Sousa-Figueiredo, José C; Betson, Martha; Atuhaire, Aaron; Arinaitwe, Moses; Navaratnam, Annalan MD; Kabatereine, Narcis B; Bickle, Quentin; Stothard, J Russell; (2012) Performance and safety of praziquantel for treatment of intestinal schistosomiasis in infants and preschool children. PLoS neglected tropical diseases, 6 (10). e1864-. ISSN 1935-2727 DOI: https://doi.org/10.1371/journal.pntd.0001864

Downloaded from: http://researchonline.lshtm.ac.uk/id/eprint/375782/

DOI: https://doi.org/10.1371/journal.pntd.0001864

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by/2.5/
Performance and Safety of Praziquantel for Treatment of Intestinal Schistosomiasis in Infants and Preschool Children

José C. Sousa-Figueiredo, Martha Betson, Aaron Atuhaire, Moses Arinaitwe, Annalan M. D. Navaratnam, Narcis B. Kabatereine, Quentin Bickle, J. Russell Stothard

Abstract

Background: In 2012 the WHO formally recognised that infants and preschool children are at significant risk of schistosomiasis and qualify for treatment with praziquantel (PZQ). Targeted surveys determining both the performance and safety of this drug are now needed in endemic areas. We have formally assessed parasitological cure and putative side-effects in a prospective cohort of Schistosoma mansoni-infected children (aged 5 months–7 years old) in lakeshore settings of Uganda.

Methodology/Principal Findings: From a total of 369 children found to be egg-patent for intestinal schistosomiasis, 305 were followed-up three to four weeks after PZQ treatment and infection status re-assessed. Separately, a previously tested side-effect questionnaire was employed before and 24 hours after PZQ treatment to assess incidence and amelioration of symptoms in young children and their mothers. While the overall observed parasitological cure was 56.4%, a significant difference was found between a sub-set of children who had a history of multiple PZQ treatments (between one and four in an 18 month period), where cure rate was 41.7%, and those who had never received treatment (cure rate was 77.6%). PZQ proved to be safe, with only mild reported side effects which cleared within a month after treatment. Prevalence of reported symptoms was significantly lower in children than in mothers, and fewer side-effects were reported upon subsequent rounds of PZQ treatment.

Conclusion/Significance: Our findings show that PZQ treatment of young children resulted in satisfactory cure rates, and marked reduction in egg-output, with only mild and transient reported side-effects. However, the cure rate is clearly lower in younger children and those with history of previous treatment. Cure rate, but not egg reduction rate, was also lower in children with heavier pre-intervention infection intensity. With chemotherapy now recommended as a long-term strategy for disease control in young children, research into optimising the periodicity of targeted treatment strategies is now crucial.

Introduction

Schistosomiasis is one of the major neglected tropical diseases (NTDs) affecting over 207 million people worldwide. Since the agreement of the eight Millennium Goals in 2000, control of the infection has gained much international interest and political commitment, leading to the World Health Assembly 54.19 recommending regular de-worming of school-aged children at risk of infection with anthelmintics [1].

Until very recently, preschool children (≤5 years) were considered at “low-risk” for infection based on the assumption that children this young have little direct contact with schistosome cercariae-infested water, and consequently they have consistently been overlooked by mapping and treatment initiatives [2]. Recent research studies conducted in Ghana, Mali, Niger, Nigeria, Sudan, Uganda, Zanzibar and Zimbabwe, however, have clearly identified that this young age-class is living at risk for both urogenital and intestinal schistosomiasis, and preliminary results showed that treatment with praziquantel (PZQ) is safe and efficacious [3–14].

For this reason, the World Health Organisation (WHO) is now recommending that young children living in endemic areas should be considered for treatment with PZQ during child health campaigns at the standard dose of 40 mg/Kg [15]. Nonetheless, there are still concerns associated with treatment of preschoolers for schistosomiasis. Namely, infants are believed to be at risk of choking, and there is a lack of prescribing information by the pharmaceutical companies on toxicity, method of administration, adverse effects and pharmacokinetics in this age group [16].
This study therefore aimed to assess the performance and safety of PZQ treatment in under seven year olds living in Schistosoma mansoni endemic areas. Two different groups were investigated – children who had never received PZQ treatment and children that had been treated during a recent cohort study, in order to evaluate the likely PZQ efficacy during a rolling programme of annual treatment of preschool-aged children. In this way, we aim to build on the present body of evidence and highlight issues that should be addressed now as treatment of infants and preschoolers is scaled-up [15].

Methods

Ethical statement, recruitment and treatment

The London School of Hygiene and Tropical Medicine, London, UK (application no. LSHTM 5338.09) and the Ugandan National Council of Science and Technology approved this study. Before selection, all families received an information leaflet (in local languages) detailing the objectives and procedures of this study. Those who chose to participate had the study explained in local languages. After collection of samples (at the time of treatment to ensure treatment was not taken on an empty stomach and improve absorption of the drug.

