The contribution of host-related factors to low cure rates of praziquantel for the treatment of Schistosoma mansoni in Senegal

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Abstract. Surprisingly low cure rates were repeatedly observed after treatment with a standard dosage of praziquantel in a recently established Schistosoma mansoni focus in northern Senegal. In 4 discrete cohorts from the same population, cure rates were 18–36% and egg count reduction rates were 77–88%. Data and material of 920 compliant subjects from all 4 cohorts were further analyzed to identify possible host-related factors associated with low cure rates. The lowest cure rates were found in the highest egg count groups. However, in low and moderate egg count groups, drug efficacy was also below normal values. Cure rates were similar in males and females, showed no seasonal variation, and were independent of previous praziquantel treatment. They were significantly higher in adults than in children, also after allowing for intensity of infection. Individual water contact behavior and specific humoral immune responses were examined in 2 extreme subgroups, either without significant egg count reduction or showing complete parasitologic cure. There was no significant difference in frequency and duration of water contact between those individuals with complete cure and those that showed little effect of praziquantel treatment. Levels of IgG, IgG1, IgG3, IgG4, IgM, and IgE against adult worm antigen were not different between the 2 subgroups. Thus, the abnormally frequent failure of treatment observed in this focus could not be associated with any host-related factor, other than age and pretreatment egg counts.

In a recently established focus of Schistosoma mansoni in the Senegal River Basin, praziquantel treatment apparently showed a lower efficacy and induced more side effects than usually reported.1 2 In an initial cohort of 400 subjects, only 18% was found to be parasitologically negative 12 weeks after treatment with the standard single oral dosage of 40 mg/kg. However, egg reduction rates were substantial (86%) in those remaining positive. Results were confirmed by determination of circulating antigens excreted by metabolically active adult worms.3 Similar low cure rates were found in 3 subsequent cohorts from the same focus (Stelma FF; unpublished data). An increased dosage of praziquantel, given at the same day, showed no significant improvement,4 while normal cure rates were found after treatment with oxamniquine,5 or after repeated treatment with praziquantel within a period of 40 days.5

Possible explanations for these findings can be divided into 3 main categories: 1) parasite-related, including reduced strain susceptibility to praziquantel, 2) drug-related, including drug quality or bioavailability, and 3) host-related, including high initial intensity of infection, prepatent infections, rapid reinfection, and still under-developed pharmacosynergistic humoral immune responses.2 This paper focuses on the third category of hypotheses. We use data from all 4 cohorts to analyze the contribution of host-related factors on the efficacy of praziquantel treatment in this area, with emphasis on the variables age, gender, history of exposure, pre-treatment intensity of infection, history of previous praziquantel treatment, individual water contact behavior, and specific antibody levels.

Population, materials, and methods

General study design. The field studies took place in Ndombo, Senegal, a community with approximately 4,000 inhabitants, close to Richard Toll. Local authorities and participating individuals were informed about the study and agreed to collaborate. The study was approved by the Ethical Commission of the European Special Program for Operational and Integrated Research (ESPOIR) within the Senegalese Ministry of Health and by the Ethical Commission of Leiden University Medical Centre. The area and the design of the study are described in detail elsewhere.16 Briefly, between August 1991 and September 1993, 4 randomly selected cohorts of approximately 400 subjects were surveyed, at eight-month intervals. From each participant, 2 stool samples, taken on two separate days within a 2-week interval, and a serum sample were collected. Numbers of S. mansoni eggs were counted by examining duplicate, 25-mg, Kato slides of each stool.7 Those who were found positive after parasitologic examination were offered treatment with 40 mg/kg of praziquantel (Distocide;8 Shin Poong, Seoul, Republic of South Korea) in a single oral dose. The tablets were swallowed under supervision of a medical team. Pregnant women were excluded and asked to return for treatment after the delivery. Serum collection and parasitologic examination was repeated at week 12 (cohort 1) or week 6 (cohorts 2–4).

