Evaluation of density-dependent fecundity in human *Schistosoma mansoni* infections by relating egg counts to circulating antigens through Deming regression

K. POLMAN[1,2]*†, S. J. DE VLAS[3], L. VAN LIESHOUT[1], A. M. DEELDER[1] *and* B. GRYSEELS[2]

[1] *Department of Parasitology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[2] *Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium*
[3] *Department of Public Health, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands*

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SUMMARY

Regression analysis of the relationship of serum circulating anodic and cathodic antigens (CAA and CCA), as a possible direct measure of worm burden, and fecal egg counts allows the study of phenomena like density-dependent fecundity in human *Schistosoma mansoni* infections. For a reliable analysis, variations in egg count measurements as well as in circulating antigen levels have to be taken into account, and an accurate estimation of these variations (represented by parameter λ in the so-called Deming regression) is of great importance. From a new, extensive data set of repeated measurements of fecal egg counts and CAA and CCA concentrations we determined the respective values for parameter λ, and (re)analysed the relationship between circulating antigens and egg counts in 3 data sets from Burundi, Senegal and Zaire by Deming regression. For comparison, ordinary linear regression was performed as well, which considerably biased the regression lines for CCA, but not for CAA. The analyses resulted in a clearly non-proportional relationship between egg counts and CAA, and, to a lesser extent, CCA. Assuming that egg counts and antigen measurements directly reflect egg production and worm burdens, respectively, our findings reinforce the indication of density-dependent fecundity in schistosomiasis mansoni, as suggested by others.

Key words: *Schistosoma mansoni*, CAA, egg counts, Deming regression, density-dependent fecundity.

INTRODUCTION

Density-dependent fecundity (i.e. decreasing egg production per female worm with higher parasite burdens) has significant implications for the epidemiological interpretation of helminth infections, e.g. with respect to the use of egg counts as a measure of worm burden, mathematical modelling of parasite dynamics, and understanding the impact of control measures on parasite transmission. Possible mechanisms leading to density dependence may be a lack of resources in the habitat within the host (e.g. space, nutrients), or immunological or non-specific host responses (Anderson & May, 1985, 1991). The evidence for density-dependent fecundity is substantial in the case of intestinal nematodes, which can be directly counted after chemotherapy. In human schistosomiasis, research on this issue is hampered due to the intravascular localization of the worms. The only available (and widely cited) direct measurements of *Schistosoma mansoni* worm burdens in humans are those provided by Cheever's autopsy studies (Cheever, 1968; Cheever *et al*. 1977), which have been analysed and interpreted by various authors with conflicting results on density-dependent fecundity (Anderson & May, 1982; Medley & Anderson, 1985; Wertheimer *et al*. 1987; Gryseels & De Vlas, 1996). An alternative direct measure of *S. mansoni* worm burdens may be the detection of serum circulating antigens excreted by metabolically active adult worms (Deelder *et al*. 1994; Agnew *et al*. 1995). The good correlation of serum circulating antigen concentrations with worm burdens in animal models and with repeated egg counts in humans, and their rapid clearance after chemotherapeutic cure suggest that serum circulating antigen levels can be interpreted as a reflection of current worm burden in humans. Consequently, relating egg counts to circulating antigens by regression analysis may thus give us more information on density-dependent fecundity in human schistosomiasis (Van Lieshout *et al*. 1995, 1998; Agnew *et al*. 1996).

For a reliable regression analysis of the relationship between circulating antigen levels and fecal egg counts, the variation in not only egg counts, but also circulating antigen measurements should be taken into consideration (De Jonge *et al*. 1989; De Vlas *et*

* Corresponding author: Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. Tel: 0032 3 2476204. Fax: 0032 3 2376731. E-mail: kpolman@itg.be
† Both authors contributed equally to this paper.

al. 1992; Polman *et al*. 1998). Ordinary linear regression only accounts for variation in the dependent variable, which may considerably bias the least-squares regression coefficients, and thus result in incorrect inferences on density-dependent fecundity. Whether or not significant errors will occur depends on the degree of variation (within-individual variation as compared to the between-individual variation) in the independent variable, i.e. CAA or CCA measurements respectively (Cornbleet & Gochman, 1979). Alternatively, regression analysis according to Deming can be applied, which allows for variation in the dependent as well as the independent variable (Deming *et al*. 1943; Cornbleet & Gochman, 1979; Ward & Cornish, 1992). This so-called Deming regression requires a parameter λ which represents the ratio of the within-individual variance of the independent variable and the within-individual variance of the dependent variable, and an accurate estimation of this parameter is thus important.

