Comparative Assessment of Health Benefits of Praziquantel Treatment of Urogenital Schistosomiasis in Preschool and Primary School-Aged Children

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Abstract

Schistosomiasis is a major public health problem in Africa. However, it is only recently that the burden of schistosomiasis has become recognised as a significant component impacting on the health, wellbeing and development of infants and preschool children (aged ≤5 years). A longitudinal study was conducted in Zimbabwean children to determine the effect of single praziquantel treatment on *Schistosoma haematobium*-related morbidity markers: microhaematuria, proteinuria, and albuminuria. Changes in these indicators were compared in 1–5 years versus 6–10 years old to determine if treatment outcomes differed by age-group.

Praziquantel was efficacious at reducing infection 12 weeks post-treatment: cure rate=94.6%; (95% CI: 87.9–97.7%). Infection rates remained lower 12 months post-treatment compared to baseline in both age-groups. Among children who received praziquantel, the odds of presenting with two markers of morbidity 12 weeks post-treatment were significantly lower compared to baseline; proteinuria: odds ratio, OR=0.54; (95% CI: 0.31–0.95), and albuminuria: OR=0.05; (95% CI: 0.02–0.14). Levels of microhaematuria significantly reduced 12 months post-treatment, and the effect of praziquantel did not differ between the two age-groups: OR=0.97; (95% CI: 0.50–1.87). Praziquantel treatment has health benefits in preschool-aged children exposed to *S. haematobium* and the efficacy of praziquantel on infection and morbidity is not age-dependent.
1. Introduction

Urogenital schistosomiasis, caused by the water-borne parasitic helminth *Schistosoma haematobium*, is an important but neglected tropical disease in Africa [1-3]. The disease causes significant paediatric health problems in endemic regions, with negative impacts on child health and development. In *S. haematobium* infections, damage caused by the parasite eggs lodged in tissues or exiting the body via the urine can result in bladder or urinary tract pathology, often characterized by blood in urine (macro- or microhaematuria), painful urination (dysuria) and proteinuria [4, 5]. High levels of albumin in urine have also been shown to be strongly correlated with urinary tract pathology due to *S. haematobium* infection [6]. Chronic infection with schistosomes in children is associated with complications such as anaemia, malnutrition, growth retardation, reduced physical fitness, and impaired memory and cognition [7, 8]. Infection and morbidity are controlled by treatment of infected individuals with the antihelminthic drug, praziquantel (PZQ) [9, 10]. Delayed or a lack of intervention can result in more severe and irreversible forms of morbidity including urinary bladder cancer (squamous cell carcinoma) and chronic kidney disease, which may eventually result in death [11-13].

In several countries implementing schistosomiasis control programmes, the control strategies follow the directive by the World Health Assembly resolutions (WHA 54.19 and WHA 65.21) in 2001 [9], involving regular school based de-worming using PZQ, aimed at reducing morbidity and promoting child health. However, a growing number of studies from Africa have shown that preschool children (aged ≤5 years old) are also at high risk of schistosomiasis through passive exposure to infection whilst being bathed with infested water [14-18]. Furthermore, recent studies have also shown that PZQ treatment of schistosome infection is safe in preschool children [16, 19]. These findings have led to a new major recommendation by the World Health Organization (WHO) in 2010, stating that preschool-aged children should be considered for treatment through the regular health services as well as in ongoing public
health intervention programmes [20]. This recent development in schistosomiasis control policy has heightened the need for a clear understanding on the health benefits of PZQ beyond the immediate reduction in infection levels in preschool-aged children in order to improve the effectiveness of interventions targeting this age group [21].

In a previous study in this population, we showed that, based on attributable fractions, albuminuria measured as the urine albumin-to-creatinine ratio (UACR) was the most reliable tool for detecting schistosome-related morbidity, followed by proteinuria and microhaematuria determined by dipsticks, visual urine inspection, questionnaires, and lastly clinical examination [22]. Thus, in this current study we focused on albuminuria, proteinuria and microhaematuria. The aim of this study was to evaluate the immediate health benefits of PZQ in preschool-aged (1–5 years) children endemically exposed to S. haematobium at 12 weeks post-treatment, and to determine if a single dose of PZQ (40 mg/kg) has sustainable impact on the health status of these children by assessing changes in infection rates and levels of schistosome-related morbidity markers (microhaematuria, proteinuria and albuminuria) 12 months after treatment. Furthermore, we sought to determine whether the impact of PZQ treatment outcome is age-dependent by comparing these indicators in preschool and primary school-aged (6–10 years) children in the same population. The findings of our present study provide an operational recommendation for future studies on the control of paediatric schistosomiasis, whilst also giving further insights into the health benefits of antihelminthic treatment in preschool-aged children.
2. Materials and Methods

2.1 Ethics Statement and Consent

Prior to commencing the study, ethical clearance was obtained from the Medical Research Council of Zimbabwe (Approval Reference: MRCZ/A/1615). In addition, the study received institutional approval from the University of Zimbabwe. Permission to conduct the study was obtained from the Provincial Medical Director, the District Educational Officer, and heads of schools in the study area. Study objectives and procedures were fully explained to the community, parents/guardians, teachers, and primary school-aged children in the local language, Shona. Samples were collected only after obtaining informed written parental consent and study participants’ oral assent. Participants were permitted to withdraw from the study at any point without further obligation.