Selected subpopulations. Individual water contact behavior and specific humoral immune responses were evaluated in well-defined subgroups, selected to show extremes in the efficacy of praziquantel treatment. Since cure rates were clearly age-related, these subgroups were selected separately for children (<16 years old) and adults (≥16 years old). Thirty children and 30 adults were randomly selected from 1) those who showed complete cure based on parasitologic diagnosis and 2) those who showed less than 60% reduction in their egg counts. Individuals who did not provide serum both before and after treatment, as well as children younger than 5 years of age, were excluded from the selection procedure.

Water contact data. Direct, quantitative water contact was observed at 5 different sites in the village, where most activities leading to exposure took place.1 Observers recruit-
ed from the village noted individual behavior of the whole population at these sites from 6:00 AM until 7:00 PM for 7 consecutive days each month during the whole study period. Each person visiting a site was personally identified and frequency and duration of water contact was noted. Retrospectively, cohort participants were identified in the notes and their data were entered in the computer and further processed. For the current study, data of 4 observation months, situated around the actual moment of praziquantel treatment, were analyzed.

**Immunoassays.** Specific antibody levels (IgG, IgG1, IgG3, IgG4, IgM, and IgE) against adult worm antigen (AWA) were determined in serum as previously described in detail. For the IgG1 assay, serum samples were diluted 1/2,000. Specific IgG2 and IgA were not measured because the previous study showed levels of these antibodies to be very low in this community. Briefly, plates were coated with AWA and then coated with bovine serum albumin (BSA) (except for IgE). Thereafter, sera were incubated in duplicate in a single dilution step. For each specific assay a standard serum was selected, which was assigned to contain 10\(^9\) arbitrary units/ml (AU/ml) of the isotype concerned. A duplicate dilution series of a standard serum was included on each plate. After incubation with specific anti-human immunoglobulin conjugates, serum reactivities were read against a standard curve and expressed as AU/ml. Plates coated only with BSA were used to determine nonspecific binding (except for IgE). For each antibody isotype assay, all samples were tested simultaneously and in random order. Sera from the same individual, collected before and after treatment, were always tested on the same plate. Between-plate variability was checked using reference sera. For each antibody isotype assay a cut-off level was determined by the mean plus 3 times the SD of the antibody activities of 20 sera from healthy Dutch blood donors tested at the same time as the Senegalese samples.

**Statistical analysis.** Egg counts, frequency and duration of water contact, and antibody levels were examined after log-transformation of the data because they approximated a log-normal distribution. Zero values were excluded from transformation. Data were described by the percentage of individuals positive for the various parameters and by the geometric means (gmean) plus 95% confidence intervals of the means (95% CIs).

Cure rates were calculated as the percentage of positive individuals becoming parasitologically negative after treatment. Percentages intensity reduction rate (IRR) were calculated as the [(gmean before treatment—gmean after treatment)/gmean before treatment] × 100 of those subjects still parasitologically positive after treatment. Associations between the various parameters were examined by Pearson’s correlation. Seasonal variation was analyzed by comparing data from different cohorts. The chi-square test, one-way analysis of variance, and 95% CI of means were used to compare groups. The effect of praziquantel treatment on antibody levels was examined by paired t-test. In addition, individual differences in pretreatment and post-treatment antibody levels were calculated for the various isotypes. Because of their highly skewed distribution, these differences were examined by nonparametric statistical methods. Statistical significance by any of the methods used was inferred as at least a \( P < 0.05 \).

**RESULTS**

**Total study population.** A total of 1,651 individuals were included in the 4 different cohorts, of which 1,423 cases (86%) were positive at parasitologic examination. Intensity of infection was extremely high with a mean egg excretion of 497 epg and 34% showing more than 1,000 eggs per gram of feces (epg). Cohorts 1 and 4 showed significantly higher pretreatment intensity of infection than cohorts 2 and 3. No other relevant or significant differences between the 4 cohorts were observed. A total of 1,204 of the parasitologically positive cases received treatment and 920 were followed-up at weeks 6–12. Compliance was highest in cohort 1 (86%) and lowest in cohort 2 (60%). Non-responders analysis showed no significant dissimilarity in age, gender, or intensity of infection between those who were followed and those who were not. This paper will further focus on these 920 treated and followed cases.

