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EVIDENCE OF LONG TERM BENEFIT OF PRAZIQUANTEL TREATMENT AGAINST *SCHISTOSOMA MANSONI* IN KIGUNGU FISHING VILLAGE OF ENTEBBE, UGANDA

Running title: Praziquantel treatment against *Schistosoma mansoni* in Kigungu fishing village of Entebbe, Uganda

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Abstract.

Praziquantel (PZQ) is efficacious against all species of schistosome: *Schistosoma mansoni*; *Schistosoma haematobium*; *Schistosoma japonicum* and other parasites like the *Taenia species*. This cross-sectional cohorts study was carried out in Kigungu fishing village along Lake Victoria shore in Entebbe Uganda. Our analysis was based on examining microscopically three slides from a single stool specimen from each of base line cohorts 945. These included children and adults, participants from both sexes in Kigungu fishing village in Entebbe Uganda. Nine hundred and one (901) of the cohorts were re-examined after six months and 625 of the same cohorts who were examined at the baseline and after six months were re-examined 18 months later. The slides were prepared using modified Kato/Katz (Odongo-Aginya) method. The infection proportion with *Schistosoma mansoni* at baseline was 448 (47.5%) but this was reduced to 244 (25.8%) 18 months after treatment with a single oral dose of praziquantel at 40mg/kg. However 495 (52.5%) were negative at the baseline study. The cure proportion after six months was significant {(P=0.00), (OR4.63) CI at 95% (3.53-6.06)}. Similarly the cure proportion after 18 months was significant {(P=0.00), (OR2.2) CI at 95% (1.87-3.34)}. The force of re-infection after six months was significant {(P=0.0001), (OR 0.47) CI at 95% (0.31-0.71)}. Nevertheless the force of re-infection was not significant after 18 months {(P=0.766), (OR 0.95) CI at 95% (0.68-1.34)} eggs excretion did not reach the level of the pre-treatment intensity. The egg reduction was 69.3%. This was associated with age and pre-treatment intensity < 400 eggs per gram (epg) of faeces and age groups ≥ 30 years. The egg reduction also resulted in marked decrease in clinical symptoms in the participants. Our study suggests evidence of long-term benefit of praziquantel in Kigungu and that the re-infection occurred more commonly in younger age group than in the older patients.

Key words: Praziquantel; *Schistosoma mansoni*; Kigungu; Entebbe; Uganda.

Introduction

Praziquantel (PZQ) is efficacious against *Schistosoma mansoni*; *Schistosoma haematobium*; *Schistosoma japonicum* and other parasites like the *Taenia species* (1). Presently in Uganda, one of schistosomiasis endemic countries in sub Saharan Africa, PZQ is the drug of choice in controlling morbidity due to schistosomiasis because the mean cost of treatment per dose per person in Uganda is \$0.3 (1, 2, 3). In spite of its low cost, Uganda is still unable to procure adequate PZQ for mass chemotherapy (2). Consequently it was found necessary to establish a treatment regimen for reevaluation study in Uganda. Previous studies in Uganda and elsewhere reevaluated the efficacy of PZQ after six months to establish the cure proportion (4, 5). Nevertheless other studies reevaluated the efficacy of PZQ at the interval of one year for a longer period (6, 7). At present, studies in different endemic settings using the single oral dose regiment of 40 (mg/kg wt) of PZQ against *S. mansoni*, *S. haematobium* and mixed infections, the efficacy of

PZQ is reported to stand at 60-90 % (8). Extensive use of PZQ in Uganda and elsewhere in the tropics has been linked to the development of parasite resistance to the drug. This is evidenced by reduced cure proportion in humans treated with PZQ in the Richard Toll area of Senegal (3). However, factors like intensity of infections and high transmission of infections have been known to influence schistosomicidal activity of PZQ(4). Pharmacologically, Praziquantel kills adult worms by penetrating and damaging tegument of the adult worms (9). Subsequently there is a transient increase of antigens released from the dead adult worms and egg antigens in the circulation, which stimulate humoral and cellular immune responses of the host (10, 11). These anti-worm and anti-egg responses in the host aid in the killing of the worms by antibody binding to the antigen exposed due to Praziquantel damaged tegument (12). This phenomenon may continue for long after the pharmacological effect of

PZQ (9). Repeated treatment with PZQ has been shown to improve cure proportion as observed in West Nile districts of Uganda (13, 14). These are districts with high transmission and high intensity of infection (13, 15).

