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Modelling heterogeneity and the impact of chemotherapy and vaccination against human hookworm

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There is a growing emphasis on the development of vaccines against helminths (worms), and mathematical models provide a useful tool to assess the impact of new vaccines under a range of scenarios. The present study describes a stochastic individual-based model to assess the relative impact of chemotherapy and vaccination against human hookworm infection and investigates the implications of potential correlations between risk of infection and vaccine efficacy. Vaccination is simulated as a reduction in susceptibility to infection and the model includes population heterogeneities and dynamical waning of protection. To help identify appropriate measures of vaccine impact, we present a novel framework to quantify the vaccine impact on the infection-associated morbidity and introduce a measure of symmetry to study the correspondence between reduction in intensity and reduction in morbidity. Our modelling shows that, in high-transmission settings, the greatest impact of vaccination will be attained when vaccine efficacy is the greatest among individuals harbouring the heaviest worm burdens, and that the decline of morbidity primarily depends on the level of protection attained in the most at risk 8–12% of the population. We also demonstrate that if risk of infection and vaccine protection are correlated, there is not always a direct correspondence between the reduction in worm burden and in morbidity, with the precise relationship varying according to transmission setting.

Keywords: mathematical modelling; human hookworm; helminths; chemotherapy; vaccination

1. INTRODUCTION

Mathematical models are often used to predict the impact of vaccines against infectious diseases and are seen increasingly as an essential part of the appraisal of candidate vaccines. Models have the advantage of being able to assess the potential impact of vaccination under a range of scenarios, conditions and assumptions and have, for example, been applied to investigate the impact of vaccines against malaria (Smith *et al.* 2006), human papilloma virus (Hughes *et al.* 2002) and dengue (Shepard *et al.* 2004). The model structure and assumptions for many infectious diseases, such as measles, rubella and varicella, are relatively straightforward because they have well-defined latent periods, followed by acute infectiousness and morbidity, and induce lifelong immunity (Anderson & May 1991). In contrast, models of vaccination against many parasitic diseases such as malaria and helminth (worm) infections must include additional complexities. For malaria, these include antigenic variation of the malaria parasite (Molineaux *et al.* 2001) and the partial immunity that develops against disease but not against infection (Ross *et al.* 2006).

Helminths have fundamentally different transmission dynamics from viral and bacterial infections since the dynamics are primarily determined by the number of worms present (the worm burden or intensity of infection) rather than the number of hosts infected (Anderson & May 1991). Thus, understanding and modelling patterns of worm burdens in communities is crucial in modelling anti-helminth vaccines. A key feature of helminth infections is the heterogeneous (aggregated) distribution of parasites within host populations, arising from a variety of exposure- and immunity-related factors (Anderson 1982; Anderson & May 1982). The effect of this aggregation is typically

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incorporated into analysis or mathematical models by assuming that the distribution of parasites between hosts is highly skewed (usually a negative binomial distribution). Such heterogeneity must be taken into account in considering the potential impact of an anti-helminth vaccine (Bundy *et al.* 1995; Brooker *et al.* 2005). A further characteristic of helminth infections is the observation that individuals appear to be predisposed to heavy (or light) infections (Schad & Anderson 1985; Bradley & Chandiwana 1990; Quinnell *et al.* 1993, 2001). Predisposition is indicated by a positive correlation between egg counts in faecal samples from individuals before successful treatment and after a period of further exposure and reinfection. The evidence of predisposition suggests that risk factors for helminth infections (whether determined by genetics, behaviour or environment) do not change significantly over the typical 1- to 5-year time duration of a reinfection study (Quinnell *et al.* 2001). Finally, the rate of egg production per worm decreases when a person has many worms, so-called density dependence (Anderson & Schad 1985).

Hookworm infections in humans are caused by two species, either *Ancylostoma duodenale* or *Necator americanus*, with *N. americanus* representing the predominant species in most developing countries (Schad & Banwell 1984). Adult worms live in the small intestine, where they cause intestinal blood loss. Eggs exit the body in the faeces and contaminate soil, where larvae emerge and moult to become infective larvae that can penetrate the skin of a new host. The larvae migrate through the lungs and trachea, from where they are swallowed before maturing into adults in the small intestine. Worldwide, hookworms are estimated to infect approximately 600 million individuals living in the tropics and subtropics, and among populations with poor underlying nutrition they are a major cause of intestinal blood loss and iron-deficiency anaemia (Hotez *et al.* 2004; Bethony *et al.* 2006). The severity of hookworm-related morbidity is strongly related to the size of the number of worms present (Lwambo *et al.* 1992).