2.2 Study Site

The study was undertaken in Murewa district, in the north-east of Zimbabwe (31°90'E; 17°63'S) where *S. haematobium* is endemic. The area has low transmission of *S. mansoni* and soil-transmitted helminths (STH) as previously reported in other studies [23, 24]. In a nationwide survey among school-aged children in 2010 in Zimbabwe, Midzi *et al* [25] reported a schistosomiasis prevalence of 31.2% in Murewa district based on parasitological diagnostic methods. To confirm this reported level of schistosome infection in the community in our study site, we initially conducted a pilot study among primary school-going children (aged 6–10 years old) as per sampling guidelines and recommendations by the WHO [26, 27]. A random sample of 50 compliant children from the two schools in the study area (25 from each school) was screened for schistosome infections by parasitology. Children found positive for infection during the pilot survey were treated with the recommended single dose of PZQ (40 mg/kg), but were then ineligible for participation in the main study.
2.3 Study Design and Population

The longitudinal study was designed to relate changes in the levels of infection and markers of schistosome-related morbidity to the two age groups of children (1–5 years vs. 6–10 years) conducted over a period of 12 months. There were two aspects to the longitudinal study design investigating; (i) the short term effects of PZQ treatment which measured the morbidity markers and infection levels before treatment/baseline (February–March 2012) and 12 weeks (May 2012) after treatment; the workflow for this aspect of the study is shown in Figure 1a and, (ii) the longer-term effects of PZQ treatment which measured the morbidity markers and infection levels before treatment and 12 months (March 2013) after treatment; the workflow for this aspect of the study is shown in Figure 1b. The 388 children who received treatment at baseline (Figures 1a/b) participated in both aspects of the study, so that the 303 children whose infection status was confirmed egg negative at the 12 weeks efficacy check survey formed the treated cohort for follow up at 12 months (Figure 1b). Untreated children (Figure 1a) were not followed up beyond 12 weeks.

Children were recruited from two primary schools (typically for 6–10 years olds) and the early childhood development centres (ECDs) for preschool-aged children located within each of the primary schools. The schools also served as recruitment centres for children aged between 1–5 years not enrolled in any of the educational programmes in the study area who were also invited for enrolment to participate in this study. The villages within the study sites share the same river systems, therefore the transmission dynamics in the two schools (and associated ECDs) were similar.

2.4 Screening and Follow-up Criteria

In order to be included in this study at baseline, children had to meet the following criteria: (i) had been life-long (i.e., permanent) residents of the study area, (ii) had no prior history of antihelminthic treatment (assessed by questionnaires administered to parents/guardians of all children), and (iii) had provided at least two urine samples collected on consecutive days for
the parasitological diagnosis of *S. haematobium* infection. For potential exclusion, children were assessed to identify: (i) pre-existing medical conditions or clinical symptoms of tuberculosis, any form of fever, or other signs of being unwell upon examination by study clinicians, (ii) recent major operation or illness as reported by parents/guardians, and (iii) infection with any of STHs or *S. mansoni*. No soil transmitted helminth infections were detected in this study cohort and 27 children positive for *S. mansoni* were offered PZQ treated but excluded based on this criterion. Participating children that were found egg-positive for *S. haematobium* at the 12 week efficacy check were treated with 40 mg/kg praziquantel but excluded from further follow-up to ensure that in addition to new infections, only ‘true’ re-infection was measured. Children who met the eligibility criteria, with informed consent, but would not accept treatment on religious grounds, or were absent on treatment survey days but voluntarily remained in our study cohort at 12 weeks effectively became untreated controls for evaluating the effect of treatment on schistosome-related morbidity markers. At the end of the study (12 months), PZQ treatment was offered to all children diagnosed egg positive for infection.

### 2.5 Parasitological Methods

At all survey time points, urine samples were collected on three consecutive days for parasitological examinations. *S. haematobium* infection was detected by microscopic examination of the parasite eggs in urine, processed using the standard urine filtration method [28]. For each child, infection intensity was expressed as the arithmetic mean of egg counts per 10 mL urine of samples collected on consecutive days. At least two stool samples were also collected over three consecutive days, processed using the Kato-Katz method [29], and subsequently examined by microscopy for the diagnosis of *S. mansoni*. In a previous study we also determined the infection status of the children by serology, details of this methodology are described elsewhere [30].
2.6 Assessment of Morbidity Markers

Three urinary markers; microhaematuria, proteinuria and albuminuria identified in our earlier published study [22] were chosen for investigating the effect of PZQ treatment on schistosome-related morbidity in this present study. Urine samples collected between 10:00h and 14:00h and processed on the first day of each survey time point were examined for microhaematuria and proteinuria, detected by dipstick reagent strip test (Uripath, Plasmatec, UK). Briefly, the reagent end of the test strip was dipped into fresh, well-mixed urine for 40 seconds. Upon removal, the test area was compared with a standard colour chart following the manufacturer’s guidelines and the dipstick test results were calibrated as either positive or negative. To assess the stability of dipstick urinalysis [31], repeated tests (at least two repetitions) were performed on a random sample (n=189) of urine specimens, allowing for time delay of up to 5 minutes between each of the repeated readings. There were no marked differences observed between repeated tests to suggest potential instabilities of urinalysis due to delays in dipstick testing. No observer bias was suggested by the urinalysis results when comparing the visually recorded dipstick readings to those read automatically using Siemens' CLINITEK Status + Analyzer (Bayer, UK). CLINITEK Microalbumin Reagent Strips (Bayer, UK) were used to determine the urine albumin-to-creatinine ratio (UACR) threshold levels (normal: UACR < 3.4 mg/mmol, abnormal: UACR ≥3.4 mg/mmol, or high abnormal: UACR ≥33.9 mg/mmol), as read from the instrument following the manufacturer’s guidelines. For each child, albuminuria as a marker of schistosome-related morbidity associated with urinary tract pathology was defined as a positive test for high abnormal UACR in each of the fresh urine samples examined [4].
2.7 Praziquantel Treatment