This study investigated the effect of PZQ on cure proportion and egg reduction 18 months after treatment in Kigungu-fishing village, a community with high exposure risk to *S. mansoni* infection in order to establish cost effective treatment regimen that could be adopted in Uganda schistosomiasis mansoni endemic foci (16).

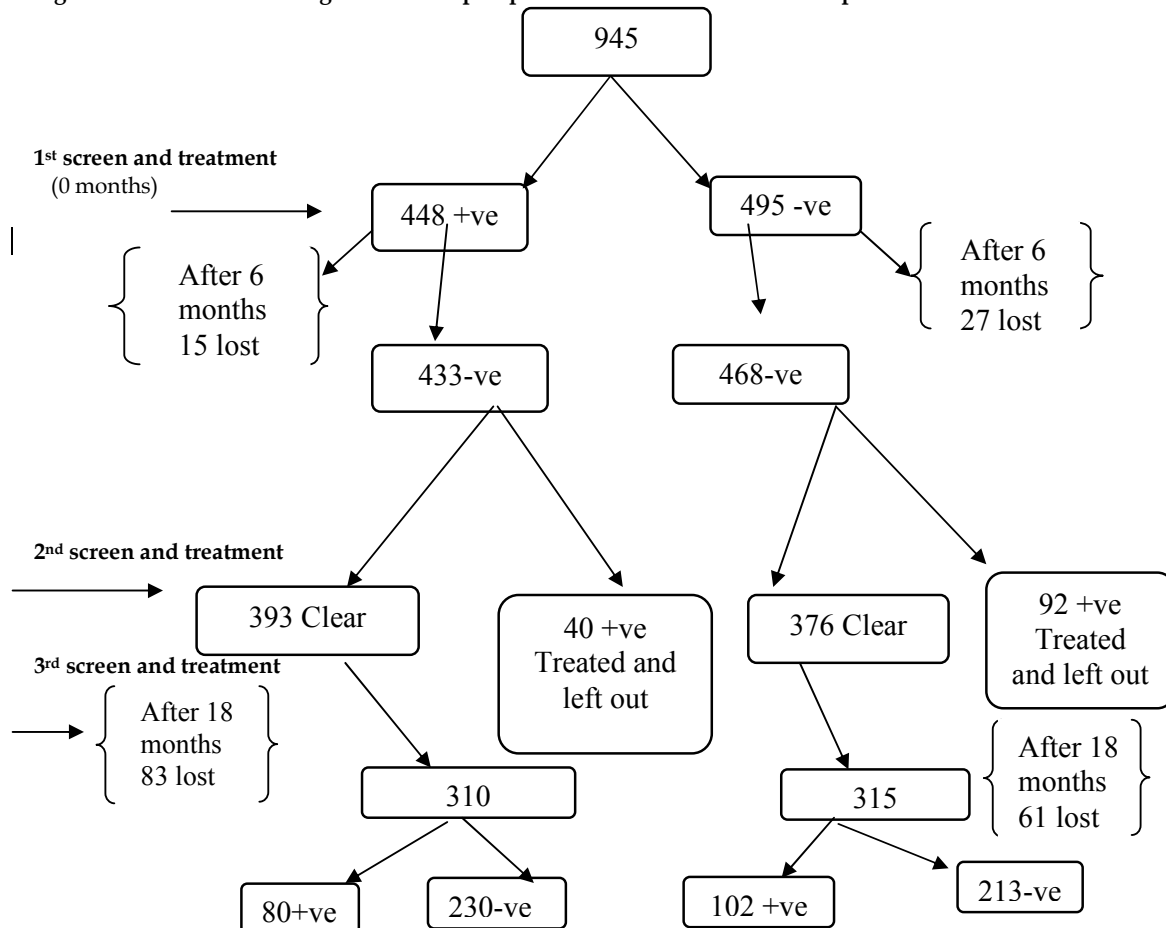
MATERIALS AND METHODS

Study sample

A baseline evaluation study was carried out on a cohort of 945 volunteer adults and children who were residents of Kigungu fishing village, Entebbe, Uganda. Enrolled 448 patients were stool positive for *S. mansoni* while enrolled 495 patients were stool negative for *S. mansoni*. Treatment was administered to the 448 *S. mansoni* positive patients. Six months later 95.5% (901/945) patients were reviewed. These consisted of 433 (96.7 %) patients from the 448 *S.*