Inclusion and exclusion criteria

In order to be included in the treatment efficacy study, all participants had to meet the following criteria: 1) aged below seven years at recruitment; 2) had been resident in study area since birth; 3) had successfully swallowed the PZQ tablets prescribed and no other medication was taken over the course of treatment. For analysis of the safety of praziquantel treatment, data from the SIMI cohort studies were used: a questionnaire was administered during the SIMI project, looking at longitudinal dynamics of schistosomiasis, malaria and associated morbidities in mothers and their preschool-aged children (≥6 year olds) [17–18].

Praziquantel efficacy study. The study involved a comparison of PZQ efficacy in (i) a random sample of 507 children (mean age 4.3, range 3 months–7 years old) from the SIMI cohort who had been found to be egg positive at the closing stages of the project or had received at least one PZQ dose in the past 18 months (“previously treated”), and (ii) a random sample of 472 children (mean age 3.7, range 3 months–7 years old) who had not been previously treated (“treatment-naive”). In total, 369 were found to be egg-patent for intestinal schistosomiasis. Of these, 305 were followed up 21–28 days later and infection status re-assessed according to WHO guidelines. Of these 125 were “treatment-naive” and 180 children were “previously treated”. For more details, see Fig. 1.

Praziquantel safety study. For analysis of the safety of praziquantel treatment, data from the SIMI cohort studies were used: a questionnaire was administered as part of the SIMI project at baseline and follow-ups (6 and 12 month), immediately before and 24 h after treatment, where mothers were asked to report whether they or their children needed any medical assistance or felt any of the following fourteen symptoms: dizziness, headache, sleepiness, fatigue, vertigo, abdominal pain, cramps, nausea, vomiting, diarrhoea, bloody stools, night fevers, sweating, lower back pain and urticaria/rash. In total, side-effects data were available from 781 children and 539 mothers at baseline, 171 children at six month follow-up and 167 children at twelve month follow-up.

Inclusion and exclusion criteria

In order to be included in the treatment efficacy study, all participants had to meet the following criteria: 1) aged below seven years at recruitment; 2) had been resident in study area since birth; 3) had successfully swallowed the PZQ tablets prescribed and no other medication was taken over the course of treatment. For analysis of the safety of praziquantel treatment, data from the SIMI cohort studies were used: a questionnaire was administered as part of the SIMI project at baseline and follow-ups (6 and 12 month), immediately before and 24 h after treatment, where mothers were asked to report whether they or their children needed any medical assistance or felt any of the following fourteen symptoms: dizziness, headache, sleepiness, fatigue, vertigo, abdominal pain, cramps, nausea, vomiting, diarrhoea, bloody stools, night fevers, sweating, lower back pain and urticaria/rash. In total, side-effects data were available from 781 children and 539 mothers at baseline, 171 children at six month follow-up and 167 children at twelve month follow-up.

Inclusion and exclusion criteria

In order to be included in the treatment efficacy study, all participants had to meet the following criteria: 1) aged below seven years at recruitment; 2) had been resident in study area since birth; 3) had successfully swallowed the PZQ tablets prescribed and no other medication was taken over the course of treatment. For analysis of the safety of praziquantel treatment, data from the SIMI cohort studies were used: a questionnaire was administered as part of the SIMI project at baseline and follow-ups (6 and 12 month), immediately before and 24 h after treatment, where mothers were asked to report whether they or their children needed any medical assistance or felt any of the following fourteen symptoms: dizziness, headache, sleepiness, fatigue, vertigo, abdominal pain, cramps, nausea, vomiting, diarrhoea, bloody stools, night fevers, sweating, lower back pain and urticaria/rash. In total, side-effects data were available from 781 children and 539 mothers at baseline, 171 children at six month follow-up and 167 children at twelve month follow-up.
rejection recorded; and 4) had provided two consecutive stool samples and a single urine sample at baseline and follow-up. Participants who had existing medical conditions or diarrhoea at baseline were excluded from the study. In order to be included in the safety study, all participants had to meet criteria 2 and 3 above, and be aged 2–7 years at recruitment so that only children who were able to verbally communicate with their mothers were included to avoid reporting bias.

Parasitological diagnosis

Parasitological diagnosis of S. mansoni was performed using double Kato-Katz thick smears prepared for two consecutive day stool samples (41.7 mg of stool per smear) [19]. Microscopy was conducted by experienced Ministry of Health technicians and supervised by a senior technician for quality control. Results were expressed as eggs per gram of faeces (epg) and infection intensities of S. mansoni were categorised as follows: 1–99 epg as light, 100–399 epg as medium and ≥ 400 epg as heavy infections according to WHO guidelines [20]. A single urine sample from each provided a 50 μl aliquot for testing of the presence of schistosome circulating cathodic antigen (CCA) with a commercially available immuno-chromatographic dipstick (Rapid Medical Diagnostics, Pretoria, South Africa), a rapid diagnostic test for intestinal schistosomiasis. [21] Trace results were considered positives.

