Worm Burdens in Schistosome Infections

B. Gryseels and S.J. de Vlas

Schistosomiasis, caused by fluke worms of Schistosoma *spp, is one of the most common tropical diseases. Despite decades of research and progress towards the control of the disease, many aspects of the dynamics of infection and immunity remain unresolved. There is, in fact, not even an approximate measure of how many worms are harboured by infected humans. Epidemiological, mathematical and biomedical arguments indicate that individual worm burdens in endemic areas number hundreds to thousands of adult schistosomes, instead of the few to dozens generally assumed on the basis of available autopsy data. As Bruno Gryseels and Sake de Vlas here discuss, this hypothesis has important consequences for research and control, as many constants in schistosomiasis research have to be reconsidered.*

In contrast to intestinal worms such as *Ascaris* and *Trichuris*, which are expelled after treatment and which can then be counted, direct quantification of *Schistosoma mansoni* in endemic situations is impossible. In control programmes and epidemiological studies, intensity of infection is estimated indirectly by counting the number of *S. mansoni* eggs in calibrated faeces samples, which are usually converted into eggs per gramme faeces (EPG). Surprisingly little effort has been made to relate EPGs to worm numbers, and to estimate actual worm burdens in endemic situations.

Quantification of worm burdens would greatly improve our understanding of the dynamics of schistosome populations, host immunity, morbidity and control. The regulation of schistosome populations is still fundamentally debated: for some authors, transmission is the main regulatory level[1], for others the host[2]. The answer has profound implications for control: should we concentrate on destroying snails or on developing vaccines? Epidemiological models that could test the various hypotheses against field data depend strongly on variables representing schistosome numbers[3–5].

Acquired immunity, developed over years of exposure, is believed to determine individual levels of susceptibility to (re)infection[6,7], and vaccine development has therefore received high priority[8,9]. However, without a measure of worm burdens, the protective effect of a vaccine will be difficult to evaluate, especially if it would also trigger anti-fecundity immunity[8]. Obviously, anti-infection and anti-fecundity effects cannot be distinguished on the basis of faecal egg counts.

Population chemotherapy has reduced morbidity in many endemic countries, so that transmission control now becomes the next objective[10]. Whether interruption or even reduction of transmission can be pursued via chemotherapy or vaccination depends crucially on pre-intervention worm burdens. Indeed, if these were in the range of hundreds to thousands, even a 99% effective drug or vaccine would leave a few worms in most infected people. The remaining light infections, often not detectable by stool examination, will then act as a continued reservoir for transmission[11]. Without additional interventions which disturb the ecologically established balance between humans and parasite populations, the latter will (after cessation of chemotherapy) inevitably re-establish their initial levels.

Commonly assumed knowledge and its consequences

The average productivity of mated female *S. mansoni* worms is about 300 eggs per day, of which about 50% will reach the faeces[12–14]. This assumption is based mainly on experiments in mice, hamsters, baboons and chimpanzees[15,16], and – perhaps questionably – extrapolated to humans. For a daily faecal production of about 150 grammes per human[17], a simple calculation shows that EPG and worm pair (WP) burdens would have a 1:1 relationship. A 'common' egg count of 200 EPG would then correspond with a burden of 200 worm pairs and at least 400 worms. Counts of 30 000 – 40 000 EPG, such as seen in some high-endemicity areas[18,19], would at first sight imply incredible numbers of worms.

However, individual counts are subject to marked variations[20,21]. We have developed a model for individual variations in worm loads and egg counts, which, after extensive fitting, testing and validating, allows the prediction of the corresponding distributions of WP burdens[11,21,22]. Figure 1 shows the consequences of this model on the population level, when a 1.0 EPG per WP ratio is assumed, for three typical endemic situations where we have worked over the past decade[18,23]. In all three villages, worm burdens over 100 (>50 WP) would be abundantly present. Also burdens over 1000 worms are a common phenomenon: even in the low-endemicity village of Buhandagaza, three percent of the population would harbour more than 1000 worms, and in the moderately endemic focus of Gihungwe, this would be 17%. In the intense-focus Makundju, eight percent of the population would harbour as many as over 10 000 worms.

