Analysis of Worm Burden Variation in Human *Schistosoma mansoni* Infections by Determination of Serum Levels of Circulating Anodic Antigen and Circulating Cathodic Antigen

Lisette Van Lieshout, Anton M. Polderman, Sake J. De Vlas, Paul De Caluwé, Frederik W. Krijger, Bruno Gryseels, and André M. Deelder

Department of Parasitology, University of Leiden, and Centre for Decision Sciences in Tropical Disease Control (CDTDC), Department of Public Health, Erasmus University Rotterdam, Netherlands; Service Médicale, Société Minière et Industrielle du Kivu, Kalima, Zaire

Serum circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) concentrations, as a possible direct measure of worm burden, were compared with fecal egg counts in a heavily *Schistosoma mansoni*–infected population from Zaire to allow differentiation between worm loads and worm fecundity in relation to age and intensity of infection. Of the 517 subjects, 95% excreted eggs and 97% demonstrated circulating antigens. Fecal egg counts showed an age-related pattern characteristic for an area in which schistosomiasis is endemic with intense transmission levels. Regression analysis showed that antigen concentrations were strongly associated with egg counts. For CAA, but not for CCA, this relation was found to be nonlinear, which would be consistent with density-dependent fecundity or crowding. The trend was uniform for all age groups, which for this particular population indicated a genuine reduction of worm loads rather than reduced worm fecundity with age of the host.

In most areas in which *Schistosoma* organisms are endemic, prevalences and intensities of infection are higher in children than in adults. This characteristic age-related egg count pattern can be explained by reduced exposure or the development of protective immunity in adults (or both). Such "acquired immunity" could be directed against incoming larvae but possibly also against fecundity of established worms [1–5].

Thus far, studies on infection levels in humans are mainly based on counting eggs in excreta. Because individual fecal egg counts show strong day-to-day fluctuations, repeated examinations are required to get reliable quantitative data [6]. However, egg counts do not allow differentiation of effects of changes in worm loads from those in worm fecundity. In addition, egg excretion itself can be influenced by numerous host- and parasite-related factors [7]. For example, on the basis of data from the few available postmortem studies on worm loads and egg counts, it has been suggested that egg production is depressed in hosts with high worm loads [8]. However, this phenomenon of density-dependent fecundity or crowding is still a point of debate [9, 10].

In this study, we assessed worm burdens by measuring serum concentrations of two schistosome-specific circulating antigens, circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) [11]. These gut-associated antigens are excreted in large quantities by the adult worms into the circulation of the host. Several experimental animal studies have shown a good correlation between worm loads and the levels of these antigens [12–14]. CAA and CCA can also be demonstrated in serum or urine of humans with *Schistosoma* infections by monoclonal antibody (MAb)–based sandwich ELISAs, showing a specificity of at least 98%, with sensitivities ranging from ~70% to 100% [15–18]. Several studies have proven the diagnostic value of these assays. Titers and concentrations are strongly correlated with egg counts and decline rapidly and strongly after successful chemotherapy [19–21]. However, most of these studies have been based on small or selected patient groups, which limits their epidemiologic interpretation.

In the present study, we investigated a large population across all age groups from an area where intense, year-round transmission of *Schistosoma mansoni* has been taking place for several decades. Regression analyses of antigen levels and egg counts were used to analyze worm burdens in relation to age, sex, and intensity of infection. Specific aims of the study were to determine whether circulating antigen levels, as a possible direct measure of worm burden, confirm the age-related pattern suggested by egg counts and to examine whether in the respective age groups the relation between circulating antigen (representing worm burdens) and egg counts provides evidence for density-dependent fecundity.

Received 7 February 1995; revised 14 June 1995.

Presented in part: III CEC/STD Meeting on Schistosomiasis Research, September 1993, Noordwijk/Leiden, Netherlands; IV International Symposium on Schistosomiasis, November 1993, Rio de Janeiro.

Samples have been collected in consultation and full cooperation with both the local authorities and the persons concerned. Persons showing *Schistosoma* egg counts were treated with praziquantel according to the protocol current at the time of sample collection.

Financial support: Research and Development Programme "Life Science and Technology for Developing Countries (STD 3)" (contract TS3-CT91-0040) of the European Communities.

Reprints or correspondence: Dr. L. Van Lieshout, Dept. of Parasitology, University of Leiden, P.O. Box 9605, 2300 RC Leiden, Netherlands.

The Journal of Infectious Diseases 1995; 172:1336–42

0022–1899/95/7205-0023$01.00

Materials and Methods

Study population. The serum samples and parasitologic data came from the population of eight mining villages in Maniema (Kivu Province, Eastern Zaire) and were collected between 1978 and 1980. The area and the local epidemiology of schistosomiasis mansoni has been described [22, 23]. Fecal egg counts, expressed as eggs per gram of feces (epg), were based on two stool examinations, with an interval of a few weeks, each consisting of a duplicate 25-mg Kato-Katz smear [22, 24]. Serum samples were collected from a random selection of the population and stored at −70°C until use. Children <1 year of age were excluded. CAA and CCA levels were determined in serum samples of 517 persons (262 male, 255 female) with a median age of 30 years (range, 1–66).

