Evaluation of the patterns of *Schistosoma mansoni* infection and re-infection in Senegal, from faecal egg counts and serum concentrations of circulating anodic antigen

K. POLMAN*,†, F. F. STELMA*,‡, S. LE CESSIE§, S. J. DE VLAS¶, S. T. M. FALCÃO FERREIRA*, I. TALLA**, A. M. DEELDER* and B. GRYSEELS†

**Department of Parasitology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
†Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium
‡Department of Epidemiology, University of Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands
§Department of Medical Statistics, Leiden University Medical Centre, P.O. Box 9604, 2300 RC Leiden, The Netherlands
¶Department of Public Health, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands
***Région Médicale de St Louis, B.P. 394, St Louis, Senegal*

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Infection and re-infection patterns were evaluated in a recent *Schistosoma mansoni* focus in northern Senegal, by determining concentrations of serum circulating anodic antigen (CAA), as a measure of worm burden, and counting eggs in faeces before, 6 or 12 weeks and 1 year after praziquantel treatment in two subsequent cohorts (cohort A and B). No differences in egg counts and CAA concentrations or their relationship were found between the cohorts, which were examined 2 years apart. Within both cohorts, CAA concentrations showed the same, typical, age-related patterns as egg counts, with a peak in children and a strong decline in adults. These trends were apparent both before and 1 year after treatment. The results indicate that an age-related resistance to infection and to re-infection has been firmly established, at a steady level, in the recent *S. mansoni* focus investigated, with no indication of a gradual development of immunity or anti-fecundity immunity over a period of 2 years.

Both shortly and 1 year after treatment, the decrease in egg counts was stronger than that in CAA concentrations, indicating that that there had been a reduction in worm fecundity after treatment. The possibility that praziquantel may induce anti-fecundity immunity has important implications for the use and interpretation of the results of (egg-count-based) re-infection studies designed to follow the development of naturally acquired immunity.

Re-infection studies are an important component of immuno–epidemiological research on the dynamics of schistosome infections. However, their intravascular localization means that adult schistosomes cannot be directly quantified in human populations. Worm burdens and their dynamics have to be assessed using indirect, quantitative, diagnostic methods, such as the counting of eggs in faeces. A major drawback of egg counts, however, is that they do not distinguish between worm load and worm fecundity. Infection and re-infection patterns based on egg counts generally show the counts peaking in children and then dropping dramatically

Reprint requests to: K. Polman, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. E-mail: kpolman@itg.be; fax: +32 3 2376731.

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into adulthood. The characteristically convex shape of age–intensity plots may reflect acquired or innate immunity. However, egg counts cannot show whether such immunity is directed against the cercariae, schistosomula and/or adult worms, or whether it reduces worm burdens and/or worm fecundity. Several vaccine candidates appear to have an impact not only on resistance to re-infection but also on the egg production of adult worms (Capron, 1998). Although those conducting vaccine trials need to be able to distinguish these two effects, they cannot do so just by counting eggs in faeces.

The detection of circulating anodic antigen (CAA) in serum is a sensitive, very specific and quantitative method for the diagnosis of *Schistosoma* infections (Deelder *et al.*, 1989, 1994). CAA is a glycoconjugate associated with the gut of the adult worm and released by the parasite into the circulation of the infected host. Generally, CAA concentrations correlate well with worm loads in laboratory animals and with repeated egg counts in humans, decreasing rapidly after successful treatment (Deelder *et al.*, 1994; Agnew *et al.*, 1995). For these reasons, determination of CAA, as a measure of worm burden, appears to be an interesting alternative to egg counts in the study of *S. mansoni* infection and re-infection patterns. There have already been a few studies in which CAA concentrations and egg counts have been investigated together, to assess both worm burden and worm fecundity in relation to age and infection intensity (Van Lieshout *et al.*, 1995, 1998; Polman *et al.*, 1995, 2000, 2001*a*, *b*; Agnew *et al.*, 1996).

