Focus

Underestimation of *Schistosoma mansoni* Prevalences

S.J. de Vlas and B. Gryseels

Field methods used for detecting Schistosoma mansoni *infection miss a certain proportion of the infections. Prevalences of infection appear to be far underestimated by faecal screening, with important consequences for control and research. Sake de Vlas and Bruno Gryseels investigate how the number of undetected infections can be statistically inferred from population surveys.*

Most control programmes and epidemiological studies on *Schistosoma mansoni* infection are based on the detection and quantification of parasite eggs by faecal thick-smear methods, such as the Kato–Katz technique[1,2]. These methods allow the examination of large numbers of samples within a short time, and provide useful quantitative epidemiological information. However, in operational conditions it is generally not possible to examine more than one stool sample per individual. Repeated examinations or the application of more sensitive methods show that infections, especially light ones, may then be missed (the so-called 'false negatives') and prevalences underestimated[2–11].

The Relevance of Missed Infections

Light infections, missed after screening, are not considered a crucial problem to morbidity control, as it is assumed that they are not associated with severe pathology[12,13]. Nevertheless, it can be useful to estimate the proportion of missed infections for several reasons. First, the relationship between egg counts and morbidity is less straightforward than generally assumed[14]. Autopsy data show that the relationship between worm load and faecal egg counts is variable, particularly in cases with severe pathology, such as pipe stem fibrosis, where egg counts may be low or even negative[15,16]. More recently, this has also been demonstrated in community-based ultrasound studies[17]. Second, infections that remain undetected and untreated may be partly responsible for the persistence of transmission after population chemotherapy, a major problem in many control programmes[11,18–20]. Third, the sensitivity of detection decreases as the intensity of infection decreases; the proportion of missed infections increases after treatment, leading to an overestimation of cure rates[8,21]. False negatives obscure the results of selective chemotherapy by showing up as positives at the subsequent screening round[11]. Fourth, persistent light infections may be crucial for the maintenance of concomitant immunity and thus also for the understanding of acquired resistance and the development of possible vaccine strategies[22–24]. In sero-epidemiological surveys, 'false positive' humoral reactions in parasitologically negative individuals cannot be properly interpreted; they may be due to light infections but also to either cross-reactions or past infections[5,10,25,26].

How Many Infections are Missed by Faecal Egg Counts?

Several authors have tried to estimate and to improve the sensitivity of egg counts by repeating stool examinations[2–11,27]. Most of these studies have been limited to three stool samples. On the basis of empirical data from eight communities, Jordan *et al.*[3] have related prevalences estimated by a filtration method in a single stool (x) to those obtained from examining three stools (y). The formula they obtained using polynomial regression

$$y = 4.987 + 1.72754 - 0.0077x^2$$

is only applicable for the comparison of results from one and three stools. For every other combination, new empirical data must be gathered and processed. Furthermore, it is not known how accurate the estimation of the 'true prevalence' with three or more examinations really is. How many more infections would show up after additional examinations? It would clearly be useful to have a generally valid estimation of true prevalences on the basis of conventional stool surveys.

The classical assumption of sensitivity and specificity being intrinsic, constant characteristics of a diagnostic test[28,29] is not applicable for most helminthic infections. The probability of detecting parasite eggs in the stools of an individual depends on the intensity of his infection[11,27]. Relationships between the sensitivity of stool examinations and prevalences, shown for example by Jordan *et al.*[3], are in fact due to the relation between prevalence and intensity of infection[21,29]. Goddard recognized that sensitivity varies with the level of endemicity, and modelled the probability of a false negative outcome as a negative exponential function of the prevalence[21]. Exploring the consequences on the interpretation of the effects of chemotherapy, he demonstrated that cure rates are overestimated when detection of infection is imperfect. However, his model was not tested with empirical data and lacked a satisfactory relationship between prevalence and intensity of infection. In order to quantify underdiagnosis, we would need an approach that takes into account this relationship and, more generally, the underlying mechanisms of egg count variations in the population.

Recently, a stochastic model has been developed which incorporates the distributions of worms and worm pairs in the population, as well as the variability of egg counts in stool samples from an individual with a given worm load[30]. The model thus explicitly distinguishes inter- and intra-individual variation in egg counts, and implies a consistent statistical relationship between prevalence and intensity of infection according to a negative binomial distribution. Empirical data based on single and repeated faecal egg counts from endemic communities in Zaire and Burundi have been used to test and validate this model[7,11,30]. Mathematical details of the model are found in Box 1.