Deming regression of circulating antigens and egg counts has previously been applied by Van Lieshout *et al*. (1995), who used a $\lambda = 0{\cdot}82$, indicating only slightly more variation in egg counts than in circulating antigen levels. This λ was calculated from a data set by De Jonge *et al*. (1989), in which both fecal egg counts and serum CAA levels were measured 3 times in 20 patients with *S. mansoni* infection. Although this was the only available dataset on repeated measurements of circulating antigens in serum at that time, it was not quite representative for the data as analysed by Van Lieshout *et al*. (1995, 1998) for 2 reasons: (1) circulating antigen levels were expressed in titres by De Jonge *et al*. (1989), while Van Lieshout *et al*. (1995, 1998) used continuous data in the form of concentrations; (2) De Jonge *et al*. (1989) only measured the variation in CAA levels, while the data as analysed by Van Lieshout *et al*. (1995, 1998) comprised CAA as well as CCA concentrations.

We have recently collected a new data set, which allows for a more accurate estimation of λ (Polman *et al*. 1998). Using Deming regression with the revised λ's, 3 existing data-sets (from Burundi, Senegal and Zaire) were (re)analysed on the relationship between egg counts and circulating antigens to address the issue of density-dependent fecundity in human schistosomiasis.

MATERIALS AND METHODS

Data sets

For the estimation of λ, a data set from Burundi consisting of 50 individuals with 3 successive measurements of fecal egg counts (i.e. 3 duplicate 25 mg Kato-Katz stool examinations) and of CAA as well as CCA concentrations in serum was used. Details on the study group, protocols and analyses are published elsewhere (Polman *et al*. 1998).

The relationship between egg counts and circulating antigens was analysed in 3 data sets from Burundi, Senegal and Zaire. The data set from Burundi consisted of 100 individuals from the Rusizi Plain, endemic for *S. mansoni*. The area and epidemiological details have been described by Gryseels & Nkulikyinka (1988). The first 50 individuals comprised the group with 3 successive egg counts and 3 antigen measurements for estimation of λ (see above); the other 50 consisted of 3 repeated egg counts and 1 CAA or CCA measurement per person. The total study group consisted of 54 females and 46 males, all of whom volunteered to participate in the study after having been diagnosed as positive in a preliminary population survey based on single 25 mg Kato-Katz slides. Their age ranged from 7 to 70 years (median 19 years).

The study groups from Zaire and Senegal have previously been described by Van Lieshout *et al*. (1995, 1998). In short, the data set from Zaire consisted of 517 individuals from Maniema, an *S. mansoni* endemic area. The group included 262 males and 255 females, with median age of 30 years (1–66). The data set from Senegal consisted of 428 individuals (195 males, 233 females), with median age of 15 years (0–77). This group was collected in Ndombo, a recently established *S. mansoni* focus.

Fecal egg counts were determined by duplicate 25 mg Kato-Katz stool examinations (Katz, Chaves & Pellegrino, 1972; Polderman *et al*. 1985), and expressed as eggs per gramme of feces (epg). CAA and CCA levels were determined by ELISA, as described by Deelder *et al*. (1989) and De Jonge *et al*. (1990) with some minor modifications. For the sake of comparability, fecal egg counts for each regression data set were based on only the first duplicate stool examination, and antigen levels on the first antigen measurement (if applicable).

Relating egg counts to circulating antigens

Regression analysis of egg counts with antigen levels was performed on log-transformed data. The respective populations were divided into 4 age-groups (0–9 years, 10–19 years, 20–39 years, 40 years and older). Analogous to Van Lieshout *et al*. (1995), the regression lines were described 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 and egg counts as a reflection of worm burden and egg production, respectively, a measure of fecundity can be introduced: $F = \text{epg}/\text{CAA} = 10^{\alpha}(\text{CAA or CCA})^{\beta-1}$. Thus, if $\beta = 1{\cdot}0$, the relationship between epg and CAA (or CCA) will be proportional, suggesting a constant fecundity. If $\beta < 1{\cdot}0$, the