Performance and safety of praziquantel treatment

The efficacy of PZQ (co-administered with ALB) for intestinal schistosomiasis was evaluated qualitatively (cure rates) and quantitatively based on a reduction in faecal egg counts (egg reduction rates) within three to four weeks after treatment. Two different cure rates (CR: the percentage of the infected population negative for infection after drug treatment) were calculated, the first using data from microscopy visualising egg-patent infections and second using urine CCA-dipsticks [22]. The outcome of the egg reduction rates (ERR) was calculated using two formulae (ERR1 according to arithmetic mean and ERR2 according to geometric mean) as described in Vercruysse and colleagues [23]. The CRs and the ERRs (1 & 2) were calculated for both sexes, age-classes (A: 1 to 3 year olds, B: 4 to 7 year olds) and for the different levels of pre-treatment egg excretion intensity.

A side-effect or incidence of symptom is defined as a symptom absent before treatment and experienced after treatment. Amelioration of a symptom is defined as a symptom that was experienced before treatment and no longer present 24 hours afterwards. To assess relative safety of PZQ (co-administered with ALB) in children, the incidence and amelioration of symptoms in children was compared to that in their mothers (all treated with PZQ/ALB combination). To assess the continued safety of PZQ (co-administered with ALB) throughout the project, the incidence and amelioration of symptoms was compared between baseline and follow-up surveys (among all treated children irrespective of egg-patency). Additionally, the incidence and amelioration of symptoms was compared between those treated with the combination therapy (PZQ/ALB) and those treated with only ALB (control group).

During the SIMI project, anti-malarials (Lonart, Bliss GVS Pharma Ltd, India), and paracetamol were administered on-site to children who tested positive for Plasmodium falciparum using ParaCheck-Pf (RDT, Orchid, India) and children who exhibited symptoms of uncomplicated malaria. The administration of both medications and prevalence of malaria were taken into consideration during the analysis as potential confounders.

Statistical analysis

Data were collected using pro-forma data sheets in the field, and then entered using Epidata (The Epidata Association, Odense, Denmark) or Microsoft Excel. The data thus collated were analysed using the R statistical package v 2.10.1 (The R Foundation for Statistical Computing, Vienna, Austria) and Microsoft Excel spreadsheet software. For percentage values, 95% confidence intervals (CI 95) were estimated using the exact method [24]. Prevalence comparisons were performed using (one-tailed) Fisher’s exact modification of the 2x2 chi-squared test [25]. For infection intensity values, the geometric mean of Williams, GMW, was chosen as the measure of central tendency due to the typical over-dispersion present in this type of data [26].

Multivariate logistic regression was carried out to ascertain factors (tested variables: age, sex, PZQ dosage administered, history of previous treatment, daily water contact and pre-treatment S. mansoni infection intensity) associated with “curing” after treatment. Furthermore, multivariate logistic regression was used to identify factors (tested variables: age, sex, pre-treatment S. mansoni (Kato-Katz) and malaria (Giemsa microscopy) prevalence and co-administration of anti-malarial and antipyretic paracetamol) associated with “incidence” and “amelioration” of symptoms after treatment. Within village intra-correlation in the data was accounted for using a generalized linear mixed model with multivariate normal random effects (the random-effects of village in our case), with penalized quasi-likelihood (function glmmPQL in R) [27]. For each variable, odds ratio (OR) and P-values were calculated, and a P-value <0.05 was considered indicative of statistical significance.

Results

Of the 1039 children recruited into the treatment efficacy study, 60 failed to provide replicate stool samples at baseline and a further 64 children from the 369 already allocated children failed to provide replicate stool samples upon follow-up; these children were excluded from the study (see Fig. 1). A total 14 children failed to provide urine samples at baseline; these children were allocated into the study groups nonetheless if found positive and only excluded during statistical analysis concerning the CCA rapid diagnostic test.

The overall prevalence of egg-patent intestinal schistosomiasis among the 979 children (5–93 months old, mean age 54 months old) surveyed was 37.7% (GM = 5.99 epg, max intensity 7284 epg), with the prevalence of high intensity infections (>399 epg) reaching 8.9%. Prevalence of urine-CCA positive individuals was significantly higher reaching 57.1% (P<0.0001). For detailed information of infection intensity categories and confidence intervals, see Table 1.

Praziquantel efficacy

Observed PZQ cure rate in the treatment-naive children (N = 125) was 77.6%, ERR1 was 92.1% and ERR2 was 99.1%. The maximum recorded intensity decreased from 7284 epg to 1524 epg, with the arithmetic mean decreasing from 394 epg to 31 epg and GM decreasing from 108 epg to 1 epg. Observed PZQ cure rate for the previously treated children (N = 180) was 41.7%, ERR1 was 72.8% and ERR2 was 92.2%. The maximum recorded intensity decreased from 3906 epg to 3228 epg, with arithmetic mean decreasing from 290 epg to 79 epg and GM decreasing from 102 epg to 8 epg. For detailed information on cure rates according to age, sex and pre-intervention infection intensity, as well as confidence intervals, see Table 2.
Observed PZQ efficacy based on CCA results in treatment-naïve children (N = 124) was 32–3% (CI95 24–1–41–2%) After treatment, there was also a significant six-fold reduction in the prevalence of strong (++/++) CCA results, from 62–4% (CI95 53–2–70–9%) at baseline to 10–4% (CI95 5–7–17–2%) after treatment. Observed PZQ efficacy in previously treated children (N = 179) was 20–7% (CI95 14–9–27–4%). Similarly, after treatment there was a significant reduction, albeit smaller (three-fold), in the prevalence of strong CCA results, from 47–4% (CI95 40–0–55–1%) at baseline to 15–6% (CI95 10–7–21–8%) after treatment.