Table 1 summarizes the results of parasitologic examination before and after treatment. Complete cure was found in 261 cases (28%). In the remaining 659 cases, mean egg counts decreased from 668 epg to 88 epg (IRR = 87%). Compared with the first cohort, cure rates were found to be significantly higher in the 3 other cohorts (\( P < 0.001 \)), which had a shorter follow-up period. There was no significant difference in cure rates between the 3 cohorts themselves (\( P = 0.53 \)) and reduction in egg counts was comparable in all 4 groups (Table 1).

Table 2 shows treatment results of all 920 cases stratified by intensity of infection and age. Cure rates were found to be higher in adults than in children (\( P < 0.001 \)), even after...
correction for intensity of infection ($P < 0.02$). Reduction in egg counts was found to be the same for the two age groups (85% and 88%). Cure rate decreased linearly with increasing pretreatment intensity of infection, there was also an increasing percentage of reduction in egg counts. Limiting the analysis to individuals of cohorts 2–4 only resulted into exactly the same findings.

No gender-related variation in cure rates (26% and 31%) or reduction in egg counts (85% and 88%) was observed. A history of praziquantel treatment was reported in 213 cases. The mean egg count of this group was lower compared with the 666 cases without history of previous treatment (419 versus 575; $P = 0.01$). However, a history of treatment had no significant influence on the parasitologic cure rate (27% versus 29%) or the reduction in egg counts (84% versus 88%) following praziquantel treatment in this study.

Selected subpopulations. Figure 1 shows individual egg counts before and after treatment of the 4 subgroups showing extreme levels in the efficacy of praziquantel treatment. It is clearly seen from this figure that failure of treatment is not restricted only to the high egg excreters. Adults with failure of treatment even showed significantly lower egg counts before treatment compared with adults with complete cure ($P < 0.001$; Table 3). No significant differences were found between the low and high cure rate groups in gender or cohort distribution or in history of treatments. Data on age, egg counts, water contact behavior, and antibody levels are shown in Table 3. Adults with failure of treatment tend to be younger in age compared with adults with complete cure, but the difference was not significant.

Water contact was recorded during the observation period for 101 individuals. The missing 19 cases were equally divided among the 4 selected groups. Frequency and total duration of water contact were highly correlated ($r = 0.88$, $P < 0.001$) and showed no seasonal variation ($P > 0.85$). Water contact was much higher in females compared with males ($P < 0.001$), especially in those between 16 and 29 years old. Neither frequency or duration of water contact was correlated with egg counts ($P > 0.2$). Water contact was slightly less in the cured cases, but the difference was not significant (Table 3).

The percentage of samples showing specific antibody levels above the cut-off value was the highest for the IgG1 assay (92%), followed by IgG4 (85%), total IgG (82%), IgE (62%), IgM (50%), and IgG3 (35%). Levels of specific antibodies in serum collected before and after treatment were significantly correlated for all isotypes ($r > 0.26$, $P < 0.004$). No seasonal variation in antibody level was observed. Levels of IgG, IgG1 and IgG4 were correlated with pretreatment egg counts ($r > 0.23$, $P < 0.005$) and negatively correlated with age ($r > 0.19$, $P < 0.02$). IgG3 showed a positive association with age ($r > 0.16$, $P < 0.04$), but no correlation with egg counts. No such associations were apparent for IgM and IgE. Following praziquantel treatment, an overall significant increase in specific antibody level was seen for all isotypes except IgG1 and IgG3. No significant differences were found between cured and not cured cases in antibody levels, either in serum collected before or after treatment (Table 3) or in the change in antibody level caused by the treatment.