In the present study, of *S. mansoni* re-infection in a recently exposed community in Senegal, CAA concentrations were determined and egg counts made. The aim was to compare the age-related distributions and concentrations of CAA and egg counts before and after treatment, and to examine whether and how their relationship changed over time, after treatment and/or during re-infection.

SUBJECTS AND METHODS

Data-sets

Data were collected in Ndombo, a recently established *S. mansoni* focus in northern Senegal. The area and the design of the study have already been described in detail (Stelma *et al.*, 1993; Gryseels *et al.*, 1994; Van Lieshout *et al.*, 1999). Briefly, every 8 months between August 1991 and September 1993, a cohort of approximately 400 subjects was randomly selected and surveyed, giving four separate cohorts. To exclude seasonal bias, the data analysed for the present study came from the first and last of these cohorts (here referred to as cohorts A and B, respectively), from which stool and blood samples were collected in the same season but 2 years apart. Faecal egg counts were based on duplicate, 25-mg, Kato smears produced from each of two stool samples, the second sample being collected 1–2 weeks after the first (Katz *et al.*, 1972; Polderman *et al.*, 1985). The total number of *S. mansoni* eggs in the four smears for each subject was multiplied by 10 to give the number of egg/g faeces (epg). CAA concentrations in serum were determined by ELISA (Deelder *et al.*, 1989; Polman *et al.*, 1995). Those who were found positive after parasitological examination were offered a single treatment of praziquantel in an oral dose of 40 mg/kg. The egg counts and CAA assays were repeated at week 12 (cohort A) or week 6 (cohort B), and again 1 year after treatment.

Data Analysis

For their data to be included in the analysis, a subject had to have been investigated fully (i.e. with four Kato smears and a CAA assay). This selection did not lead to any statistically significant change in age, sex, egg counts or CAA concentrations (compared with those of the full cohorts). Complete baseline data were available from 505 individuals, of whom 450 received treatment, 331 were investigated on week 6 or 12, and 169 were investigated 1 year after treatment.

Non-responder analysis showed no significant dissimilarity in age, sex or intensity of infection between those who were successfully followed-up and those who were not.

As they both showed skewed distributions, the CAA concentrations and egg counts were normalized by log transformation. To allow for zeros in the analyses, log-transformation was applied after adding a value equal to half of the detection limit of the particular assay to each count/concentration. Data were characterized as geometric means (GM), ranges and 95% confidence intervals (CI). Cure 'rates' were calculated, separately for the CAA assays and egg counts, as the percentages of positive individuals becoming negative after treatment. For those subjects still positive after treatment, intensity-reduction 'rates' (IRR) were also calculated separately for the CAA assays and egg counts, as the [(GM before treatment − GM after treatment)/GM before treatment] × 100. The associations between CAA concentrations and egg counts were examined by calculating Pearson's correlation coefficients (r).

For further comparison, the population was divided into four age-groups (0–9, 10–19, 20–39, and ⩾40 years) and regression analysis performed. [Previous studies on the relationships between egg counts and antigen concentrations, such as those of Van Lieshout *et al.* (1995, 1998), have used a Deming regression. This was not used in the present study, however, since the results of a recent study by Polman *et al.* (2001*a*) indicate that for CAA (in contrast to circulating cathodic antigen) an ordinary linear regression can be applied just as well.]

To check for any changes that may have occurred in the relationship between CAA concentrations and egg counts shortly after treatment (week 6 or 12) and/or 1 year after treatment, a linear mixed model, with age, sex, and cohort serving as covariates, was used, so that the repeated-measures nature of the data could be taken into account (Verbeke and Molenberghs, 1997). The relationship between egg counts and CAA concentrations at each time-point was described by the equation $\log(\text{epg}) = \alpha + \beta \log(\text{CAA})$, where α is the intercept and β the slope, or, equivalently, as the antilog: $\text{epg} = 10^{\alpha}(\text{CAA})^{\beta}$. Cases with negative results for both egg excretion and CAA were considered to be uninfected and excluded from the regression analysis. The exclusion of these 'double zeros' may introduce some bias, especially shortly after treatment when their number is highest. However, analysis of the data with inclusion of the 'double zeros' demonstrated that this bias was only minor and did not affect the main results.

