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Dynamics of egg counts and circulating antigen levels in a recent *Schistosoma mansoni* focus in northern Senegal

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Summary Serum circulating anodic antigen (CAA) levels were compared with faecal egg counts in four subsequent population samples, randomly selected at 8-month intervals, in a recent *Schistosoma mansoni* focus in northern Senegal. In all four samples, antigen levels showed the same age-intensity profiles as egg counts, with a strong decline in adults. Also across population samples, a consistent relationship was found between egg counts and antigen levels. Assuming the level of CAA to be a direct reflection of worm burden, these findings support the idea that the observed egg count patterns and levels indeed reflect dynamics of worm burdens, and not of egg excretion or worm fecundity. Remarkably similar levels of both egg counts and CAA were observed in the first and last sample, collected in the same season (August–September), but 2 years apart. This suggests that a steady state of *S. mansoni* infection had already been reached shortly after the onset of the epidemic in this focus (3 years). Significantly lower infection levels were found in the intermediate population samples collected in January and April. The differences in infection levels across the four population samples may be because of seasonal transmission patterns. They would indicate a substantial turnover of worm populations, with an estimated average life span of only 7 months, probably less, in this recently emerged, intense *S. mansoni* focus.

keywords *Schistosoma mansoni,* circulating anodic antigen (CAA), faecal egg counts, seasonality, worm longevity, Senegal

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Introduction

We found very high prevalences and intensities of infection in Ndombo, the epicentre of a new focus of *Schistosoma mansoni* in northern Senegal. In spite of the recent introduction of *S. mansoni* and the assumed absence of acquired immunity in this community, egg counts and circulating antigen levels were declining strongly in adults (Stelma *et al.* 1993; Polman *et al.* 1995). To follow up the evolution of *S. mansoni* infection in what would be expected a dynamically evolving focus, we subsequently studied four random cross-sectional population samples at 8-month intervals. Preliminary parasitological results suggested no specific evolution in infection levels or patterns over the 2-year study period. Instead, mean egg counts showed considerable variations, which seemed to fluctuate with the seasons (Gryseels *et al.* 1994). These findings may have important epidemiological implications, as they suggest that a steady state of *S. mansoni* infection would have developed in this recently exposed community; that worm burdens show seasonal fluctuations; and that a high turnover of worm populations has to be assumed.

In the present study, these assumptions were examined in more detail. As egg count variations over time may also be caused by changes in worm fecundity or egg excretion instead of worm load variations, we assessed *S. mansoni* worm burdens by quantification of serum circulating anodic antigens (CAA), next to faecal egg counts. CAA is a

glycoconjugate excreted from the gut of the metabolically active adult *Schistosoma* worm into the host circulation (Deelder *et al.* 1989, 1994). The significant correlation between serum CAA concentrations and worm burdens in animal models and with repeated egg counts in humans, and their rapid clearance after chemotherapeutic cure, indicate that serum CAA levels are a reflection of current worm burdens in humans (Deelder *et al.* 1994; Agnew *et al.* 1995). By comparing and relating CAA levels to egg counts within and between the respective population samples in this focus, we examined whether and to what extent the observed patterns genuinely reflect variations in worm load and/or in worm fecundity or egg output.

Materials and methods

Data sets

The field studies took place in Ndombo, a village close to Richard Toll, northern Senegal. Ethical clearance was obtained from 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 the Leiden University Medical Centre. All studies were conducted under the supervision and authority of the local and regional health services.

The area and the design of the study are described in detail elsewhere (Stelma *et al.* 1993; Gryseels *et al.* 1994). Briefly, between August 1991 and September 1993 four randomly selected cross-sectional population samples of approximately 400 subjects each were surveyed, at 8-month intervals: August 1991 (end of rainy season), April 1992 (hot dry season), January 1993 (cold dry season) and September 1993 (again end of rainy season). This design was chosen to approximate a longitudinal follow-up of an untreated population. Faecal egg counts (expressed as epg, i.e. eggs per gram of faeces) were determined by two stool examinations, each consisting of duplicate 25-mg Kato examinations, within a time interval of 1–2 weeks (Katz *et al.* 1972; Polderman *et al.* 1985). Consistencies of stools were inspected immediately after collection and categorized as hard, formed, mushy or diarrhoeic. CAA levels in serum (expressed as concentrations of pure CAA) were determined by ELISA as described by Deelder *et al.* (1989) and Polman *et al.* (1995). Those found positive after parasitological examination were treated with 40 mg/kg praziquantel in a single oral dose. The follow-up after treatment and re-infection will be reported elsewhere. We will further refer to each population sample as cohorts 1–4, in order of their selection date.