Determination of circulating antigens. Sera were pretreated with trichloroacetic acid (TCA) to dissociate immune complexes and to remove interfering proteins [25]. CAA and CCA concentrations were determined by time-resolved immunofluorometric assays (TR-IFMAs), based on the highly specific MAb-based sandwich CAA and CCA ELISAs [15, 16]. In brief, CAA was captured onto MAb 120-1B10-A–coated microtitration plates (Maxisorp; Nunc, Roskilde, Denmark) and detected using biotin-labeled MAb 120-1B10-A. The lower detection limit of this assay was ~15 pg of CAA/mL. CCA was captured onto MAb 54-5C10-A–coated microtitration plates and detected using biotin-labeled MAb 8-3C10. The lower detection limit was ~60 pg of CCA/mL. After the biotin-labeled MAb step, streptavidin-europium conjugate (DELFIA; Pharmacia, Uppsala, Sweden) 1000 times diluted in DELFIA assay buffer was added. In both assays, all incubation steps except coating were shortened to 15 min by applying a shaking incubator system [26]. The last washing step was done with DELFIA washing buffer, after which enhancement solution (DELFIA) was added. Plates were read on a time-resolved fluorometer ARCUS-1234 (Pharmacia).

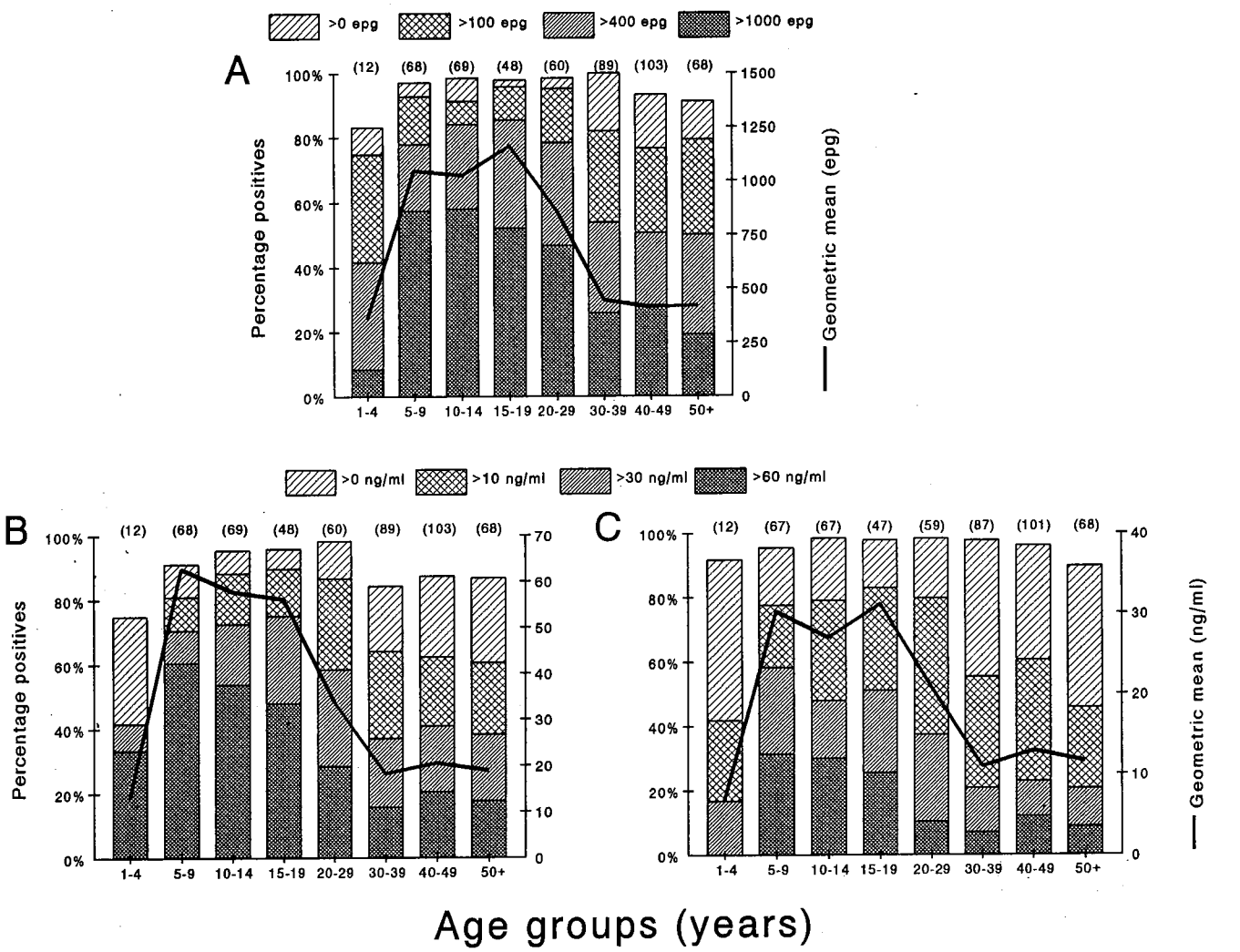

Figure 1. *S. mansoni* prevalence and intensity of infection, in different age classes, measured by fecal egg counts (**A**), serum CAA concentration (**B**), or serum CCA concentration (**C**). Bars represent percentages positive; lines represent geometric mean eggs per gram of feces (epg) or antigen levels (ng/mL) of those positive. Numbers of observations are shown at top of each bar.

Serum samples were tested in duplicate (final dilution, 8×) in assay buffer (0.2 *M* TRIS-HCl, pH 7.7, 0.15 *M* NaCl, 0.5% [wt/vol] bovine serum albumin) [27]. Serial dilutions of the TCA-soluble fraction of adult worm antigen (AWA-TCA) were assayed simultaneously on each plate to calculate CAA and CCA concentrations. AWA-TCA contains ~3% (wt/wt) CAA and ~3% CCA, as determined by using immunopurified antigen preparations [28, 29]. Samples were considered positive if measured counts were higher than the mean of 8 buffer controls plus 3 times the SD. If antigen concentrations fell outside the linear range (>250 ng/mL), samples were tested again at a higher dilution.

Data analysis. As circulating antigen levels and egg excretion showed skewed distributions, data were normalized by applying a $\log_{10}$ transformation on all positive values. Data were characterized by ranges, geometric means, and 95% confidence intervals (CI) of the geometric mean for all positive persons. The association between circulating antigen concentrations and egg counts was first examined by Pearson's correlation coefficient. The population was divided into 8 age categories and the variation of intensity of infection with age was calculated with one-way analysis of variance (*F* test).

