

## The Susceptibility of *Biomphalaria* spp. from Mwea Irrigation Scheme in Kenya against *Schistosoma mansoni* Miracidia Infection

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### ABSTRACT

Schistosomiasis as a neglected disease is second to malaria in its adverse effect to public health and socioeconomics impact in the tropics and sub-tropical of the developing countries where 90% of 249 million people affected are found in Africa. Mwea irrigation scheme in Kenya is infested with *Biomphalaria* spp. and the research set out to find their susceptibility to *Schistosoma mansoni* miracidia. Vector snails' samples were taken from endemic region of Mwea irrigation farmlands and were morphological identified and then cultured. The miracidia exposed snails were transferred into aquaria and after four weeks of exposure they were examined for cercaria shedding twice every week under direct sunlight illumination. It was found that Mwea irrigation scheme was dominantly infested by *Biomphalaria pfeifferi*. From the morphological parameters of *B. pfeifferi* it was found that there was no statistical difference in physical characteristics between resistant and susceptible populations. The results in this study showed that the mean value of infection for the Field, F1 and F2 snail samples were  $36.6 \pm 3.72$ ,  $1.93 \pm 1.46$ ,  $0.36 \pm 0.049$  respectively and the infection rate decreased from the field snail samples through F2 snail samples. This suggested that the exhibited resistant traits may be due to snail internal defense mechanisms rather than morphological characteristics and this could be thought that the various levels of *B. pfeifferi* susceptibility to *S. mansoni* is attributed to genetic variations within a population. Finally, the findings generated in this study, under laboratory condition, suggest that *S. mansoni* resistant population of *B. pfeifferi* can be isolated and mass reared with a view of diversifying biological control measures of the vector in Mwea irrigation scheme in Kenya.

## 1. INTRODUCTION

Schistosomiasis is a major neglected disease in the tropical region and has been found second to malaria in its adverse effect to public health and socioeconomics impact the tropics and sub-tropical of the developing countries. 249 million people are affected by the diseases worldwide and of these 90% requiring treatment reside in the Africa [1, 2]. Schistosomiasis is referred to as a “silent pandemic” because of problems caused by it and the reduced productivity of the affected people [3, 4] *Biomphalaria*, *Bulinus* and *Oncomelania* are the three genera which are the main intermediate host of human Schistosoma parasites. The distribution of intestinal causing *Schistosoma mansoni* all over the world is assisted by the broad geographical range of susceptible snails species of its intermediate host of the Genus *Biomphalaria*. The *Biomphalaria* spp. is the obligatory hosts for miracidia, which causes infection in humans and other primate hosts [3, 5, 6].

Schistosomiasis endemic is in 78 tropical and sub-tropical countries according to the global epidemiological data [2]. About 779 million people are at risk of schistosomiasis globally and a further 250 million are infected of which 20 million suffer from debilitating illnesses associated with schistosomiasis [7, 8]. Africa accounts for majority of disease incidence with poverty associated with schistosomiasis and Kenya has about more than six million people infected, accounting for approximately 23% of the total population, who are infected with urinary or intestinal schistosomiasis [7, 9].

There are numerous intermediate host snail species that serve as obligatory hosts for the schistosome parasite larval stage (Miracidia) which infects humans. They act as environmental reservoirs of the disease and ensuring sustained transmission cycle. *Biomphalaria sudanica* and *B. pfeifferi* are the known major vectors of *S. mansoni* while *Bulinus africanus* and *Bulinus globosus* for *S. haematobium* [10]. These snail intermediate hosts are predominantly found in fresh water bodies like lakes, ponds, streams, rivers, irrigation canals and dams. In Kenya, Schistosomiasis is endemic with a 100% of human population being considered at risk for contracting the disease by 1995 [7]. Estimates for the proportion of the population infected have remained at 23% since the first estimation in 1986. The agents responsible for both *S. mansoni* and *S. haematobium* the causative agents of intestinal schistosomiasis and urinary schistosomiasis respectively are endemic in Kenya [11, 12].