RESULTS

The data collected before and after treatment are summarized for both cohorts in the Table. Before treatment, no significant differences were observed between cohort A and B in terms of age, sex or prevalence or intensity of infection (as determined either by egg counts or CAA assays). The cure 'rates' shortly after treatment with praziquantel were lower in cohort A (checked at week 12) than in cohort B (checked at week 6), whether based on egg counts (17% *v.* 25%) or detectable CAA (15% *v.* 39%). At the same times, the intensities of infection were found to be markedly lower than those recorded pre-treatment, with IRR based on egg counts of 87% for cohort A and 93% for cohort B, and corresponding IRR based on CAA concentrations of 75% and 91%, respectively. Although the intensities observed 1 year after treatment were higher than those recorded soon after treatment, they had not returned to their pre-treatment levels. As observed before treatment, there were no significant between-cohort differences in the prevalences or intensities of infection 1 year post-treatment, whether these were based on egg counts or CAA concentrations. When the data for both cohorts were pooled, significant correlations were observed between egg counts and CAA concentrations before treatment ($r = 0.64$; $P < 0.001$), 6–12 weeks

TABLE. *Summary of the egg-count and circulating-anodic-antigen (CAA) data collected before treatment, 6 (cohort B) or 12 weeks (cohort A) after treatment, and 1 year after treatment. The two cohorts were enrolled in the same season but 2 years apart: cohort A in August 1991 and cohort B in September 1993*

Time-point	Cohort A	Cohort B	Both cohorts	*P*
BEFORE TREATMENT				
No. of subjects	223	227	450	–
% egg-positive	100	100	100	>0.05
Geometric mean egg count (eggs/g)*	738.7	868.8	807.8	>0.05
% CAA-positive	87.9	88.5	88.2	>0.05
Geometric mean CAA concentration (ng/ml serum)*	5.63	6.71	6.10	>0.05
SHORT-TERM FOLLOW-UP				
No. of subjects	196	135	331	–
% egg-positive	83.7	74.8	80.1	0.047
Geometric mean egg count (eggs/g)*	67.1	36.7	52.5	0.006
% CAA-positive	83.2	55.6	71.9	<0.0001
Geometric mean CAA concentration (ng/ml serum)*	1.92	0.491	1.11	<0.0001
ONE-YEAR FOLLOW-UP				
No. of subjects	130	39	169	–
% egg-positive	90.0	84.6	88.8	>0.05
Geometric mean egg count (eggs/g)*	141.5	137.4	139.5	>0.05
% CAA-positive	70.8	66.7	69.8	>0.05
Geometric mean CAA concentration (ng/ml serum)*	1.49	1.25	1.42	>0.05

*Geometric means were calculated for all of the subjects, including those who had zero egg counts and/or non-detectable CAA concentrations.

after treatment ($r = 0.60$; $P < 0.001$) and 1 year after treatment ($r = 0.62$; $P < 0.001$).

The age-related intensities of infection before and after treatment and of re-infection are shown in Figures 1 and 2. Both cohorts showed the characteristic age-related pattern of egg counts whenever they were sampled, with peak intensity in childhood. Similarly, CAA concentrations at all three time-points showed a rapid increase in childhood (peaking between the ages of 5–20 years) followed by a decline in adulthood.