By making inferences about the worm and worm pair distributions, the model can be applied to analyze the underestimation of prevalences in any surveyed population. The *true* prevalence of active infection is defined here as the proportion of individuals with at least one worm pair, and is

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Box 1. A Model for Variations in *S. mansoni* Egg Counts

We consider three sources of variation responsible for the egg count distribution in a population:

(1) variation in exposure, acquired immunity, and susceptibility; the resulting probability for a member of the population of having n worms is indicated by $P_N(n)$,

(2) mating of male and female worms; the probability of having x worm pairs for an individual with worm load n is indicated by $P_{X|N}(x|n)$,

(3) variation in repeated egg counts from the same individual, which depends on several processes such as day-to-day fluctuations of egg production, the amount and consistency of stools, etc.; the probability of counting y eggs in a stool sample from an individual harbouring x worm pairs is represented by $P_{Y|X}(y|x)$.

The distribution of the number of worm pairs in the population is a mixture of (1) and (2) and is expressed by:

$$P_X(x) = \sum_n P_{X|N}(x|n) \cdot P_N(n) \qquad (1)$$

The joint distribution of egg counts $y_1, y_2, \ldots y_w$ in w consecutive stool samples is now described by:

$$P_{Y_1 Y_2 \ldots Y_w}(y_1, y_2, \ldots y_w) = \sum_X \left[\prod_{i=1}^{w} P_{Y_i|X}(y_i|x)\right] \cdot P_X(x) \qquad (2)$$

In *S. mansoni* infection, theoretical and empirical considerations support the use of the negative binomial distribution for describing both the variation in worm numbers and the individual variation in faecal egg counts[30,33]. We consider schistosomes to be monogamous with a male:female ratio of 1:1, and we therefore use a binomial distribution with $p = 0.5$ for describing the mating process. Under the assumption that egg excretion is not density dependent, a linear relationship between worm pair load, x, and the mean number of eggs per sample is used[30].

The model now enables the estimation of the worm and worm pair distribution in the population, $P_N(n)$ and $P_X(x)$, respectively, on the basis of actual egg counts, which provides insight into the 'true prevalence'.

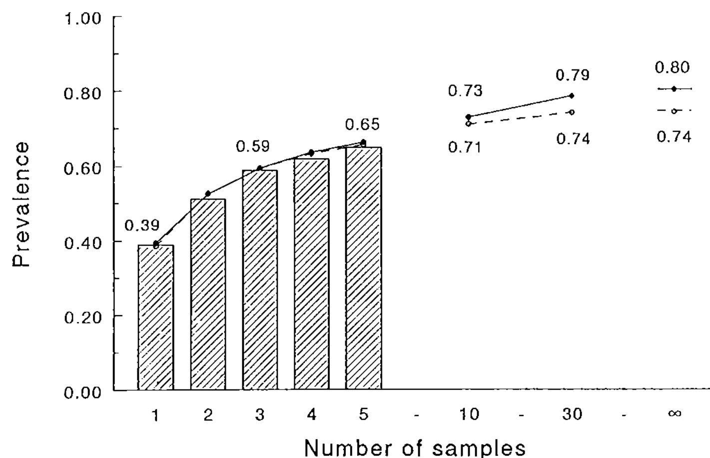

Fig. 1. Prevalences of S. mansoni *infection over successive surveys. The data concern 131 inhabitants of the village of Buhandagaza in Burundi, aged 20 and older, of whom five consecutive egg counts in 50 mg Kato slides[1] were obtained[11]. Bars indicate the observed proportion of positively diagnosed individuals. The model (Box 1) has been fitted to all quantitative egg counts of each individual. The two curves represent predicted prevalences, shown for two values of the expected number of eggs per sample per worm pair:* h = 0.05 *(solid curve) and* h = 0.14 *(broken curve). The value* h = 0.05 *was obtained by maximum likelihood estimation from the complete Buhandagaza data[30] and equals the mean value based on experiments in several non-human mammals[34]. The corresponding values of mean* (M) *and index of aggregation* (k) *of the negative binomial distribution of worm numbers are* M = 78 *and* k = 0.40. *The value* h = 0.14 *is the upper limit of the confidence interval from the Buhandagaza data set, and approximates the results of an Egyptian autopsy study[35]; the corresponding parameter values are* M = 29 *and* k = 0.46. *The index of aggregation for the negative binomial distribution of repeated individual egg counts (r) is 0.87 (Ref. 30). The model predictions agree very well with the observed prevalences. The true prevalence is theoretically obtained by examining an infinite number of samples. The results differ slightly for the two values of* h, *but in both cases the true prevalence would be about twice as high as the prevalence measured in a single survey.*