Various control interventions have been used to block transmission and reduce the disease burden, including mass drug administration (MDA) using praziquantel drug, snails habitat modification, improving sanitation and use of molluscicides. However, schistosomiasis has remained a major public health problem, more so in sub-Saharan African rural areas [13].

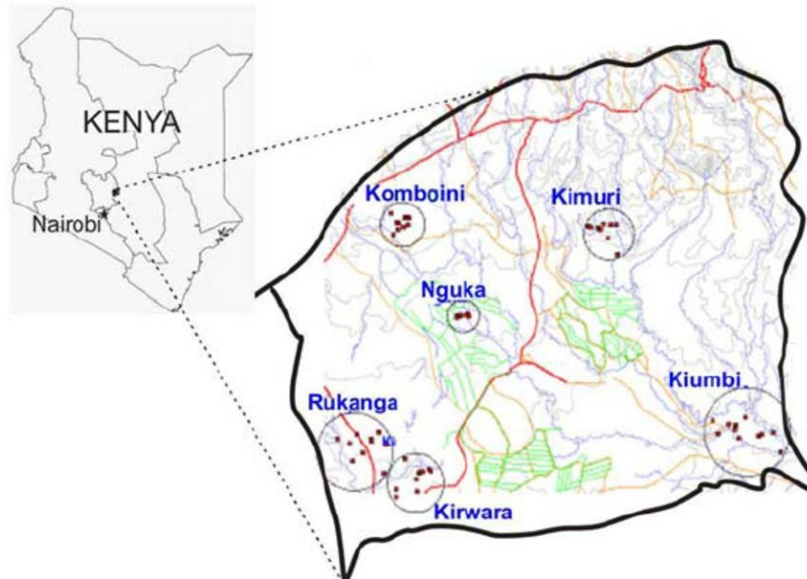
While Schistosomiasis can be treated with praziquantel drug, people often get re-infected after treatment if they come into contact with water containing infectious cercariae. According Hotez [13] the increasingly use of praziquantel MDA campaign in schistosomiasis in endemic regions has led to the appearance of reduced efficacy indicating selection of drug-resistant forms of parasite. The use of synthetic molluscicides on the other hand is increasing becoming unpopular due to adverse effects on environment and its high costs. Currently, all Schistosomiasis endemic countries are encouraged to increase snail vector control efforts and move towards elimination as required by the World Health Organization (WHO) roadmap for the global control of the neglected tropical diseases [14].

With the current interest in eliminating schistosomiasis, various snails control strategies need to be devised. Breaking the life cycle of the parasites will offer an option to decrease transmission, by targeting intermediate host snails [15, 16]. The use of genetically resistant snail vector to displace susceptible populations has been suggested [17, 18]. The aim of this study therefore was to identify resistance and susceptible of vector snails populations in Kenya Mwea rice irrigation region.

## 2. MATERIALS AND METHODOLOGY

### 2.1. Study Site

The sampling site was within Mwea Irrigation Scheme, Kirinyaga County which is about 1100 - 1200 m above sea level and stretching between latitudes 0°37'S and 0°45'S and longitudes 37°14'E and 37°26'E, see Figure 1.



**Figure 1.** The map shows the sampling area at Kirinyaga county.

## 2.2. Vector Snail Sampling

Vector snails' samples were taken from schistosomiasis endemic region of Mwea irrigation farmlands. Live snails were collected and put on wet cotton wool containers which were perforated and transported to aquaculture facility at Institute of Primate Research (IPR) within one day.

## 2.3. Vector Snails Identification

Field collected samples of snails were morphologically identified at National Museum of Kenya (NMK). The snails Shell morphometric, tool for identification of mollusc taxonomy and ecological studies was used to determine species of representative population of snails collected. A total of nine measurements were taken for each shell. These were total length of shell, width of shell, basal width of shell spire, length of aperture, width of aperture, total shell area, area of spire, area of aperture, and angular.

