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Two years impact of single praziquantel treatment on infection of urinary schistosomiasis in the Barombi Kotto focus, Cameroon

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Key words: Schistosoma haematobium, prevalence and intensity, praziquantel, two years impact, Barombi Kotto focus, Cameroon.

doi: <http://dx.doi.org/10.12692/ijb/3.3.98-107>

Article published on March 28, 2013

Abstract

To evaluate the impact of a single dose praziquantel on urinary schistosomiasis in the Barombi Kotto focus, urine samples were collected from 306 participants (279 school children and 27 volunteer parents) of the Barombi Kotto health area from May 2007 to May 2009 and examined using the filtration technique. A malacological survey was conducted to identify and follow up the infection rates of the intermediate snail host. The overall prevalence (69.17 %) of *Schistosoma haematobium* was significantly different between boys (73.22%) and girls (66.66%; $P = 0.03$), same as between the island (84.3 %) and mainland (60.45 %; $P = 0.0001$) quarters. The prevalence reduced significantly by 55.03 % (from 69.17 % to 31.10 %; $P = 0.0001$) and intensity of infection by 76.16% (from 212.1 to 50.56 eggs/10 ml urine; $P = 0.01$) at 2 years post treatment. Heavy *S. haematobium* infections in school children decreased from 23.31 % to 2.12% at 2 years post treatment. The infection rates of the intermediate snail hosts (*Bulinus truncatus* and *B. camerunensis*) identified reduced from 3.7 % to 0.9 %. These results show a significant impact of a single dose praziquantel in reducing *S. haematobium* infections after two years. The general uptrend of the prevalence and intensity of infection observed requires a continued monitoring of the disease transmission, repeated treatment and availability of adequate sanitation facilities in the Barombi Kotto focus.

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Introduction

Three main forms of human schistosomiasis exist in Africa (intestinal, rectal and urinary), caused by the species *Schistosoma mansoni*, *S. intercalatum*, and *S. haematobium*, respectively. Schistosomiasis remains a significant health burden for many parts of the world, especially where health resources are limited (King, 2010). Out of the 239 million people with active *Schistosoma* infection in 2009 (King *et al.*, 2011), 85% lived in sub-Saharan Africa, where about 112 and 54 million are infected with urinary and intestinal schistosomiasis respectively and the number of subjects at risk of infection stands superior to 600 million (Martyne *et al.*, 2007). Estimated data show that 200.000 deaths/year where caused by schistosomiasis (Zhang *et al.*, 2007).

The WHO recommendation on schistosomiasis control is based on morbidity control through chemotherapy with praziquantel (WHO, 2002; 2006). Schistosome morbidity is mainly caused by eggs trapped in various parts of the human body; therefore the fundamental aim of morbidity control is to reduce intensity of infection by drug treatment (Touré *et al.*, 2008). Although praziquantel has been available as an effective treatment for *Schistosoma* infection for nearly 30 years, treatment may not be fully curative and questions remain about the best possible timing and frequency of praziquantel dosing for optimal control of infection and morbidity (King *et al.*, 2011). It has been observed in some studies that preventive chemotherapy using praziquantel has resulted in significant reduction of infection and morbidity one year post treatment (Koukounari *et al.*, 2006; Zhang *et al.*, 2007; Nkengazong *et al.*, 2009) and two years post treatment (Saathoff *et al.*, 2004; Nsawah-Nuamah *et al.*, 2004; Touré *et al.*, 2008). Previous findings suggest that the WHO recommended treatment strategies of schistosomiasis should be adopted with some degree of flexibility according to the different epidemiological and geographical settings (Nsawah-Nuamah *et al.*, 2004; Touré *et al.*, 2008).

In Cameroon, the three forms of human schistosomiasis exist with unequal distribution foci. Intestinal and urinary Schistosomiasis are more prevalent in the Northern part of the country, while only a few foci are found in the Southern part (Ratard *et al.*, 1990). Schistosomiasis due to *S. haematobium* is sparsely distributed in the south west Cameroon. Lake Barombi Kotto in Barombi Kotto village and Barombi Mbo in Kumba serve as the main transmission foci, where transmission is assured by *Bulinus truncatus* and *B. camerunensis* (lake Barombi Kotto) and *Bulinus truncatus* (lake Barombi Mbo). Multiple outbreaks of the disease have been observed in the Barombi Kotto focus despite the control done in the mid-1970s (Duke and Moore, 1976) and the treatment given after each survey, with the highest prevalence and infection intensity observed in children living around the transmission site (Moyou *et al.*, 1987; Ratard *et al.*, 1990; Ndamukong *et al.*, 2001; Nkengazong *et al.*, 2009). Past control done in this focus has mainly focused on the treatment of school-age children (Moyou *et al.*, 1987; Ndamukong *et al.*, 2001), but it has not been well defined for adults resident in the same vulnerable area.