For a more detailed analysis of the relationship between egg counts and CAA concentrations and of possible changes in this relationship shortly after treatment or during re-infection, a linear mixed model was fitted. In this model, log(epg) was the dependent variable, subject number the random factor, time the fixed factor, log(CAA) the covariate, and log(CAA) and time the interaction. Treatment and follow-up had a clear effect on the relationship, with significant differences between the intercepts for the data collected before, 6–12 weeks after and 1 year after treatment ($P < 0.0001$). The interaction between log(CAA) and time was not significant, indicating that the slope of the regression line of log(CAA) on log(epg) did not differ significantly between the three time-points. Similarly, adding cohort ($P = 0.22$), age ($P = 0.29$) or sex ($P = 0.73$) to the model did not have a significant effect on the relationship between egg counts and CAA concentrations across the three time-points. Figure 3 shows the scattergrams of the individual egg counts and CAA concentrations for each cohort at all three time-points, and also the regression lines of the relationship between log(CAA) and log(epg) at each time-point. For each of the cohorts, the levels of the three regression lines differed considerably, with the highest line representing the relationship between egg counts and CAA concentrations before treatment, the lowest for the relationship 6–12 weeks after treatment, and the intermediate line for the relationship 1 year after treatment. The

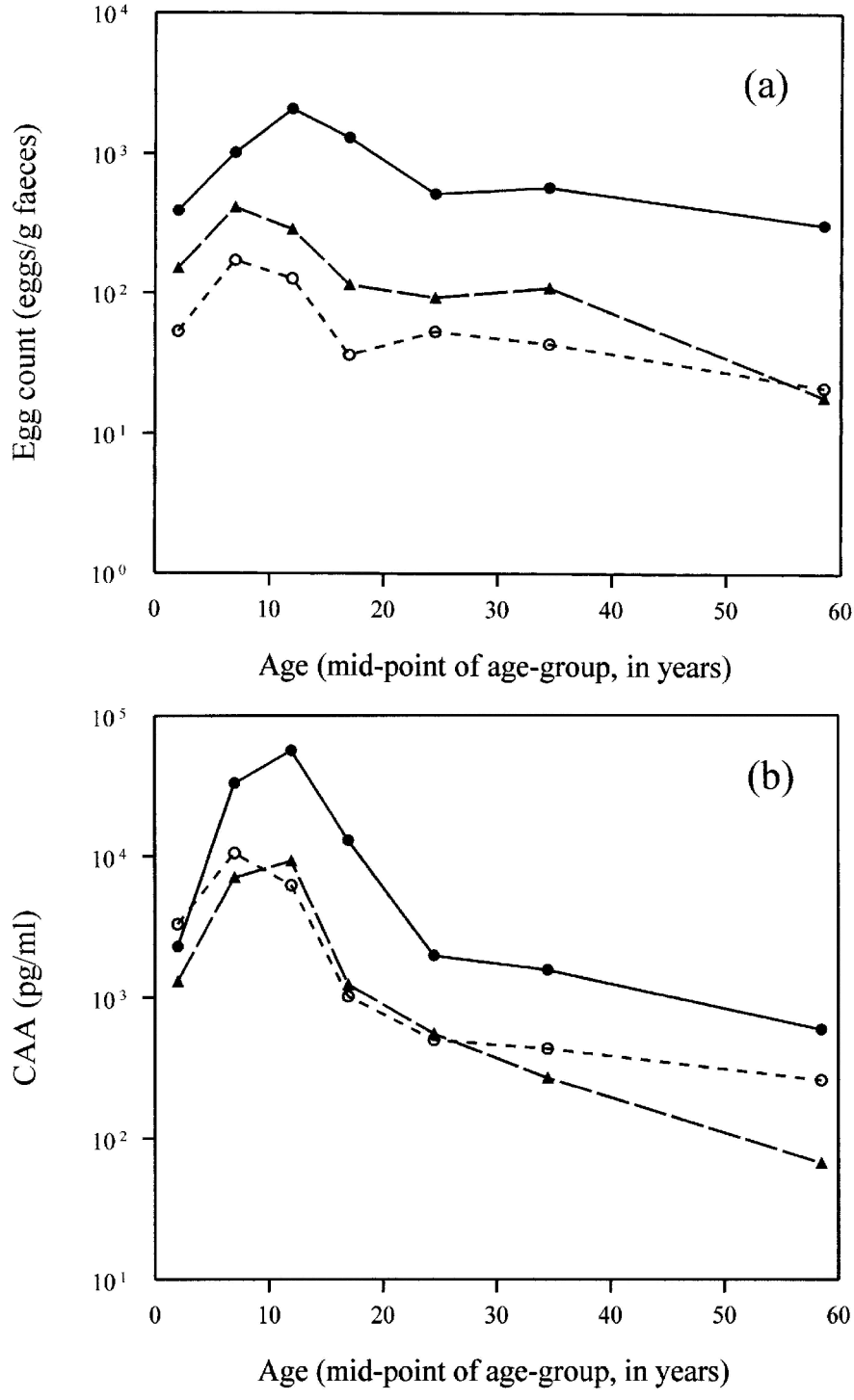

FIG. 1. Age–intensity plots for infection with *Schistosoma mansoni* in cohort A, as measured before treatment (●), 12 weeks after treatment (○), and 1 year after treatment with praziquantel (▲). Intensity was measured both as egg excretion (a) and as serum concentrations of circulating anodic antigen (CAA) (b). The age-groups were 0–4, 5–9, 10–14, 15–19, 20–29, 30–39 and ⩾40 years.