calculated from the (estimated) distribution of worm pair numbers (Eqn 1, Box 1). The *observed* prevalence, defined here as the proportion of individuals that would show at least one positive egg count in a number of samples, is projected from Eqn 2. People who have no or monosexual infections will always show negative egg counts, ie. true negatives. However, as a result of the intraindividual variation in egg counts, negative counts can also be found in samples from people with low numbers of worm pairs, ie. false negatives. The difference between true and observed prevalence is the proportion of false negatives in a population survey.

Fig. 1 shows an application of the model for selected data from Burundi. The prevalence measured in a single stool survey was 39%, increasing to 59% after three surveys and to 65% after five. The agreement between measured and projected prevalences after one to five surveys shows that the model fits the data very well. Further extrapolation with the model predicts that, in such an endemic situation, the true prevalence would be between 74% and 80%. Thus, only about half of all infected individuals would have been detected if only one survey had been done.

Fig. 2 demonstrates the consequences of the model in a more general way, by showing the relationship between the prevalence and the proportion of false negatives. The relation with intensity is incorporated in the value of k. The graphs show that, particularly in low-prevalence areas, the underestimation of prevalences can be extremely important. An observed prevalence of 20% from a single survey would actually (for $k = 0.5$) correspond to a true prevalence higher than 70%. As may be expected, the proportion of false negatives decreases after examining multiple stools, but a considerable number of infections would still show up after more than three egg counts; even after 30 surveys, previously undetected infections may be found (Fig. 1).

The consequences of the underestimation of prevalences for the interpretation of control results are shown by the following example. A prevalence reduction from 40% to 20%, based on one sample per individual, appears considerable. Assessment of the true prevalences, assuming moderate over-dispersion ($k = 0.5$), indicates a much less spectacular reduction from 85% to 71%. The proportional prevalence reduction in this case would thus be 14% instead of 50%. It may be argued that