## 2.4. Snails Culturing and Infection with Schistosome Larvae (Miracidia)

The snails samples (*B. pfeifferi*) collected from field were housed for two weeks in glass aquaria containing snail-conditioned water to acclimatize to laboratory conditions. At the culture facility the snails were feed on tetramine fish food, lettuce leaves, and calcium carbonate, in a conducive environmental condition, using the method described by Eveland [19].

Schistosome eggs were collected from stool of experimentally infected primate (baboons) in an on-going schistosomiasis experiments at Institute of Primate Research (IPR) colony were exposed to light for 2 - 3 hours to stimulate hatching into miracidia. In 10 ml beakers, individual snails were exposed to 8 - 10 newly hatched miracidia in direct sunlight for 3 - 4 hours. The miracidia exposed snails were kept under laboratory condition at room temperature as previously described by [19-21].

The miracidia exposed snails were transferred into aquaria each containing 48 snails replicated 10 times in a Complete Randomized Design. They were kept under laboratory condition at room temperature.

The snails were individually examined after four weeks of exposure to the parasite for cercarial shedding twice in direct light illumination procedure in which individual snails were put in beaker containing 10 ml of water and cercaria shedding was observed on 10 X magnification -microscope. Cercaria shedding was determined using the method direct illumination method [22] in which the exposure larvae infected snails to white light caused emergence of cercaria into the water and lastly snail infection and mortality

rates were determined.

Snail Infection Rate was arrived at by dividing the cercaria shedding snails by total exposed snails. The resistant and susceptible snails' percentage was determined as illustrated by [23] as follows:

$$\text{Infection rate} = \frac{\text{Total positive and shedding crushed snails in each subgroup}}{\text{Total snails exposed in each subgroup}} \times 100$$

Snail Mortality Rate was determined by counting the dead snails starting from the day of exposure to the parasite divided by exposed snails in each subgroup as follows: -

$$\text{MR}\% = \frac{\text{Total snails which dead in each subgroup}}{\text{Total snails exposed in each subgroup}} \times 100$$

## 2.5. Determining Resistance and Susceptibility of Snail's Infection in Laboratory Bred Snails

Populations of laboratory adapted *B. pfeifferi* snails at IPR were tested for resistance/susceptibility to *S. mansoni* infection. The snails were reared under suitable laboratory conditions according to by [19] where they were kept in glass aquaria with snail-conditioned water and fed on lettuce leaves, tetramine fish food and calcium carbonate. Source and maintenance of the parasite *S. mansoni* miracidia were collected from infected primate host (Baboons) at IPR schistosomias on-going experiments.

Snails were individually exposed to miracidia larvae and selection of susceptible and resistant snail isolates determined by actual biological shedding of cercaria. The field sample snails that remained uninfected after 4 - 6 weeks of exposure to infection were reared separately. Their progeny (F1) were selected and reared under the same conditions as the resistant group [24].

## 2.6. Miracidial Infection Tests to Resistant Isolates (F1) Generation

In 10 ml beakers, 480 resistant isolates snails were exposed individually to 8 - 10 newly hatched miracidia and the beakers were placed under direct sunlight for 3 - 4 hours. The exposed snails were maintained in aquaria each with 48 snails replicated 10 times in a Complete Randomized Design experimental design. The snails were kept under laboratory condition at room temperature as was illustrated by [19-21].

Each snail was checked for cercarial shedding two times a week for four weeks post-exposure to the miracidia and repeated for three weeks. The cercaria shedding or non-shedding from individual snail was determined using a direct light illumination procedure where individual snails were put in 10 ml water in beaker and cercaria shedding determined by observation on 10× magnification stage microscope. During the shedding period of three weeks were kept in darkness throughout [23, 24]. The snail infection and mortality rates were determined.

## 2.7. Miracidial Infection Tests to Resistant Isolates (F2) Generation

The resistant F1 snails isolates that were exposed to miracidia in the above (2.6) experiment and did not shed cercaria were kept separately for propagation. Their progeny (F2), were selected for miracidial infection. The experiment set-up was repeated as described in 7section (2.6) and the same parameters determined.