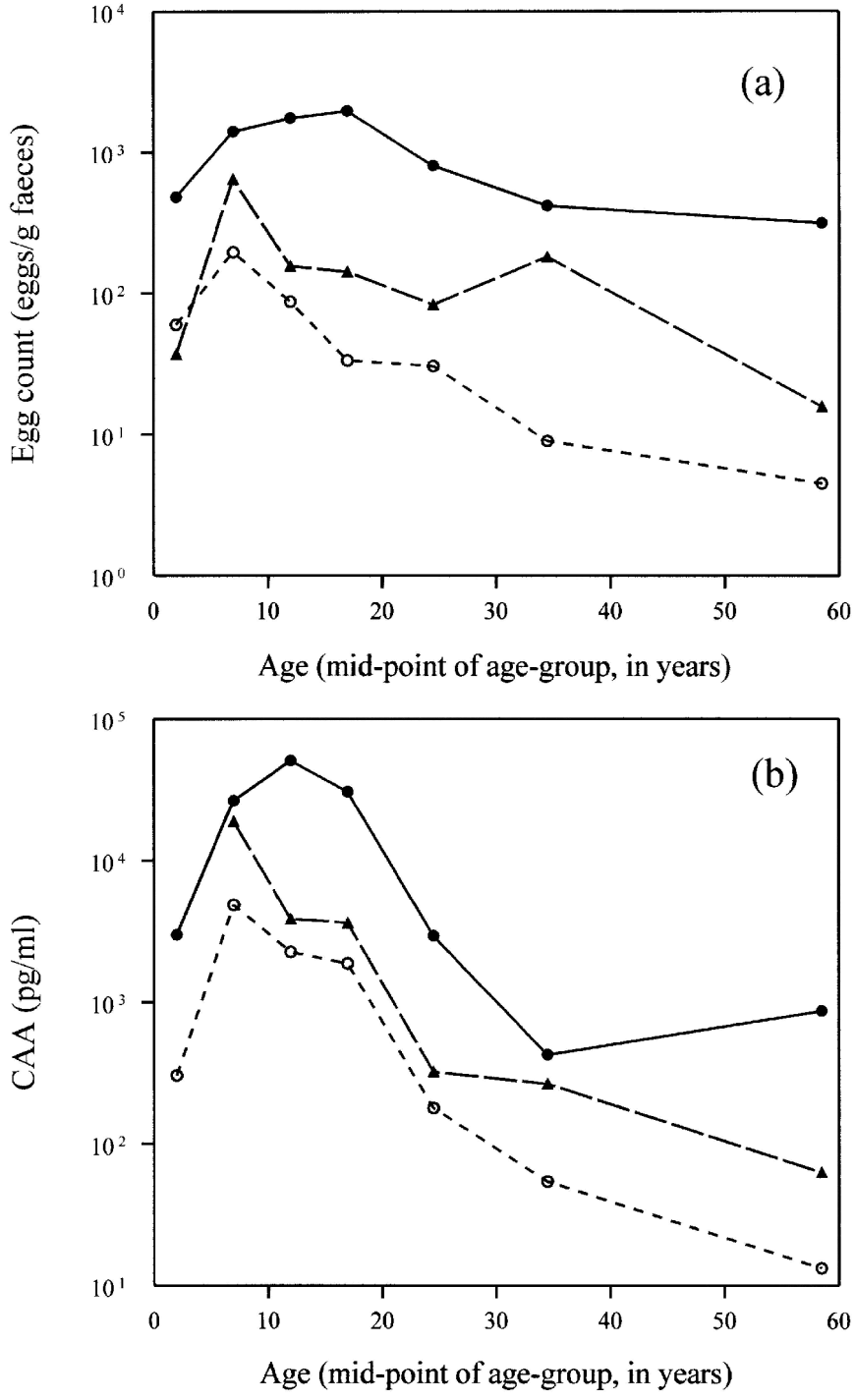

FIG. 2. Age–intensity plots for infection with *Schistosoma mansoni* in cohort B, as measured before treatment (●), 6 weeks after treatment (○), and 1 year after treatment (▲) with praziquantel. Intensity was measured both as egg excretion (a) and as serum concentrations of circulating anodic antigen (CAA) (b). The age-groups were 0–4, 5–9, 10–14, 15–19, 20–29, 30–39 and ⩾40 years.

