Exposure, infection and immune responses to *Schistosoma haematobium* in young children

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SUMMARY

Behavioural, parasitological and immunological data were obtained from 48 children up to 6 years old, resident in a *Schistosoma haematobium* endemic area in Zimbabwe. The children averaged more than 1 contact with infective water bodies every 3 days and all showed immunological evidence of exposure (an anti-cercarial and/or anti-egg antibody response). IgM was the dominant isotype and appeared in the youngest children, followed by IgA, IgE and IgG3. However, only 38 children showed evidence of infection (an anti-egg response or eggs in urine) and only 14 were excreting eggs. The best estimates from these data are that less than 1 in 100 contacts result in infection and less than 1 in 1000 result in egg output. This suggests that there may be substantial attrition of invading cercaria even in naïve individuals.

Key words: antibody, cercaria, epidemiology, schistosomiasis, water contact, Zimbabwe.

INTRODUCTION

Despite numerous field studies of human infection with schistosomes, the precise relationships between contact with potentially infective water bodies, exposure to cercariae, the development of mature worms and the excretion of eggs remain poorly understood. Various field studies of endemic *Schistosoma haematobium* infection in communities in Zimbabwe have demonstrated high transmission rates – as indicated by observed rates of water contact, densities of infected intermediate host snails, densities of cercariae, infection rates in sentinel animals or rates of human reinfection following chemotherapy (Chandiwana, 1987; Chandiwana, Woolhouse & Bradley, 1991; Mutapi *et al.* 1999). These studies did not concentrate on young children but the available information indicates that, although children less than 6 years old frequently have high water contact rates, their prevalence of infection is typically of the order of 25%. The question of how apparently heavily exposed children avoid early infection with *S. haematobium* has never been quantitatively addressed. Here, a detailed study of water contact, exposure, infection, egg excretion and immune responses is presented for young children resident in a community where *S. haematobium* infection is endemic. The data are used to derive first estimates of the probability of schistosome infection per water contact.

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MATERIALS AND METHODS

Study site

The study was carried out in March 1997 among the children from a community of farm workers at Nyamikari and Sunnyvale farms in the Burma Valley district of eastern Zimbabwe. *S. haematobium* infection is endemic in this area and a high prevalence of infection had previously been reported in this community (Chandiwana *et al.* 1991).

Sample collection

Children below school age (i.e. up to 6 years old) who were lifelong residents of the farms were enrolled in the study with the informed consent of their mothers. A total of 84 children presented. These were required to provide at least two 10 ml urine specimens (most provided 4), 1 stool specimen and a 1–2 ml blood sample. After sample collection all presenting children were offered antihelminthic treatment with praziquantel at 40 mg/kg body weight.

Urine specimens were examined immediately by filtration through a nitrocellulose filter and *S. haematobium* eggs were enumerated using a dissec-

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ting microscope (Mott, 1983). Stool specimens were examined using the Kato–Katz method (Katz, Chaves & Pellegrino, 1972); 2 children excreting other helminth eggs were excluded from the study to avoid possible cross-reactivity in serological assays. Blood samples were allowed to clot at room temperature and stored at 4 °C overnight. After removal of the clot, sera were centrifuged at 3000 rpm for 10 min and stored in Nunc cryotubes at –20 °C.

Water contact

Water contact observations were carried out over a 1 month period in March/April 1997. Trained and supervised observers were placed at each of the sites used by the community daily between 10.00 and 18.00 hours. The observers recorded details of all contacts by children up to 6 years old: identity of the child, activity (e.g. playing, being washed), time of contact and duration of contact (Chandiwana & Woolhouse, 1991).

The presence of intermediate host snails was confirmed at each site although no quantitative surveys of snail abundance or cercarial shedding were carried out. Transmission at this site is known to be seasonal and to vary in intensity between sites (Chandiwana *et al*. 1991 and other unpublished data).