Hard evidence

It is quite remarkable that these consequences of handbook knowledge are in such flagrant conflict with the only available (and widely cited) direct measurement of worm burdens in humans. In 1968, Cheever published the results of perfusion and dissection of 197 cadavers of people who had died of different causes in a hospital in Salvador da Bahia, Brazil, of which 103 were infected with *S. mansoni*[24]. Worms were collected and counted, and faecal and tissue egg counts were determined from each cadaver. Figure 2 summarizes his findings with respect to faecal

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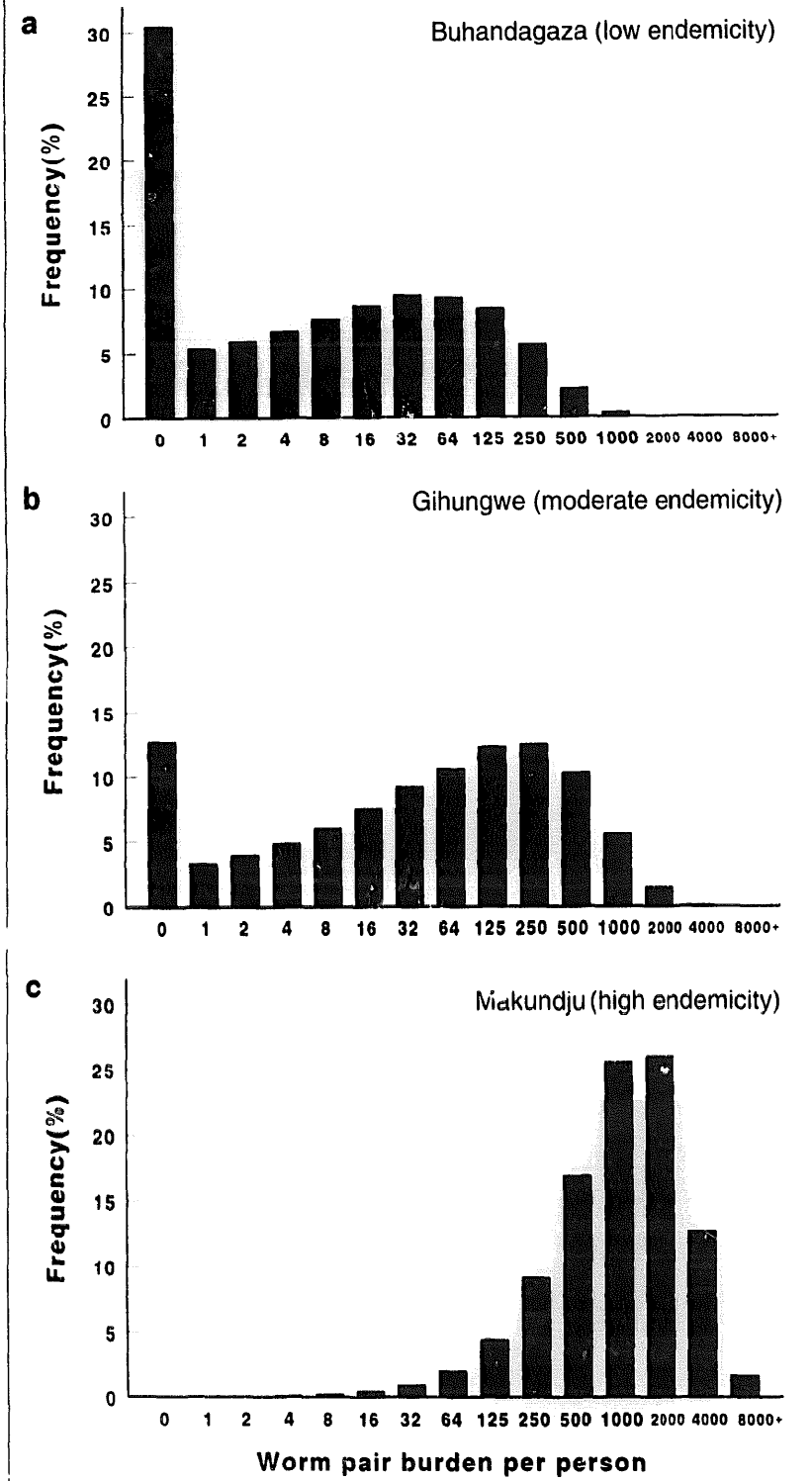

Fig. 1. Predicted distribution of *Schistosoma mansoni* worm pairs among individuals from three different endemic communities in Burundi and Eastern Zaire: Buhandagaza, low endemicity (38%, 80 EPG) (a); Gihungwe, moderate endemicity (61%, 149 EPG) (b); and Makundju, high endemicity (96%, 791 EPG) (c). The values between parentheses represent the observed prevalences (%) and geometric means of positive egg counts (EPG), respectively. These observations were based on 50 mg Kato-Katz thick smears[35]. On the x-axis, the lower limits of the predicted worm pair burden intervals are indicated. Predictions are obtained after fitting a model for egg count variation[11,21,22] to the observed data. The distribution of individual worm pair burdens is characterized by a negative binomial distribution of total worm burdens with mean (M) and aggregation parameter (k), and assuming monogamous mating with a male:female ratio equal to 1:1. The estimated parameter values are for Buhandagaza $M = 147$, $k = 0.23$, for Gihungwe $M = 561$, $k = 0.33$ and for Makundju $M = 4288$, $k = 1.2$.

egg counts and WP loads. The majority of the cases had <100 WP. Only ten cadavers harboured >100 WP; the highest number was 1608 (3669 worms) found in a four-year-old child who had died from ulcerative colitis.