# 3. RESULTS AND DISCUSSION

## 3.1. Vector Identification

Snails shell morphometric identification determined populations dominating the Mwea irrigation scheme in Kenyan highland were *B. pfeifferi*. This observation concurs with other previous works by Mutuku [25]. The snails had mean Shell Height of 9.9 and 10.0 mm and shell width of 9.5 mm and 9.5 mm for resistant and susceptible snails respectively. The morphological parameters of *B. pfeifferi* indicated that there are no significant differences in physical characteristics of resistant and susceptible populations of

*B. pfeifferi* snails against *S. mansoni* from the study region.

These findings point to demonstrate that the exhibited resistant traits may not as a result of snails morphological characteristics but probably due to snail internal defense mechanisms. Similar of observations have been reported by Nacif-Pimenta and Negrão-Corrêa *et al* [26, 27]. Earlier, Webster and Newton had reported that susceptibility of *Biomphalaria* spp. to *S. mansoni* infection was inherited character [28, 29].

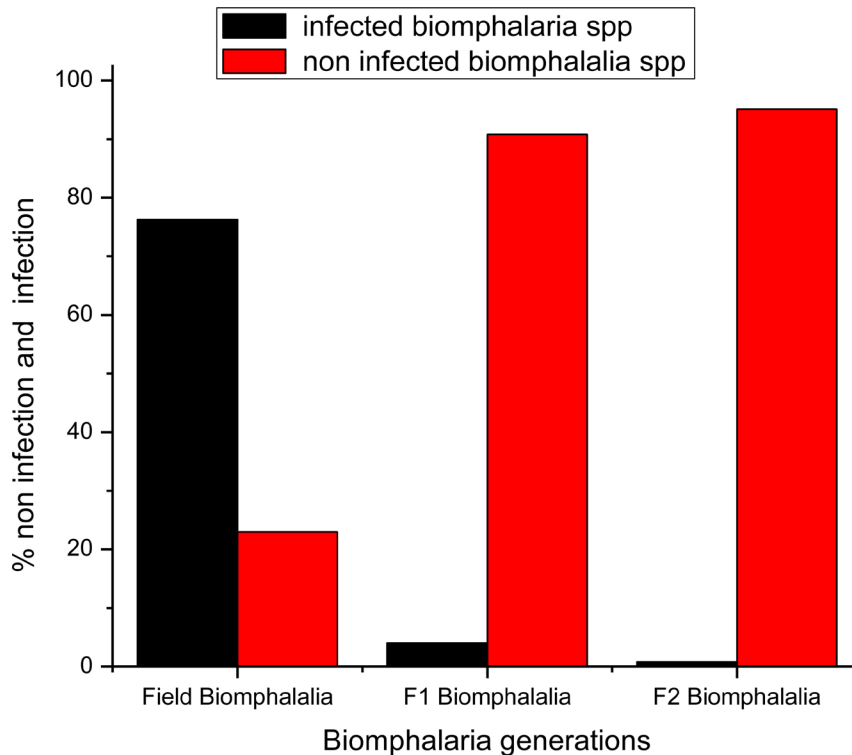
### 3.2. Susceptibility of *B. pfeifferi* against *S. mansoni* Infection

The snails populations collected from field exhibited significantly high rate of infection at 76% shedding cercaria while 24% of the snails showed some resistance. However, these observations significantly differed with infections rates exhibited by resistant snails' progeny of F1 and F2. In F1 generation, susceptible snails dropped to 4% while 90.8% were resistant. In F2 generation susceptibility decreased even further to only 0.8% and that of resistant group increased further to 95.1%, see **Figure 2**.