Immunological assays

The sera were tested using ELISA assays for IgA, IgE, IgG1, IgG2, IgG3, IgG4 and IgM responses directed against cercarial (CAP), soluble worm (SWA) and soluble egg (SEA) antigens. All monoclonal antibodies were horseradish peroxidase (HRP) conjugated. IgA, IgG and IgM were from Sigma, IgE from Dako, and IgG subclasses from Serotec. For all ELISAs, optimum dilutions for antigen, sera and monoclonal antibodies were determined after a series of antibody titrations. For ELISAs, Imulon 4 plates (Dynatec Ltd) were coated with 100 μl of antigen at 5 μg/ml (for CAP and SWA) or 10 μg/ml (for SEA) diluted in carbonate–bicarbonate buffer (pH 9·6) per well and left overnight at 4 °C. All plates except those for IgE were emptied and blocked with 200 μl per well of 5% skimmed milk for 1 h at room temperature and then washed 3 times.

For ELISAs against CAP, after washing the plates, 100 μl of the sera were plated out in duplicate at the following dilutions, 1:100 for IgA, 1:200 for IgM and IgG4, 1:40 for IgE, IgG1 and IgG3, and 1:20 for IgG2. All plates were incubated for 2 h at room temperature (overnight for IgE plates at 4 °C), washed 3 times and 100 μl of monoclonal antibody added at the following dilutions; 1:2000 for IgA, IgM, IgE, IgG2 and IgG3, 1:400 for IgG1 and 1:4000 for IgG4. Plates were incubated for 1 h at room temperature (overnight for IgE plates at 4 °C), washed out 6 times and 100 μl of OPD in phosphate citrate buffer (pH 5) were added. The reaction was allowed to take place in the dark for 15 min before being stopped with 25 μl of 10% H_2SO_4. The absorbance was read at 492 nm.

For ELISAs against SEA, the sera dilutions were 1:200 for IgA and IgM, 1:40 for IgE, IgG2, IgG3 and IgG4, and 1:20 for IgG1. The monoclonal antibody dilutions were 1:4000 for IgA, 1:400 for IgG1, and 1:2000 for all other isotypes. For ELISAs against SWA, sera dilutions were 1:200 for IgA, IgM, IgG2 and IgG3, 1:40 for IgE, 1:20 for IgG1, and 1:100 for IgG4. Monoclonal antibody dilutions were 1:1000 for IgA, IgG2 and IgG4, 1:2000 for IgM, 1:4000 for IgE and IgG3, and 1:200 for IgG1.

All dilutions and washes were carried out using PBS (pH 7·4) unless otherwise stated. On all plates for the 3 antigens, 4 standards were run (also in duplicate), a positive control, made from a pool of 10 sera from people presenting the highest egg counts across the age range, a negative control, made from a pool of sera from 10 British children below the age of 6, a negative control made from an immunocompromised British adult who did not produce antibodies and a blank control containing no sera. The ELISA reader was zeroed using the blank control.

Data analysis

For every child meeting the inclusion criteria, data were available on water contact behaviour, parasitology and antibody responses as well as census information.

Raw data on antibody responses were expressed as absorbances at 492 nm. Children were categorized as responders or non-responders where a responder was defined as having an absorbance more than 2 standard deviations above the mean estimated from a pool of 10 sera from the negative controls (Fig. 1).

Each child was also categorized as exposed or unexposed, infected or uninfected and egg positive or egg negative. Exposure was defined as either a positive anti-CAP response for any isotype and/or a positive anti-SEA response for any isotype and/or the presence of urine eggs. Infection was defined as a positive anti-SEA response for any isotype and/or the presence of urine eggs. Egg positive was defined as the presence of eggs in at least 1 urine sample. (Note that anti-SWA responses were not considered in defining these categories; see below.)