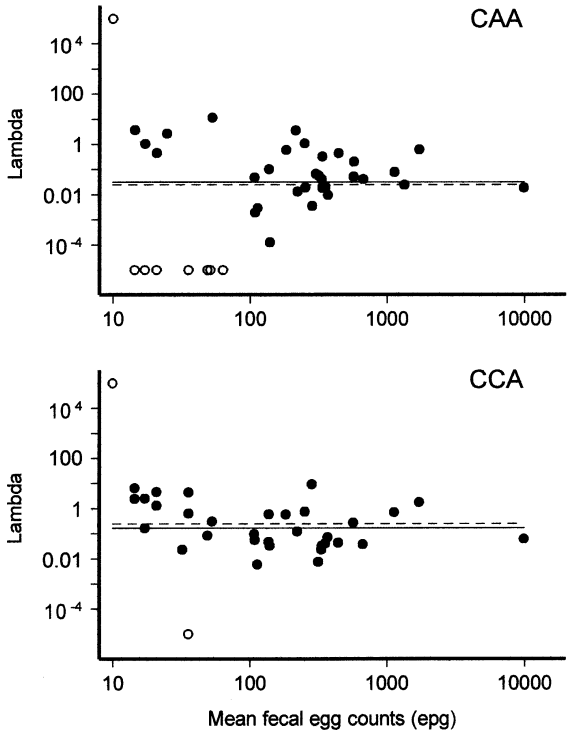

Fig. 1. Individual values of lambda ($\lambda = S^2_{ex}/S^2_{ey}$) plotted against the mean egg counts. Individual λ values were calculated for all individuals who had complete measurements for egg counts and for circulating antigens, and were positive for at least 1 measurement of egg counts or (CAA or CCA). (○) Cases with a within-individual variance of 0 for either epg or (CAA or CCA), and thus a log λ of either $+\infty$ or $-\infty$. The geometric mean λ (———) was calculated after leaving out an equal number of highest and lowest values until no infinite numbers were left; 'bootstrap' was used to determine 90% confidence intervals (CI). The resulting geometric mean λ (90% CI) is $\lambda = 0{\cdot}025$ (0·008–0·072) for CAA and $\lambda = 0{\cdot}25$ (0·13–0·47) for CCA. (– – – –) Corresponding median λ of all values. It is shown that the individual λ's show less variation with higher intensities, and that the mean λ does not depend on the intensity of infection.

relationship between the two variables will be non-proportional, suggesting a reduction in fecundity at high worm loads (i.e. circulating antigen levels), i.e. density-dependent fecundity. Cases with negative results for both egg excretion and CAA (or CCA) determination were considered to be uninfected and excluded from the regression analysis. Individuals with negative results for only 1 of these indicators were included. They were assumed to have light infections missed by one of the diagnostic methods, and leaving them out would thus lead to bias due to differences in sensitivity. To allow for these zeros in the analyses, log-transformation was applied after adding a value equal to half the detection limit of the particular assay to the data. A limited number of outlying points (i.e. > 3 times the standard deviation from the regression line) were rejected from the analysis.

Deming regression

Regression analysis according to Deming was used, allowing for variation in both the dependent $y =$ log(epg) and the independent variable $x =$ log(CAA or CCA) (Deming, 1943; Cornbleet & Gochman, 1979; Ward & Cornish, 1992). Calculation of the Deming slope

$$\beta = U + \sqrt{U^2 + (1/\lambda)},$$

where

$$U = \frac{\sum_{i=1}^{N}(y_i - \bar{y})^2 - (1/\lambda)\sum_{i=1}^{N}(x_i - \bar{x})^2}{2\sum_{i=1}^{N}(y_i - \bar{y})(x_i - \bar{x})} = \frac{S_y^2 - (1/\lambda)S_x^2}{2S_{xy}},$$

requires the ratio

$$\lambda = \frac{S^2_{ex}}{S^2_{ey}},$$

of the within-individual variance S^2_{ex} of the independent variable x and the within-individual variance S^2_{ey} of the dependent variable y. Also here, to allow for zeros, log-transformation was applied after adding a value equal to half of the detection limit of the particular assay to the repeated measurement data from Burundi.