Evidently, this autopsy series does not represent a normal endemic community, particularly with regard to the higher range of worm burdens. Most terminal patients belonged to older age groups, in which infections are generally less intense[10,12–14]. Because of their illness and hospitalization, they probably had not been exposed to transmission for a considerable time anyway. Furthermore, most subjects came from around Salvador, a (sub)urban area with low transmission. It is therefore safe to assume that worm burdens in more typical, rural endemic communities will be considerably higher than those reflected in Fig. 2.

Nevertheless, the autopsy studies could help to clarify the relationship between worm pairs and faecal egg counts. Figure 2 shows a significant positive correlation, although individual ratios of EPG per WP vary strongly. From the data, the overall EPG per WP ratio can be calculated to be 5.5 (with 95% confidence limits 3.9–7.9), significantly higher than the 1.0 assumed above. However, there are a number of reasons why this autopsy relationship may not be valid in natural endemic situations as well. First, a worm recovery rate of 100% could not possibly be achieved[24,25]; in six cadavers with positive tissue-egg counts, for example, no worm pairs were recovered. Second, by definition the study population consisted of terminally ill hospital patients, mostly with chronic diseases, in whom faeces production is usually reduced[24]. The stools from the cadavers indeed contained less debris and required less concentration than those of live patients[24]. This factor would probably be even more important in comparison to patients from a rural endemic setting, where diets are usually rich in fibres. Third, Cheever corrected the cadaver EPG count for mushy and liquid stools with a factor 1.9 to 2.6, a correction which is usually not made in field observations. Without this correction the ratio would be 3.4 EPG per WP (with 95% confidence limits 2.4–4.9).

The ratio of EPG per WP in normal endemic situations is thus most probably several factors lower than

that derived from the autopsy studies. Cheever himself noted that the EPG per WP ratio in his autopsy series was unusually high, as compared to mice[24]. From a later autopsy study on *S. haematobium* patients in Egypt[26,27], in which also many light infections with *S. mansoni* were encountered, an EPG per WP of 2.8 was calculated (without correction for stool consistency)[21], but these data were much less complete than the Brazilian set[24].

A complication in relating egg counts to worm numbers is that in humans with high worm loads, female worms may become less fertile. Some authors saw evidence in the Brazilian autopsy data for such density-dependent egg production[28,29]. However, they excluded nine infected individuals with zero egg counts from their analysis and based their statistical analysis on individual EPG per WP ratios, which is a questionable approach. Their conclusion was therefore challenged by both Cheever[25] and, based on a re-analysis including the Egyptian autopsy data, by Wertheimer *et al.*[30] Keymer and Slater[31] previously drew attention to pitfalls in deriving density dependence from parasite fecundity data in general. Figure 2 shows that, with a log-transformation, conditions for linear regression can be met, which is not the case with EPG per WP ratios. As the slope does not differ significantly from 1.0, density dependence cannot be confirmed from the Brazilian autopsy data. Density dependence might still exist for worm burdens higher than shown in Fig. 2, but worm loads corresponding to high EPGs would then be even higher.