Data on exposure, infection, urine eggs, water contacts and age were related using a set of mathematical models. Using urine eggs as an example the model has the form

$$P_i = 1 - (1-q)^{(1-w)\,ma_i + wc_i a_i},$$

where P_i is the probability that child i is egg positive, q is the probability that a single exposure

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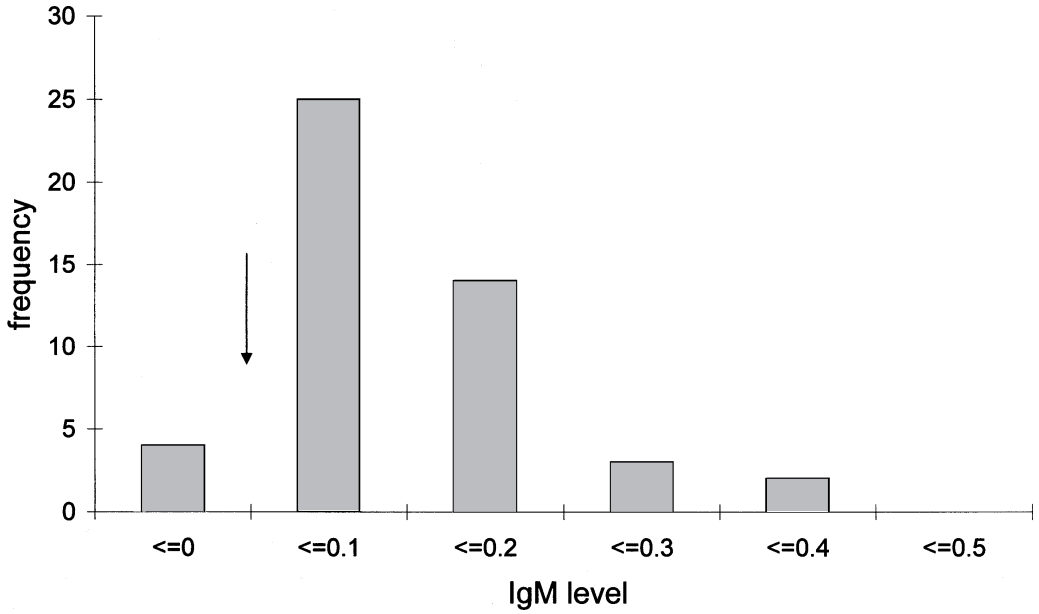

Fig. 1. Frequency distribution of anti-CAP IgM levels as absorbancies at 492 nm relative to 2 standard deviations above the mean absorbancy of negative controls (see text) so that values greater than zero (cut-off shown by the arrow) correspond to a response ($n=48$).

leads to an egg positive infection, a_i is the age of child i (scaled as age minus 3 months to allow for worm maturation), m is the mean rate of water contact, c_i is the observed rate of water contact by child i, and w weights the contributions of individual contact rates and average contact rates to life-long cumulative exposure. For exposure or infection P_i and q are redefined accordingly. The models assume no loss of anti-CAP responses (consistent with frequent boosting of the response – see below), or of anti-SEA responses or egg output (consistent with a life-expectancy for schistosomes of 3–6 years; Fulford *et al.* 1995). The models also ignore maternal antibodies, although these are unlikely to have been present in any but the very youngest children. The models also ignore any acquired immunity, which is not generally considered to have developed in such young children (Hagan, Ndhlovu & Dunne, 1998).

The models were fitted using maximum likelihood to data for individual children. The dependent variable was P_i and the independent variables were m, a_i and c. Fitted parameters were q and w. Goodness of fit and 95% confidence limits (CLs) on parameter estimates were obtained from χ^2 values as -2(log maximum likelihood). This analysis was done using the 'Solver' function in Microsoft Excel; all other statistical analyses were performed using the SAS or SPSS packages.

RESULTS

A total of 48 children met the inclusion criteria, the main reasons for exclusion were that the children were not lifelong residents or failure to obtain a blood sample. The youngest was aged 4 months, the oldest 68 months.

Water contact

There was no change in contact rate with age up to 5 years old (linear regression with $\log(x+1)$ transformed number of contacts: $F_{1,40}=2{\cdot}8$, $P=0{\cdot}10$). The mean observed contact rate for children in this age range was 11·6 contacts per month but the variance was large with 1 child making 72 contacts (Fig. 2A). There was a good correlation between total number and total duration of contacts ($R^2=0{\cdot}96$, $P<0{\cdot}001$) which was robust to the inclusion of age as a covariate (Fig. 2B). The average duration of contact was 5·1 min. There were no clear patterns of variation in either the type of activity (the main activity was playing) nor the time of day of contact. Overall, number of contacts was considered a fair index of exposure for these children. Contact rates among children over 5 years old were higher but were not considered relevant to the present analysis which is concerned with lifelong cumulative exposure.