The main intervention strategy against hookworm is periodic chemotherapy using benzimidazole anthelmintics (mainly albendazole and mebendazole), with pre-school and school-age children as well as pregnant women as the priority groups (Bundy *et al.* 2006; Hotez *et al.* 2006). Such chemotherapy should ideally be implemented in the context of ongoing improvement of sanitation and health education, the ultimate long-term control strategy. In the short term, however, concerns about the variable efficacy of mebendazole against hookworm and possible development of drug resistance have stimulated efforts to develop new control tools, including new anthelmintic drugs and anti-hookworm vaccines (Hotez *et al.* 2006). Recent studies have demonstrated the potential of a hookworm vaccine based on recombinant larval and adult hookworm antigens including *Ancylostoma* secreted protein-2 of *N. americanus* (*Na*-ASP-2; Bethony *et al.* 2005; Loukas *et al.* 2006) and a glutathione S transferase of *N. americanus* (*Na*-GST-1; Asojo *et al.* 2007), respectively, which are currently undergoing cGMP manufacture and clinical testing in Brazil. Because the *Na*-ASP-2 hookworm vaccine candidate targets only the infective larval stage of the hookworm life cycle, it will still be necessary to treat individuals for existing infections using anthelmintics before vaccination.

To help inform the design and cost-effective implementation of a future hookworm vaccine, in this paper, we develop a mathematical framework to model and explore the potential impact of a vaccine against hookworm infection relative to chemotherapy alone. To model effectiveness, we expand upon a deterministic model used to evaluate the effect of community treatment on the morbidity due to ascariasis (caused by *Ascaris lumbricoides* or roundworm; Medley *et al.* 1993). Our model includes epidemiological features specific to hookworm and considers the importance of epidemiological heterogeneities, such as aggregation and predisposition, in evaluating the impact of vaccination. In particular, we study the impact on the intensity of infection of a risk-stratified population and consider a scenario in which the vaccine is given only to a randomly selected population sample, and it elicits a risk-of-infection-correlated heterotypic immune response and subsequent vaccine efficacy (i.e. of a magnitude depending on the risk group). We subsequently use our results to discuss the potential measures of vaccine impact to be used in future vaccine trials. We show that, in the early stages of a trial, assessing the impact of a helminth vaccine on the worm population and assessing the health benefit associated with the vaccine are two distinct problems.

2. EXPECTED MODE OF ACTION

To model the potential impact of a vaccine, we need to understand how it acts within a population and which factors may influence its impact. In general, vaccines can have different modes of action. Two rather extreme cases are represented by the 'all-or-nothing' and 'leaky' models (Smith *et al.* 1984). In the former, vaccinated individuals are either completely protected against new infections or remain as susceptible as if they had not been vaccinated; in the latter case, all vaccinated individuals have some degree of immune response to the vaccine, but protection against infection is only partial so that they remain somewhat susceptible, although are less likely to acquire the infection (or to develop the associated disease). In leaky vaccines, characteristics of the exposure may also influence the efficacy of a vaccine. Some vaccines, e.g. pertussis, appear to be less effective if transmission is between household members, suggesting that protection can be 'overwhelmed' by a large infective inoculum (Fine & Clarkson 1988).

In regard to the *Na*-ASP-2 and *Na*-GST-1 hookworm vaccines, animal studies suggest a leaky type mode of action (Bethony *et al.* 2005; Zhan *et al.* 2005; Ghosh *et al.* 2006), such that vaccinated individuals would still be susceptible to hookworm infection. The *Na*-ASP-2 vaccine is expected to induce an immune response that will prevent invading larvae from developing and reaching the gastrointestinal tract so that they cannot develop into adult blood-feeding worms (Bethony *et al.* 2005). If given with chemotherapy, the vaccine should prolong the benefit of the treatment by reducing the reinfection rate.