To study the effect of age on the relationship between antigen level and egg excretion in more detail, we divided the population into 4 age groups for regression analysis. The regression lines are given by the equation $\log(\text{epg}) = \alpha + \beta\log(\text{CAA or CCA})$, with intercept α and slope β, or equivalently by taking the antilog: $\text{epg} = 10^{\alpha}(\text{CAA or CCA})^{\beta}$. Regarding antigen levels as a reflection of worm burden, a measure of fecundity can be introduced by calculating egg production per CAA (or CCA) unit: $F = \text{epg/CAA} = 10^{\alpha}(\text{CAA})^{\beta - 1}$. These equations clearly show that if $\beta = 1.0$, the relationship between epg and CAA (or CCA) will be linear, indicating a constant fecundity. If $\beta < 1.0$, the relationship between the two variables will be nonlinear, indicating a reduction in fecundity at high worm loads (antigen levels).

Although the variation in serum antigen concentrations is comparatively lower than the well-documented variation in egg counts, it still cannot be neglected [6, 7, 20, 22] (Polman K, personal communication). This will lead to an underestimation of the slopes, when using the standard least square regression analysis, with circulating antigen levels as the independent and egg counts as the dependent variable. To overcome this problem, we applied regression analysis according to Deming, which is an extension of the conventional linear regression analysis, allowing variation in the dependent but also in the independent variable [30, 31]. The required ratio of the within-variance of both parameters was calculated from the data of a previous study, in which both egg counts and serum CAA levels were measured three times in 20 schistosomiasis mansoni patients from the same area as the present study [20]. This ratio was 0.82, indeed indicating more variation in egg counts than in circulating antigen levels.

The few subjects negative for both egg excretion and circulating antigen determination were considered noninfected and left out of the regression analysis, although it is possible that some of them had light infections missed by both diagnostic methods. Persons negative for only one of these parameters were included. They were assumed to have light infections missed by one of the diagnostic methods [11, 32]. Exclusion of these cases could lead to a bias because of the difference in sensitivity of the respective methods. To properly include them in the analysis, a value equal to half the detection limit of the particular assay was assigned to persons concerned. Although the number of singly positive cases was not large, they might be essential for the outcome of the analysis, as they are on the outskirts of the regression line. For comparison and reference, regression analysis was also done with the data from persons positive for both tests only. A limited number of outlying points, >3 times the SD from the regression line, were rejected from the analysis. The effect of age was tested by covariance analysis. No corrections were made for age-related differences in blood volume, but likewise no corrections were made for the age-related daily stool production.

Results

S. mansoni eggs were found in the stools of 95% of the study population, 38% of which excreted >1000 epg. Positive egg counts ranged from 10 to 13,182 epg, with a geometric mean of 650 (95% CI = 578–731). No significant differences were found between male and female subjects.

Figure 1A shows the age–egg count pattern, which is characteristic for an area with intense *Schistosoma* transmission. The prevalence was already 83% in the youngest children, reached

Table 1. Percentage of subjects positive for circulating antigens and mean CAA and CCA concentration per egg count class.