Other detected infections or disorders were also treated or referred adequately. The primary health care station of the village and the nearby district health centre were provided with all due diagnostic and therapeutic means to treat clinical cases. Based on the results of the studies, an area-wide intensive control programme was initiated.

Data analysis

First, the data were analysed for faecal egg counts and serum CAA levels separately. Only individuals with full data (two duplicate Kato examinations and a serum CAA determination) were included. This resulted in a data set of 921 individuals, with about the same number of persons per cohort (Table 1). As CAA levels and egg output showed skewed distributions, data were normalized by log-transformation. Parametric statistical methods were used to describe and analyse the results. To allow for zeros in the analyses, log-transformation was applied after adding a value equal to half the detection limit of the particular assay to all measurements. Data were described by geometric means, ranges and 95% confidence intervals (CI). Chi-square tests were applied to test variations in age and sex between cohorts. Associations between CAA concentrations and egg counts were examined by Pearson's correlation test. Variations in intensity of infection by cohort were determined by analysis of variance, controlling for age. Bonferroni corrections were used to adjust for multiple testing.

To study possible cohort differences in the relation between egg counts and CAA levels, multiple linear regression analysis was performed, with $\log_{(epg)}$ as response variable, $\log_{(CAA)}$ as covariate, and cohort and age as categorical variables. In contrast to previous studies on the relation between egg counts and antigen levels (e.g. Van Lieshout *et al.* 1995, 1998), we did not use Deming regression, as recent findings indicate that for CAA [but not for circulating cathodic antigen (CCA)] ordinary linear regression can be applied just as well (Polman *et al.* 2001).

Based on the pocket chart developed by De Vlas *et al.* (1993), all individuals in this focus can be considered as infected. Therefore, even cases with negative results for both egg excretion and CAA determination were included in the regression analysis. For comparison and reference, regression analysis was also performed with the data from only those who were positive for at least one of the indicators (Van Lieshout *et al.* 1995, 1998).

Results

The data are summarized per cohort in Table 1. Although all ages were represented in each cohort, individuals with complete CAA and egg count data from cohort 2 were on

Table 1 Comparison of CAA and egg count (epg) data of four subsequent cohorts from Ndombo (Senegal), selected between 1991 and 1993, at 8-month intervals

	Cohort 1 (August 1991)	Cohort 2 (April 1992)	Cohort 3 (January 1993)	Cohort 4 (September 1993)	Total	*P*
Nr examined	246	172	244	259	921	–
Males (%)	47	42	53	48	48	0.245
Age (years)*	18 (1–77)	30 (1–84)	18 (3–80)	18 (2–80)	20 (1–84)	<0.0001
epg						
Positives (%)	96	94	95	94	95	0.810
GM (epg)†	563	257	334	625	436	<0.0001
CAA						
Positives (%)	87	58	75	85	78	<0.0001
GM (ng/ml)^2	4.65	0.866	2.61	5.63	3.10	<0.0001

*Median (range).
†Geometric mean including zeros.

the average older ($P < 0.001$). There were no significant sex differences between cohorts ($P > 0.05$).

Of all participants, 95% had positive egg counts, and mean egg excretion was 436 epg. The consistency of the stools varied substantially. In cohort 1 and 4 more hard stools were collected (40 and 27%, respectively) than in cohorts 2 and 3 (21 and 12%, respectively). Thirty-four per cent of the stools in the third cohort were diarrhoeic (Figure 1).

Of all study subjects 78% had positive CAA levels, and mean CAA levels were 3.10 ng/ml. In all cohorts, CAA levels correlated well with egg counts ($r = 0.57$; $P < 0.01$), and positivity rates increased with rising egg counts (data not shown).

Figure 2 shows the age-related intensity patterns per cohort, as determined by egg counts (a) and CAA levels (b). In all four cohorts, both egg counts and CAA levels peaked in individuals aged 10–20 years, showing a less pronounced peak in cohort 3 than in the other cohorts. Intensity levels decreased strongly in adults (> 20 years), and appeared to vary between cohorts as well, with higher levels in cohort 1 and 4 than in cohort 2 and 3.