mansoni positive patients in the base line survey (fifteen were lost). Out of the 433, forty were excreting eggs of *S. mansoni* in their stool. They were treated and left out of the study. Therefore people who remained eligible for the study in this group were 393. Similarly, 468 (94.5%) among the 495 negative patients at the baseline were reviewed (see flow chart below). Ninety two of these 468 previously negative patients were subsequently (6 months later) also found with eggs of *S.mansoni* in their stool. They were also treated with 40mg/kg body weight and left out of the study. Therefore people who remained eligible for the study in this group were 376. Of the 393 *S. mansoni* positive reviewed after six months, 83 people were lost to follow up only 310 patients came for follow-up and 80 patients were stool positive for *S. mansoni* while patients 230 were still stool negative for *S. mansoni*. Of the 376 *S. mansoni* negative after six months (not treated), 61 people were lost to follow up only 315 patients came for follow-up, and 102 patients had become infected with *S. mansoni* while 213 patients remained stool negative for *S. Mansoni* throughout the 18 months' follow up. The participants were registered in the three studies using their study code numbers, names, sex, age, home locations and the names of head of the families.

Figure 1: Flow chart showing the follow up of patients from first recruitment up to 18 months



Study area

This study was conducted in Kigungu fishing village, situated along Lake Victoria in Entebbe peninsula. This village is located to the extreme end of the peninsula, at latitude 35° to 38° East and 03° to 07° North. It is about 15 kilometers from Entebbe Municipality and about half a kilometer from the Entebbe international airport. This fishing village was selected because it had base line data on prevalence and intensities of *S. mansoni* and other soil-transmitted helminths (16). The population of Kigungu is estimated to be 6,000 people with nearly an equal sex 1:1 ratio. Their main occupation is fishing. Besides fishing, they do a little subsistence farming mostly for food crops. Their water exposure is high and hence is the source of infection and reinfection.

Procedure of the study

Informed consent was obtained from all the participants. Residents who had consented to participate in the study and children between 5 and 18 years old who had been granted permission to participate in the study by their parents/guardians and have not taken antischistosomal treatment six months prior to the base line study were registered. This explains the 55 people who were excluded from the base line registration. Those giving their consent and were literate, were asked to sign an official form showing acceptance. Meanwhile the illiterate patients used thumb prints on official form showing acceptance. All participants who consented to the study and were found *S. mansoni* positive were administered free antischistosomal therapy and treatment for any other ailments. Patients unwilling to participate in the study were not penalised in any way and normal clinical services and treatments including antischistosomal therapy were not conditioned to the patient's participation in the proposed research work. Medical doctors, and nurses, carried out health examinations and recorded the various clinical symptoms. Medical laboratory technologists carried out laboratory investigations and the social worker coordinated the interviews and recruitment. All patients infected with *S. mansoni* were treated at the study site with praziquantel (Medochemie Ltd. Limassol-Cyprus Europe) at 40 mg per kg body weight. Illnesses, other than schistosomiasis, detected during examination were appropriately treated or referred to other health facilities. Patient's privacy was duly respected.

Residents for the study reported to the clinic between 9 a.m and 2 p.m of our publicised workdays. They were interviewed and examined by a physician and a nurse during initial screening. The physical examination included special attention to status of the abdominal organs commonly affected by *S. mansoni* worm. Anaemia and fever were also recorded. Patients with body temperature greater than 37.5°C had blood smear test for malaria parasites done. On each working day,

thirty consecutive patients in the order of their arrival at the clinic were registered into the study.

Determination of intensity of infestation by intestinal worms

A stool container labelled with individual code number, name, age and sex was given to each patient to return with about 5-10 gram of stool specimen. Eggs in the stool were quantified using modified Kato-Katz method (17). Essentially, each stool specimen was initially strained through a stainless steel sieve of 250 µm mesh size to remove artefacts. The stained sample was filled in a template, a device measuring 41.7 mg of stool. Each of three separate aliquots of 41.7 mg stool was sampled on a different glass microscope slide and processed for examination as follow. About 10 µl of compound stain consisting of eosin 5% eosin, and 7.5% nigrosin in 10% formalin was added to stool smear on each slide. The stain was stirred in the stool smear on the slide using tooth pick. A wettable cellophane cover slip (32 x 41 mm) pre-soaked in 50% glycerine was carefully placed on the stained stool smear and gently pressed down. The excess stain from the smear on slide was blotted out with absorbent paper before the prepared slide was read using objective x 10 (17, 18). The arithmetic mean of the eggs counted in three slides was recorded as the count in 41.7 milligram of stool. To convert the mean egg count into egg per gram of faeces a factor of 24 was multiplied by the mean of the eggs counted {i.e. number of eggs (n) x 1000 mg/ 41.7 mg = 24 x n = eggs per gram faeces}. Intensity of infection was classified as follows: low = 1-100 eggs per gram, medium = 101-400 and high = ≥ 401 eggs per gram of stool (6).