After collection of samples at baseline, compliant children were offered treatment with PZQ at the standard oral dosage of 40 mg/kg body weight. The PZQ drug was procured from Pharmaceutical and Chemical Distributors (Pvt) Ltd., Harare, Zimbabwe, a company registered and licensed to sell the antihelminthic drug in Zimbabwe. The tablets were swallowed with squash juice to reduce their bitter taste and a slice of bread to reduce the side effects of PZQ [16, 32]. For the very young children, the tablets were crushed according to the current recommendation by the WHO [20].

2.8 Sample Size Calculations

The relationship between schistosomiasis infection levels and indicators of morbidity is still unclear. We thus based our sample size calculations under the expectations that PZQ treatment reduces re-infection rates by more than 50% after 12 months. These expected effects are consistent with data from previous studies [16, 25], and are of sufficient magnitude to be of practical interest. Allowing for dropouts, our simulation studies using StatXact v.8 (Cytel Software Corp, Cambridge, MA, USA) indicated that the sample sizes shown in Figures 1a and 1b will give us more than 80% power to detect age-related and treatment-related differences with significance level, $\alpha = 0.05$.

2.9 Data Management and Statistical Analysis

Infection intensity was log-transformed: $\log_{10}(\text{egg count} + 1)$ to meet the distributional assumptions of parametric test-statistics. Treatment efficacy against *S. haematobium* infection was assessed by means of cure rates (CR) and egg reduction rates (ERR), defined as: $\text{CR}= (\text{number of children } S. \text{ haematobium } \text{egg-negative at 12 weeks post-treatment/ number of children confirmed egg-positive at baseline}) \times 100$; $\text{ERR}= (\text{arithmetic mean egg count at baseline } – \text{arithmetic mean egg count at 12 weeks post-treatment/ arithmetic mean egg count at baseline}) \times 100$. The 95% confidence intervals (95% CI) for the ERR were calculated using a
bootstrap resampling method with 1000 replicates in R 3.1.2 (R Development Core Team, Vienna, Austria), package="eggCounts". A Chi-square test ($\chi^2$) or the Fisher's exact test in the case of small expected frequencies (n<5) was used for comparison of infection prevalence and cure rates between the two age groups. For infection intensity, a paired $t$-test was used to compare levels of infection pre- vs. post-treatment. For each of the three urinary markers of schistosome-related morbidity (microhaematuria, proteinuria, and albuminuria), the outcome of interest was a dichotomous variable indicating whether the child presented with morbidity or not at a given time point (morbidity: 0=negative; 1=positive). The main predictor variables included the host factors sex (male vs. female) and age group (1–5 years vs. 6–10 years), treatment group (untreated vs. PZQ treated) and time (pre- vs. post-treatment). To assess the effect of treatment (pre/post) on morbidity markers, we used the method of generalized linear mixed model (GLMM) with a random effect to account for the correlation between children recruited from the same primary school/ECD as described earlier. The model-building process involved backward stepwise inclusion of the main effect covariate terms and their two-way interactions. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The GLMMs were run using “PROC GLIMMIX” with a logit-link function to model the log odds of the probability of a child presenting with morbidity marker upon examination, and the parameter estimation was implemented using the method of penalized quasi-likelihood to account for over-dispersion [33]. Comparisons for the binary morbidity marker responses between different sub-groups of predictor variables were implemented using the contrast options within the “GLIMMIX” procedure. In all the analyses, multiple pairwise comparisons were adjusted for family-wise type I error using the less conservative (i.e. has low rate of false negatives) simulation-based approach [34]. The level of significance was set at $P<0.05$ for all the statistical tests performed. To aid the interpretation of the relative effect of
treatment on infection levels and morbidity markers, standard errors (SE), 95% CI and adjusted odds ratios (OR) were presented along with the significance test statistics.
3. Results

3.1 Demographic Characteristics and Pre-treatment Infection Levels

Of the eligible children screened at baseline (see Figure 1a and Figure 1b), a total of 508 children were included in the study, with 388 receiving PZQ treatment and 120 remaining untreated. Infection prevalence in the 508 children was 24.2% (95% CI: 20.5–28.0%), 6.7% (95% CI: 3.5–9.9%) in 1–5 year olds (n=239) and 39.8% (95% CI: 33.9–45.7%) in 6–10 year olds (n=269). In a subgroup of the 508 children, serological analysis showed that 63.0% (95% CI: 57.7–68.2%) of the children were seropositive for *S. haematobium* egg antigens, 39.8% (95% CI: 33.9–45.7%) were 1–5 year olds (n=131) and 79.1% (95% CI: 73.4–84.8%) were 6–10 year olds (n=201). The characteristics of the current study population are shown in Table 1. When considering the children treated at baseline and followed up at 12 weeks (n=303, Figure 1a), the baseline infection prevalence determined by parasitology was 30.4% (95% CI: 25.2–35.6%) compared to 18.0% (95% CI: 8.1–28.0%) in the children who were not treated at baseline (n=61, Figure 1a) but voluntarily remained in the study at 12 weeks.