The study reported here aimed to evaluate the parasitological impact of a single treatment on urinary schistosomiasis in the Barombi Kotto health area after two years by: (1) assessing the prevalence and parasite intensity at base line, prevalence and intensity reduction at one and two years post treatment among school children aged 3-22 years; (2) Identifying and following- up the infection rates of the intermediate snail hosts following the treatment of the human population. The results obtain will be vital in determining the timing of treatment in the area and other endemic areas.

Materials and methods

Study area

The study was conducted in the Barombi kotto health area, where the principal transmission site (Lake Barombi Kotto) is found.

The Barombi Kotto village (4° 28' 4"N; 9° 15', 2"E) is located in Mbonge subdivision (South West Cameroon). The crater lake Barombi Kotto (altitude of 400 m) is situated at about 41 km from Kumba, the head quarter of the division (Moyou *et al.*, 1987). This village belongs to the equatorial forest zone, Cameroon-type climate having one long rainy season (9 months) and one short dry season (3 months). The total annual rain fall varies between 2000-10000 mm. Most water bodies are perennial while few others dry up for only brief periods (Greer *et al.*, 1990, Ratard *et al.*, 1990). The Crater Lake Barombi Kotto has a width of about 1 km on its largest diameter. Two small streams meet in the lake, one emptying into it (the inlet) and the other flowing out of it (outlet). Some of the inhabitants in Barombi Kotto village live in a small island situated in the middle of the lake, while the majority lives around the lake and along the road leading to Kumba through other villages in the middle of the equatorial forest. This village is characterised by absence of good water sources (wells, taps, forages), poorly disposed garbage and inadequate sanitation facilities; this means that most human activities necessitating water (laundry, bathing, fishing) are done only in the lake.

The residents of the island cross the lake every day for their activities (Farming, marketing, schooling, etc) in the other villages. Because of the great number of patients diagnosed, this village was qualified as a highly endemic area (Moyou *et al.*, 1987; Ndamukong *et al.*, 2001, Nkengazong *et al.*, 2009).

Malacological study

Three water points were chosen for the collection of snails: Point I - situated at the mainland shoreline of the lake, Point II- with multiple sub-points situated at the island shoreline of the lake and Point III- situated at about 1.5 km away from the lake in a cocoa plantation (figure 1). These water points were the ones used by the population for multiples activities (laundry, bathing, fishing, and farming).

Snails were collected at each point following the method described by Njiokou *et al.* (2004) during 7 surveys (first, second, third, forth, fifth, sixth and seventh) respectively in the months of May and October 2007, January, February, March, September and November 2008. Snails collected were brought to the laboratory for test of natural infestation and for species identification following the morphology of their shells (Mimpfoundi and Ndassa, 2005). The test of cercarial shedding and observation was done during one month according to Njiokou *et al.* (2004).

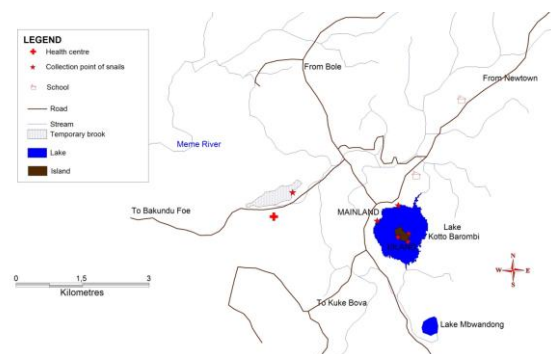


Fig. 1. Map showing the different collection points of snails.

Study subjects

The study was conducted from May 2007 to May 2009. Out of the 380 school children contacted, 279 (153 girls and 126 boys), 197 (109 girls and 88 boys) and 151 (81 girls and 70 boys) of ages between 3 and 22 years, participated respectively for the initial, one and two year post treatment surveys. An additional 21 volunteer parents participated in the longitudinal survey. The infection status in these parents should give information on the quality of community-based treatment in the focus.

Subject consent

Administrative authorities (Chief of health District, school directors and traditional leaders) were informed about the project and they gave their verbal consent for the study to be undertaken. A written informed consent that met the standards of the National Ethical Commission was obtained from the pupils or the guardians of the young children that accepted to participate in the study.