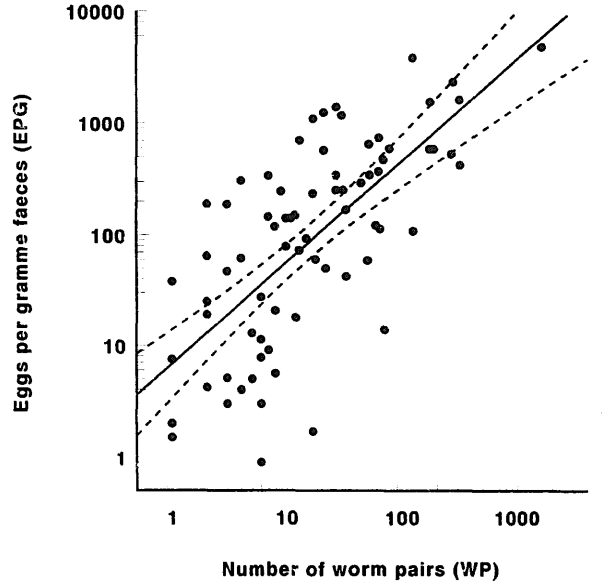

Fig. 2. Relation between eggs per gramme faeces (EPG) and number of *Schistosoma mansoni* worm pairs recovered by autopsy from 74 out of 103 infected cadavers described by Cheever[24]. In total, 29 cases were excluded for lack of egg counts (16), no worm pairs recovered (7), use of antimonial drugs before investigation (3) or incomplete autopsy (3). In nine cases, zero egg counts in infected cadavers were assigned a value of one-half the minimum number of eggs that could have been detected by the procedures used; these differ as EPGs were based on examination of variable amounts of faecal material (50–200 mg). Log-transformations of both EPGs and worm pair burdens clearly satisfy normality and homoscedasticity conditions to apply regression. The straight line represents the best fitting equation for the relationship between number of worm pairs (WP) and EPG = $6.83.WP^{0.92}$, with 95% confidence limits (dashed lines). The slope 0.92 suggests a slight density dependence in fecundity, but the trend is far from significant ($p>0.25$). We can therefore calculate a single value for egg production per worm pair, EPG per WP = 5.5 (within 95% confidence limits 3.9–7.9) at the geometric mean of 16 WP. In the text, we discuss the representativity of these values for endemic situations.

Anecdotal evidence

Goldsmith *et al.*[32] reported the results of extracorporeal blood filtration of living patients during splenectomy. Worm pair counts of the three cases (67, 383 and 69) were highly inconsistent with egg counts (125, 100 and 1100 EPG, respectively). If anything, they show that low EPGs can be found in people with high worm burdens. Worm counts were certainly underestimated with this method, as it is unlikely that all worms were passing through the shunt or withheld by the filter mesh[32].

There are very little data on the cercarial challenge an individual in an endemic community is actually confronted with. The results of the few available natural mice exposure experiments vary widely. In St Lucia, Sturrock[33] found only a few infections in over 11000 naturally exposed mice, illustrating the erratic character of transmission in such low endemicity areas. In foci with continuous, intense transmission such as Makundju, quite different observations have been made. Mice exposed for a few hours in natural waters in which women and children dwelled for hours daily, contracted dozens to hundreds of worms (Table 1). It is thus quite probable that, in such conditions, individuals are challenged by hundreds of cercariae per day and carry thousands of worms. A simple comparison of blood volumes between humans and mice makes clear, moreover, that five WP in mice is, relatively, a heavier infection than 5000 WP in adult humans.

Conclusions

There is a discrepancy between commonly assumed EPG per WP ratios and those found in autopsy series.

Table 1. Results of mice exposure and perfusion experiments in the intense focus of Makundju (Maniema, Zaire)[a]

Duration of exposure (min)	Total no. of mice	Dead/agony[b]	Without WP	Mean no. of WP[c]
16	8	0	2	19 ± 11
60	8	2	0	82 ± 35
120	9	5	1	38 ± 27

[a]Data from Ref. 18.
[b]Mice with severe agony or which had already died could not be properly perfused.
[c]The mean number of worm pairs (WP) recovered from only those mice that harboured at least one worm pair (within 95% confidence limits) is indicated. The maximum number of worms recovered was 344 in one hour.

Table 2. Predicted range in *Schistosoma mansoni* worm pair (WP) burden for a given egg per gramme faeces (EPG) count in three different endemic situations[a]

EPG	Buhandagaza (low endemicity)[b]	Gihungwe (moderate endemicity)	Makundju (high endemicity)
0	0–72	0–192	11–2977
100	22–427	28–863	83–3553
400	86–727	108–1423	222–4220
1000	183–1032	238–1977	422–4947
10000	942–2563	1326–4662	1972–8778

[a]See Fig. 1 and Refs 18, 23 for epidemiological characteristics.
[b]Indicated are 90% ranges; ie. 5% chance that WP is below and 5% that WP is above this range. The calculations are based on a model for egg count variation[11,21,22], assuming a productivity of 1.0 EPG per WP.

There are good reasons to believe that the EPG per WP ratio of about 1:1 is a realistic starting point. This would imply that endemic worm counts are much higher than generally assumed (Fig. 1).