3.2 Treatment Efficacy on Infection Levels at 12 weeks Post-treatment

Twelve weeks after treatment, PZQ was efficacious at reducing *S. haematobium* infection among treated children in both age groups, as shown by high cure and egg reduction rates in Table 2. The overall cure and egg reduction rates in our study were 94.6%; (95% CI: 87.9–97.7%) and 97.9% (90.6–99.5%), respectively. There was no significant difference (Fisher’s exact test, *P*=0.481) in cure rates between the two age groups (1–5 years vs. 6–10 years) 12 weeks after treatment shown in Table 2.

3.3 Effect of Treatment on Infection/re-infection rates at 12 months Post-treatment

Following initial treatment at baseline, children who had successful curative treatment were further followed-up to determine the re-infection rates and proportion of new infections 12
months after intervention. Table 3 shows the percentage proportion of treated children who were infected at baseline and then got re-infected (n=7) and those newly infected (n=11) within 12 months following treatment. The infection prevalence 12 months after treatment was low (i.e. <10% according to WHO classifications) as shown in Table 3. Furthermore, the proportion of infected/re-infected children did not significantly differ ($\chi^2=0.37; P=0.542$) between the two age groups: 1–5 years, n=5: 6.3% (95% CI=2.7–13.8%) vs. 6–10 years, n=13: 8.5% (95% CI: 4.8–14.4%). Similarly, the mean infection intensity level 12 months post-treatment (mean=0.74 egg/10 mL urine; SE=0.27) was significantly lower (paired $t$-test=$-7.95; P<0.001) compared to the baseline level (mean=14.3 egg/10 mL urine; SE=4.63) in this study, with all egg-positive children carrying light infection intensities only.

### 3.4 Effect of Treatment on Morbidity Markers at 12 weeks Post-treatment

The effect of PZQ on levels of morbidity markers was assessed in those children that had successfully cleared the infection at 12 weeks. Praziquantel significantly reduced levels of proteinuria and albuminuria 12 weeks after treatment (Figure 2). In addition, the odds of children who received praziquantel presenting with each these markers of morbidity 12 weeks after treatment were lower compared to the odds before treatment, adjusting for sex and age group; proteinuria: OR=0.54; (95% CI: 0.31–0.95) and albuminuria: OR=0.05; (95% CI: 0.02–0.14). Furthermore, all the treated children aged 1–5 years successfully cleared albuminuria at 12 weeks and a significant reduction from baseline was observed among the 6–10 year olds (Figure 2). A mild decrease in the levels of microhaematuria among the treated preschool-aged children was also observed, however, these changes were not significant. Changes from baseline in the levels of the three markers of schistosome-related morbidity in untreated children at 12 weeks were not significant (Figure 2). Furthermore, when evaluating the overall changes in morbidity markers among the treated group (pre- vs. post-treatment) compared to the changes in untreated children, it was observed that the odds of albuminuria were lower:
OR=0.43 (95% CI: 0.19–0.98; \( P=0.045 \)), adjusting for sex and age group.\textbf{3.5 Effect of Treatment on Morbidity Markers at 12 months Post-treatment}

Following curative treatment of infection at 12 weeks, as assessed by parasitology, children were further followed-up and examined for morbidity at 12 months. Our analyses showed that when morbidity marker levels were investigated relative to baseline levels, microhaematuria significantly dropped in both age groups at 12 months after treatment (Figure 3). Furthermore, the results of GLMMs weighted by age-group sample sizes showed that the odds of treated children presenting with microhaematuria 12 months post-treatment did not significantly differ between 1–5 years vs. 6–10 years old children: OR=0.97; (95% CI: 0.50–1.87). In the case of proteinuria and albuminuria at 12 months, there was a significant reduction relative to baseline in the older age group, but not in 1–5 year olds (Figure 3). This was despite the initial reduction in albuminuria levels in this age group observed 12 weeks after treatment, as was shown in Figure 2.
4. Discussion

Praziquantel is currently the recommended antihelminthic drug of choice by the WHO for treating schistosomiasis, and is effective against all the major schistosome species infecting humans [9]. As of present, the health benefits of PZQ treatment in children aged 5 years and below has not yet been extensively evaluated. Thus, in this study we sought to determine if PZQ treatment improves the current health status of children aged 1–5 years old. The pre-treatment infection levels reported in this study confirmed that preschool-aged children are exposed to schistosome infection, in concurrence with findings from other recent studies [16, 30], and further support the premise that if left untreated, these children are at an increased risk of developing severe morbidity that may cause serious health consequences and negatively impact their future quality of life [18, 35, 36]. In this study there were few children aged between 1 and 5 years who were excreting parasite eggs, but the prevalence of 6.7% is typical in this age group. More importantly, we and others [17, 30], have shown that the widely utilised egg count diagnostic method greatly underestimates infection prevalence in young children. Indeed, in a seroprevalence study of a subgroup of the 508 children in this group, the seropositive rate for *S. haematobium* egg antigen was 39.8% in 1–5 year olds. Thus, it is important to investigate the effects of treatment on other markers with health implications other than just the egg counts.