3. A MATHEMATICAL MODEL FOR THE TRANSMISSION OF HOOKWORM INCORPORATING THE IMPACT OF CHEMOTHERAPY AND VACCINATION

In this section we present details of the mathematical model developed to explore the transmission of hookworm and the relative impact of chemotherapy (which removes existing infections) and vaccination (which reduces susceptibility to new infections) on the overall worm burden in a community. Our approach builds on a model previously developed to evaluate the transmission dynamics and control of ascariasis using anthelmintic chemotherapy (Medley *et al.* 1993), but has been developed as an individual-based simulation to allow more flexibility in population stratification and vaccine definitions (see §4). The model tracks the establishment and removal (through worm mortality) of parasites in hosts within a community. Previous studies show that at the level of the community the distribution of worm burdens among hosts in the population is overdispersed such that a minority of individuals harbour high worm burdens and a majority harbour relatively low worm burdens (Anderson & May 1985). The precise mechanism that underlies this observed distribution is not fully understood; however, it is typically incorporated into models by assuming that it is driven by variation in susceptibility to infection (Dietz 1988). The rate at which individuals acquire new parasites will therefore depend on host susceptibility and on the number and distribution of hookworm larvae in the community, which in turn depends directly on the rate at which eggs are expelled by infected hosts (Anderson & May 1991). Since there is a nonlinear relationship between an individual host's worm burden and female worm fecundity (and hence faecal egg counts), this density-dependent constraint is incorporated in the model through a function that decreases the community-level reproductive rate as the total worm burden in the community increases (Anderson & Schad 1985).

We then adapt the framework to explore the impact that a vaccine may have on the risk of acquiring hookworm infection. It is assumed that the vaccine acts by reducing individual susceptibility to infection and that its protective effect wanes at a constant rate (owing to failure of individual direct protection, a gradual reduction of protection or a combination of both effects).

3.1. Model structure

Let $W_j(t)$ be the worm burden of individual j and $W(t)=\sum_{j=1}^{N} W_j(t)/N$ the mean worm burden in a community of size N at time t. The rate of acquisition of new worms in a host depends on the basic reproductive number of the worm R_0 (the number of female parasites produced by an average female parasite in the absence of density-dependent constraints), a community density-dependent constraint $f(\cdot)$ and the susceptibility of host j to the acquisition of new worms h_j. The functional dependence between the worm reproductive rate and the density of parasites is described by a power-law function rather than the exponential weight function assumed by Medley *et al.* (1993). This assumption is consistent with field and laboratory studies that have investigated the relationship between hookworm density and fecundity (Anderson & Schad 1985). Let $\boldsymbol{W}(t)=\{W_1(t),\ldots,W_N(t)\}$ denote the vector of individual worm burdens in the community, which can be transformed into a probability distribution of worm densities $P_n(t)$ representing the probability that a host in the community has a worm burden n at time t. Then, taking into account the fact that single worms cannot reproduce, $f(\cdot)$ takes the form

$$f(\gamma, P_n(t)) = (W(t) - P_1(t)) \sum_{n=2}^{n_{\max}} n^{-\gamma} P_n(t), \qquad (3.1)$$

where γ is the power-law exponent and $n_{\max}=\max\{W_1(t),\ldots,W_N(t)\}$ is the maximum number of worms harboured by a single person. Thus, the rate at which new worms are acquired by host j is given by $\mu R_0 h_j f(\gamma, P_n(t))$ and are cleared in host j at rate μ, the parasite death rate.

If $\Delta W_j(t)$ denotes the change in worm burden in individual j occurring between time t and $t+\delta t$, then an individual's worm burden dynamics are described by the following equations:

$$\Delta W_j(t) = \tilde{p}_j(t) - \tilde{d}_j(t), \qquad (3.2)$$

$$\tilde{p}_j(t) \in \text{Poisson}(\mu R_0 h_j f(\gamma, P_n(t))\delta t), \qquad (3.3)$$

$$\tilde{d}_j(t) \in \text{binomial}(W_j(t), 1-\mathrm{e}^{-\mu\delta t}). \qquad (3.4)$$