### 3.3. Parameters of Statistics-F-Test

The F-test statistics of *B. pfeifferi* in infection rates revealed highly significant differences between the Field Collected snails, F1 and F2 generations. The mean value for the Field, F1 and F2 snail samples were  $36.6 \pm 3.72$ ,  $1.93 \pm 1.46$ ,  $0.36 \pm 0.049$  respectively. Comparing the Field samples and F1 generation for the infected snails,  $F = 6.48$  and  $P = 0.00$  at 0.05 confidence level, the two populations variations were found to be significantly different. The Field and F2 snail samples for the infected snails,  $F = 57.59$  and  $P = 0.00$  at 0.05 confidence level, the two populations variations were found to be significantly different. Lastly, the F1 and F2 generation of infected were found to have an  $F = 8.88$  and  $P = 0.00$  and at 0.05 confidence level were found to be significantly different, see **Table 1**.

In the non-infected, the Field samples and F1 generation snails,  $F = 2.54$  and  $P = 0.01$ . At 0.05 confidence



**Figure 2.** Shows percentage of infected and non-infected *B. pfeifferi* snails in field sample population through F1 and F2 generation.

**Table 1. Means infection rates for the infected and non-infected snails for the Field, F1 and F2 snails.**

Field snails and F1 infected		N	Mean	S.D	Variance	F	Number DF	Denom. DF	Prob > F
Infected Snails	Field snails	30	36.6	3.72	13	6.48	29	29	2.76E-06
	F1 Snails	30	1.93	1.46	2.1				
	Field snails	30	36.6	3.7	13.8	57.588	29	29	1.48E-06
	F2 Snails	30	0.37	0.049	0.24				
	F1 Snails	30	1.93	1.46	2.13	8.88	29	29	7.78E-06
	F2 Snails	30	0.37	0.49	0.24				
Non-Infected snails	Field snails	30	11.6	4	16.06	2.54	29	29	1.40E-06
	F1 Snails	30	43.6	2.5	6.32				
	Field snails	30	11.07	4	16.06	3.133	29	29	2.95E-06
	F2 Snails	30	45.67	2.264	5.13				
	F1 Snails	30	43.6	2.513	6.317	1.23	29	29	5.77E-06
	F2 Snails	30	45.67	2.26	5.126				

level, the two populations variations were found to be significantly different. The field and F2 snail samples for the non-infected snails,  $F = 3.13$  and  $P = 0.00$  and at 0.05 confidence level, the two populations variations were found to be significantly different. Lastly, the F1 and F2 snail samples non-infected were found to have an  $F = 1.23$  and  $P = 0.058$  and at 0.05 confidence level were found to be significantly different. Since there was significant difference between the field F1 and F2 generations there might be a gene enhancement as one observes the generations' field, F1, F2, and So on.

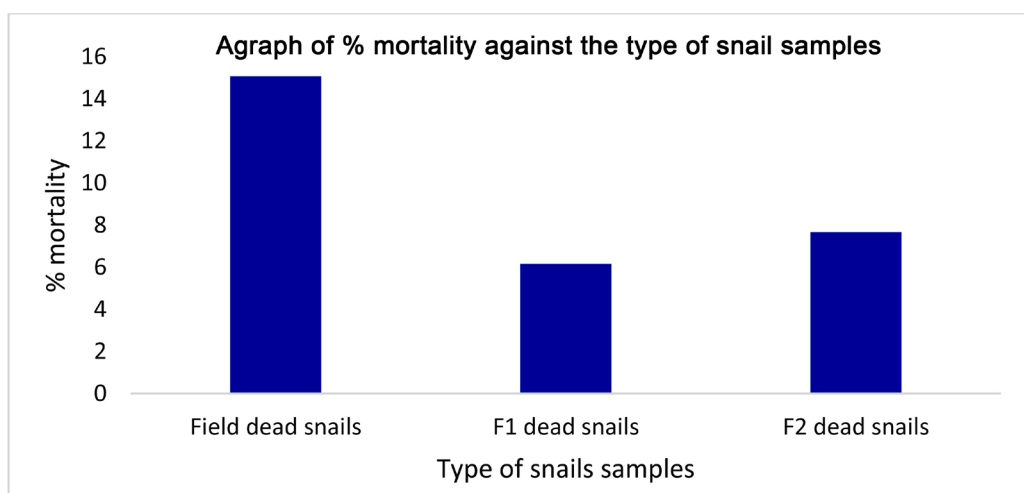
These laboratory observations of infections rates of *B. pfeifferi* from Mwea irrigation scheme against *S. mansoni*, seems to support previously reported work that suggests there are enhancement of resistant genes individual snails progressed through the F1 and F2 generations. Similar trend was noted by Paraense on determination of phenotypic resistance of F1 and F2 progeny [30]. In the work of Lewis and Iman while investigating the genic flux of the resistance traits in F1 progeny obtained by crossbreeding susceptible and resistant strain of the same species of *B. glabrata*, it was observed that resistant individuals predominantly occurred in all groups [31, 32]. Previous investigations by Richards and Merritt documented dominant resistance heritability in *B. glabrata* snails [33]. Rosa reported two dominant genes determine resistance in *B. tenagophila*, [34] while Negra-Correa reported that the factors that influence the susceptibility may be genetically determined by the activities of the Snails internal defense system [27]. Webster and Woolhouse found that both resistant and susceptibility to *Schistosoma* infection were heritable [28].