Our study results at 12 weeks after chemotherapy showed that a single standard dosage of PZQ was efficacious against *S. haematobium* infection in both 1–5 year olds and 6–10 year olds. The ranges of cure and egg reduction rates observed in this study for both age groups are consistent with data reported in the literature that have also shown high PZQ treatment efficacy within six weeks after treatment [16, 37, 38]. More interestingly, our study further revealed that PZQ treatment was effective in reducing *S. haematobium* infection levels in preschool-
aged children as was observed in their older counterparts (6–10 year olds), further supporting their inclusion in current schistosomiasis control programmes [20, 39].

Infection prevalence remained lower 12 months after treatment compared to baseline levels, and the proportion of infected/re-infected children did not significantly differ between the two age groups. In addition, our study showed significant reductions in mean *S. haematobium* egg counts 12 months after treatment compared to baseline. These results are in agreement with those of previous studies from different endemic areas that also reported a lower risk of *S. haematobium* re-infection after annual school-based treatment [40, 41], indicative of the benefit of treatment in the prevention of infection through reduced parasite transmission at population level [40]. This combination of findings on the benefits of PZQ chemotherapy against infection provides some strong support for the need for inclusion of preschool-aged children in ongoing schistosomiasis control programmes, in order to increase the effectiveness of coverage of those infected, also recently highlighted in a study by Garba *et al* [1].

Since schistosome-related morbidity is cumulative and progressive [42], decrease in current morbidity can reduce the long-term schistosomiasis sequelae. At 12 weeks after the first treatment, there was a significant decrease in the levels of the morbidity markers proteinuria and albuminuria in children successfully treated for infection. Interestingly, the effects of treatment were found not to be age-related, with microalbuminuria completely reversed in preschool-aged children at 12 weeks. The study also revealed that the prevalence of morbidity, diagnosed by presence microhaematuria, declined slowly, with a significant reduction observed after 12 months post-treatment. Our findings on the immediate health benefits of PZQ treatment were further reinforced by the results of the untreated group, showing no significant change in the levels of markers of schistosome-related morbidity at the 12 weeks follow-up.
The results showing persistently high levels of microhaematuria 12 weeks after treatment differ from some published studies that reported a considerable drop in microhaematuria within eight weeks after PZQ treatment [5, 43, 44]. However, most of these studies focused on older school-aged children who may have developed chronic infection. The high sensitivity of dipstick reagent strips in detecting microhaematuria, as previously reported [45], could also be one possible reason for these findings in our study. Another possible explanation for this delayed decrease in microhaematuria may be that most of the observed microhaematuria in these children may have likely been due to other health conditions other than schistosome infection.

In an earlier study of this population we showed that the proportion of microhaematuria attributable to schistosome infection was less than that of albuminuria and proteinuria [22]. These results therefore need to be interpreted with caution.

One of the main objectives of schistosomiasis control programmes in endemic areas is preventative chemotherapy to combat the development of severe morbidity [20]. Thus, more efforts are needed to ensure that the immediate health benefits of chemotherapy are sustained in the targeted populations for effective control [1]. The current study revealed that a single PZQ treatment had sustained effects on the reduction of schistosome-related morbidity, as indicated by the levels of urinary markers that remained low 12 months after treatment. Furthermore, it is interesting to note that both the preschool and primary-school age groups demonstrated improved health beneficial treatment effect in terms of reduced morbidity burden measured by microhaematuria 12 months following single treatment with PZQ. In view of the current observations showing no age-related differences in treatment efficacy, it is thus practically possible for control programmes in endemic areas targeting preschool-aged children to be implemented using the existing treatment strategies designated for school-aged children.

Nevertheless, our study has limitations that must be considered when interpreting these results. Firstly, participation of the controls was on a voluntary basis for ethical reasons, hence leading
to a small sample size for this group. This could have introduced additional uncertainties in the levels of infection and schistosome-related morbidity markers leading to a potential bias in the effects of treatment reported in this study. To minimise the effects of this potential bias, a random effect was included in the statistical models to account for some of the uncertainty. Secondly, since the majority of the children carried light infections, the parasitological cure rates may have been overestimated. Nonetheless, it is reassuring that efficacy rates reported in our study were comparable with those observed from other previous epidemiological studies in the same age range [1, 16].

5. Conclusions

In this study, we have demonstrated that praziquantel treatment does not only effectively reduces *S. haematobium* infection levels, but also the levels of related morbidity in both preschool and primary school-aged children, with the reduction in some morbidity markers recorded within 12 weeks of treatment being sustained over a period of one year. In conclusion, praziquantel treatment has immediate health benefits in preschool-aged children exposed to *S. haematobium*, and the effects of praziquantel on infection and morbidity measures is not age dependent. These findings are important for practitioners, policy makers and stakeholders involved in the control of schistosomiasis and timely because of the current global drive to address the health iniquity created by the paucity of information on the impact of praziquantel treatment on schistosome-related morbidity in children aged 5 years and below.
Conflict of Interests Disclosure

The authors declare that they have no competing interests.