	CAA			CCA		
Eggs/g of feces	No.	% positive	Concentration (95% CI)	No.	% positive	Concentration (95% CI)
0	26	35	3.6 (1.0–12.6)	25	76	2.4 (1.4–4.2)
1–100	47	72	4.9 (2.7–8.9)	46	91	5.1 (3.5–7.3)
101–400	106	91	14.6 (11.4–18.8)	104	96	9.6 (7.7–11.9)
401–1000	140	96	37.2 (31.0–44.8)	138	99	18.9 (15.6–22.8)
>1000	198	98	62.1 (53.5–72.1)	195	98	35.8 (30.6–41.9)
Total	517	90	31.3 (27.5–35.7)	508	96	16.4 (14.5–18.5)

NOTE. Concentration is geometric mean (ng/mL); CI, confidence interval.

99% in adolescents, and remained high in adults. The mean egg excretion showed a peak in the age groups of 5–19 years ($F = 8.9$, $n = 491$, $P < .001$).

Table 1 summarizes the results obtained with the two antigen assays, arranged by egg count class. Ninety percent of the total population was positive for CAA (concentration range, 0.056–514 ng/mL), 96% for CCA (range, 0.177–676 ng/mL), and 97% for at least one of these two antigens. Only 6 cases (1.2% of the study population) were negative in both antigen determination and egg counts.

For both CAA and CCA, the percentage of positive results and the mean circulating antigen level rose with increasing egg excretion (table 1). Although more persons were positive for CCA, CAA concentrations were generally higher, particularly in persons with high egg counts. CAA and CCA levels strongly correlated with each other ($r = .77$, $n = 452$, $P < .001$). Also, the correlation between egg excretion and circulating antigen level was significant, for both CAA ($r = .56$, $n = 457$, $P < .001$) and CCA ($r = .53$, $n = 469$, $P < .001$).

Antigen concentrations were divided into 4 categories, with about the same number of persons per category. Figure 1B and 1C show the age-related profiles for CAA and CCA, respectively. The age-related positivity rates mirrored the egg count pattern: >75% of young children, rising to at least 95% in adolescents, and remaining high in adults. The mean concentration of both antigens also showed a peak in the age groups of 5–19 years (CAA: $F = 10.1$, $n = 465$, $P < .001$; CCA: $F = 8.2$, $n = 487$, $P < .001$).

Figures 2 and 3 show scattergrams of the individual data for CAA and CCA, respectively, including the regression lines of the relation between log antigen level and log egg counts. For every given antigen concentration, an about equal distribution of egg counts can be noticed (and vice versa). Herewith, the statistical conditions of homoscedasticity and normality are