Data management and statistical analysis

Data were double entered using Microsoft Excel and crosschecked by different researchers. The arithmetic means egg counts of the individuals were categorised according to infection intensities as follows 0-100; 101-200; 201-300; 301-400; and ≥ 401. Similarly age group was also categorised into five groups 5-10; 11-20; 21-30; 31-40 and ≥ 41. The percent of cured proportion was calculated from individuals who had no *S. mansoni* egg in their stool after treatment divide by the total number of individuals who had *S. mansoni* egg in their stool before treatment multiply by 100%. The percentage of egg count reduction was calculated from the arithmetic mean egg count after and before treatment. Pearson's Chi-X² was used to find out associations between a pair of categorical data at 95% level of confidence while Pearson's and Spearman's rank correlations were used for comparison of continuous data.

RESULTS

In this study, we recruited nine hundred and forty five residents, adults and children of both sexes. They

were all from Kigungu village of Entebbe in Uganda. Four hundred and forty eight (47.5%) were *S. mansoni* positive at the baseline survey and they were treated with a single dose of praziquantel at 40 mg/kg body weight. Four hundred and ninety five (52.5%) were negative for *S. mansoni*. Six months later, the study participants were reviewed for treatment success in clearing the infection. Nine hundred and one (95.5%) of the people who participated in the baseline survey came back for the review but 4.5% (mostly school children relocated themselves elsewhere). A very small proportion (2.4%) of the 448 patients who were *S. mansoni* positive at the baseline survey continued to excrete eggs of *S. mansoni* in their faeces. Similarly 2.5% of the 468 negative participants at the baseline survey were infected. The *S. mansoni* positive participants were again treated with 40 mg/kg body weight and were left out of the study. After follow up of participants from the seventh to the eighteenth months, 625 (310 from positive +315 from negative) of

them came back for the third evaluation while additional 276 participants were lost to follow up. Table 1, shows the distribution of intensity of infection among 80 male and female participants who were found infected in the baseline study, treated six months later and still got re-infected and thus found to be stool positive at end of 18 months of follow up. At the same time, the force of clearance by PQZ was 230 (74.2%) (i.e. 230 from 310) this was the total number of patients who were negative for *S. mansoni* after 18 months. Table 2, Compares difference between those who remained negative after treatment with those who were infected in the baseline study the force of clearance of PZQ showed significant difference (P= 0.00). Similarly the force of clearing the infection of PZQ after 18 was also significant (P=0.00). While the re-infection rate was significant (P=0.0001) after six months there was no significant difference in the re-infection rate after 18 months (P= 0.766).

TABLE 1: INTENSITY OF S. MANSONI AMONGST 80 PATIENTS AT RECRUITMENT AND 18 MONTHS LATER ACROSS AGE GROUP IN YEARS.

S. MANSONI AT RECRUITMENT, N=80				REINFECTION WITH S. MANSONI 18 MONTHS LATER, N=80		
Age group	N	%	†(mean)	N	%	†(mean)
5-10	14	17.50	9,592 (685.1)	14	17.50	4,851 (346.5)
11-20	45	56.25	20,283 (450.7)	45	56.25	9,853 (219.0)
21-30	12	15.00	9,845 (820.4)	12	15.00	2,857 (238.0)
31-40	7	8.75	6,129 (875.6)	7	8.75	1447 (206.7)
≥ 41	2	2.50	2,096 (349.3)	2	2.50	144 (72.0)
TOTAL	80	100	47945 (599.3)	80	100	16197 (225.0)

Legend: % = Percentage of infections in the age group. †: Total number of eggs excreted by each age group before and 18 months after the third treatment. The figures in parenthesis represent arithmetic mean of the egg count. N: the total number individuals infected with *S. mansoni* before and after the third treatment.