**DISCUSSION**

Praziquantel has been considered a safe and efficacious drug for the treatment of *S. mansoni* for many years, generally resulting in cure rates of 70–95%.$^9$ Therefore, much attention was paid to the initial finding that parasitologic cure rates were < 20% after treatment of the first cohort at Ndombo.$^2$ The low efficacy of praziquantel treatment in this

### Table 2

<table>
<thead>
<tr>
<th>Intensity before treatment</th>
<th>Children</th>
<th>Adults</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;401 epg</td>
<td>n % CR</td>
<td>N % CR</td>
<td>n % CR</td>
</tr>
<tr>
<td>401–1,000 epg</td>
<td>151</td>
<td>33</td>
<td>203</td>
</tr>
<tr>
<td>&gt;1,000 epg</td>
<td>81</td>
<td>16</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>459</td>
<td>20</td>
<td>461</td>
</tr>
</tbody>
</table>

* n = number examined; % CR = percentage cure rate; epg = eggs per gram of feces.
† Age ranging from 0 to 15 years (mean = 8 years).
‡ Age ranging from 16 to 89 years (mean = 35 years).

![Figure 1](image_url)  
**Figure 1.** Individual egg counts before and after praziquantel treatment in 4 groups of 30 subjects each. Groups were selected separately for children and adults based on either complete parasitologic cure or less than a 60% reduction in egg counts. For further details, see section on selected subpopulations. Epg = eggs per gram of feces; NC = not cured; C = cured. The small horizontal lines indicate median values, and the broken line shows the negative/positive cut-off value.
recently exposed community was confirmed in subsequent cohorts, as shown in this paper.

Several hypotheses have been put forward to explain these observations.² The possibility of the parasite strain being tolerant or having developed resistance to praziquantel has drawn particular attention.¹⁰,¹¹ Fallon and others observed diminished susceptibility to praziquantel in a schistosome isolate from Senegal, but this may partly be explained by a lower maturation rate of this strain.¹²,¹³ Field trials with oxamniquine resulted in normal cure rates, but so did repeated treatment with praziquantel.⁵ We do not yet consider the evidence as conclusive and continue both in vivo and in vitro studies.

No pharmacokinetic studies have been performed in patients from this focus. The praziquantel batches used were of unsuspectable quality and showed normal cure rates in experimental studies.² Poor bioavailability due to genetic or dietary factors cannot be excluded, but is difficult to study in a non-clinical setting. We observed similar cure rates during different seasons (Table 1), when food patterns of the population differ substantially.

In this study, we concentrated on the possible contribution of host-related factors. A first set of factors relates to the high levels of transmission and the recent nature of the focus. Both prevalence and intensity of infection, as indicated by pretreatment egg counts, were found to be extremely high in the community of Ndombo.¹ Most infected subjects harbor high worm burdens and are continuously exposed to new infections. Thus, the observed low cure rates could be explained by the possibility that many individuals carry prepatent infections not susceptible to praziquantel, and/or are rapidly reinfected after treatment. At follow-up, even shortly after treatment, these new infections may be diagnosed and identified as treatment failures. Indeed, cure rates improved when the follow-up period was shortened from 12 (cohort 1) to 6 weeks (cohorts 2–4). On the other hand, cure rates remained unusually low (< 40%) and were completely similar in the 3 subsequent cohorts (Table 1), despite variations in transmission intensity in the different seasons.¹ Analysis of individual water contact behavior could not relate failure of treatment to high exposure levels; no differences were found in frequency and duration of water contact between those who showed complete parasitologic cure and those who showed no effect of treatment (Table 3). Furthermore,
water contact was much higher in women, while there was no gender-related difference in the efficacy of treatment.