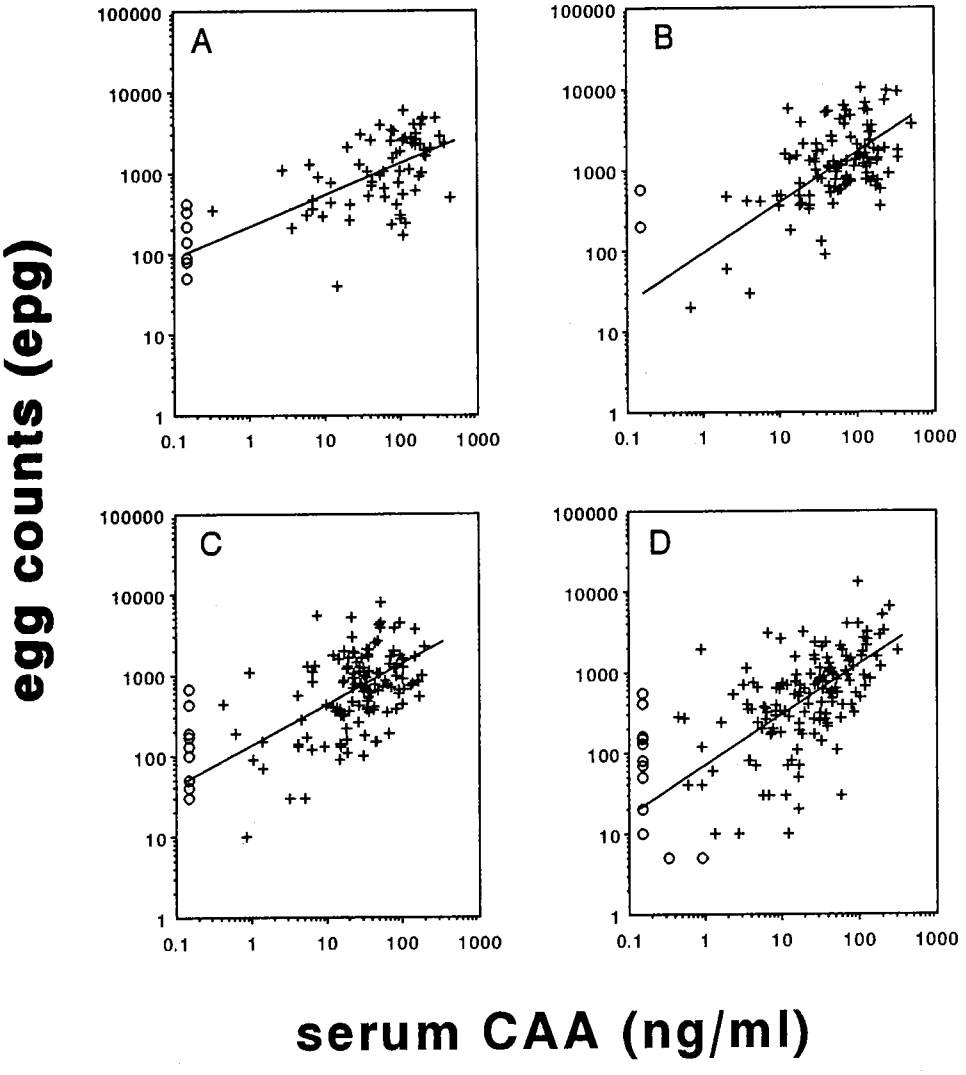

Figure 2. Egg counts per gram of feces (epg) plotted against serum CAA levels for 4 age groups: **A**, 1–9 years ($n = 76$); **B**, 10–19 years ($n = 112$); **C**, 20–39 years ($n = 140$); and **D**, ≥40 years ($n = 158$). Persons negative for both parameters ($n = 17$) and outliers ($n = 14$) were excluded. ○, persons negative for 1 of 2 parameters, in which case value was assigned representing half detection limit of particular assay. Each line represents outcome of regression analysis of all data points, given by $\log(epg) = \alpha + \beta\log(CAA)$, with slope ($\beta \pm$ 95% confidence interval) of: **A**, 0.39 ± 0.09; **B**, 0.62 ± 0.12; **C**, 0.51 ± 0.09; **D**, 0.63 ± 0.09.