After controlling for age, analysis of variance revealed no significant differences in egg counts or CAA levels between cohort 1 and 4 ($P > 0.05$), which were collected in the same season, but 2 years apart. Significant differences were found between mean egg counts and CAA levels of cohort 2 and 3 compared with cohort 1 and 4, and of mean CAA levels of cohort 2 compared with cohort 3 ($P < 0.001$). Table 2 shows that mean intensities in cohort 1 and 4 (collected in the rainy season) were always higher than for cohort 2 and 3 (collected in the dry season) in

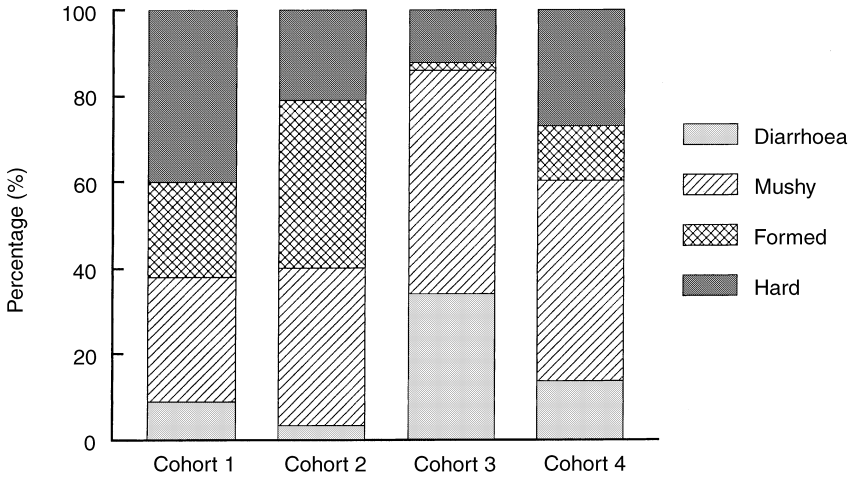

Figure 1 Consistency of stools in the respective cohorts of Ndombo, Senegal.

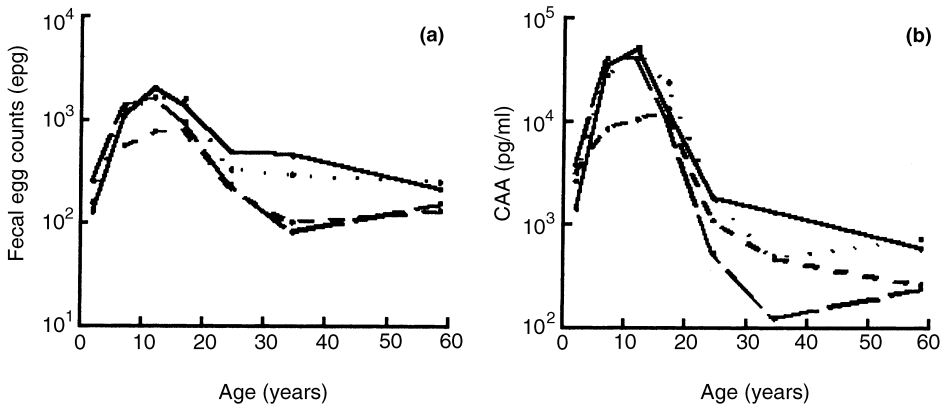

Figure 2 Age-related *Schistoma mansoni* intensity of infection, as determined by (a) mean egg counts (in epg) and (b) serum CAA concentrations (in pg/ml), in the four cohorts from Ndombo, Senegal. (——) cohort 1; (— —) cohort 2; (- - - -) cohort 3; (·····) cohort 4.

Table 2 Geometric mean egg counts and CAA levels for children (5–19 years) and adults (≥20 years) in the four cohorts of Ndombo, Senegal. Rank: ranking order of cohorts, from highest to lowest mean intensity. In both age groups, the highest mean egg counts and CAA levels were always found in cohort 1 and 4, and the lowest in cohort 2 and 3

		Mean intensities				
	Age (years)	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Rank
Egg counts (epg)	5–19	1421	1149	713	1457	4–1–2–3
	≥20	352	155	149	287	1–4–2–3
CAA levels (ng/ml)	5–19	29.0	17.7	10.6	33.4	4–1–2–3
	≥20	1.08	0.277	0.530	0.952	1–4–3–2

children (5–19 years) and adults (20 years and older), for egg counts as well as CAA levels.

Figure 3 shows the scattergrams of the individual CAA and egg count data in all four cohorts, including the regression lines of the relation between $\log_{(CAA)}$ and $\log_{(epg)}$. Regression analysis revealed a consistent relationship between egg counts and CAA levels across the cohorts, with no significant differences in slopes or intercepts ($P > 0.05$). Similar results were found after analysis of only those who were positive for at least one of the indicators (data not shown).