Parasitology

Thirty-three of the children provided 4 urine specimens, 11 provided 3 and 4 provided 2; there was no clear relationship between number of specimens and probability of detecting urine eggs ($\chi^2_{(2)}=3{\cdot}78$, $P=0{\cdot}15$). The overall prevalence of urine eggs was 29% and prevalence increased with age, though this was not statistically significant (logistic regression: Wald statistic 1·86, $P=0{\cdot}17$) (Fig. 3A). For further analysis the children were divided into 3 age groups: 0–24, 25–48 and 49–72 months.

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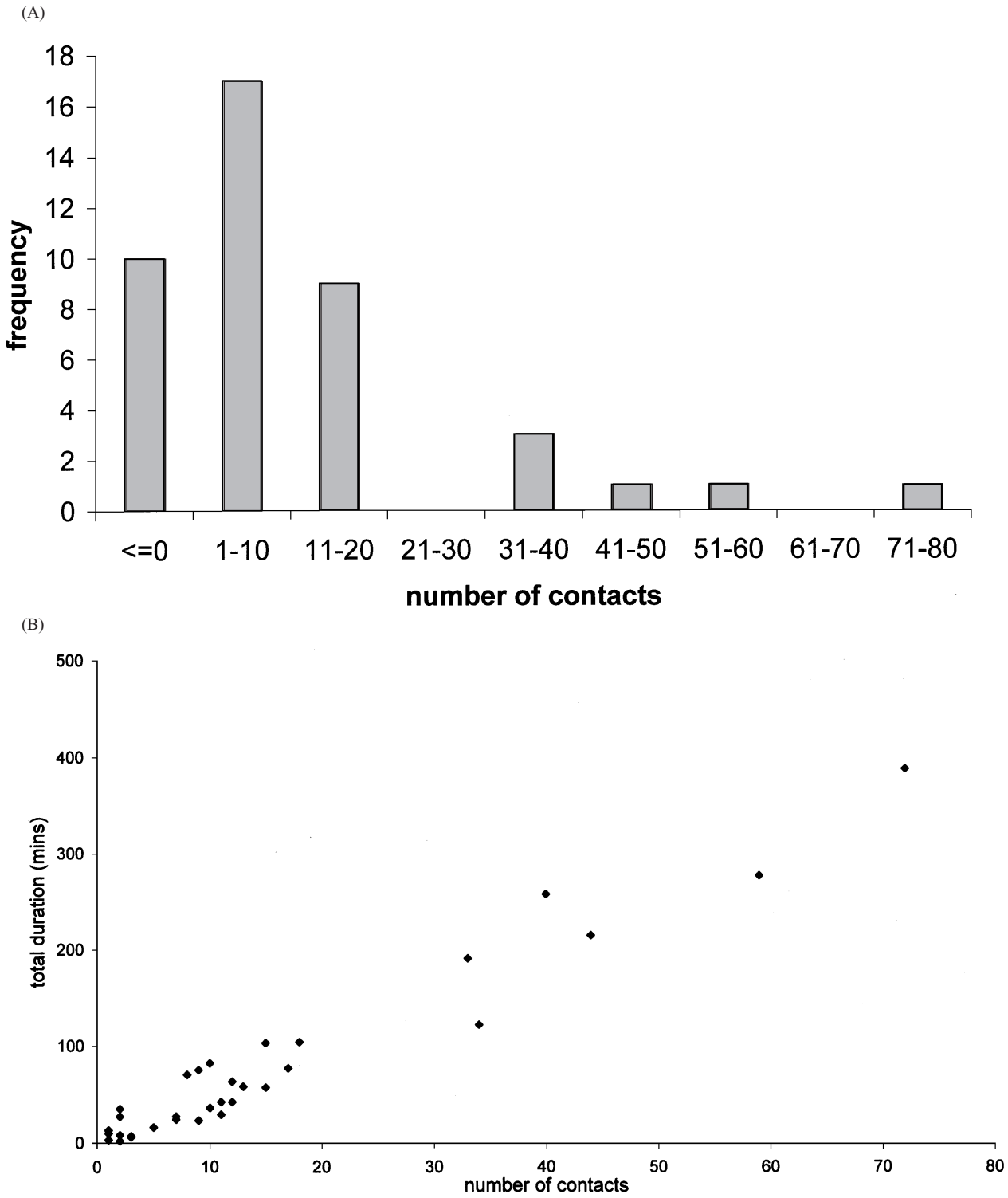