Parasitological study

One urine sample was collected from each participant in a 10 ml plastic screw-cap vials between 10 am and 2 pm. The samples were transported to the General Biology Laboratory (Faculty of Science) at the University of Yaounde I and examined using the filtration technique (Plouvier *et al.*, 1975). The urine was filtered, stained with 1% Lugol solution, eggs counted under a light microscope at 10X magnification and their number in 10 ml urine recorded (e/10ml). The entire population (children and parents: ≥ 3 years) living in the Barombi Kotto health area were treated two weeks after the initial survey with a single dose of praziquantel (40 mg/kg

of body weight). The drug impact was evaluated one and two years later by collecting urine samples from all those who participated during the initial survey. The samples were examined using the same method described above

Data analysis

The parameters assessed during the study were the prevalence (Margolis *et al.*, 1982), egg load, egg reduction rate (Touré *et al.*, 2008; Nkengazong *et al.*, 2009), the level of infection intensity (Kihara *et al.*, 2007) and the natural infection of intermediate snail hosts.

Table 1. Distribution and infection rates of *Bulinus* species collected.

Collection Period	Species and sites*						Total*			Overall total	Infection rate (%)
	<i>B. truncatus</i>			<i>B. camerunensis</i>			I	II	III		
	I	II	III	I	II	III					
May 2007	-	-	-	52 (2)	112 (4)	-	52 (2)	112 (4)	-	164 (6)	3.7
October 2007	5	117	2	25	187	6	30	304	8	342	0.0
January 2008	2	298	-	51	48	10	53	346	10	409	0.0
February 2008	-	6	-	81	256	21	81	262	21	364	0.0
March 2008	18 (1)	43 (2)	-	49	312 (2)	32	67 (1)	355 (3)	32	454 (4)	0.9
September 2008	11	489	3	9	171	15	20	660	18	698	0.0
November 2008	-	315	-	26	68	7	26	383	7	416	0.0
Total	36	1268	5	293	1154	91	329	2422	96	2847	0.4

I, II and III: Collection sites for mainland, island and in cocoa plantation respectively

*Values in parentheses indicate the numbers of snails infected

The Chi-square test was used to compare the prevalence in relation to sex, quarters and between the different survey periods while one - way ANOVA or Kruskal-Wallis (Sokal and Rohlf., 1981) tests were used to compare the egg load in relation to sex, quarters, egg load and the level of infection intensity during the different survey periods. The level of statistical significance was at 95% ($P < 0.05$). Participants who dropped out or missed either of the two follow-up surveys were not included in the longitudinal analysis.

Results**Malacology**

During the malacological survey a total of 2847 snails, all intermediate snail hosts of *S. haematobium* were collected. This number included 1309 (46.0 %) *B. truncatus* and 1538 (54.02 %) *B. camerunensis*. Snails collected during the first (May 2007) and the fifth (March 2008) surveys all from the lake Barombi Kotto emitted *S. haematobium* forked-tailed cercariae with their respective infection rates being 3.7 % and 0.9 %. Those collected during the other surveys did not emit forked-tailed cercariae

during one month period of exposure in the laboratory (Table 1).

Parasitology

Baseline results

The global prevalence of *S. haematobium* among 279 children examined at baseline was 69.17 %. Children from the island (84.3 %) were significantly more infected than those from the mainland (60.45 %; P =

0.0001). A significant difference of prevalence was observed between boys (73.22%) and girls (66.66%) (P = 0.03). The global intensity of infection was 212.1 e/10ml urine. This value was significantly higher in children from the island (280.1 e/10 ml urine) than the mainland (130.2 e/10 ml urine) (P= 0.01). The intensity of infection did not vary significantly between boys and girls (Table 2).

Table 2. Prevalence and intensity of *S. haematobium* infection before and after treatment