Discussion

In this study, CAA levels were compared and related with egg counts in four subsequent cohorts, each of which was assumed to be a representative sample of the same community of Ndombo, exposed to intense *S. mansoni* for only 3 (cohort 1) to 5 years (cohort 4) at the onset of the study (Talla *et al.* 1990, 1992). Egg counts were consistently lower in adults than in children within each cohort, and did not show an overall downward pattern across cohorts, as would be expected on the basis of a gradual development of immunity in this recently exposed community. Instead, significant egg count variations were observed between the cohorts.

These observations suggested fluctuations in worm burdens, but could also be caused by factors concerning worm fecundity or egg excretion. The stools in this study were generally harder during the surveys in the first and the fourth cohort, whereas in cohort 3 relatively many stools were diarrhoeic. Variations of the consistency and/or volume of stools (e.g. because of the changes in diet, fluid balance or other intestinal infections) may affect the concentration of eggs in the stools and thus the number of egg counts at parasitological examination (Scott 1937; Hall 1982; Teesdale *et al.* 1985). The egg count variations might thus have been caused by seasonal variations of stool consistencies. However, CAA levels showed the same levels and patterns as egg counts within each cohort. Also, a consistent relationship between egg counts and antigen levels was observed across the cohorts. The antigen levels

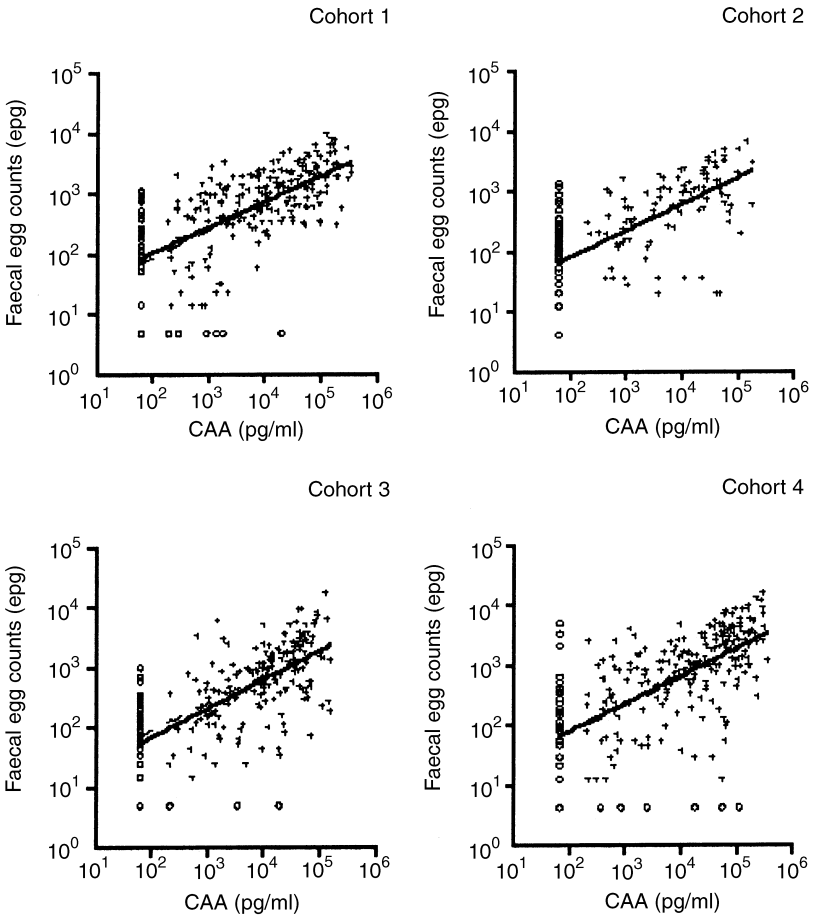

Figure 3 Faecal egg counts plotted against serum CAA levels for the four cohorts. For each cohort, the regression line of the relation between $\log_{(CAA)}$ and $\log_{(epg)}$ is shown, given by $\log_{(epg)} = \alpha + \beta \log_{(CAA)}$. The dashed line represents the outcome of the regression analysis on the combined data of all four cohorts. The intercepts α (±95% CI) and slopes β (±95% CI) of the regression lines were: $\alpha = 1.2$ (±0.3) and $\beta = 0.42$ (±0.07) for cohort 1 ($n = 246$); $\alpha = 1.1$ (±0.2) and $\beta = 0.44$ (±0.08) for cohort 2 ($n = 172$); $\alpha = 0.9$ (±0.2) and $\beta = 0.48$ (±0.06) for cohort 3 ($n = 244$); $\alpha = 1.1$ (±0.3) and $\beta = 0.46$ (±0.07) for cohort 4 ($n = 259$); $\alpha = 1.1$ (±0.1) and $\beta = 0.45$ (±0.03) for all four cohorts ($n = 921$).

thus support the idea that the observed egg count variations indeed reflect variations of worm burdens, and not of egg excretion or worm fecundity.