Authors’ Contribution

Conceived and designed the experiments: Francisca Mutapi, Nicholas Midzi, and Takafira Mduluza. Performed the experiments, participated in the fieldwork, supported experiments, and contributed to draft manuscript editing/reviewing: Welcome Mkululi Wami, Norman Nausch, Nicholas Midzi, Reggis Gwisai, Takafira Mduluza, Mark Woolhouse, and Francisca Mutapi. Statistical analyses of the data: Welcome Mkululi Wami, with inputs from Mark Woolhouse, and Francisca Mutapi. All authors contributed to the revisions and approved the final version of the manuscript.

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References

1. Garba A, Barkiré N, Djibo A, Lamine MS, Sofo B, Gouvras AN, et al. Schistosomiasis in infants and preschool-aged children: Infection in a single *Schistosoma haematobium* and a mixed *S. haematobium*-*S. mansoni* foci of Niger. *Acta Tropica*. 2010;115:212-219.

2. Samuels AM, Matey E, Mwinzi PN, Wiegand RE, Muchiri G, Irerri E, et al. *Schistosoma mansoni* morbidity among school-aged children: a SCORE project in Kenya. *The American Journal of Tropical Medicine and Hygiene*. 2012;87(5):874-82.

3. Hotez PJ, Fenwick A. Schistosomiasis in Africa: an emerging tragedy in our new global health decade. *PLoS Neglected Tropical Diseases*. 2009;3(9):e485.

4. Stothard RJ, Sousa-Figueiredo JC, Simba Khamis I, Garba A, Rollinson D. Urinary schistosomiasis-associated morbidity in schoolchildren detected with urine albumin-to-creatinine ratio (UACR) reagent strips. *Journal of Pediatric Urology*. 2009;5(4):287-91.

5. Kahama AI, Odek AE, Kihara RW, Vennervald BJ, Kombe Y, Nkulila T, et al. Urine circulating soluble egg antigen in relation to egg counts, hematuria, and urinary tract pathology before and after treatment in children infected with *Schistosoma haematobium* in Kenya. *The American Journal of Tropical Medicine and Hygiene*. 1999;61(2):215-9.

6. Rollinson D, Klinger EV, Mgeni AF, Khamis IS, Stothard JR. Urinary schistosomiasis on Zanzibar: application of two novel assays for the detection of excreted albumin and haemoglobin in urine. *Journal of Helminthology*. 2005;79(3):199-206.

7. Shaw JG, Friedman JF. Iron deficiency anemia: focus on infectious diseases in lesser developed countries. *Anemia*. 2011;2011:260380.

8. WHO. "Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity". WHO/NMH/NHD/11.1. Geneva: World Health Organization, 2011.

9. WHO. "Prevention and control of schistosomiasis and soil-transmitted helminthiasis". Report No.: 0512-3054. Geneva: World Health Organization, 2002.
10. Richter J. The impact of chemotherapy on morbidity due to schistosomiasis. *Acta Tropica*. 2003;86(2-3):161-83.

11. King CH, Dangerfield-Cha M. The unacknowledged impact of chronic schistosomiasis. *Chronic Illness*. 2008;4(1):65-79.

12. Olveda DU, Olveda RM, McManus DP, Cai P, Chau TN, Lam AK, et al. The chronic enteropathogenic disease schistosomiasis. *International Journal of Infectious Diseases*. 2014;28:193-203.

13. Smith JH, Christie JD. The pathobiology of *Schistosoma haematobium* infection in humans. *Human Pathology*. 1986;17(4):333-45.

14. Bosompem KM, Bentum Ia, Otchere J, Anyan WK, Brown Ca, Osada Y, et al. Infant schistosomiasis in Ghana: a survey in an irrigation community. *Tropical Medicine & International Health*. 2004;9(8):917-22.

15. Odogwu SE, Ramamurthy NK, Kabatereine NB, Kazibwe F, Tukahebwa E, Webster JP, et al. *Schistosoma mansoni* in infants (aged < 3 years) along the Ugandan shoreline of Lake Victoria. *Annals of Tropical Medicine and Parasitology*. 2006;100(4):315-26.

16. Mutapi F, Rujeni N, Bourke C, Mitchell K, Appleby L, Nausch N, et al. *Schistosoma haematobium* treatment in 1-5 year old children: safety and efficacy of the antihelminthic drug praziquantel. *PLoS Neglected Tropical Diseases*. 2011;5(5):e1143.

17. Stothard JR, Sousa-Figueiredo JCD, Sousa-Figuereido JC, Betson M, Adriko M, Arinaitwe M, et al. *Schistosoma mansoni* infections in young children: when are schistosome antigens in urine, eggs in stool and antibodies to eggs first detectable? *PLoS Neglected Tropical Diseases*. 2011;5(1):e938.

18. Dabo A, Badawi HM, Bary B, Doumbo OK. Urinary schistosomiasis among preschool-aged children in Sahelian rural communities in Mali. *Parasites & Vectors*. 2011;4:21.
19. Sousa-Figueiredo JC, Pleasant J, Day M, Betson M, Rollinson D, Montresor A, et al. Treatment of intestinal schistosomiasis in Ugandan preschool children: best diagnosis, treatment efficacy and side-effects, and an extended praziquantel dosing pole. *International Health*. 2010;2(2):103-13.