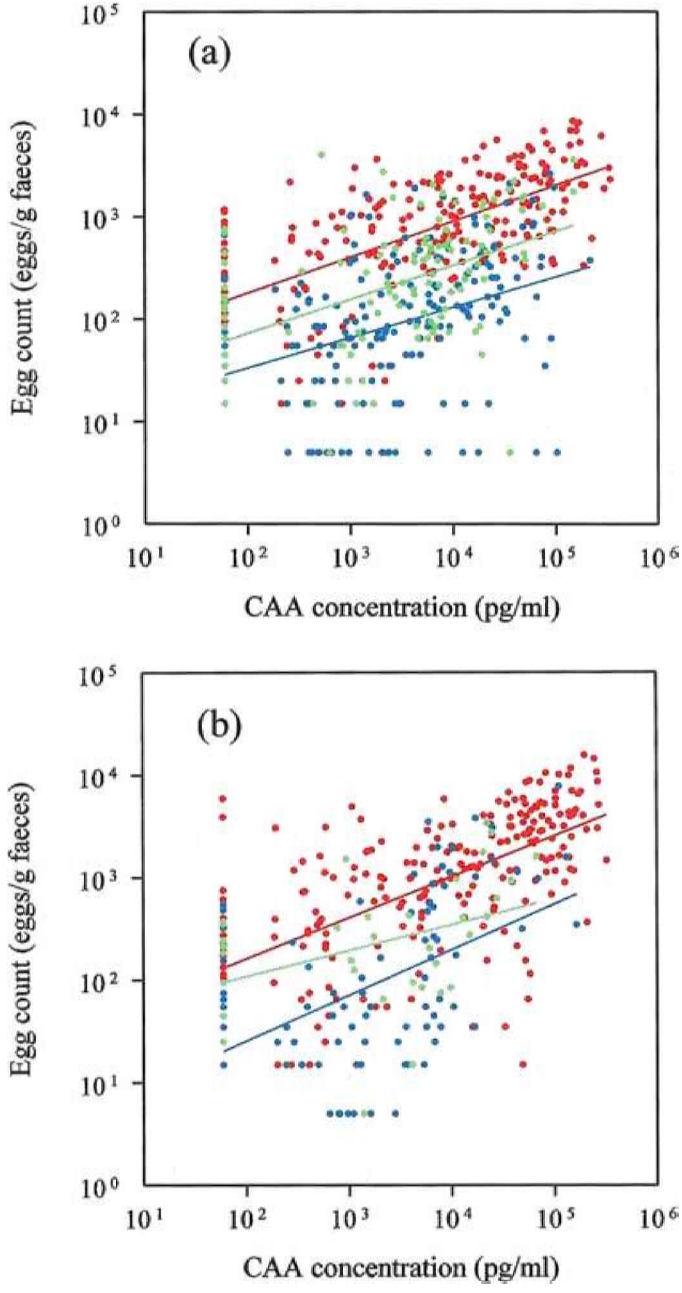

FIG. 3. Faecal egg counts plotted on a log–log scale against serum concentrations of circulating anodic antigen (CAA), as measured in cohorts A (a) and B (b) before treatment (●), 12 (cohort A) or 6 (cohort B) weeks after treatment (●), and 1 year after treatment (●). The lines running through the scatterplots represent the outcome of regression analysis of the relationships between log(CAA concentration) and log(egg count), given by log(egg count) = $\alpha + \beta$ log(CAA concentration), before (—), shortly after (—) and 1 year after treatment (—). For cohort A, the values of the intercepts (α) and slopes (β) and (their 95% confidence intervals) were, respectively, 1.6 (0.2) and 0.35 (0.05) before treatment ($N = 223$), 0.9 (0.4) and 0.30 (0.10) 12 weeks after treatment ($N = 183$), and 1.2 (0.4) and 0.33 (0.10) 1 year after treatment ($N = 119$). For cohort B, they were, respectively, 1.4 (0.3) and 0.40 (0.06) before treatment ($N = 227$), 0.5 (0.4) and 0.44 (0.12) 6 weeks after treatment ($N = 109$), and 1.5 (0.7) and 0.25 (0.21) 1 year after treatment ($N = 34$).

decrease seen in egg counts after treatment was thus stronger than that seen in CAA concentrations; this effect was largest shortly after treatment and became smaller with longer follow-up.

DISCUSSION

In the present study, CAA concentrations were related to egg counts before and after treatment. The main aim was to determine whether the patterns seen in age-related infection, cure and re-infection were entirely the result of variations in worm load or if variations in worm fecundity were also contributing to the patterns.