For comparison, least-squares regression lines of the relationship between egg counts and CAA and CCA were also determined. Ordinary regression assumes no variation in the independent variable and thus corresponds with Deming regression with $\lambda = 0$. This may lead to an underestimation of slope β, and thus overestimation of a potential density-dependent fecundity effect. To determine whether application of Deming regression is required, depends on the degree of variation in the independent variable (within-individual variation as compared to the between-individual variation). A useful rule of thumb was given by Cornbleet & Gochman (1979): $S_{ex}/S_x > 0{\cdot}2$, i.e. significant error in the least-squares slope estimation occurs when the ratio of the standard deviation of measurement of a single x value (near the mean x) to the standard deviation of the x-data set exceeds 0·2. Otherwise, least-squares regression analysis may do.

RESULTS

Figure 1 shows the individual λ's plotted against the mean egg count. It is shown that the mean λ does not depend on the intensity of infection, and only becomes more stable (i.e. individual λ's show less variation) with higher intensities. The resulting geometric mean $\lambda = 0{\cdot}025$ for CAA and $\lambda = 0{\cdot}25$ for CCA, representing a variation in serum circulating antigens which is much lower than in fecal egg counts.

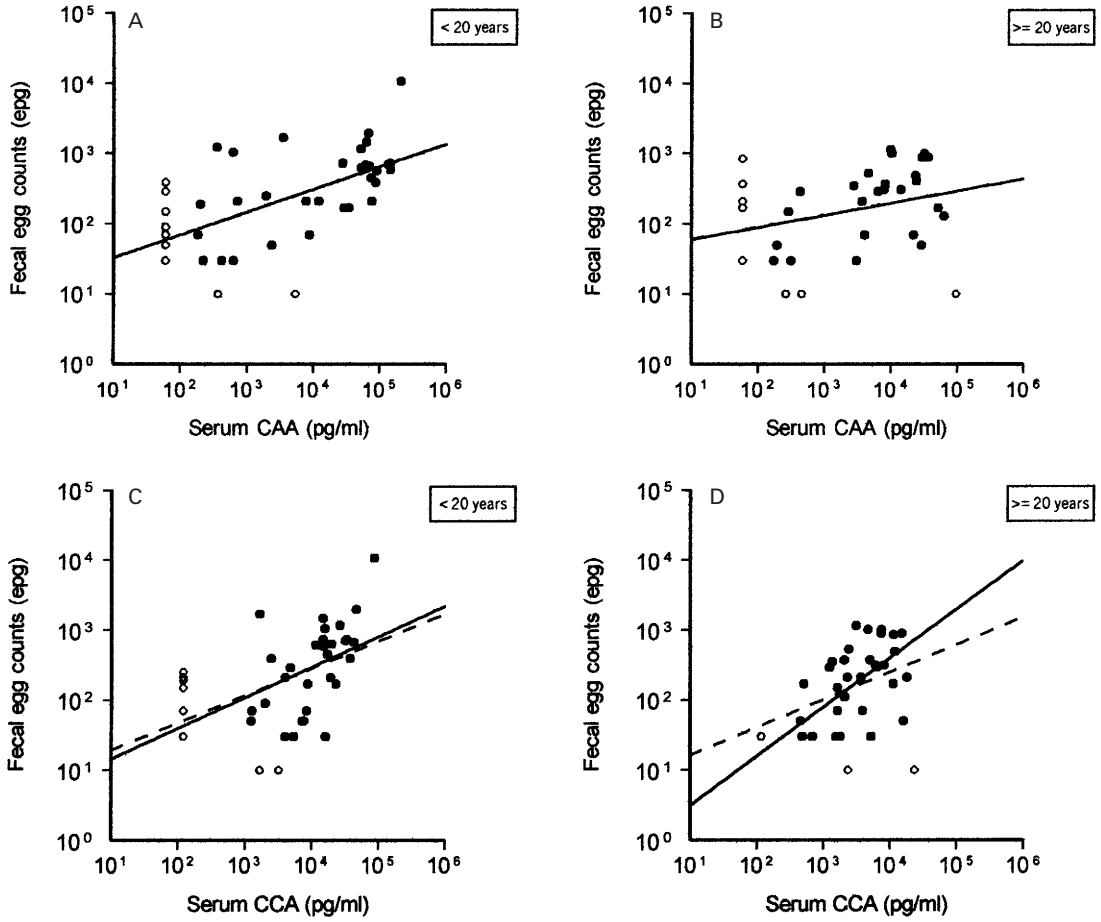

Fig. 2. Fecal egg counts plotted against serum circulating antigen levels for 2 age groups in Burundi: (A) CAA, 0–19 years ($n = 44$); (B) CAA, $\geqslant 20$ years ($n = 34$); (C) CCA, 0–19 years ($n = 39$); (D) CCA, $\geqslant 20$ years ($n = 34$). Individuals negative for both egg counts and CAA ($n = 12$) or CCA ($n = 13$), and outliers (none for CAA; $n = 1$ for CCA, both for Deming and least-squares regression) were excluded from the analysis. (———) Outcome of Deming regression; (– – – –) least-squares regression lines. It is shown that for CCA the least-squares slope is considerably lower than the Deming slope, while for CAA they are virtually the same.