20. WHO. "Report of a meeting to review the results of studies on the treatment of Schistosomiasis in preschool-age children". Report No.: 2011.7. Geneva: World Health Organization, 2011.

21. Mutapi F. Changing policy and practice in the control of pediatric schistosomiasis. *Pediatrics*. 2015;135(3):536-44.

22. Wami WM, Nausch N, Midzi N, Gwisai R, Mduluza T, Woolhouse M, et al. Identifying and evaluating field indicators of urogenital schistosomiasis-related morbidity in preschool-aged children. *PLoS Neglected Tropical Diseases*. 2015;9(3):e0003649.

23. Midzi N, Sangweme D, Zinyowera S, Mapingure MP, Brouwer KC, Kumar N, et al. Efficacy and side effects of praziquantel treatment against Schistosoma haematobium infection among primary school children in Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2008;102(8):759-66.

24. Reilly L, Magkrioti C, Mduluza T, Cavanagh DR, Mutapi F. Effect of treating *Schistosoma haematobium* infection on *Plasmodium falciparum*-specific antibody responses. *BMC Infectious Diseases*. 2008;8:158.

25. Midzi N, Mduluza T, Chimbari MJ, Tshuma C, Charimari L, Mhlanga G, et al. Distribution of schistosomiasis and soil transmitted helminthiasis in Zimbabwe: towards a national plan of action for control and elimination. *PLoS Neglected Tropical Diseases*. 2014;8(8):e3014.

26. Nagelkerke NJ, Borgdorff MW, Kalisvaart NA, Broekmans JF. The design of multi-stage tuberculin surveys: some suggestions for sampling. *The International Journal of Tuberculosis and Lung Disease*. 2000;4(4):314-20.
27. WHO. "Preventive chemotherapy in Human Helminthiasis. Coordinated use of Anthelminthic Drugs in Control Interventions: a Manual for Health Professionals and Programme Managers". Geneva: World Health Organization, 2006.

28. Mott K, Baltes R, Bambagha J, Baldassini B. Field studies of a reusable polyamide filter for detection of Schistosoma haematobium eggs by urine filtration. *Tropenmedizin Und Parasitologie*. 1982;33(4):227-8.

29. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical de São Paulo*. 1972;14:397-400.

30. Wami WM, Nausch N, Bauer K, Midzi N, Gwisai R, Simmonds P, et al. Comparing parasitological vs serological determination of Schistosoma haematobium infection prevalence in preschool and primary school-aged children: implications for control programmes. *Parasitology*. 2014;141(14):1962-70.

31. Froom P, Bieganiec B, Ehrenrich Z, Barak M. Stability of common analytes in urine refrigerated for 24 h before automated analysis by test strips. *Clinical Chemistry*. 2000;46(9):1384-6.

32. Sousa-Figueiredo JC, Betson M, Atuhaire A, Arinaitwe M, Navaratnam AM, Kabatereine NB, et al. Performance and safety of praziquantel for treatment of intestinal schistosomiasis in infants and preschool children. *PLoS Neglected Tropical Diseases*. 2012;6(10):e1864.

33. Molenberghs G, Verbeke G. *Models for Discrete Longitudinal Data*. New York: Springer; 2005.

34. Edwards D, Berry JJ. The efficiency of simulation-based multiple comparisons. *Biometrics*. 1987;43(4):913-28.

35. Stothard JR, Sousa-Figueiredo JC, Betson M, Green HK, Seto EYW, Garba A, et al. Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control
of schistosomiasis in African infants and preschool-aged children. *Parasitology.* 2011;138(12):1593-606.

36. Botelho MC, Machado A, Carvalho A, Vilaca M, Conceicao O, Rosa F, et al. *Schistosoma haematobium* in Guinea-Bissau: unacknowledged morbidity due to a particularly neglected parasite in a particularly neglected country. *Parasitology Research.* 2016;115(4):1567-72.

37. Tchuente LA, Shaw DJ, Polla L, Cioli D, Vercruysse J. Efficacy of praziquantel against *Schistosoma haematobium* infection in children. *The American Journal of Tropical Medicine and Hygiene.* 2004;71(6):778-82.

38. Coulibaly JT, N’Gbesso Y K, Knopp S, Keiser J, N’Goran EK, Utzinger J. Efficacy and safety of praziquantel in preschool-aged children in an area co-endemic for *Schistosoma mansoni* and *S. haematobium*. *PLoS Neglected Tropical Diseases.* 2012;6(12):e1917.

39. Stothard JR, Sousa-Figueiredo JC, Navaratnam AM. Advocacy, policies and practicalities of preventive chemotherapy campaigns for African children with schistosomiasis. *Expert Review of Anti-infective Therapy.* 2013;11(7):733-52.

40. King CH. Long-term outcomes of school-based treatment for control of urinary schistosomiasis: a review of experience in Coast Province, Kenya. *Memorias do Instituto Oswaldo Cruz.* 2006;101 Suppl 1:299-306.

41. Ahmed AM, Abbas H, Mansour Fa, Gasim GI, Adam I. *Schistosoma haematobium* infections among schoolchildren in central Sudan one year after treatment with praziquantel. *Parasites & Vectors.* 2012;5:108.