According to multivariate logistic regression, becoming egg negative after PZQ treatment was associated with age (1–3 year olds less likely to cure than 4–7 year olds, OR = 3–53, P<0.001). Additionally, children heavily infected with S. mansoni at baseline were less likely to cure after treatment (OR = 0–47, P = 0.028). Finally, history of treatment also influenced treatment outcome, with children who had been treated once in the 18 months preceding this study being less likely to cure than those naive for treatment (OR = 0–35, P = 0.003), and even lower odds for those with history of two (OR = 0–06, P<0.0001) or more (OR = 0.08, P<0.0001) treatments in the 18 months preceding this study. In fact, the cure rate for children treated once (N = 93) in the past was 60–0% (CI95 48–4–70–8%), for those treated twice (N = 65) was 25–0% (CI95 14–9–36–9), and for those treated three times or more (N = 61) was 29–2% (CI95 17–0–44–1%). This model controlled for sex, dosage of PZQ received and daily time spent in water (proxy for exposure), and neither were found to be significantly associated with becoming egg-negative after treatment. For more details, see Table 3.

### Safety of praziquantel

Side-effect data were available for 781 children and 539 mothers from SIMI’s baseline survey (raw data are available in Supplementary Tables S1 and S2). Prevalence levels of egg-patent S. mansoni infection in these study participants were 32–4% (CI95 29–1–35–8%) and 48–6% (CI95 44–3–52–9%), respectively for children and mothers. Mothers reported a higher prevalence of new symptoms post-treatment than their children for all listed symptoms with the exception of bloody stools. On the other hand, mothers reported a lower percentage of improvement of existing symptoms after treatment than their children for all listed symptoms with the exception of vomiting and bloody stools (Fig. 2A-B).

Prevalence of egg-patent S. mansoni infection in the children was 32–4% (CI95 29–1–35–8%), 34–6% (CI95 27–2–42–5%) and 32–5% (CI95 25–4–40–3%), respectively for baseline, 6 month and 12 month follow-ups. As the cohort study progressed, the number of cases of dizziness, sleepiness, fatigue, cramps, nausea, sweating and and night fevers post-treatment decreased significantly compared to baseline. No symptom became more prevalent with time. As for amelioration, with time children were more likely to clear abdominal pain, bloody stools, sweating, night fevers and urticaria/rash after treatment compared to baseline. Confidence intervals and information on proportion of new and improving symptoms are presented in Fig. 2 C–D.

Side-effect data were available for 529 children treated with PZQ and ALB combination therapy and 370 children treated with ALB monotherapy only. Prevalence levels of egg-patent S. mansoni infection in these study participants were 23–4% (CI95 19–7–27–3%) and 6–6% (CI95 4–2–9–8%), respectively for combination and monotherapy. Children who received PZQ were more likely to report new cases of dizziness, abdominal pain, cramps, nausea and diarrhoea, while children who received ALB monotherapy were more likely to report night fevers. As for amelioration, children who received PZQ were less likely to clear pre-existing cases of abdominal pain and diarrhoea (Fig. 2E–F).
According to multivariate logistic regression, presence of egg-patent schistosomiasis in a child was associated with incidence of dizziness (OR = 1.6, P = 0.049), headache (OR = 1.7, P = 0.077), abdominal pain (OR = 2.1, P = 0.002), bloody stools (OR = 2.0, P = 0.052) and night fevers (OR = 2.8, P < 0.0001) after PZQ treatment. Similarly, presence of heavy (>399 epg) *S. mansoni* infection in a child was associated with incidence of abdominal pain (OR = 3.6, P = 0.005), vomiting (OR = 4.7, P < 0.001), diarrhoea (OR = 3.7, P = 0.004), bloody stools (OR = 3.1, P = 0.044) and night fevers (OR = 3.1, P = 0.044) after PZQ treatment.

**Discussion**

Intestinal schistosomiasis in infants and preschool children from the shoreline villages in our study areas is clearly a chronic public health problem, where the need for PZQ treatment is especially obvious. In these areas young children currently remain untreated, and these untreated infections acquired in early childhood may contribute to the worsening of their longer term clinical picture [17]. In the case of intestinal schistosomiasis, hepato-splenomegaly and perportal fibrosis regress very slowly post treatment, which emphasises the necessity for prompt treatment before the intensity of the infection becomes significant [28].

With children as young as one year of age affected by this disease, recent WHO guidelines now endorse anti-schistosomal treatment of preschool children [15]. However, some research topics remain to be explored, such as pharmacokinetics of the drug in young children, appropriate dosing and treatment periodicity. Our findings provide clear evidence that PZQ (40 mg/Kg) can confer benefit to the infants in terms of significant cure rates and marked reduction in egg reduction rates likely to avert development of chronic morbidity. However, our results also show potentially important differences in relation to age, previous treatment and infection intensity.