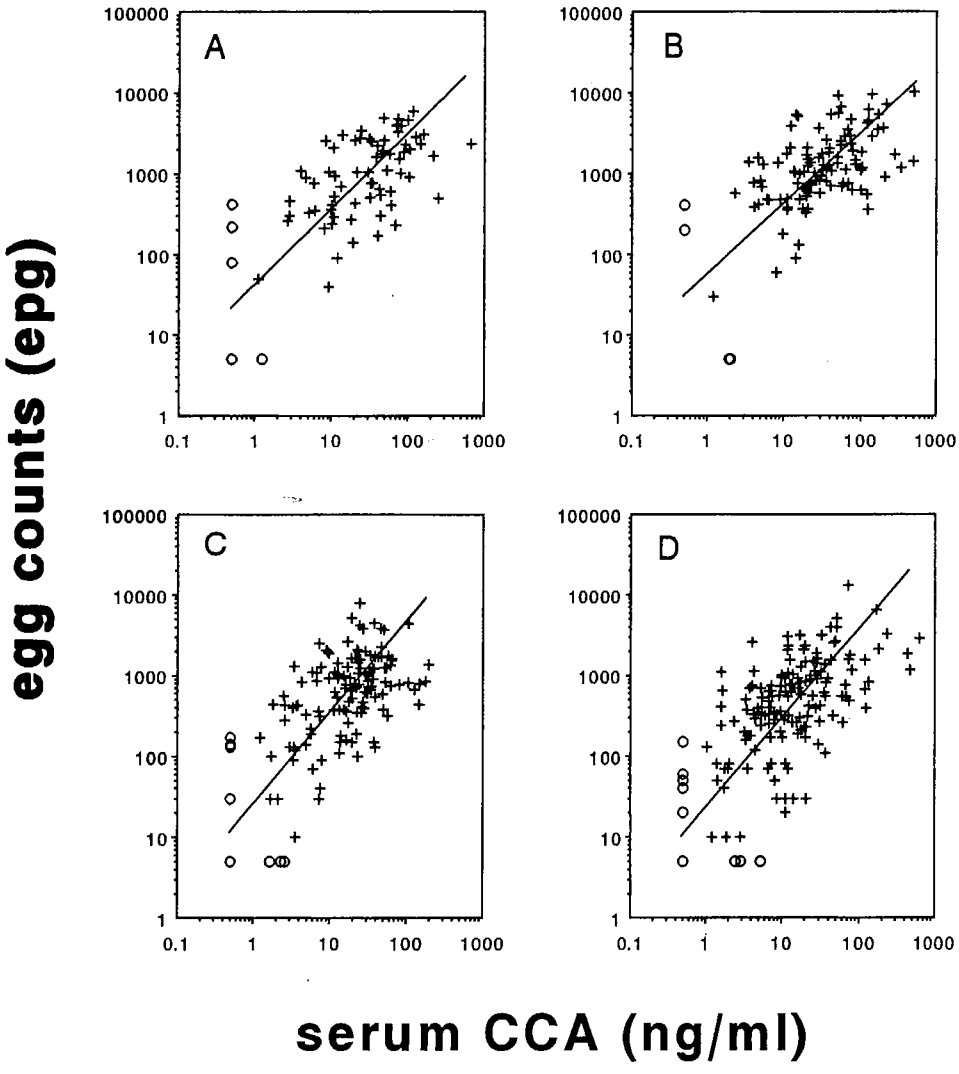

Figure 3. Egg counts per gram of feces (epg) plotted against serum CCA levels for 4 age groups: **A**, 1–9 years (n = 77); **B**, 10–19 years (n = 109); **C**, 20–39 years (n = 139); and **D**, ≥40 years (n = 158). Persons negative for both parameters (n = 6) and outliers (n = 19) were excluded. (See also figure 2.) Each line represents outcome of regression analysis of all data points, given by equation $\log(\text{epg}) = \alpha + \beta\log(\text{CCA})$, with slope ($\beta \pm 95\%$ confidence interval) of: **A**, 0.93 ± 0.13; **B**, 0.86 ± 0.13; **C**, 1.14 ± 0.12; **D**, 1.10 ± 0.11.

reasonably fulfilled. The slopes of the regression lines are given in the legends of the figures. For CAA, slope β_{CAA} was significantly <1.0 in all 4 age classes, indicating that the egg counts do not increase as rapidly as the levels of CAA (figure 2). Although to a lesser extent, β_{CAA} was still significantly <1.0 when analyzing only the persons positive at both the parasitologic examination and the antigen assay. For CCA, the relationship between egg counts and antigen level was about linear, with β_{CCA} slightly >1.0 in those 20–39 years and slightly <1.0 in those 10–19 years (figure 3). Analyzing just the subjects positive by both tests, β_{CCA} equaled 1.0, except again for those 10–19 years old.

Covariance analysis revealed no significant influence of age on the relationship between circulating antigen concentrations and egg counts. For the total study population, β_{CAA} (±95% CI) was 0.56 (±0.05) and β_{CCA} was 1.04 (±0.08). No differences were found between male and female subjects.

Discussion

In the present study, CAA and CCA concentrations were determined by TR-IFMA, which compared with ELISA has the advantage of a longer linear range [33]. Sera could therefore be tested in one standard dilution instead of dilution series. The TR-IFMA for the detection of CCA was developed and applied here for the first time. For both antigens, the detection limit of the TR-IFMA was comparable to that of the standard ELISA, which means that we were not able to reproduce the detection limit of 20 pg of AWA-TCA/mL reported earlier for CAA [27].

Both CAA and CCA are heavily glycosylated and are extremely stable, as illustrated by the fact that CAA was still demonstrable in tissue of a 5000-year-old Egyptian mummy [34]. This allowed us to readdress serum samples that had been collected ~15 years before.

We found 97% of all egg excreters to be positive for CAA or CCA (or both) in serum. For the remaining 3% negative for both antigens, egg counts were generally <400 epg. These results confirm the diagnostic applicability of CAA and CCA determination, as shown previously [17, 19, 21, 35, 36].

The main goal of our study was to use circulating antigen quantification to allow a differentiation between worm reduction and reduced fecundity over age. As judged by the good correlation between the number of *S. mansoni* worms and CAA or CCA concentrations, as shown in animal studies, antigen levels supposedly reflect actual number of parasites [13, 14]. On the other hand, it is likely that the production and clearance of these antigens is affected by several partly host-related mechanisms. The production of CAA and CCA (as a reflection of worm metabolism) may be closely related to egg excretion, both mirroring the general well-being of the parasites. Also, the efficiency of the immune system to clear these antigens may be influenced by the age, health status, or sex of the host, although no differences between male and female subjects were found in this study. Even taking these limitations into account, estimation of worm burdens in humans by circulating antigen determination seems to be a valuable approach because it is, for the time being, the only alternative to the measurement of egg counts.

In general, the age-intensity curves of antigen levels resembled those of egg counts. Regression analysis according to Deming was applied to study the relation between these two variables in detail, with the emphasis on density-dependent fecundity or crowding. This phenomenon was first described by Medley and Anderson [8] from autopsy data of 65 persons with light to moderate *S. mansoni* infections. The population we studied here not only has a high number of participants but, more important, also represents a large range of infection intensities, including persons with extremely high egg counts. This implies that if density dependence is of any importance, it should certainly be demonstrable in a population as described here.