Figure 2 shows the scattergrams of the individual egg count data against serum CAA and CCA measurements from Burundi, including the Deming and least-squares regression lines of the relation between log antigen level and log egg counts. For the relationship between egg counts and CAA, least-squares and Deming's slopes are almost similar, while the least-squares slopes for the relationship between egg counts and CCA appear to be considerably underestimated, especially in those of 20 years and older. Applying the rule of thumb by Cornbleet & Gochman (1979), the S_{ex}/S_x ratio was 0·24 (< 20 years) and 0·30 ($\geqslant$ 20 years) for CCA and 0·08 (< 20 years) and 0·10 ($\geqslant$ 20 years) for CAA, respectively, indicating that for CCA Deming regression should be used, while for CAA ordinary linear regression may actually suffice.

Figure 3 shows the slopes (β) of the regression lines describing the relationship between egg counts and circulating antigens (CAA and CCA) in the data sets from Senegal, Zaire, and Burundi, using Deming regression with $\lambda = 0{\cdot}025$ for CAA and $\lambda = 0{\cdot}25$ for CCA. It is shown that β is significantly < 1·0 for CAA, indicating that egg production is suppressed in individuals with higher worm burdens for all 4 age classes in Senegal, Zaire and Burundi. The slopes for CCA are also consistent with density-dependent fecundity, although to a lesser extent than for CAA. These findings still hold when using the upper limits of the 90% confidence intervals of the respective λ's, i.e. $\lambda = 0{\cdot}072$ for CAA and $\lambda = 0{\cdot}47$ for CCA.

DISCUSSION

The low levels of slope β in Senegal and Zaire confirm and even strengthen the non-linear relationship between CAA and egg counts as found by Van Lieshout *et al.* (1995, 1998), implying that egg counts do not increase proportionately with CAA levels. Moreover, they also suggest non-proportionality for CCA, while Van Lieshout *et al.* (1995) observed an approximately linear relationship between CCA levels and egg counts. Van Lieshout *et al.* (1995) already mentioned that their estimate of λ might be rather high and that more studies with

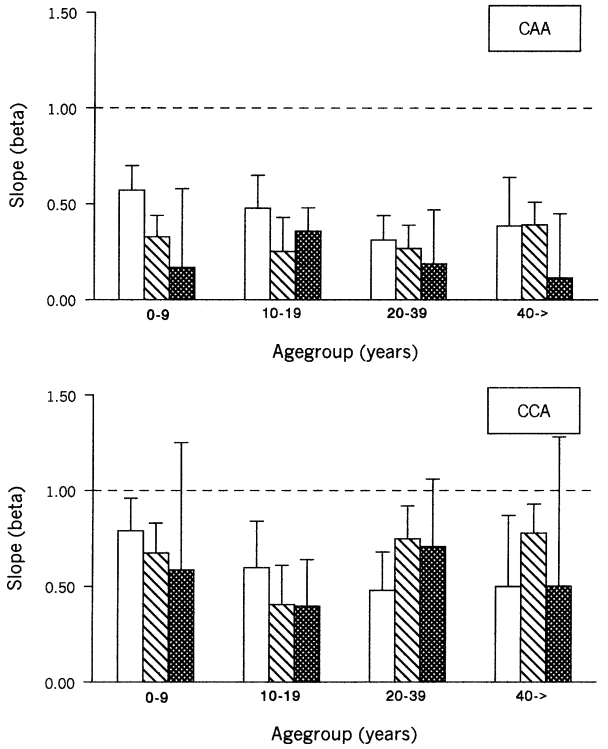

Fig. 3. Slopes (β) of the regression lines describing the relationship between egg counts and circulating antigens in Senegal (□), Zaire (▨) and Burundi (▩), using Deming regression with $\lambda = 0{\cdot}025$ for CAA and $\lambda = 0{\cdot}245$ for CCA. The error bars show the upper limit of the 95% confidence intervals of the slopes, given the λ's. (– – – –) Slope $\beta = 1$, which would mean a perfect proportional relationship between egg counts and CAA (or CCA). It is shown that slope β is $< 1{\cdot}0$ for CAA and CCA, indicating non-proportionality in all age classes in Senegal, Zaire and Burundi.