**Table 2.** Cure and egg reduction rates (CR and ERR, respectively) as determined by egg output, in % (and CI 95), for treatment with a single dose of praziquantel (40 mg/Kg) against intestinal schistosomiasis in very young children (5 months–7 year olds); (1) is ERR of arithmetic mean, and (2) is ERR of geometric mean.

| History of previous treatment          | No. allocated | No. followed-up | CR   | ERR (1) | ERR (2) |
|----------------------------------------|---------------|-----------------|------|---------|---------|
| None                                   | 150           | 125             | 77.6 | 92.1    | 99.1    |
|                                        |               |                 |      | (69.3–84.6) | (89.0–94.6) | (94.9–100.0) |
| At least one treatment in the last 18 months | 219          | 180             | 41.7 | 72.8    | 92.2    |
|                                        |               |                 |      | (34.4–49.3) | (67.2–77.8) | (85.1–96.6) |
| Single round in 18 months              | 93            | 80              | 60.0 | 75.6    | 96.5    |
|                                        |               |                 |      | (48.4–70.8) | (70.0–80.6) | (90.0–99.3) |
| Two rounds in 18 months                | 65            | 52              | 25.0 | 52.0    | 82.6    |
|                                        |               |                 |      | (14.0–38.9) | (45.6–58.4) | (73.4–89.7) |
| Three rounds or more in 18 months      | 61            | 48              | 29.2 | 84.8    | 91.0    |
|                                        |               |                 |      | (17.0–44.1) | (80.8–88.3) | (85.4–95.0) |
| Age class*                             |               |                 |      |         |         |
| 1–3 years                              | 85            | 71              | 40.8 | 75.4    | 96.3    |
|                                        |               |                 |      | (29.3–53.1) | (69.5–80.7) | (89.4–99.2) |
| 4–7 years                              | 271           | 222             | 60.8 | 82.9    | 97.3    |
|                                        |               |                 |      | (54.1–67.3) | (78.7–86.6) | (92.3–99.4) |
| Sex*                                   |               |                 |      |         |         |
| Boy                                    | 164           | 135             | 60.0 | 88.3    | 97.1    |
|                                        |               |                 |      | (51.2–68.3) | (84.5–91.6) | (91.8–99.4) |
| Girl                                   | 200           | 167             | 53.9 | 76.8    | 95.2    |
|                                        |               |                 |      | (46.0–61.6) | (71.9–81.2) | (89.2–98.4) |
| Pre-intervention infection intensity   |               |                 |      |         |         |
| Low                                    | 191           | 155             | 61.3 | 34.2    | 89.3    |
|                                        |               |                 |      | (53.1–69.0) | (19.6–51.3) | (71.7–97.7) |
| Moderate                               | 91            | 79              | 56.9 | 70.2    | 98.0    |
|                                        |               |                 |      | (45.3–68.1) | (63.6–76.3) | (95.0–99.5) |
| High                                   | 87            | 71              | 45.1 | 88.4    | 99.0    |
|                                        |               |                 |      | (33.2–57.3) | (86.4–90.3) | (98.0–99.5) |
| Total                                  | 369           | 305             | 56.4 | 82.2    | 96.2    |
|                                        |               |                 |      | (50.6–62.0) | (77.7–86.2) | (90.4–98.9) |

*Information on sex and age of the child not available for all children.

doi:10.1371/journal.pntd.0001864.t002

According to multivariate logistic regression, presence of egg-patent schistosomiasis in a child was associated with incidence of dizziness (OR = 1.6, P = 0.049), headache (OR = 1.7, P = 0.077), abdominal pain (OR = 2.1, P = 0.002), bloody stools (OR = 2.0, P = 0.052) and night fevers (OR = 2.8, P < 0.0001) after PZQ treatment. Similarly, presence of heavy (>399 epg) *S. mansoni* infection in a child was associated with incidence of abdominal pain (OR = 3.6, P = 0.005), vomiting (OR = 4.7, P < 0.001), diarrhoea (OR = 3.7, P = 0.004), bloody stools (OR = 3.1, P = 0.044) and night fevers (OR = 3.1, P = 0.044) after PZQ treatment.
While it is often assumed that the performance of PZQ is relatively homogenous across the age classes, we have identified factors influencing the outcome of treatment in very young children which have not been previously highlighted in other age ranges. Our results indicate that cure rate is lower in children under the age of four, although ERR was less affected. Note that infection intensities were significantly higher in 4–7 year olds comparing to 1–3 year olds (GMw 111 epg v. 80 epg, respectively, \( P < 0.0001 \)). This could also be related to different pharmacokinetics in infants, as an issue set to be explored in the initiative by the Merck Serono to develop a PZQ paediatric formulation. Another possible explanation for the lower cure rates in younger children could be lower anti-worm immune responses, since chemotherapy in experimental animals has been shown to be immune-dependent [29].