For CAA, we found slope β_{CAA} to be significantly <1.0, which may indicate a reduced fecundity at high infection levels. However, other explanations are still possible, such as increased egg retention in tissue. In contrast to CAA, the relationship between CCA levels and egg counts was almost linear. There are some indications that worm burdens are somewhat better reflected by the level of serum CAA than of CCA. For instance, CCA seems to be more efficiently cleared into the urine than CAA, as urine levels are much higher for CCA than for CAA [17, 18] (van Dam G, personal communication). In addition, specific IgM antibodies, as measured in an immunofluorescence assay against *Schistosoma* gut–associated antigens, are >90% directed against CCA, which indicates also that antibody-dependent clearance probably occurs much more efficiently for CCA than for CAA [37].

For both antigens, the slopes were calculated by using a ratio of the within-variance of the two variables based on a data set with comparably stable egg counts [20]. Future data sets will most likely show a lower ratio, automatically leading to a lower value of β. Therefore, more studies with repeated measurements of circulating antigen levels are needed to allow a thorough evaluation of their relative stability.

In conclusion, we found the relationship between CAA or CCA levels and egg counts to be consistent, independent of the age of the host. This suggests that in this heavily and chronically infected population, the decline in egg counts after adolescence purely results from a reduction in worm load and not from reduced worm fecundity, as suggested by others [1, 3, 38, 39]. For CAA, but not for CCA, our data are consistent with density-dependent fecundity. However, other mechanisms affecting production and clearance of the circulating antigens cannot be excluded, and to corroborate our findings, further research on these issues is needed.

Acknowledgments

We thank G. van Dam and D. Kornelis for helpful discussions and critical reading of the manuscript.

References

1. Capron A. Immunity to schistosomes. Curr Opin Immunol **1992**;4:419–24.
2. Butterworth AE, Dunne DW, Fulford AJC, et al. Human immunity to *Schistosoma mansoni:* observations on mechanisms, and implications for control. Immunol Invest **1992**;21:391–407.
3. Grzych JM, Grezel D, Xu CB, et al. IgA antibodies to a protective antigen in human schistosomiasis mansoni. J Immunol **1993**;150:527–35.
4. Woolhouse MEJ. Immunoepidemiology of human schistosomes: taking the theory into the field. Parasitol Today **1994**;10:196–202.
5. Gryseels B. Human resistance to *Schistosoma* infections: age or experience? Parasitol Today **1994**;10:380–4.
6. De Vlas SJ, Gryseels B, Van Oortmarssen GJ, Polderman AM, Habbema JDF. A model for variations in single and repeated egg counts in *Schistosoma mansoni* infections. Parasitology **1992**;104:451–60.
7. Teesdale CH, Fahringer K, Chitsulo L. Egg count variability and sensitivity of a thin smear technique for the diagnosis of *Schistosoma mansoni.* Trans R Soc Trop Med Hyg **1985**;79:369–73.
8. Medley G, Anderson RM. Density-dependent fecundity in *Schistosoma mansoni* infections in man. Trans R Soc Trop Med Hyg **1985**;79:532–4.
9. Wertheimer SP, Vermund SH, Lumey LH, Singer B. Lack of demonstrable density-dependent fecundity of schistosomiasis mansoni: analyses of Egyptian quantitative human autopsies. Am J Trop Med Hyg **1987**;37:79–84.
10. Gryseels B, De Vlas SJ. Worm burdens in schistosome infection. Parasitol Today **1995** (in press).
11. Deelder AM, Qian ZL, Kremsner PG, et al. Quantitative diagnosis of *Schistosoma* infections by measurement of circulating antigens in serum and urine. Trop Geogr Med **1994**;46:233–8.
12. Qian ZL, Deelder AM. *Schistosoma japonicum:* immunological characterization and detection of circulating polysaccharide antigens from adult worms. Exp Parasitol **1983**;55:168–78.
13. Barsoum IS, Colley DG, Kamal KA. *Schistosoma mansoni:* detection of circulating antigens in murine schistosomiasis by antigen-capture

sandwich ELISA using a monoclonal antibody. Exp Parasitol **1990**;71:107–13.