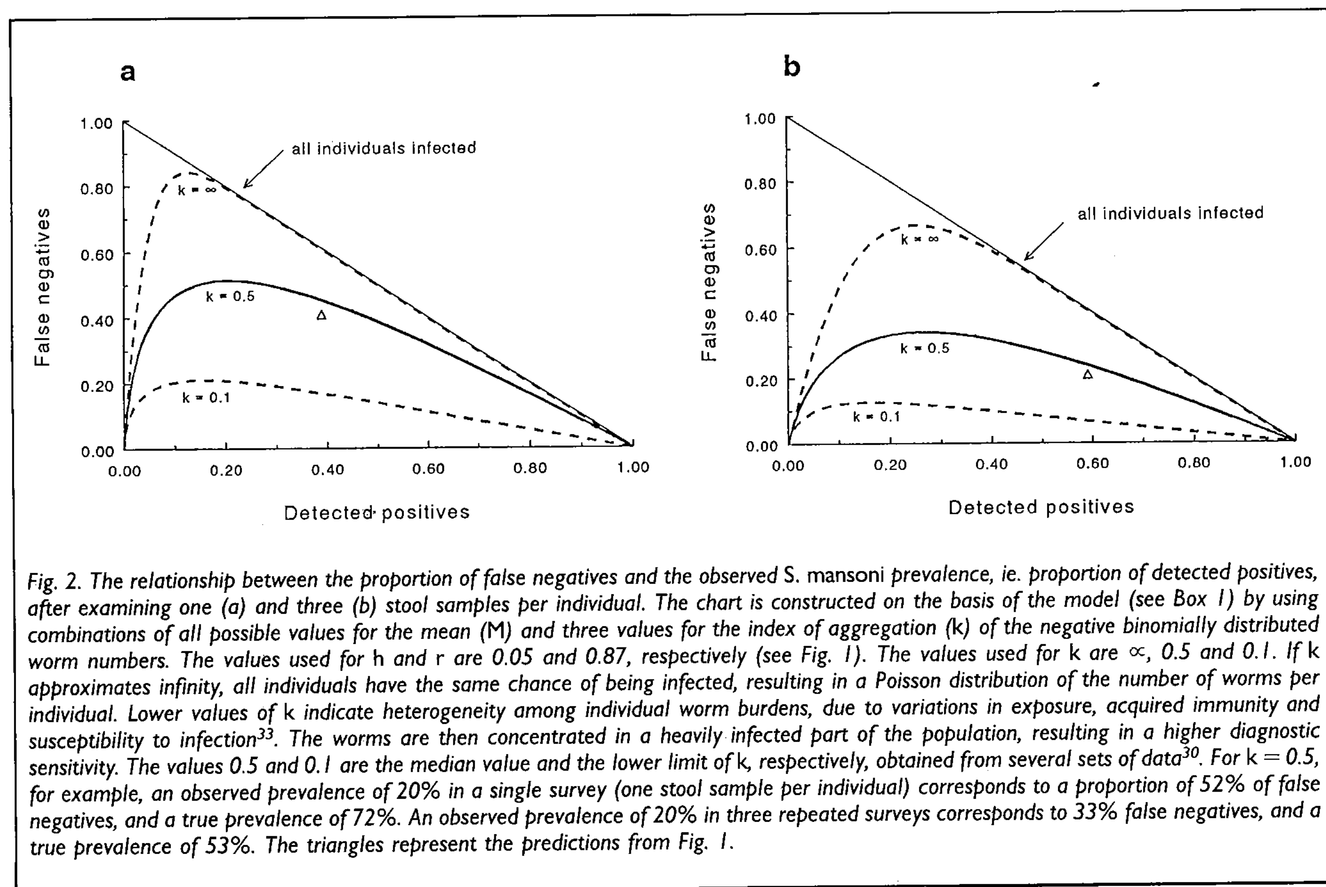

Fig. 2. The relationship between the proportion of false negatives and the observed S. mansoni *prevalence, ie. proportion of detected positives, after examining one (a) and three (b) stool samples per individual. The chart is constructed on the basis of the model (see Box 1) by using combinations of all possible values for the mean (M) and three values for the index of aggregation (k) of the negative binomially distributed worm numbers. The values used for h and r are 0.05 and 0.87, respectively (see Fig. 1). The values used for k are ∝, 0.5 and 0.1. If k approximates infinity, all individuals have the same chance of being infected, resulting in a Poisson distribution of the number of worms per individual. Lower values of k indicate heterogeneity among individual worm burdens, due to variations in exposure, acquired immunity and susceptibility to infection[33]. The worms are then concentrated in a heavily infected part of the population, resulting in a higher diagnostic sensitivity. The values 0.5 and 0.1 are the median value and the lower limit of k, respectively, obtained from several sets of data[30]. For k = 0.5, for example, an observed prevalence of 20% in a single survey (one stool sample per individual) corresponds to a proportion of 52% of false negatives, and a true prevalence of 72%. An observed prevalence of 20% in three repeated surveys corresponds to 33% false negatives, and a true prevalence of 53%. The triangles represent the predictions from Fig. 1.*

the negative binomial distribution at the basis of these projections may not be valid shortly after population treatment. However, reduction of worm loads in heavily infected individuals would decrease overdispersion, giving a higher value of *k*, and would result in an even lower sensitivity of detection (Fig. 2).

Concluding Remarks

We have attempted to formalize the obvious point that a single or even multiple faecal egg counts reveal only a proportion of the infections with *S. mansoni* in an endemic community. More sensitive parasitological methods, such as elaborate concentration techniques or rectal biopsies, are not very suitable for field work. Indirect techniques such as haematuria dipsticks, which have shown their merit in surveys and control of urinary schistosomiasis[31], are unfortunately not available for schistosomiasis mansoni. Conventional immunodiagnostic assays to detect antibodies are not specific enough to provide a valid alternative, although antigen detection may be a more promising approach[32].

Previous attempts to determine underdiagnosis mathematically were not very satisfactory as they were either specific for particular data sets or did not make allowance for underlying egg count variation. Our approach shows that statistical methods can make a valuable contribution to the quantification of true prevalences. The results reveal that underestimation of prevalences, even by repeated egg counts, may be surprisingly large. Although our results provide no 'gold standard', comparative graphs such as those in Fig. 2 may help field workers to estimate at least the order of magnitude by which they underestimate prevalences by conventional screening methods.