42. King CH. Lifting the burden of schistosomiasis-defining elements of infection-associated disease and the benefits of antiparasite treatment. *The Journal of Infectious Diseases.* 2007;196(5):653-5.
43. Sacko M, Magnussen P, Traore M, Landoure A, Doucoure A, Reimert CM, et al. The effect of single dose versus two doses of praziquantel on *Schistosoma haematobium* infection and pathology among school-aged children in Mali. *Parasitology*. 2009;136(13):1851-7.

44. Stete K, Krauth SJ, Coulibaly JT, Knopp S, Hattendorf J, Muller I, et al. Dynamics of *Schistosoma haematobium* egg output and associated infection parameters following treatment with praziquantel in school-aged children. *Parasites & Vectors*. 2012;5:298.

45. King CH, Bertsch D. Meta-analysis of urine heme dipstick diagnosis of *Schistosoma haematobium* infection, including low-prevalence and previously-treated populations. *PLoS Neglected Tropical Diseases*. 2013;7(9):e2431.
Table 1: Demographic characteristics of the study cohort followed-up at 12 weeks and 12 months post-treatment with complete parasitology data for *S. haematobium* infection.

| Variable               | Characteristic | Treated group | Untreated controls<sup>a</sup> |
|------------------------|----------------|---------------|-------------------------------|
|                        |                | Baseline/12 weeks | 12 months | Baseline/12 weeks |
| Sample size            | n              | 303            | 233       | 61               |
| Sex                    | M/F            | 142/161        | 108/125   | 29/32            |
| Age (years)            | Mean age       | 6.6 (1–10)     | 6.9 (1–10) | 4.9 (1–10)       |
|                        | (range)        |                |           |                  |
|                        | Median         | 6              | 7         | 5                |
| Age group, n (%)       | 1–5 years      | 109 (36%)      | 80 (34%)  | 40 (66%)         |
|                        | 6–10 years     | 194 (64%)      | 153 (66%) | 21 (34%)         |

<sup>a</sup> The data on voluntary untreated controls was only collected at baseline and 12 weeks follow-up surveys.
Table 2: Treatment efficacy of a single dose of praziquantel (40 mg/kg) against *S. haematobium* infection by age group at 12 weeks post-treatment. SE=standard error; CR=cure rate; ERR=egg reduction rate, with 95% confidence intervals (95% CI).

| Age group | Baseline (pre-treatment) | 12 weeks post-treatment | Treatment efficacy |
|-----------|--------------------------|-------------------------|--------------------|
|           | Number screened and treated | Number diagnosed positive | Mean egg count (SE) | Number of cases cured | Mean egg count (SE) | CR (95% CI) | ERR<sup>a</sup> (95% CI) |
| 1–5 years | 109 | 8 | 2.7 (1.79) | 8 | 0.0 (–) | 100.0% (67.7–100.0%) | 100.0% (–) |
| 6–10 years | 194 | 84 | 28.8 (8.40) | 79 | 0.6 (0.45) | 94.0% (86.8–97.4%) | 97.9% (89.9–99.5%) |

<sup>a</sup>The 95% confidence intervals for the ERRs were calculated using a bootstrap resampling method with 1000 replicates.
Table 3: Levels of *S. haematobium* re-infection and new infection rates among treated children in the study 12 months following treatment with a single dose of 40 mg/kg praziquantel. Percentage proportions, with 95% confidence intervals (95% CI) of children infected with *S. haematobium* detected by parasitology.

| Infection group | Sample size (n) | Number egg-positive | Prevalence (95% CI) |
|-----------------|-----------------|---------------------|---------------------|
| Re-infections\(^a\) | 76              | 7                   | 9.2% (4.5–17.8%)    |
| New infections\(^b\) | 157             | 11                  | 7.0% (4.0–12.1%)    |

\(^a\)Egg-positive at baseline and re-infected 12 months after treatment.

\(^b\)Uninfected at baseline, and found egg-positive 12 months after treatment.
List of Figure Legends

Figure 1a: Study design flowchart showing the number of children included for the final analysis to assess the effect of praziquantel treatment efficacy 12 weeks after treatment. Participants who preferred not to receive treatment but voluntarily remained in the study at 12 weeks were utilised as untreated controls.

Figure 1b: Study design flowchart to assess the effect of praziquantel 12 months after treatment.

Figure 2: Effect of praziquantel (PZQ) treatment on the levels of urinary markers of schistosome-related morbidity 12 weeks after treatment. (A) Microhaematuria, (B) Proteinuria, and (C) Albuminuria. The error bars indicate the 95% confidence intervals. The P-values for pairwise comparisons are from generalized linear mixed models investigating the probability of a child presenting with morbidity marker pre- vs. post-treatment adjusted for sex and age group. Significant P-values are highlighted in bold. *Contrast P-value determined using the Binomial exact test.

Figure 3: Effect of praziquantel treatment on levels of urinary markers of schistosome-related morbidity 12 months post-treatment. (A) Microhaematuria, (B) Proteinuria, and (C) Albuminuria. Error bars indicate 95% CI and the P-values for pairwise comparisons are from the generalized linear mixed models investigating the probability of a child presenting with morbidity markers pre- and post-treatment, adjusted for sex and age group. Significant P-values are highlighted in bold.