Variable	Baseline	1 year post-treatment	2 years post-treatment	Overall reduction (%)	
				1 year post-treatment	2 years post-treatment
Prevalence (%)					
Overall prevalence	69.17 (193; n= 279)	13.7 (27; n= 197)	31.10 (47; n= 151)	80.19	55.03
By quarter					
Mainland	60.45 (107; n=177)	8.8 (11; n= 125)	27.05 (n= 85)	85.44	55.33
Island	84.3 (86; n= 102)	22.2 (16; n= 72)	33.36 (n= 66)	73.66	60.42
By sex					
Boys	73.22 (91; n= 126)	14.80 (13; n= 88)	28.60 (20; n= 70)	79.80	60.93
Girls	66.66 (102; n= 153)	12.84 (14; n= 109)	33.33 (27; n= 81)	80.73	50.0
Egg load (e/10ml urine)					
Overall mean	212.1 (n= 193)	32.65 (n= 27)	50.56 (n= 47)	84.60	76.16
By quarter					
Mainland	130.2 (n= 107)	41.8 (n= 11)	20.96 (n= 23)	67.90	83.90
Island	280.1 (n= 86)	24.31 (n= 16)	61.41 (n= 24)	91.32	78.08
By sex					
Boys	213.7 (n= 91)	36.07 (n= 13)	62.61 (n= 18)	83.12	70.70
Girls	210.53 (n= 102)	29.23 (n= 14)	29.13 (n= 29)	86.11	86.16

n: Total

The proportion of light (≤ 99 e/10ml urine), moderate (100-399 e/10ml urine) and heavy (≥ 400 e/10ml urine) infections of the school children examined were 54.4 %, 22.27 % and 23.31 % respectively (figure 1). The level of heavy infection did not vary significantly by sex and quarter of residence. Of the 27 volunteer parents examined, six (22.22%) were positive for *S. haematobium* eggs among whom four (14.81%) were heavily infected.

Post treatment results

One round of mass Praziquantel treatment significantly reduced the prevalence in the cohort children from 69.17 % at baseline to 13.7 % and 31.10 % at one year and two years post treatment respectively (P = 0.0001) with the corresponding overall reduction rates of 80.19 % and 55.03 %. A significant decreased of prevalence was observed at baseline compared to one and two years post treatment, with a significant increase from one to

two years post treatment ($P= 0.01$). The overall intensity of infection significantly reduced from 212.1 at baseline to 32.65 and 50.56 e/10 ml urine at one and two years post treatment respectively ($P = 0.01$), with the corresponding overall reduction rates of 84.60 % and 76.16 % (Table 2). No significant difference Though a high prevalence was observed in females with a high intensity in males at one and two years post treatment, no significant difference was observed.

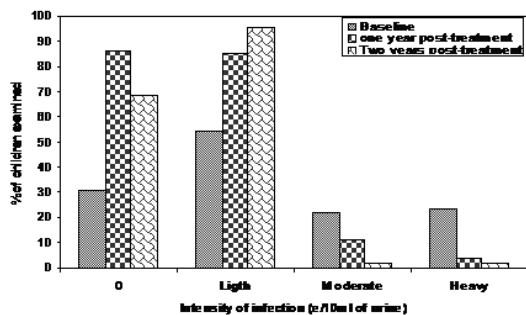


Fig. 2. Categories of *S. haematobium* intensity before ($n= 279$), one year post treatment ($n= 197$) and two years post treatment ($n=151$): 0 = number of persons uninfected.

Importantly, heavy infections decreased to 3.7% at 1 year post treatment and remained at 2.12% at two years' post treatment. Moderate infection was maintained at 11.10% and 2.12% and light infections at 85.20% and 95.74% respectively at one and two years post treatment (figure 2). Four parents were found infected at one and two years post treatment among whom one who was negative at base line was heavily infected during the two follow-up periods.

Discussion

The malacological survey conducted permitted to identify two water points in the Barombi Kotto village harbouring the intermediate snail hosts of *S. haematobium* (*B. truncatus* and *B. camerunensis*) known since the discovery of the focus. *B. camerunensis* was more abundant than *B. truncatus* during our survey confirming the results observed by Duke and Moore (1976), Moyou *et al.* (1987) and Greer *et al.* (1990). This shows stability in the Gastropod population in the Barombi Kotto focus.

The cercariae observed from snails collected during the first and fifth survey from the lake confirm that the lake remains the main transmission site of urinary schistosomiasis in the Barombi Kotto health area. The absence of cercariae during the second, third and fourth surveys could likely be due to the fact that both children and parents were treated, leading to a reduction of the level of snail infection. The sixth and seventh surveys were conducted during the rainy season, when the human population had another water source (rain water) what could limit the frequency of contact with the transmission site. This is in conformity with the result of Sturrock *et al.* (1979) who showed that the rate of natural infestation of snails in a transmission focus of schistosomiasis could be linked to the pressure of snail infestation in a transmission site or to the frequency of water contact by infected people. However, most water points are only accidentally contaminated at particular periods, as contamination is determined by human behaviour for specific purposes. This makes snails infestation to be a discontinuous process, taking place only at particular moments in such a way that a given snail population can be uninfected completely. This may explain the case of snails collected in the third water point located in a cocoa plantation where people come into contact with water point only during cocoa harvesting and clearing of the farm.