The lower cure rates in previously treated children (41.7% for those receiving any prior treatment and 29.2% for those having received three treatments) compared with treatment-naïve children (78%) was intriguing and requires explanation. This is not explained by the child’s age or intensity of infection since the mean and age ranges and infection intensity in the two groups were essentially similar. These results suggest that there could have been selection of parasites with lower sensitivity to PZQ as a result of repetitive treatment, a phenomenon previously observed in other settings [30–31]. Interestingly, results from the longitudinal cohort project show that for certain individuals egg and urine-antigen excretion never cease despite six rounds of treatment in a two-year period (results not shown). While the possibility of putative resistance to the drug is of concern, what should also be considered is that these individuals could be immune-incompetent either due to host-specific factors or due to co-infections.

In our study, CR results were significantly lower when using urine-antigen detection as diagnostic tool, even though previous work using an enzyme-linked immunosorbent assay (ELISA)-format to detect CCA in sera samples has shown levels decreasing within one week after treatment in adults [32]. Although lack of specificity of the CCA could be an explanation for the disparity with the parasitology we consider that relative lack of sensitivity of the Kato Katz in detecting low intensity persisting infections a more likely explanation [33–35]. Alternatively this novel commercially available test could also be indicating a cessation of egg production despite parasites remaining active and metabolising in the host’s vascular system after PZQ treatment. The issue of diagnostic performance by either test used in this study requires further evaluation. To this end, a larger data set is being analysed looking at longitudinal dynamics (before and during mass treatment) of CCA evaluation. To this end, a larger data set is being analysed looking at longitudinal dynamics (before and during mass treatment) of CCA evaluation. To this end, a larger data set is being analysed looking at longitudinal dynamics (before and during mass treatment) of CCA evaluation. To this end, a larger data set is being analysed looking at longitudinal dynamics (before and during mass treatment) of CCA evaluation.

Another factor to consider is that sensitivity to PZQ is dependent on the maturity of the parasite, and in younger children where a larger proportion of cases possibly result from newly contracted infections, and especially if no second round of treatment was given between baseline and follow-up [36], parasite maturity could be contributing to reduction of observed drug performance [37]. However, both groups of children included in this study are from the same villages, meaning both groups have an equal exposure potential to cercariae-infested waters. Additionally, daily water contact (as proxy for risky behaviour or exposure) was taken into consideration when modelling the outcome “cure”, and was found to be statistically insignificant (\( P \)-value = 0.24; see Table 3). Therefore the likelihood of recently acquired infections at baseline or follow-up (i.e. presence of immature parasites) should be biasing group results equally (lower than expected PZQ efficacy), as opposed to explaining the marked difference in treatment efficacy identified here.

In our setting, PZQ/ALB integrated therapy proved to be safe for preschool children, with fewer reported side-effects than for their mothers, who are currently targeted by mass drug administration campaigns. In fact, the few side-effects reported at heightened levels compared to children receiving ALB monotherapy were found to be associated with the presence and intensity of infection, and all cleared soon after, i.e. treatment of uninfected individuals leads to
no adverse reactions. There are likely to be biases in parental reporting of symptom on behalf of children, but this approach has been used successfully in previous studies [5,7].

Our results clearly highlight a need for further research into understanding the human factors influencing clearance of infection post-treatment. While there are tools already available for inclusion of younger children in mass treatment campaigns, such as an extended version of the current WHO dose pole [38], this study brings to light the potential problem of low cure rate (41.7%) in preschool children with history of previous treatment. Our observation that preschoolers receiving repetitive treatment in a period of 18 months are less likely to clear infection than children naïve to treatment indicates that treatment of children this young should be conducted under different periodicity than that for school-aged children. The potential for non-cure should not go overlooked, since the emergence of resistance to the only commercially available drug for schistosomiasis would undermine ongoing African control programmes. Nevertheless, the recent change in international policy, the availability of tools for pragmatic dosing of

Figure 2. Percentage of symptoms reported after treatment (left column) and percentage of symptoms ameliorated (right column). A side-effect is defined as a symptom absent before treatment and experienced after treatment; amelioration of a symptom is defined as a symptom that was experienced before treatment and no longer present 24 hours afterwards. A–B: children (N = 781) v. mothers (N = 539); C–D: baseline (N = 781) v. follow-ups (children only, N = 171 and 167, respectively for 6 and 12 month follow-ups); E–F: PZQ+ALB integrated chemotherapy (N = 529) v. ALB monotherapy (N = 370, children only). doi:10.1371/journal.pntd.0001864.g002
the young child and the results reported here on the performance, albeit far from perfect, and safety of PZQ are encouraging premises for improvement of global child health.

Supporting Information

Table S1 Number of children (2–7 year olds) and mothers reporting symptoms after treatment during the SIMI project. These data were used to compile Figure 2; symptom legend: Diz. = Dizzy, Head. = Headache, Sleep. = Sleepy, Abd. Pain = Abdominal Pain, Cra. = Cramps, Nau. = Nausea, Vom. = Vomiting, Diar. = Diarrhoea, Blo. Sto. = Bloody Stools, Swe. = Sweating, Nig. Fev. = Night Fevers, Lo. Ba. Pa. = Lower Back Pain, Urt./Rash = Urticaria/Rash.