14. Agnew AM, Fulford AJC, De Jonge N, et al. The relationship between worm burden and levels of circulating anodic antigen (CAA) of five species of *Schistosoma* in mice. Parasitology **1995**;111:67–76.
15. Deelder AM, De Jonge N, Boerman OC, et al. Sensitive determination of circulating anodic antigen in *Schistosoma mansoni*–infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. Am J Trop Med Hyg **1989**;40:268–72.
16. De Jonge N, Kremsner PG, Krijger FW, et al. Detection of the schistosome circulating cathodic antigen by enzyme immunoassay using biotinylated monoclonal antibodies. Trans R Soc Trop Med Hyg **1990**;84:815–8.
17. Van Lieshout L, De Jonge N, El Masry NA, Mansour MM, Krijger FW, Deelder AM. Improved diagnostic performance of the circulating antigen assay in human schistosomiasis by parallel testing for circulating anodic and cathodic antigens in serum and urine. Am J Trop Med Hyg **1992**;47:463–9.
18. Krijger FW, Van Lieshout L, Deelder AM. A simple technique to pretreat urine and serum samples for quantitation of schistosome circulating anodic and cathodic antigen. Acta Trop (Basel) **1994**;56:55–63.
19. De Jonge N, Gryseels B, Hilberath GW, Polderman AM, Deelder AM. Detection of circulating anodic antigen by ELISA for seroepidemiology of schistosomiasis mansoni. Trans R Soc Trop Med Hyg **1988**;82:591–4.
20. De Jonge N, De Caluwé P, Hilberath GW, Krijger FW, Polderman AM, Deelder AM. Circulating anodic antigen levels in serum before and after chemotherapy with praziquantel in schistosomiasis mansoni. Trans R Soc Trop Med Hyg **1989**;83:368–72.
21. Van Lieshout L, De Jonge N, El Masry NA, et al. Monitoring the efficacy of different doses of praziquantel by quantification of circulating antigens in serum and urine of schistosomiasis patients. Parasitology **1994**;108:519–26.
22. Polderman AM, Mpamila K, Manshande JP, Bouwhuis-Hoogerwerf ML. Methodology and interpretation of parasitological surveillance of intestinal schistosomiasis in Maniema, Kivu Province, Zaire. Ann Soc Belge Med Trop **1985**;65:243–9.
23. Polderman AM, Mpamila K, Manshande JP, Gryseels B, Van Schayk O. Historical, geological and ecological aspects of transmission of intestinal schistosomiasis in Maniema, Kivu Province, Zaire. Ann Soc Belge Med Trop **1985**;65:251–61.
24. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo **1972**;14:397–400.
25. De Jonge N, Fillié YE, Deelder AM. A simple and rapid treatment (trichloroacetic acid precipitation) of serum samples to prevent non-specific reactions in the immunoassay of a proteoglycan. J Immunol Methods **1987**;99:195–7.
26. Mushens RE, Scott ML. A fast and efficient method for quantification of monoclonal antibodies in an ELISA using a novel incubation system. J Immunol Methods **1990**;131:83–9.
27. De Jonge N, Boerman OC, Deelder AM. Time-resolved immunofluorometric assay (TR-IFMA) for the detection of the schistosome circulating anodic antigen. Trans R Soc Trop Med Hyg **1989**;83:659–63.
28. Van Dam GJ, Bergwerff AA, Thomas-Oates JE, et al. The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis x as repeating unit. Eur J Biochem **1994**;225:467–82.
29. Bergwerff AA, Van Dam GJ, Rotmans JP, Deelder AM, Kamerling JP, Vliegenthart JFG. The immunologically reactive part of immunopurified circulating anodic antigen from *Schistosoma mansoni* is a threonine-linked polysaccharide consisting of →6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal-*p*NAc-(1→ repeating units. J Biol Chem **1994**;269:31510–7.
30. Cornbleet PJ, Gochman N. Incorrect least-squares regression coefficients in method-comparison analysis. Clin Chem **1979**;25:432–8.
31. Ward LC, Cornish B. Use of a spreadsheet program for Deming's linear regression analysis. Comput Methods Programs Biomed **1992**;37:101–5.
32. De Vlas SJ, Gryseels B. Underestimation of *Schistosoma mansoni* prevalences. Parasitol Today **1992**;8:274–7.
33. Roberts IM, Jones SL, Premier RR, Cox JC. A comparison of the sensitivity and specificity of enzyme immunoassays and time-resolved fluoroimmunoassay. J Immunol Methods **1991**;143:49–56.
34. Deelder AM, Miller RL, de Jonge N, Krijger FW. Detection of schistosome antigen in mummies. Lancet **1990**;335:724–5.
35. Feldmeier H, Nogueira-Queiroz JA, Peixoto-Queiroz MA, et al. Detection and quantification of circulating antigen in schistosomiasis by monoclonal antibody. II. The quantification of circulating antigens in human schistosomiasis mansoni and haematobium: relationship to intensity of infection and disease status. Clin Exp Immunol **1986**;65:232–43.
36. Barsoum IS, Kamal KA, Bassily S, Deelder AM, Colley DG. Diagnosis of human schistosomiasis by detection of circulating cathodic antigen with a monoclonal antibody. J Infect Dis **1991**;164:1010–3.
37. Deelder AM, Van Zeyl RJM, Fillié YE, Rotmans JP, Duchenne W. Recognition of gut-associated antigens by immunoglobulin M in the indirect fluorescent antibody test for schistosomiasis mansoni. Trans R Soc Trop Med Hyg **1989**;83:364–7.
38. Xu CB, Verwaerde C, Grzych JM, Fontaine J, Capron A. A monoclonal antibody blocking the *Schistosoma mansoni* 28-kDa glutathione S-transferase activity reduces female worm fecundity and egg viability. Eur J Immunol **1991**;21:1801–7.
39. Capron A, Riveau G, Grzych JM, Boulanger D, Capron M, Pierce RJ. Development of a vaccine strategy against human and bovine schistosomiasis. Background and update. Trop Geogr Med **1994**;46:242–6.