Fig. 2. Water contact patterns for children ⩽60 months old during the 1 month observation period. (A) Frequency distribution of number of contacts ($n=42$). (B) Relationship between number of contacts and net duration of contact for children making at least 1 contact ($n=32$).

Immune responses

The age profiles for anti-CAP and anti-SEA IgA, IgE, IgG3 and IgM antibody responders are shown in Fig. 4. Few or no children showed either anti-CAP or anti-SEA IgG1, IgG2, or IgG4 responses (not shown). Results for anti-SWA responses showed similar patterns to those for anti-SEA responses but were much more variable (as reported previously for other field studies; Mutapi *et al*. 1997) and, as cross-reactivity between responses to these antigens is expected, only anti-SEA results were analysed.

The dominant antibody was IgM and the fraction of children showing anti-CAP and anti-SEA IgM

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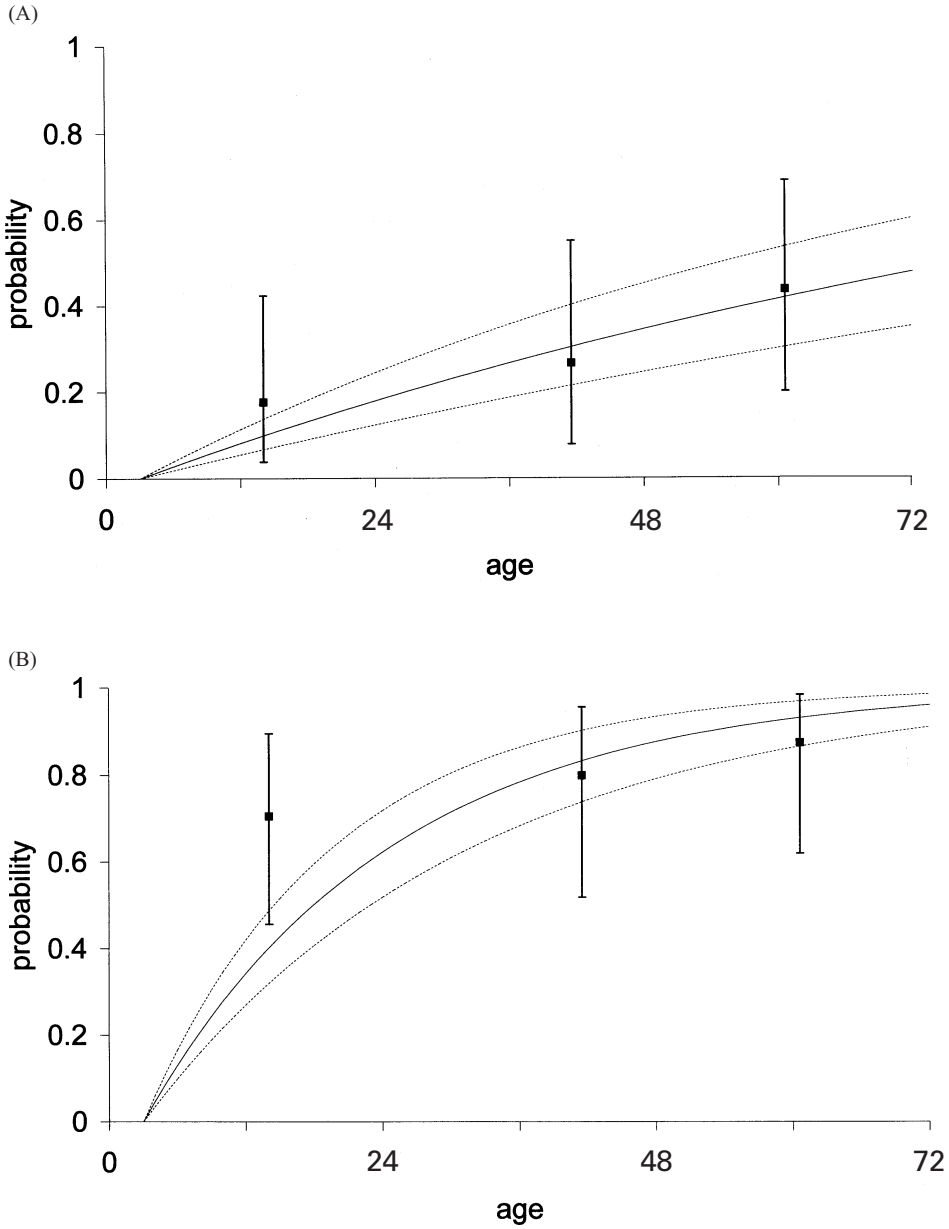