Table S2 Number of children (2–7 year olds) and mothers reporting improvements in symptoms after treatment during the SIMI project. These data were used to compile Figure 2; symptom legend: Diz. = Dizzy, Head. = Headache, Sleep. = Sleepy, Abd. Pain = Abdominal Pain, Cra. = Cramps, Nau. = Nausea, Vom. = Vomiting, Diar. = Diarrhoea, Blo. Sto. = Bloody Stools, Swe. = Sweating, Nig. Fev. = Night Fevers, Lo. Ba. Pa. = Lower Back Pain, Urt./Rash = Urticaria/Rash.

Acknowledgments

We wish to thank the children and mothers who took part in the study. We also thank the Ministry of Health of Uganda, Vector Control Division, and all their staff for their support during the SIMI project. We would particularly like to acknowledge the work of the local Vector Control Division officers, Mr. Juma Nabonge, Mr. Ashuman Babyesiza, Mr. Perez Estigoma and Mr. Chris Byalero for making cohort studies possible in areas where populations are constantly migrating. Mr. Chris Byalero regretfully passed away shortly beforehand submission of this manuscript and we would like to express our regret for the irreplaceable loss of a friend and colleague. JCSF and JRS would like to acknowledge Prof. David Rollinson and the Natural History Museum, London, for continued support during the SIMI project.

Author Contributions

Conceived and designed the experiments: JCSF QB MB AA JCSF. Performed the experiments: JCSF MB AA JCSF. Analyzed the data: JCSF MB AA JCSF. Contributed reagents/analysis tools: AMDN NBK QB JRS. Participated in revising the final article: JCSF MB AA MA JRS. Coordinated diagnostic procedures in the field: MA JCSF. Responsible for literature research: AMDN. Wrote the first draft of the manuscript: JCSF A JCSF MB AA MA JRS. Contributed to the recruitment, clinical care, and follow-up of patients: JCSF MB AA MA JRS. Coordinated diagnostic procedures in the field: MA JCSF. Analysed and managed the data: JCSF. Responsible for literature research: AMDN. Wrote the first draft of the manuscript: JCSF A JCSF MB AA MA JRS. Coordinated diagnostic procedures in the field: MA JCSF.