When hookworm is endemic in a community, the endemic distribution of individual worm burdens P_n is best described by a negative binomial distribution whereby relatively few people harbour most of the parasites. This heterogeneity is achieved in the model by assuming that hosts have different levels of susceptibility h_j. Drawing the establishment rate $\Delta W_j(t)$ for a host j from a Poisson distribution with a mean equal to $h_j \Delta W(t)$, the susceptibility h_j from a gamma distribution with variance $1/k$, describing the dynamics of the mean parasite burden W with an immigration-death process (with a birth rate $\mu R_0 h_j f(\gamma, P_n(t))$ and a death rate μ), ensures that the individual distribution of parasites can be described by a negative binomial with mean W and variance $\sigma^2 = W + (W^2/k)$ (Hadeler & Dietz 1983; Anderson & May 1985; Anderson & Medley 1985). In the absence of drug treatment and ignoring possible seasonal variations (since the lifespan of a hookworm typically exceeds 1 year) and when the infection is at endemic equilibrium, the total worm burden in the community is constant. Thus, by imposing the stationarity condition $\sum_{i=1}^{N} \Delta W_i(t=0) = 0$, the power exponent γ can be derived from the model parameters.

3.2. Chemotherapy

Chemotherapy acts by killing a fraction of the worms hosted in the population that receives the treatment. We assume that this happens within a few days after treatment and hence is instantaneous on the time scales

of the model (which is in months). Thus, if chemotherapy is introduced at time t_c, the worm burden of treated individuals will change as follows:

$$W_j(t_c) \rightarrow W_j(t_c) - \tilde{b}_j, \tag{3.5}$$

$$\tilde{b}_j \in \text{binomial}(W_j(t_c), \alpha), \tag{3.6}$$

where $\tilde{b}_j$ is the number of worms killed by chemotherapy within an individual harbouring $W_j(t_c)$ worms at time t_c and α is the probability that an adult worm is killed by chemotherapy.

3.3. Vaccination

In this model, we assume that the vaccine reduces the individual susceptibility to reinfection following chemotherapy. The effects of vaccination are nonlinearly dependent on the efficacy and coverage of the vaccine owing to the nonlinearity introduced by the density-dependent constraints within the host. We allow vaccine efficacy to vary by host susceptibility to infection h_j. Vaccine efficacy, defined as the reduction in susceptibility to infection, in host j with susceptibility h_j at time t is denoted as $\text{VE}(h_j, t)$. Following vaccination, susceptibility to infection in host j is therefore reduced by

$$h_j \rightarrow h_j(1 - \text{VE}(h_j, t)). \tag{3.7}$$

We assume that the vaccine efficacy wanes exponentially so that, on average, $\text{VE}(h, t) = \text{VE}(h, 0)\, \text{e}^{-t/T}$ where T is the average duration of protection and $\text{VE}(h, 0)$ is the direct protection that would be measured in a population with susceptibility h in the absence of density-dependent effects or waning of vaccine efficacy. Waning is implemented in the model by allowing 'individual vaccine protection' to fail (i.e. to change from $\text{VE}(h, 0)$ to zero) at any one time step δt with probability $\delta t/T$. Alternative mechanisms, such as a gradual reduction of individual protection ($\text{VE}(h, t) = \text{VE}(h, 0)\, \text{e}^{-t/T}$), were also explored but did not substantially affect the results.

In our model, vaccine efficacy is therefore defined in terms of ratios of the risk of infection in vaccinated to unvaccinated groups

$$\text{VE}(h, 0) = 1 - \frac{r_V(0)}{r_U(0)}, \tag{3.8}$$

where $r_V(0)$ and $r_U(0)$ are the instantaneous risks of infection for vaccinated and unvaccinated individuals at the time of vaccination. For a hookworm vaccine, $\text{VE}(h, 0)$ therefore measures the relative reduction of the average reinfection rate within an untreated endemically infected population in the absence of waning of vaccine efficacy, worm density dependence and indirect effects.

3.4. Model parameters and simulation

Current estimates of the basic reproductive number for hookworm in the published literature are few, indirect and model dependent: estimates for a study in India suggest an R_0 of 2–3 (Schad & Anderson 1985). In our baseline scenario, defined by the parameter values contained in table 1, we assumed an R_0 of 3. We also test the results for sensitivity to R_0 by taking $R_0 = 2$ and 3.5; however, the results are not critically dependent on the assumed value of R_0. The worm mortality rate μ is assumed to be 0.4 (Anderson & May 1991). To model overdispersion, we use previous estimates ($k = 0.34$) for the aggregation parameter of the (negative binomial) distribution of parasites (Bradley *et al.* 1992). We also test the sensitivity of our results for $k = 0.2$–0.8. The endemic mean worm burden is assumed to be $W = 20$ worms/person, which corresponds to a hyper-endemic scenario (Bundy 1990). The parameter γ is entirely determined by R_0, k and W, with a baseline value of approximately 0.26.