No differences in egg counts or CAA concentrations (or in their relationship) were found between cohort A and cohort B, although the members of cohort A were enrolled 2 years before those of cohort B. Within both cohorts, CAA concentrations correlated strongly with egg counts, and the antigen concentrations and egg counts showed similar age-related distributions: low intensities in young children, rising to a peak in older children and young adults and declining to low levels in older adults. This trend was apparent both before and 1 year after treatment (i.e. for infection and post-treatment re-infection). The convex shape of the age–intensity plots (Figs 1 and 2) is typical of *S. mansoni* infection in endemic areas. It has been seen in pre-treatment data from Ndombo before, in spite of the continued exposure and assumed absence of acquired immunity in adults in this recent focus (Stelma *et al.*, 1993; Gryseels *et al.*, 1994; Polman *et al.*, 1995, 2001*b*). The present re-infection data, from egg counts and CAA concentrations, support the view that adults have a strongly reduced susceptibility to schistosome infection. The similarity between the present (re)infection data from cohort A and those from cohort B indicates that the level of this resistance remained unchanged over a 2-year period. The consistent relationship between (re)infection egg counts and CAA concentrations across both cohorts gives no indication that anti-fecundity immunity has developed in the study population. Taken together, the present results indicate that, during the period of the study, an age-related resistance to infection (and re-infection) was firmly established, at a steady level, in Ndombo. Since the study area was then a recent focus of intestinal schistosomiasis, immunity acquired through a prolonged history of exposure cannot explain the patterns observed. The age-related patterns must be the result of other mechanisms, such as the unusually quick development of acquired immunity, perhaps as a result of the extremely high levels of transmission in this focus (Woolhouse and Hagan, 1999; Mutapi, 2001), or other, possibly innate, mechanisms of resistance (Butterworth, 1994; Gryseels, 1994).