Fig. 3. Age–infection profiles. Children are placed in 3 age groups: 0–24 months ($n=17$); 25–48 months ($n=15$); 49–72 months ($n=16$). Prevalences of infection with 95% CLs (symbols and bars) are shown at the mean age (in months) for children in each age group, and model fits with 95% CLs (lines) are compared (see text). (A) Fraction egg positive (as eggs in 2–4 ten ml urine samples). (B) Fraction with mature worm infections (as an anti-SEA response and/or urine eggs).

responses appeared to increase with age (Fig. 4), although this was not statistically significant ($F_{1,47}=1{\cdot}70$, $P=0{\cdot}20$).

The age profiles for the anti-CAP and anti-SEA responses differed significantly for IgG3 (log-linear analysis: $\chi^2_{(2)}=7{\cdot}33$, $P=0{\cdot}026$) but not for any other isotype. For IgG3 there was an increase in anti-SEA responders with age but a decrease in anti-CAP (Fig. 4). The major isotypes differed in the relative frequencies of anti-CAP or anti-SEA responders ($\chi^2_{(3)}=12{\cdot}83$, $P=0{\cdot}005$); this effect was robust to age and was largely due to IgA, which alone has a higher fraction of anti-SEA than anti-CAP responders.

Exposure and infection profiles

All children showed evidence of exposure to infection: 46 had an anti-CAP antibody response and the other 2 had an anti-SEA response and/or urine eggs (implying a maximum 96% sensitivity for anti-CAP responses as a marker of exposure). The best estimate for the probability of exposure per water contact is therefore 1·0 although the lower 95% CL (using the estimate of w obtained below) is 0·027.

However, only 79% (38/48) of children showed evidence of infection: 37 had an anti-SEA antibody

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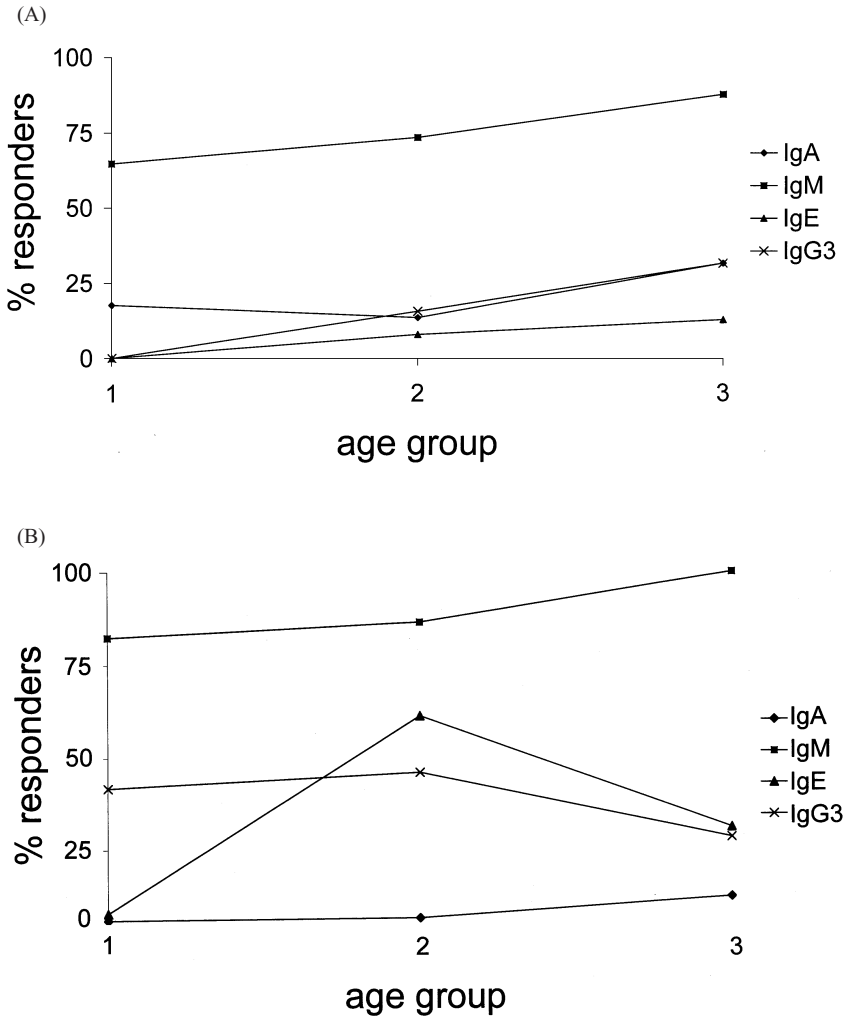