The proportion c of the population that receives either chemotherapy and vaccine or chemotherapy alone is assumed at baseline to be 0.2, and we explore the sensitivity of our results for $c = 0.1$–0.9. The efficacy of chemotherapy α (the probability of eliminating a worm harboured by an individual that has been treated with chemotherapy) is set as 0.7 (Bennett & Guyatt 2000). The duration of the vaccine protection T is taken to equal 3 years, although we do not, at present, have any reliable estimate of the duration nor we do have a good estimate of vaccine efficacy. In formulae (4.2) and (4.3), we make the simple assumption that the number of people in both the vaccinated group N_V and the unvaccinated group N_U are equal to cN, and W_{MT} is the threshold for morbidity, which is consistent with previous analyses (Lwambo *et al.* 1992) showing that hookworm-induced iron deficiency is typically found in individuals carrying at least 40 worms. Interventions (chemotherapy and vaccination) are assumed to take place at time $t_c = 0$ and the follow-up to last for $T_F = 3.5$ years (in general, we find that the vaccine impact is best assessed if T_F is equal to or slightly greater than the average duration of protection T).

For each scenario, we compute mean statistics over 1000 realizations, each of which involves 10 000 individuals, followed over a period of 3.5 years. The time evolution is attained via synchronous updating of the worm burden of each individual and the force of infection at each run, assuming that contemporaneous infective episodes involving different individuals are independent. The time step between two runs of the simulation is $\delta t = 0.2$ years. This is a trade-off between a high-resolution time dynamics, requiring a time step much smaller than the average lifespan of the parasite ($\delta t \ll 1/\mu$), and the constraints set by the Markovian structure of the model. In fact, as it may take up to 9–12 weeks for an egg deposited in the soil to develop into a sexually mature adult in a new host's gastrointestinal tract (Schad & Banwell 1984), for time steps significantly smaller than this, the intermediate stages of the hookworm life cycle should be explicitly included in the dynamics. Moreover, this choice of δt is compatible with several possible dynamics and modes of transmission (namely, clumped or multiple single transmission events; Cornell *et al.* 2004). The qualitative results presented in this paper are not sensitive to the value of δt (for positive values smaller than 1);

Table 1. Baseline model parameters.

parameter	description	values	comment	reference
VE	vaccine efficacy	0.25, 0.5, 0.75	average reduction in susceptibility to infection	present study
T	vaccine waning time	1, 3, 5 years	average duration of protection	present study
T_F	duration of follow-up	3.5 years	post-intervention observation time	present study
W_{MT}	threshold for morbidity	40 worms/person	individual worm burden above which anaemia occurs	Lwambo *et al.* (1992)
c	coverage	20%	proportion of the population that receives either chemotherapy and vaccine or chemotherapy alone	present study
α	chemotherapy efficacy	70%	probability of killing a worm harboured by an individual that has been treated with chemotherapy	Bennett & Guyatt (2000)
R_0	basic reproductive number	2, 3, 3.5	average number of female offspring that reach sexual maturity, produced by an adult female worm in the absence of density-dependence constraints	present study, based upon Anderson & May (1991)
μ	worm mortality rate	0.4/year	defined as the inverse of a worm average lifespan	Anderson & May (1991)
W	mean worm burden	20 worms/person	average number of worms harboured by individuals in an endemically infected population	present study
k	aggregation parameter	0.34	inverse measure of overdispersion	Bradley *et al.* (1992)

however, as one would expect, the quantitative results depend smoothly on the value of δt.

We use the model to simulate the effect of the vaccine allocated to a cohort, randomly selected from a larger, endemically infected and untreated population. As a baseline scenario, we assume that the targeted subpopulation comprises 20% of the whole population and that chemotherapy and vaccination coverage is high (100%) within this subpopulation. This is based on the current plans for phase 2 clinical testing of the vaccine and the current thinking that vaccination will be administered to a selected group, namely school-aged children, who are the target of current treatment programmes. We then simulate the same scenario, assuming that only chemotherapy is provided to this group. Our first objective is to explore the potential impact of a vaccine whose mode of action is to reduce the population risk of infection.

