank 3 (2.5 g L^{-1} of $CaCl_2$ (0.90 g Ca^{2+})), maximum growth was recorded in the 3rd week of the study after which a steady growth rate was recorded. In the control culture, maximum growth was obtained only after 9 weeks. Maximum growth rates in cultured tanks with 2.0 and 1.0 g L^{-1} $CaCl_2$ were recorded in the 6 and 8th weeks, respectively (Table 2). This implies that the higher the concentration of Ca ion, the faster the rate of growth of the snails. This statement is in agreement with the findings of Harrison *et al*. (1979) and Okwuosa and Ukoli (1980).

Temperature played an important role in the propagation of *Biomphalaria* under laboratory conditions. A temperature range that is close to that of their natural habitat i.e., 26-29°C was maintained in the water tanks throughout the course of this study. At temperatures lower than 25°C and higher than 32°C, the rate of growth reduced drastically. This finding agrees with that of Southgate *et al*. (2001).

In general, it was observed that the growth rate of snails cultured in different media increased with age and was concentration dependent. In spite of growth inhibitory condition posed by concentration of $CaCl_2$, the snails attained maximum size of 1.1 mm in diameter.

Calcium chloride was considered adequate as the source of Ca in this study because *Biomphalaria* spp. have been found to be tolerant to chlorides better than other intermediate hosts and also because the chloride ion ionizes in water if left for some days as also observed by Okwuosa (1982) and de Kock *et al*. (2004). The snail intermediate hosts tolerate high alkalinity and hardness than low corroborating the findings of Okwuosa and Ukoli (1980).

Moreover, calcium chloride is known to give hardness to natural water amongst others hence, it was found to be suitable for this experiment. Nduku and Harrison (1976) found that $CaSO_4$ which gave very poor results was not suitable for raising the snail intermediate hosts in the laboratory. The growth rate of snails in highest concentration (2.5 g L^{-1} $CaCl_2$) is about 0.2 mm per week while in the control tank (with no Ca added), the growth rate is 0.05 mm per week.

Survival or mortality rate of the snail intermediate hosts of *S. mansoni* is also affected by concentration of Ca in the culture media. The highest survival rate was recorded in the medium with concentration of 1.0 g L^{-1} $CaCl_2$, while highest mortality rate was recorded in the tank with highest concentration of 2.5 g L^{-1} $CaCl_2$. It is also found that the optimum calcium concentration for survival of the snails is between 0 and 0.360 g L^{-1} as also reported by Okwuosa (1982) that a range of 0-0.37 g L^{-1} of Ca was found in water bodies inhabited by the intermediate hosts of schistosomiasis.

This study has shown that deficiency of calcium ion could reduce the survival rate of the snail intermediate hosts. Similar reports were given that calcium deficiency reduced snail survival and the schistosomes developmental stages (Badger and Oyerinde, 2004). The shells of the snails cultured in the control tank were found to be whiter and more fragile than the shells of snails grown in media which contain calcaum ion. This is also reported by Broderson and Madsen (1991) that snails raised at very low concentrations were smaller and had thinner shells with low crushing resistance. The stability in the survival rate in the last 5 weeks of the study may be due to the fact that the snails got used to the

various $CaCl_2$ concentrations with time and this increases their survival rate. The bionomics and population dynamics of *S. mansoni* which are required for proper understanding of transmission of schistosomes can be looked at in further research.

In conclusion, it has been found that calcium in low concentrations is necessary for optimum growth and survival of *B pfeifferi*. It is found in this work that addition of Ca in optimal concentration (0-0.36 g L^{-1}) increases the growth of *B. pfeifferi* significantly at $p<0.05$.

High concentrations are lethal to the snails. Optimum concentration above 0.1 g L^{-1} and below 0.5 g L^{-1} Ca is suggested for proper growth and survival rates of the snail intermediate hosts. Based on the outcome of this study, calcium chloride ($CaCl_2$) can therefore be used as an additional source of calcium for laboratory culture of *B. pfeifferi* for adequate shell formation, high rates of growth and survival which are necessary for the completion of the life cycle of *S. mansoni*, a basis for the eradication of schistosomiasis.

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