Table of Contents

1. Introduction
2. Materials and Methods
3. Results
4. Discussion
5. Conclusion

Supporting Information

References

1. WHO (2001) The World Health Assembly Resolution (WHA 54.19). Geneva: World Health Organization. Available: http://www.who.int/inf-ptr-2001/en/pr2001WHA-6.html.
2. Stothard JR, Gabrielli AF (2007) Schistosomiasis in African infants and preschool children: to treat or not to treat? Trends Parasitol 23: 83–96.
3. Bosompem KM, Bentum IA, Ofotey AA, Anyan WK, Brown CA, et al. (2004) Infant schistosomiasis in Ghana: a survey in an immigration community. Trop Med Int Heal 9: 917–922.
4. Garba A, Barkine N, Djibo A, Lamine MS, Sofi B, et al. (2010) Schistosomiasis in infants and preschool-aged children: Infection in a single S. haematobium-S. mansoni foci of Niger. Acta Trop 115: 212–219.
5. Sousa-Figueiredo JC, Amin J, Lapageria J, Day M, Betson M, Rollinson D, et al. (2010) Treatment of intestinal schistosomiasis in Ugandan preschool children: best diagnosis, treatment efficacy and side-effects, and an extended praziquantel dosing pole. Int Health 2: 103–113.
6. Dabo A, Badawi HM, Baby B, Doumbo OK (2011) Urinary schistosomiasis among preschool-aged children in Sahelian rural communities in Mali. Parasite Vector 4.
7. Mutapi F, Rujeni N, Bourke C, Mitchell K, Appleby L, et al. (2011). Schistosoma haematobium infection in 1–5 year old children: safety and efficacy of the anthelminthic drug praziquantel. PLoS Negl Trop Dis 5: e1143.
8. Sousa-Figueiredo JC, Basazhe MG, Mgeni AF, Khanmian IS, Rollinson D, et al. (2008) A parasitological survey, in rural Zanzibar, of pre-school children and their mothers for urinary schistosomiasis, soil-transmitted helminthiases and malaria, with observations on the prevalence of anaemia. Ann Trop Med Para 102: 679–692.
9. Maifana CF, Ekpo UF, Ojo DA (2003) Urinary schistosomiasis in preschool children in settlements around Oyan Reservoir in Ogun State, Nigeria: implications for control. Trop Med Int Health 8: 78–82.
10. Deribe K, Eldaw A, Haadzabshil S, Kallef E, Omer MD, et al. (2011) High prevalence of urinary schistosomiasis in two communities in South Darfur, implications for interventions. Parasit Vectors 4: 14.
11. Ekpo UF, Laja-Dele A, Ohuwale AS, Sam-Wobo SO, Maifana CF (2010) Urinary schistosomiasis among preschool children in a rural community near Abeokuta, Nigeria. Parasit Vectors 3: 58.
12. Odogwu SE, Ramamurthy NK, Kabaterine NB, Kazibwe F, Tukahesa E, et al. (2006) Schistosoma mansoni in infants (aged <3 years) along the Ugandan shoreline of Lake Victoria. Ann Trop Med Para 100: 315–326.
13. Sousa-Figueiredo JC, Day M, Betson M, Rowell C, Wamboko A, et al. (2011) Field survey for strongyloidiasis in eastern Uganda with observations on efficacy of preventive chemotherapy and co-occurrence of soil-transmitted helminthisis/intestinal schistosomiasis. J Helminthol 85: 325–333.
14. Navaratnam AM, Sousa-Figueiredo JC, Stothard JR, Kabaterine NB, Fenwick A, et al. (2012) Efficacy of praziquantel syrup versus crushed praziquantel tablets in the treatment of intestinal schistosomiasis in Ugandan preschool children, with observation on compliance and safety. Trans R Soc Trop Med Hyg 106: 400–407.
15. WHO (2011) Report of a meeting to review the results of studies on the treatment of schistosomiasis in preschool-age children. Geneva: World Health Organization. Available: http://whqlibdoc.who.int/publications/2011/9789241501880_eng.pdf.
16. Montresor A, Stoltzfus RJ, Albonico M, Tieghem JL, Rice AL, et al. (2002) Is the exclusion of children under 24 months from anthelmintic treatment justifiable? Trans R Soc Trop Med Hyg 96: 197–199.
17. Stothard JR, Sousa-Figueiredo JC, Betson M, Green HK, Seto EY, et al. (2011) Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children. Parasite 18: 1593–1606.
18. Betson M, Sousa-Figueiredo JC, Rowell C, Kabaterine NB, Stothard JR (2010) Intestinal schistosomiasis in mothers and young children in Uganda: investigation of field-applicable markers of bowel morbidity. Am J Trop Med Hyg 83: 1048–1055.
19. Katz N, Chavez A, Pellegrino J (1972) A simple device for quantitative stool thick smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo 14: 397–400.
20. WHO (2006) Preventive Chemotherapy in Human Helminthiasis. Coordinated use of Anthelmintic Drugs in Human Interventions: a Manual for Health Professionals and Programme Managers. Geneva: World Health Organization.
21. Stothard JR, Kabaterine NB, Tukahesa EM, Kazibwe F, Rollinson D, et al. (2006) Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. Acta Trop 97: 219–228.
22. Bennett A, Guyatt HL (2000) Reducing intestinal nematode infection: efficacy of albendazole and mebendazole. Parasitol Today 16: 71–74.
23. Vercruysse J, Behnke JM, Albonico M, Ame SM, Angebault C, et al. (2011) Assessment of the anthelminthic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. PLoS Negl Trop Dis 5: e948.
24. Armitage P, Berry G (1994) Statistical Methods in Medical Research. Oxford: Blackwell Scientific Publications.
25. Fisher RA (1922) On the interpretation of Chi-square from contingency tables and the calculation of P. J R Stat Soc 85: 87–94.
26. Williams CB (1937) The use of logarithms in the interpretation of certain entomological problems. Ann Appl Bio 24: 404–414.
27. Breslow NE, Clayton DG (1993) Approximate inference in generalized linear mixed models. J Am Stat Assoc 88: 9–25.
28. Veniervald BJ, Duine DW (2004) Morbidity in schistosomiasis: an update. Curr Opin Infect Dis 17: 439–447.
29. Doehn Hoff MJ, Coles D, Utzinger J (2008) Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis 21: 659–667.
30. Melman SD, Steinauer ML, Cunningham C, Kubatko LS, Mwangi IN, et al. (2002) Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. Acta Trop 97: 219–228.

Checklist S1 STROBE checklist for cohort studies. (DOC)
32. Nibbeling HA, Van Lieshout L, Deelder AM (1998) Levels of circulating soluble egg antigen in urine of individuals infected with Schistosoma mansoni before and after treatment with praziquantel. Trans R Soc Trop Med Hyg 92: 675–677.

33. Knopp S, Rinaldi I, Khamis IS, Stothard JR, Rollinson D, et al. (2009) A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections. Trans R Soc Trop Med Hyg 103: 347–354.

34. Glinz D, Sihue KD, Knopp S, Lohourignon LK, Yao KP, et al. (2010) Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for Schistosoma mansoni and soil-transmitted helminths. PLoS Negl Trop Dis 4: e734.

35. Knopp S, Speich B, Hattendorf J, Rinaldi L, Mohammed KA, et al. (2011) Diagnostic accuracy of Kato-Katz and FLOTAC for assessing anthelmintic drug efficacy. PLoS Negl Trop Dis 5: e1036.

36. Renganathan E, Cioli D (1998) An international initiative on praziquantel use. Parasitol Today 14: 390–391.

37. Doenhoff MJ, Hagan P, Cioli D, Southgate V, Pica-Mattoccia L, et al. (2009) Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. Parasitol 1: 1–11.

38. Sousa-Figueiredo JC, Betson M, Stothard JR (2012) Treatment of schistosomiasis in African infants and preschool-aged children: downward extension and biometric optimization of the current praziquantel dose pole. Int Health 4: 95–102.