However, egg counts cannot straightforwardly be translated into worm burdens. The extensive within-individual variation in schistosome egg counts[20,21] results in a wide range of corresponding worm burdens. Furthermore, this range is dependent on the characteristics of the endemic situation. In an area of low endemicity, a high EPG is likely to be due to an incidental peak in egg excretion of this individual, whereas in an intense focus a high egg count more probably reflects a genuine high worm burden. Based on the model outlined above, we have calculated the range of worm burdens corresponding to given EPGs in different endemic situations (Table 2). In a low-endemicity community such as Buhandagaza, the possible range corresponding with 1000 EPG has 1000 WP as an upper limit. However, in a high-endemicity village such as Makundju 1000 EPG may reflect up to 5000 WP. On the other hand, in such an intense focus, negative egg counts most likely correspond to positive worm burdens. Of course, these predictions assume satisfaction of modelling conditions, like perfect negative binomial distributions of egg counts and worm burdens, and no disturbances by, for example, migration or chemotherapy interventions[21].

There are good reasons to believe that worm numbers in endemic communities are significantly higher than is usually perceived. Clearly, fundamental questions in the dynamics of schistosome infections remain unanswered, hampering the development of epidemiological models, and consequently of tools and strategies for control. Hopefully, new techniques for the assessment of worm burdens, such as antigen detection assays[34], will improve our diagnostic and epidemiological toolbox. Meanwhile, we have to resort to statistical and mathematical approaches to solve, or at least to identify, such crucial gaps in our knowledge.

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Schistosomes and Serpins: A Complex Business

J. Modha, M.C. Roberts and J.R. Kusel

The view of the schistosome host–parasite relationship has changed in the past two decades. Previously, it was thought the parasite simply defended itself in the face of a hostile host environment. However, it is now realized that the host–parasite interaction is much more of a dynamic interplay, where the parasite is able to exploit host homeostatic mechanisms for survival, maturity and transmission. Here, Jay Modha, Clare Roberts and John Kusel discuss the recent identification of serine protease inhibitors (serpins) on the schistosome surface and suggest how their properties might be exploited by the parasite.

Proteases play a key role in the schistosome life cycle, being involved in penetration of host skin, parasite nutrition, immune evasion and egg excretion[1,2]. Mammalian hosts, in defence against the threat of uncontrolled proteolytic activity, possess serine protease inhibitors (serpins), whose function it is to inactivate not only stray, excess and/or redundant proteolytic activity from endogenous sources, but also that from exogenous sources (eg. from parasites and microorganisms)[3,4].

Serpin identification and properties

A comparison of the properties and features of schistosome-associated serpins is given in Table 1. Blanton *et al.*[5] have screened a *Schistosoma haematobium* cDNA library with species-specific human antisera and have identified a serpin (SHSPI) with a native molecular mass of 54–58 kDa. They also report a similar molecule in *S. mansoni*. Ghendler *et al.*[6] on the other hand, have purified Smpi56, a 56 kDa serpin, from adult *S. mansoni* worm extracts. In addition, contrapsin[7] has been identified in homogenates[8] and on the surface[9] of *S. mansoni* worms from mice. Contrapsin is also present in homogenates of *S. japonicum* and *S. bovis* worms[8], but whether this is true of Smpi56 and SHSPI remains to be determined. The presence of contrapsin and Smpi56 in *S. haematobium* has not been reported. As far as stage specificity is concerned, all three serpins are present in or on the adult worm, but their origin seems to be different. Recombinant DNA- and metabolic-labelling techniques show that SHSPI and Smpi56, respectively, are synthesized by the schistosome. Contrapsin, however, may be acquired from host blood, since its forms in adult worm homogenates and in mouse plasma share immunological identity[9]. Uptake of host molecules is a common feature of *S. mansoni*, and contrapsin may be added to the catalogue of other host-derived molecules associated with the parasite[10–14].

Distribution and complexes

The distribution of serpins on the worm surface is also varied. Anti-contrapsin antibodies label the surface (but not the tubercles) and subtegumental cells intensely, whereas anti-SHSPI antibodies localize on the spines and bind only weakly to the rest of the worm surface. SHSPI appears to be an integral membrane protein and is not reported to be present in subtegumental cells[5], but contrapsin is present in the aqueous phase of a Triton X-114 fractionation[9], indicating that it is unlikely to be an integral membrane protein[15]. The distribution of Smpi56 is not described, although it is present in tegumental extracts[6]. All three serpins are present in complex form, either on the worm surface, or in tegumental extracts, but the molecules complexing with the serpins have not been identified in all cases. Undoubtedly, the cognate protease for Smpi56 is the 28 kDa schistosome elastase[16] (G.R. Newport, Abstract*), but the worm molecules complexing with contrapsin and SHSPI remain to be identified. The inhibition of complex formation of contrapsin following pretreatment of worm homogenate with phenylmethyl-sulphonyl fluoride and

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