The differences between cohorts were apparent in children (5–19 years) as well as in adults (≥20 years). As the study population had only recently been exposed to *S. mansoni* infection, variations over time in egg counts and CAA levels may be caused by the instability of the worm populations in the transition phase from epidemic to endemic state. However, the high similarity of egg counts and CAA levels in the first and fourth cohort, collected in the same season (August/September) but 2 years apart, strongly suggests that a steady state of *S. mansoni* infection has already developed. Infection intensities were consistently higher in cohort 1 and 4, which were examined just after the peak transmission season. The second and third cohort were surveyed in spring and winter, respectively, when snails are less abundant and the exposure less intense (Gryseels *et al.* 1994). An explanation for the apparent worm burden variations may thus be in seasonal transmission patterns, but this hypothesis should be confirmed by a more detailed and comprehensive analysis of the transmission dynamics in this focus.

Our findings would indicate a relatively strong turnover of the worm populations in this focus. Assuming a stable situation with only within-year fluctuations, arithmetic mean intensities in our population decreased to about 50% for both egg counts and CAA levels within 5 months: August–September (cohort 1 and 4) *vs.* January (cohort 3). Assuming an exponential survival of the worms, and no new infections within this period, the average life span of the worm would be only 7 months (Goddard & Jordan 1980; Anderson & May 1991). Taking new incoming infections into account, the average life span of the worm would be even shorter. A number of attempts to estimate the adult *S. mansoni* worm's life span have been made in the past, with lifespan estimates ranging from 3 to 10 years (Warren *et al.* 1974; Goddard & Jordan 1980; Hiatt *et al.* 1980; Vermund *et al.* 1983; Fulford *et al.* 1995). The adult schistosome life span in this recently

emerged, intense *S. mansoni* focus would thus appear considerably shorter than in older endemic or less intense situations, which the other studies were based upon.

In the only other study performed on seasonal fluctuations of circulating antigens, De Bont *et al.* (1996) observed a seasonal pattern in CAA levels, not in egg counts, of *S. matthei*-infected cattle. Here, however, a significant increase of CAA was seen in the second half of the dry, low transmission season. Variations in antigen clearance rate with the physiological condition of the host were suggested as a possible explanation. It should indeed be taken into account that unknown mechanisms affecting production and clearance of CAA may bias its correlation with worm burdens. As in our study, similar fluctuations in, and a consistent relationship between, egg counts and CAA levels was observed – such mechanisms do not seem to affect our conclusions.

Our findings in this recently infected, intense *S. mansoni* focus do not support the gradual development of protective immunity over a period of 2 years. In fact, they suggest that endemic-like infection levels and distribution patterns were reached already within 3 years after the start of the epidemic in this focus. On the other hand, strong intermediate variations in infection levels do indicate a substantial turnover of worm populations, with an estimated average life span of 7 months, probably less. The dynamics may reflect seasonal transmission patterns, but further analysis of the transmission dynamics in this focus is needed. In any case, our findings show that antigen detection is a valuable additional tool for assessing the dynamics of worm populations, and that these dynamics are still far from being understood.

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References

Agnew AM, Fulford AJC, De Jonge N *et al.* (1995) The relationship between worm burden and levels of a circulating antigen (CAA) of five species of *Schistosoma* in mice. *Parasitology* **111**, 67–76.

Anderson RM & May RM (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford.

De Bont J, Van Lieshout L, Deelder AM, Ysebaert MT & Vercruysse J (1996) Circulating antigen levels in serum of cattle naturally infected with *Schistosoma mattheei*. *Parasitology* **113**, 465–471.

De Vlas SJ, Gryseels B, Van Oortmarssen GJ, Polderman AM & Habbema JDF (1993) A pocket chart to estimate true *Schistosoma* prevalences. *Parasitology Today* **9**, 305–306.