repeated measurements of circulating antigens were needed to allow a thorough evaluation of their relative stability. Given that such studies are very intrusive because of the multiple invasive blood samplings per individual within a short period, it is not surprising that so far they have not yet been available. We believe that our data set satisfies the need and generates more reliable estimates of λ, and consequently, allows a more solid analysis of the relationship between circulating antigens and egg counts.

The values of λ do not seem to be dependent on the intensity of infection, which allows us to apply the same values in other study populations for which repeated antigen measurements are not available. Provided that the amount of feces examined is about 50 mg (e.g. a 2×25 mg or a standard 1×43 mg Kato-Katz stool examination), these λ's are thus applicable to any endemic situation. If, however, the amount of feces deviates considerably from 50 mg or repeated stool examinations are performed, a new λ should be calculated.

As the λ value for CAA was almost equal to zero, the question arises if it is really necessary to compute Deming's regression lines, or if least-squares regression analysis may actually suffice. Indeed, for the relationship between egg counts and CAA, the Deming's and least-squares slopes were virtually similar in all age groups from Senegal, Zaire and Burundi. Also, according to the rule of thumb by Cornbleet & Gochman (1979), no significant error would occur in the least-squares slope estimation of the regression line relating CAA and egg counts in Burundi. This would mean that in future studies on the relation between CAA and egg counts, least-squares regression can be applied just as well. For CCA, however, not using Deming regression would lead to overestimation of regression slopes.

Although Deming regression is frequently used to analyse method-comparison data in clinical chemistry, where results obtained by a test method are compared with those obtained by a gold-standard or reference method, it has not (yet) received much recognition in parasite epidemiology. Typical examples where Deming regression should be considered, other than the present study, are to compare new quantitative diagnostic field tests with conventional methods; to compare individual parasitological data before and after intervention in treatment (or vaccination) studies; to relate reinfection and infection data to study predisposition to infection. When the same quantitative measure is used both as dependent and independent variable (e.g. egg counts before and after treatment or reinfection), then the λ value for Deming regression will be (close to) 1.

The observed invariably non-proportional relationship between egg counts and CAA (and to a lesser extent CCA) in 3 different communities, indicating that egg production is suppressed in individuals with higher worm burdens, corroborates the hypothesis of density-dependent fecundity in human schistosomiasis. The difference in the extent of non-proportionality between CAA and CCA might be the consequence of differences in production and clearance mechanisms of these two antigens, as indicated by previous studies (e.g. Kestens *et al.* 1988*a*, *b*; Van Lieshout *et al.* 1995; Van Dam *et al.* 1996). More generally, it should be noted that the fitted relationship depends very much on the accuracy with which worm burdens are reflected by circulating antigens and egg production by egg output. Other biological mechanisms affecting the production of eggs or clearance of antigens, or even methodological/assay-related mechanisms may bias the relationship. For example, higher egg production may result in relatively more eggs retained in the tissue (due to clumping of eggs) and thus less eggs excreted in the feces, yielding a non-proportional relationship between egg output and worm burden, while the relationship between egg production and worm burden may actually be proportional. Another possibility is that with higher

circulating antigen levels, the clearance of the antigen may become less efficient, resulting in relatively higher measured antigen concentrations, which may yield a non-proportional relationship with egg output as well. Although these alternative mechanisms are hypothetical, they cannot be excluded, and biological research on these issues is still essential. Nevertheless, relating egg counts to circulating antigens is so far the only, feasible, alternative to study (density-dependent) *S. mansoni* worm dynamics and fecundity in human populations. Particularly in vaccine trials, a solid analysis of this relationship will be indispensable to allow differentiation between vaccine-induced worm reduction and anti-fecundity effects.

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