Fig. 4. Age–antibody profiles. Age classes are as defined for Fig. 3. Percentage responders are shown for specific IgA (◆), IgE (▲), IgG3 (×) and IgM (■). (A) Anti-SEA; (B) Anti-CAP.

response with 1 child having urine eggs but no detectable anti-SEA response (implying a maximum 97% sensitivity for anti-SEA responses as a marker of infection). This fraction increased slightly with age although it was already high in the 0 to 24-month-old class (Fig. 3B). Estimates of the probabilities of infection per contact and of an egg positive infection per contact were made using infection data and urine egg data but assuming a common value of w. This procedure gave best estimates (and 95% CLs) for the probability of infection per contact of 0·0079 (0·0036–0·0165) and for the probability of egg positive infection per contact of 0·00085 (0·00033–0·00185). The corresponding best estimate of w was 0·34 (0·04–0·66). The model was a satisfactory fit to both data sets grouped by age classes (infection data: $\chi^2_{(2)} = 2{\cdot}48$, $P = 0{\cdot}29$; urine egg data: $\chi^2_{(2)} = 2{\cdot}14$, $P = 0{\cdot}34$).

DISCUSSION

In this community young children typically accompany their mothers to water contact sites and rates of contact for these children are high, equivalent to an average of more than 1 contact every 3 days and to an average total of almost 700 contacts by 5 years of age. Despite this, the prevalence of egg positive *S. haematobium* infections in these children was low, the observed value of 29% being quite typical for 0 to 6-year-olds in this region.

How accumulated contacts are expected to translate into the prevalence of infection depends on the degree of predisposition (where predisposition is any characteristic – e.g. behavioural or physiological – of an individual that influences the likelihood of their acquiring or not acquiring an infection). This is because of the high variance in numbers of contacts recorded during a short observation period. If there in no predisposition then, in the long term, this variation averages out between children and the cumulative number of contacts will have a Poisson distribution. But if this variation is exactly reproduced from month to month (full predisposition) then even after several years some children will still have had very few contacts. Full predisposition therefore leads to a different age profile: the long-term prevalence will be lower but the rise in

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prevalence in the youngest children will be steeper. The parameter w, as defined here, is related to the degree of predisposition for contact rate, but it is likely that other factors contribute to predisposition, resulting in an underestimate of the convexity of the age profiles.

Regardless of the degree of predisposition there is clear evidence that the observed high contact rates do translate into high exposure rates. All children, from as young as 4 months old, showed evidence of exposure to infection as indicated by their specific antibody responses. The proportion of water contacts resulting in skin penetration by cercariae is very uncertain simply because of the high numbers of contacts involved. It could be as low as 1 in 37 contacts, reflecting both the brief average duration of contacts (approximately 5 min) and any spatial or temporal patchiness in cercarial densities. Clearly, further study of exposure to schistosome cercariae would have to concentrate on children under 12 months of age.