Deelder AM, De Jonge N, Boerman OC *et al.* (1989) Sensitive determination of circulating anodic antigen *in Schistosoma mansoni* infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. *American Journal of Tropical Medicine and Hygiene* **40**, 268–272.

Deelder AM, Qian ZL, Kremsner PG *et al.* (1994) Quantitative diagnosis of *Schistosoma* infections by measurement of circulating antigens in serum and urine. *Tropical and Geographical Medicine* **46**, 235–238.

Fulford AJC, Butterworth AE, Ouma JH & Sturrock RF (1995) A statistical approach to schistosome population dynamics and estimation of the life-span of *Schistosoma mansoni* in man. *Parasitology* **110**, 307–316.

Goddard MJ & Jordan P (1980) On the longevity of *Schistosoma mansoni* in man on St. Lucia, West Indies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 185–191.

Gryseels B, Stelma FF, Talla I *et al.* (1994) Epidemiology, immunology and chemotherapy of *Schistosoma mansoni* infections in a recently exposed community in Senegal. *Tropical and Geographical Medicine* **46**, 209–219.

Hall A (1982) Intestinal helminths of man: the interpretation of egg counts. *Parasitology* **85**, 605–613.

Hiatt RA, Cline BL, Ruiz-Tiben E, Knight WB & Berrios-Duran LA (1980) The Boqueron Project after 5 years: a prospective community-based study of infection with *Schistosoma mansoni* in Puerto Rico. *American Journal of Tropical Medicine and Hygiene* **29**, 1228–1240.

Katz N, Chaves A & Pellegrino J (1972) A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Revisto Do Instituto de Medicina Tropical de São Paulo* **14**, 397–400.

Polderman AM, Mpamila K, Manshande JP & Bouwhuis-Hoogerwerf ML (1985) Methodology and interpretation of parasitological surveillance of intestinal schistosomiasis in Maniema, Kivu Province, Zaire. *Annales de la Société Belge de Médicine Tropicale* **65**, 243–249.

Polman K, De Vlas SJ, Van Lieshout L, Deelder AM & Gryseels B (2001) Evaluation of density-dependent fecundity in human *Schistosoma mansoni* infections by relating egg counts to circulating antigens through Deming regression. *Parasitology* **122**, 161–167.

Polman K, Stelma FF, Gryseels B *et al.* (1995) Epidemiologic application of circulating antigen detection in a recent *Schistosoma mansoni* focus in northern Senegal. *American Journal of Tropical Medicine and Hygiene* **53**, 152–157.

Scott JA (1937) The regularity of egg output of helminth infestations, with special reference to *Schistosoma mansoni*. *American Journal of Hygiene* **27**, 176–195.

Stelma FF, Talla I, Polman K *et al.* (1993) Epidemiology of *Schistosoma mansoni* infection in a recently exposed community

in northern Senegal. *American Journal of Tropical Medicine and Hygiene* **49**, 701–706.

Talla I, Kongs A, Verlé P, Belot J, Sarr S & Coll AM (1990) Outbreak of intestinal schistosomiasis in the Senegal River Basin. *Annales de la Société Belge de Médicine Tropicale* **70**, 173–180.

Talla I, Kongs A & Verlé P (1992) Preliminary study of the prevalence of human schistosomiasis in Richard-Toll (the Senegal River Basin). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 182.

Teesdale CH, Fahringer K & Chitsulo L (1985) Egg count variability and sensitivity of a thin smear technique for the diagnosis of *Schistosoma mansoni*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 369–373.

Van Lieshout L, Polderman AM, De Vlas SJ *et al.* (1995) Analysis of worm burden variation in human *Schistosoma mansoni* infections by determination of serum levels of circulating anodic antigen and circulating cathodic antigen. *Journal of Infectious Diseases* **172**, 1336–1342.

Van Lieshout L, Polman K, Gryseels B & Deelder AM (1998) Circulating anodic antigen levels in two areas endemic for schistosomiasis mansoni indicate differences in worm fecundity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 115–119.

Vermund SH, Bradley DJ & Ruiz-Tiben E (1983) Survival of *Schistosoma mansoni* in the human host: estimates from a community-based prospective study in Puerto Rico. *American Journal of Tropical Medicine and Hygiene* **32**, 1040–1048.

Warren KS, Mahmoud AAF, Cummings P, Murphy DJ & Houser DB (1974) Schistosomiasis mansoni in Yemeni in California: duration of infection, presence of disease and therapeutic management. *American Journal of Tropical Medicine and Hygiene* **23**, 902–909.