Cure rates were found to be clearly associated with intensity of infection, although failure of treatment was not limited to those individuals only with high egg excretion (Table 2 and Figure 1). In those not completely cured, the average reduction in egg counts was more than 80% (Table 1). Thus, it can safely be assumed that in this focus praziquantel still kills most, but not all, of the worms. In communities with high worm burdens such as Ndombo, even normal worm killing rates could be mistaken as failure of treatment: the (normal) proportion of worms surviving treatment is sufficient to maintain positive, albeit low, egg counts in most subjects. Unfortunately, not many post-treatment data are available for similar intense foci. A cure rate of 60% was reported 6 weeks after treatment of an equally heavily infected population in Maniema (former Zaire).14

We also found cure rates to be significantly lower in children compared with adults (Table 2), a phenomenon that has been previously described in other areas.9,14 There are no indications that this is due to different pharmacokinetics in children.15 Children have higher worm loads than adults, but the difference in cure rates was still significant after stratifying in different egg count groups (Table 2). However, the biologic significance of egg-count related cure rates should be interpreted with caution. Egg counts show important in-biologic significance of egg-count related cure rates should be interpreted with caution. Egg counts show important

Alternatively, intensity of infection can be determined by measuring schistosome antigens in the circulation, such as the adult worm–associated circulating anodic antigen (CAA).19 Serum levels of CAA have shown to be more stable than fecal egg counts and following successful treatment, CAA is cleared from the circulation within a period of 6 weeks.20,21 So far, only 10% of the treated subjects in cohort 1 and a small group of schoolchildren became CAA-negative 6–12 weeks after 40 mg of praziquantel treatment.2,3 This indicates actual cure rates to be even lower than the percentages found by parasitologic diagnosis.

Animal studies have shown the humoral immune system to be essential for optimal efficacy of praziquantel,22,23 but the possible extrapolation to the human situation is still unclear. In this study, we found drug efficacy to be similar in cohorts 2–4 (Table 1), while improvement of cure rates may have been expected if immune maturation would play a role. Previous treatments may boost the immune system with possible target antigens, but we found no differences in cure rates between those with or without previous praziquantel treatment.

We found no association between levels of specific antibodies and failure of praziquantel treatment (Table 3). We tested serum samples for different isotypes since experimental studies showed both the IgG- and non-IgG-containing serum fractions to be involved.22 Crude adult worm antigen preparations were used in our immunoassays as they include a whole range of tegumental components, including those identified as possible target antigens for immunopharmacologic synergy.23 It cannot be excluded that antibodies recognizing specific target epitopes are missed. However, antibody levels against the crude preparation significantly correlated with those against a recombinant preparation of the outer tegument antigen Sm22.6 (kindly provided by D. Dunne, Department of Pathology, Cambridge University, Cambridge, United Kingdom)

Similar to what has been previously published on subjects in cohort 1 only, we found levels of IgG, IgG1, and IgG4 to be correlated with pretreatment egg counts.6 Most isotypes showed an increase in absolute levels following praziquantel treatment, since release of antigens boosts the immune system. We found no consistent linear trend in antibody levels between the 4 cohorts, indicating an immunologically stable situation, in parallel with the parasitologic data.

In conclusion, of the several host-related factors evaluated in this study, only pretreatment egg counts and age were found to be associated with low cure rates. However, egg counts may be too crude to allow for the true impact of treatment on (heavy) worm burdens to be accurately evaluated, since they are only an indirect, statistically complex reflection of worm burden. Low and high egg counts may reflect different ranges of worm burdens in different endemic situations, or before and after treatment.16 The age and egg-count related cure rates may therefore be statistically, rather than biologically, relevant. The low cure rates as such cannot be attributed to other host-related factors, including under-developed pharmacosynergistic humoral immune responses, rapid reinfection, or prepatent infections.

Financial support: This study received support from the INCO-DC program “Scientific and Technological Cooperation with Developing Countries” of the European Commission, the Netherlands Organization for Scientific Research in the Tropics (NWO/WOTRO), and the ESPOIR program in St. Louis, Senegal.

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