In line with previous studies, high prevalence and intensity of *S. haematobium* was found at baseline in the Barombi Kotto health area (Moyou *et al.* 1987; Ndamukong *et al.* 2001) with the prevalence rate of 76.0 % and 75.9 % respectively. Even though our prevalence obtained was slightly lower compared to that of previous authors, it confirms the high endemicity of schistosomiasis in this area. These differences may reflect the difference in the number of years spent since the last mass treatment before each of the three surveys. Our results are similar to those of King (2006) who showed that villages without pipe-borne water access maintained a high level of infection. Our results confirm the well documented observation that children living near

the transmission site of schistosomiasis are more infected (Moyou *et al.*, 1987; Ndamukong *et al.*, 2001; Saathoff *et al.*, 2004; Kabatereine *et al.*, 2011), and that boys are generally more active in water collection than girls (Tchuem Tchuente *et al.*, 2003; Njiokou *et al.*, 2004).

Following treatment, the proportion of heavy infections was greatly reduced, which is particularly important as high intensity of *S. haematobium* infection has been shown to contribute to morbidity, including anaemia in children (Koukounari *et al.*, 2007; Green *et al.*, 2011; Sousa-Figueiredo *et al.*, 2012). In previous studies conducted on *S. haematobium* control in eastern Africa, an annual treatment strategy was predominantly used, with varying results (Magnussen, 2003; Satayathum *et al.*, 2006). However, Nsawah-Nuamah *et al.* (2004) in southern Ghana and Touré *et al.* (2008) in Burkina Faso observed an important reduction of prevalence and parasite intensities at two years post treatment, while Garba *et al.* (2004) in Niger observed a significant reduction of prevalence and parasite intensities after three years post treatment. Our results are in line with the results of these studies, suggesting that a more spaced treatment strategy is highly effective on *S. haematobium* infections, even in highly endemic areas. However, the effectiveness of such strategy may depend on the local epidemiological and geographical settings of each area.

Though one dose of praziquantel reduced the level of infection significantly at one and two years post treatment, it has been shown that between infection intensity and morbidity risk, even light infection may impose risk of serious disease (Wamachi *et al.*, 2004). Given the association between schistosome infection and anaemia and other disability-related outcomes, infection at any level may impose a significant burden on local health (King *et al.*, 2005). Our results showed that one parent was found heavily infected at one and two years post treatment. This may be a reflection of the observation that targeted school-age treatment can suppress

transmission in some, but not all *S. haematobium* infected communities, because one individual usually uses multiple water sites, and a single infected person can maintain local transmission for an indefinite period (Woolhouse *et al.*, 1997; 1998).

The significant increase of prevalence observed from one to two years post treatment compared to baseline and one year post treatment may reflect the period of treatment which was in the rainy season (June); the availability of rain water probably reduces human contact with the transmission site during the first year post treatment, compared to the second year. Nevertheless, the general uptrend of the prevalence and intensity of infection shown two years post treatment could be a sign of a potential rebound of *S. haematobium* should drug distribution be interrupted, as reported by Touré *et al.* (2008); it could also be a reflection of the fact that multiple villages interact with multiple water contact sites, thus favouring transmission of infection or contamination of water sites (King, 2006). In general, our results highlight the importance of continued effort in monitoring schistosomiasis transmission and repeated treatment in the Barombi Kotto focus, as already shown in some studies (Mduluzza *et al.*, 2001; King *et al.*, 2011).

Conclusions

This study has shown a high prevalence and intensity of *S. haematobium* infection in the Barombi Kotto health area, a significant impact of a single dose of praziquantel in reducing prevalence and intensity of *S. haematobium* after two years, a high pace of re-infection and the presence of other water sites harbouring the intermediate snail hosts of *S. haematobium* beside the main transmission site (lake Barombi Kotto). It is therefore necessary to: (i) carry out a continuous monitoring of the disease transmission to maintain infection prevalence and intensity at a low level, (ii) repeat treatment once every year at the start of the dry season, involving both children and parents with high coverage (iii) organise a survey regarding knowledge, attitude, and practices (KAP) of the people living in the area, (iv)

improve the socio-economic conditions of the area (availability of good water sources in all villages).

Acknowledgments

We thank the headmasters and teachers of Presbyterian and Government primary schools of Barombi Kotto, the Barombi Kotto Chief of District Health Centre for the facilities given during this work, Mr Nchonganyi Ignatius and Mrs Forcha Florence for their financial support.

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