### 3.4. Snails Mortality Rate

Statistically there were no significant differences in mortality rates between snails samples from Field, F1 and F2. The mean values for the number of dead snails in the Field, F1 and F2 snail samples were  $6.033 \pm 2.025$ ,  $2.467 \pm 2.53$ ,  $3.067 \pm 1.96$  respectively. Comparing the number of the dead snails for the Field and F1 samples,  $F = 1.62$  and  $p = 0.22$  at 0.05 confidence level, the two populations variations were not significantly different. In the case of Field and F2 snail samples,  $F = 1.06$  and  $P = 0.87$  at 0.05 confidence level, the two populations variations were not significantly similarly to comparison of F1 and F2 at  $F = 0.65$  and

**Table 2.** Shows the means for the dead snails for the Field, F1 and F2 snails.

Mortality rate	N	Mean	S.D	Variance	F	Number DF	Denom DF	Prob > F
Field and F1 dead snails	Field	30	6.03	2.025	4.103	1.62	29	29
	F1	30	2.47	1.591	2.533			
Field and F2 dead snails	Field	30	6.03	2.025	4.1	1.06	29	29
	F2	30	3.07	1.96	3.85			
F1 and F2 dead snails	F1	30	2.47	1.59	2.53	0.65	29	29
	F2	30	3.07	1.94	3.86			



**Figure 3.** Mortality rate in *B. pfeifferi* under laboratory conditions.

P = 0.26 were not significantly different, see [Table 2](#).

Though not statistically significant differences were observed, the percentage mortality rates in the Field, F1 and F2 snails samples were determined as 15%, 6% and 7.6% respectively. This finding is similar mortality in snails as reported by Alvine [35] under the same conditions. The observed mortality rates may be due to natural causes, see [Figure 3](#).

Overall the findings generate in this study under laboratory condition points to suggest that *S. mansoni* resistant population of *B. pfeifferi* can be isolated and mass reared with a view of diversifying biological control measures of the vector. In line with observations [36, 37] posit that desirable long-term solution to control of schistosomiasis is to build up the field resistant or the less susceptible strains to infection, which is an ecologically safer means of breaking transmission cycles. As described by [38], one of promising measures of biological control is the introduction of parasite resistant snails into endemic areas to replace susceptible strains and avoid often destructive changes to the local ecosystem that accompany other control methods of snails, this current study could inform such interventions in schistosomiasis endemic areas in Kenyan highlands.

#### 4. CONCLUSION

Susceptible and resistant *B. pfeifferi* snail populations can be isolated when the vector is challenged with *S. mansoni* larvae. Various levels of *B. pfeifferi* susceptibility to *S. mansoni* is attributed to genetic

variations within a population. Further investigation at molecular level and phylogenetic characteristics of *B. pfeifferi* will provide useful insights. More studies concerning genetic variability of *B. pfeifferi* with different degrees of susceptibility to *S. mansoni* infection could add to further impetus to the development of control strategies for schistosomiasis.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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