Shortly after treatment, the egg-count results were generally confirmed by the results of the CAA assays. With both measures, week-6 cure 'rates' (cohort B) were higher than the week-12 'rates' (cohort A), although all of them were lower than usually reported. The low efficacy of praziquantel in the recently exposed community of Ndombo has been extensively reported and evaluated elsewhere (Gryseels *et al.*, 1994, 2001; Stelma *et al.*, 1995, 1997; Guissé *et al.*, 1997; Cioli, 1998; Van Lieshout *et al.*, 1999); it has largely been attributed to the high initial worm loads and the intense level of transmission in this focus (Gryseels *et al.*, 2001).

Although, at each corresponding time-point, the relationship between egg counts and CAA concentrations was similar in cohorts A and B, it changed considerably between time-points. Both shortly and 1 year after treatment, the intercept of the regression line relating egg counts with CAA concentrations was significantly lower than that before treatment, indicating a lower egg production at any given CAA concentration and thus a reduction in worm fecundity after treatment. Praziquantel treatment is known to induce changes in the host's specific antibody responses to antigens from both the adult

schistosomes and their eggs (Grogan *et al.*, 1996; Mutapi *et al.*, 1998*a*, *b*). The effects of these changes on schistosomes and/or their fecundity, and how they relate to levels of re-infection, are not yet clear. The data collected 1 year post-treatment during the present study may indicate the existence of a chemotherapy-induced anti-fecundity immunity. Kremsner *et al.* (1994) collected data that 'point in the same direction'; in a longitudinal study in Cameroon, they found that the median egg output observed 1 year post-treatment was only 7% of that documented before treatment, whereas the median CCA concentrations in urine and sera were then 39% and almost 100% of their pre-treatment values, respectively. Although praziquantel may be inducing anti-fecundity immunity, alternative explanations for the temporal changes in the relationship between egg output and CAA concentration should also be considered. It may be that praziquantel kills only a certain proportion of the worms, but temporarily paralyses or otherwise adversely affects the metabolism of the others, resulting in a reduced egg production. During the year after treatment, the egg production of the worms may slowly recover. If this were the case, however, a simultaneous reduction and subsequent slow increase in CAA production might be expected, as CAA excretion is also an indication of worm metabolism. The temporal changes seen, in the present study, in the relationship between egg output and CAA concentration do not seem to fit this hypothesis. Another possibility is that, after treatment, most individuals carry immature or relatively young worms (because they had non-susceptible, prepatent infections when treated or were rapidly re-infected in the intense focus in which they live). The apparent reduction in worm fecundity post-treatment would then be the result of the immaturity of the worms and not of any anti-fecundity immunity.

The results of a recent study indicate that — particularly for low or undetectable antigen concentrations — the relationship between (measured) CAA concentration and worm burden in human *S. mansoni* infections may not be as straightforward as usually assumed (Polman *et al.*, 2000). Although the underlying processes and their relative impact on the estimation of CAA concentrations are still unknown, they may interfere with the observed relationship between egg counts and CAA concentrations, especially during short-term follow-up (such as the checks, in the present study, at 6–12 weeks post-treatment), when most subjects have low or undetectable levels of egg excretion and CAA. Further research on these mechanisms and their effects is needed.

Although it is clear that CAA detection can provide important supplementary information on the dynamics of worm loads in schistosome infections, these dynamics, and in particular the role of immunity, are still unclear. The possibility that praziquantel may induce anti-fecundity immunity has important implications and calls for caution when interpreting egg-count results from re-infection studies, particularly those designed to explore the nature and development of naturally acquired immune responses. It is recommended that CAA detection is included in immuno–epidemiological treatment/re-infection studies, to allow chemotherapy-induced worm reduction to be distinguished from reductions in fecundity.

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