However, these high exposure rates do not lead to high infection rates. The best estimate is that only 1 in 127 contacts results in a mature worm infection (indicated by anti-SEA responses and/or urine eggs). This estimate will be influenced by imperfect sensitivity (leading to an under-estimate of the probability of infection per contact) and by imperfect specificity (leading to an over-estimate). Although sensitivity is apparently very high for egg-positive infections it cannot be estimated for true egg-negative infections and may be lower for such infections. Similarly, specificity cannot be estimated for either exposure or egg-negative infection.

There is also some evidence that contacts are even less likely to result in an egg-positive infection, the best estimate is 1 in 1176 contacts. This may be an under-estimate as some egg-positive infections may be missed during screening (low sensitivity), though this will have been too few to substantially affect the results. A more problematic complication is the possibility that egg-positive infections can result from the accumulation of single worms independently acquired. There is no good evidence from animal models that this occurs in practice (Basch, 1991) and it may be that near simultaneous infection is required to produce mated worm pairs. Even so, the infection model can be readily adapted to allow the possibility: this gives a revised estimate of the probability of any infection per contact of 0·0037 (0·0024–0·0055) but the model is no longer statistically a satisfactory fit to the data.

Patterns of exposure and infection are also reflected in the types of antibody responses found. Isotype responses to both cercariae and soluble egg antigens appear to develop in a definite sequence: IgM responses appear early, followed by IgA and then IgE and IgG3, with IgG1, IgG2 and IgG4 responses not appearing until later. Few children showed any deviation from this pattern. Some responses may be of short duration: there is some indication that anti-cercarial IgG3 (and possibly anti-cercarial IgE) responses have peaked by 25–48 months old while anti-egg responses for the same isotypes are still increasing. IgA responses appear to be directed more against mature than immature worms; this is consistent with this isotype being directed particularly against epitopes expressed on the schistosome egg. This sequence of responses is entirely consistent with isotype age-profiles reported for children and adults reported for a neighbouring community (Mutapi *et al*. 1997). In particular, an early IgA response but the absence of an early IgG1 response is consistent with the reported switch from a childhood IgA response to an adult IgG1 response (Mutapi *et al*. 1998). Beyond age 60 months there is an increase in water contact rate associated with independence from the mother (Chandiwana & Woolhouse, 1991), and a concomitant increase in the prevalence and intensity of infection with up to 90% of older children excreting schistosome eggs (Chandiwana *et al*. 1991; Mutapi *et al*. 1997). The subsequent fall off in egg output in older children or young adults is widely regarded as being at least partly due to acquired immunity (Hagan *et al*. 1998). The analyses described here assume that acquired immunity does not cause significant loss of worms or reduction in egg output in younger children. The goodness of fit of the models is consistent with this assumption.

Overall, the results are consistent with the view that a small fraction of exposures to cercariae result in mature worm infections. Attrition of cercariae has been reported for experimental infections using mice (30–50% cercariae surviving to adulthood; Dean, 1983), baboons (78%; Wilson *et al*. 1996) and pigs (1·5–30%; Willingham *et al*. 1996). In naïve human hosts the precise site(s) and mechanisms of attrition have yet to be defined. In this study, attrition may have been increased by any acquired immunity to cercariae, as occurs in mice (Dean, 1983), although this would still require that there was a low probability of a mature infection resulting from first exposure. The analysis also suggests that, further, only a small fraction of these infections result in egg excretion. This result can be explained because most are single-sex infections, because some worm pairs fail to produce eggs or because the eggs fail to be excreted.

The very low probability of infection per contact in a known high transmission area is of interest because cases of schistosomiasis in tourists indicate that it is possible for heavy infections to result from even a single exposure (Corachan *et al*. 1997). Whether or not this reflects differences in innate susceptibility to infection has yet to be established but it is important to note that in such studies the denominator is undefined as only exposures resulting

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in clinical infection are normally reported; it is possible that the risk per contact is as low as that reported here.

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