Our findings have substantial implications for the control of *S. mansoni*, that can also be extended to *S. japonicum*. Control programmes based on screening and selective chemotherapy will inevitably leave a large number of infected people untreated. Some of these may have severe pathology, and a potential reservoir for reinfection will always remain untouched. Both considerations support the case for non-selective mass treatment[14]. Furthermore, the evaluation of control results from estimations of prevalence may lead to unwarranted optimism, as such estimates are inherently biased by a reduction of diagnostic sensitivity. In epidemiological and immunological research, true 'negative controls' in endemic areas cannot be reliably selected on the basis of stool examinations. Defining and modelling resistance on the basis of 'absence of infection' should thus be treated with great caution[22]. Foolproof assessment of the sensitivity and specificity of antibody-based serological assays will probably never be possible. Clearly, the development of more sensitive and specific diagnostic tools for the detection of *S. mansoni* infection deserves high priority.

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Why Does Liver Fibrosis Occur in Schistosomiasis?

D.J. Wyler

Schistosomiasis is one of the most prevalent of several chronic inflammatory diseases in which morbidity results primarily from tissue scarring. New concepts regarding the molecular pathogenesis of scar formation are being applied in research efforts to define the basis of liver fibrosis in schistosomiasis. Such investigations have led to the identification of an apparently novel lymphokine, fibroblast stimulating factor-1 (FsF-1), produced in the egg granulomas. FsF-1 and other granuloma-derived fibrogenic cytokines may represent the molecular links between periovular granulomatous inflammation and hepatic fibrosis. Here, David Wyler postulates that the unmodified production of these fibrogenic signals may be responsible for the development of severe hepatic fibrosis in the subpopulation of infected individuals who develop this complication.

The classical viewpoint that scar tissue is simply a pathological dead-end has been replaced by the perspective that fibrogenesis is a dynamic, potentially preventable and reversible process comprised of discrete biological steps that are amenable to specific analysis[1,2]. Scar tissue is typically comprised of an excessive number of mesenchymal cells, most notably fibroblasts. Endothelial cell proliferation (during wound healing)[3] and vascular smooth muscle cell hyperplasia (in arteriosclerosis)[4] are other mesenchymal cell responses that can be considered as variable components of fibrosis. The excessive deposition of extracellular matrix macromolecules, most notably the collagens[5], fibronectin[6] and the glycosaminoglycans[7], is also significant.

One of the most important developments in fibrosis research in the past two decades has been the identification of proteins that influence a variety of the mesenchymal cell functions involved in scar formation[8,9]. Several of these are cytokines produced by macrophages and lymphocytes, the cells that constitute chronic inflammatory lesions. Evidence accumulated in the study of schistosomiasis[10], rheumatoid arthritis[11], systemic sclerosis[12] and pulmonary fibrosis[13] supports the notion that these cytokines are important in the pathogenesis of the tissue fibrosis that is a major cause of serious morbidity in these chronic inflammatory diseases.

The fibrogenic cytokines can influence a variety of relevant responses of fibroblasts, including their migration[14] and growth[15], the production of extracellular matrix macromolecules[16] and the synthesis of collagenases[17]. The fact that most of these fibroblast responses can be elicited, *in vitro*, with picomolar concentrations of cytokines is one argument for their physiological significance. Since inhibitors of the fibroblast responses have also been identified[18–20], there is good reason to believe that fibrosis is homeostatically regulated, that is, the prevention of fibrosis in normal connective tissue, the initiation of fibrosis during wound healing or in association with chronic inflammation, the limitation in the extent of fibrosis, and ultimately the involution of scar (when it occurs) may all be the expression of a carefully controlled balance in the stimulatory and inhibitory signals.

Why Study Fibrogenesis in Schistosomiasis?

The new developments in fibrosis research generally may assist in uncovering the pathogenesis of liver fibrosis in schistosomiasis and, conversely, insights into this pathogenesis may prove to have broadly relevant implications. The motivation to study liver fibrosis in schistosomiasis derives from the indisputable fact that the important morbidity that results from infection with *Schistosoma mansoni* and *S. japonicum* is due to complications of liver fibrosis[21]. Indeed, without such fibrosis, most patients remain asymptomatic. Since only a small subpopulation of infected individuals develop clinically significant liver fibrosis (less than 10% in schistosomiasis mansoni), it would be valuable to understand the molecular and genetic basis for this selection. Furthermore, while anthelmintic therapy has successfully prevented and

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