TABLE 2: CURE RATES AND REINFECTION RATES

Cure rates after 6 months		P -value	OR (CI)
393/433	92/468	0.00	4.63 (3.53 - 6.06)
Cure rates after 18 months			
210/310	102/376	0.00	2.20 (1.87 - 3.34)
Re infection rate after 6 months			
40/432	92/469	0.0001	0.47 (0.31 - 0.71)
Re infection rate after 18 months			
80/310	102/376	0.766	0.95 (0.68 - 1.34)

Legend: cure rate after 6 months and 18 months against force of reinfection in six months and 18 months after treatment

DISCUSSION

Several studies of infection and reinfection with *Schistosoma mansoni* after treatments with PZQ of residents living in endemic areas have shown that after treatment, prevalence and intensity of parasite infestation due to re-infection are lower than before treatment. In other words, the re-infection prevalence and intensity have been shown never to equal before treatment level (5, 7). In this cohort study to establish regimens for PZQ treatment against *S. mansoni* among the residents of Kigungu, showed that there was reduction in the percentage of infection from 47.4% (448 / 945) to 25.8% (80 / 310) and eggs excretion was reduced by 92.7% . This was 18 months after the initial treatment with PZQ 40mg/kg/wt.

We deliberately set a period of 18 months in total with the first six months for the assessment of the success of baseline treatment. We subsequently followed the cohorts twelve months later to allow the study residents exposures to infection. This was to find out the level of resistance and susceptibility developed after treatment. Follow up studies to establish the force of reinfection after treatment with PZQ after short interval is well known, (5, 7, 8). Schistosomiasis control Programmes notably Uganda and in other Tropical countries are based on the single dose administration of PZQ to large communities. Repeated treatment to re-infected individuals is recommended depending on the degree of transmission in the areas (8). In spite of the low cost of PZQ most counties in Africa are unable to procure adequate PZQ for mass chemotherapy. In Uganda, the mean cost of treatment per dose per person is \$0.3 (2, 19). Study of this kind helps to establish treatment regimens for communities in schistosomiasis endemic areas in the tropical countries with meagre budget for helminth control programmes. Mutapi in their study of changes in specific anti-egg antibody levels with PZQ followed their patients after 9 months (20). In addition, Correa-Oliveira in their natural versus drug induced resistance in *S. mansoni* infections study followed their patients for five years at an interval of one year (7). Repeated treatment with PZQ within short intervals was also found to have no effect on the re-infection period in children in their first decade of life (7). Nevertheless, the cure proportion of PZQ is greater if the treatment is repeated within a short period but the cost of the treatment remains relatively high (6, 10). In this study, in spite of a long period of 18 months after treatment, we were still able to detect a cure proportion of 67.7% (210 out of 310). This observed cure proportion, compare well with others of 60- 90% frequently accepted in endemic areas in the tropic using the same PZQ regimen (12, 21). On the other hand lower cure proportion of 18-39% was observed in very intense focus of *S. mansoni* transmission in northern Senegal (4, 22). A set up of this kind may need shorter intervals for re-intervention (7). Pre treatment infections categorised according to the levels of intensity showed that 151 patients in Kigungu were in

low intensity levels (1-100 epg), 99 of them were in the middle levels of intensity (101-400epg) and only 60 of them excreted high egg count greater than 401 epg. The observation that 213 patients remained uninfected in all studies points out to an interesting situation in which some of the residents live all their lives in *S. mansoni* endemic areas but do not get the infection. This group of patients commonly known as endemic normal (putative resistance) was always stool negative for *S. mansoni* eggs (23). The putative resistant people of Kigungu have been found to be stool negative for *S. mansoni* before and after treatment (23, 24). Concurrent infection with other intestinal helminths has been shown to affect cure of Praziquantel against schistosome (25). We have observed mixed infection of *S. mansoni* and other intestinal parasites like hookworm (species identification was not done), *Ascaris lumbricoides*, and *Trichuris trichiura*. Our stool analysis was based on a single stool specimen from each patient. In most community-based studies, cure proportion have been estimated based on only one or two slides Kato/Katz reading usually derived from a single stool sample (26). The multiple stool samples procedure is particularly relevant when the overall geometric mean egg count is likely to be low, because it increases the chances of estimating true cure proportion (21).

An unsuccessful effort to eradicate schistosomiasis in the Tropical countries using integrated control methods by World Health Organisation (WHO), lead to the development of morbidity control strategy using antiparasite drugs. However the regimens for treatment for re-infections in different endemic settings remain unclear. In this context we followed our cohorts for 18 months intervening chemotherapeutically using PZQ at base line, after six and 18 months. Reduction in the percentage of infection from 47.4% to 25.8% and reduction of eggs excretion by 69.3% after 18 months raises hope about the long term benefit of praziquantel after treatment in Uganda for large-scale treatment of schistosomiasis.

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