4. VACCINE EFFICACY

4.1. Vaccine efficacy and overdispersion

While the theoretical efficacy (the efficacy that would be measured in the absence of density-dependent effects) of the vaccine is easily defined, it has major limitations. For helminth infections, there is no clear way to measure the instantaneous risk of reinfection in the absence of density-dependent effects in a community. In fact, vaccinated individuals are likely to have helminths in the gut and this may remain the case even if they are treated before vaccination because anthelmintic treatment is not 100% effective and therefore cannot be assumed to kill all worms. Thus, a practical measure of vaccine efficacy should also take into account the levels of infection (worm burdens) prior to vaccination. Furthermore, owing to the density-dependent infection process and the highly skewed distribution of worm burden in the population, the overall impact of vaccination at a population level will depend in a nonlinear manner on the theoretical vaccine efficacy and coverage.

4.2. Measures of vaccine efficacy for hookworm vaccines

A common measure of efficacy of interventions against hookworm and other helminths is the reduction in the mean worm burden, indirectly assessed by faecal egg counts (Anderson & May 1991). A natural definition of vaccine efficacy is therefore the relative reduction in mean worm burden in vaccinated compared with unvaccinated subjects, assuming that the treatment occurs immediately prior to vaccination in both groups:

$$\mathrm{VE}_{\mathrm{MWB}}(T_F) = 1 - \frac{\sum_{t=0}^{T_F} W_V(t)}{\sum_{t=0}^{T_F} W_U(t)}, \tag{4.1}$$

where $W_i(t), i = U, V$ is the mean worm burden in the unvaccinated and vaccinated groups, respectively, at time t after vaccination and T_F is the duration of follow-up. However, such a definition does not capture changes in morbidity that are determined by the prevalence or intensity of infection above a certain threshold (Lwambo *et al.* 1992).

The major clinical manifestations of hookworm disease are the consequences of chronic intestinal blood loss. Iron-deficiency anaemia occurs and hypoalbuminaemia

develops when blood loss exceeds the intake and reserves of host iron and protein (Beaver *et al.* 1984). A hookworm burden (i.e. the intensity of infection or the number of worms per person) above 40 worms is associated with anaemia (Lwambo *et al.* 1992; Hotez *et al.* 2004). Therefore, a reduction in the mean worm burden is likely to translate into a reduction in morbidity only if it corresponds to a significant reduction of the number of people that harbour 40 or more worms. We therefore also consider two alternative measures of efficacy. The first ($\mathrm{VE_{MP}}$), termed vaccine efficacy of morbidity prevalence, captures the reduction in the prevalence of morbidity by measuring the proportional reduction in the overall worm burden restricted to the subset of the population who experience a worm burden above a given threshold. This is given by

$$\mathrm{VE_{MP}}(T_\mathrm{F}) = 1 - \frac{\sum_{t=0}^{T_\mathrm{F}} \sum_{j=1}^{N_\mathrm{V}} \frac{\theta_j(t)}{N_\mathrm{V}}}{\sum_{t=0}^{T_\mathrm{F}} \sum_{j=1}^{N_\mathrm{U}} \frac{\theta_j(t)}{N_\mathrm{U}}}, \tag{4.2}$$

where

$$\theta_j(t) = \begin{cases} 1 & \text{if } (W_j(t) - W_\mathrm{MT}) \geq 0, \\ 0 & \text{otherwise} \end{cases}$$

is an indicator function denoting whether individual j has a worm burden above the threshold $W_\mathrm{MT} = W_\mathrm{MT}(a_j, x_j, \nu_j)$, which is dependent on the age, gender and nutritional status of j; N_V and N_U are the number of people in the vaccinated and unvaccinated cohorts, respectively; and $W_j(t)$ is the worm burden of person j at time t as before.

The second ($\mathrm{VE_{MI}}$), termed vaccine efficacy of morbidity intensity, provides a measure of the proportional reduction in the intensity of infection within the vaccinated group, but only takes into account heavily infected individuals (those above the morbidity threshold as defined above)

$$\mathrm{VE_{MI}}(T_\mathrm{F}) = 1 - \frac{\sum_{t=0}^{T_\mathrm{F}} \sum_{i=1}^{N_\mathrm{V}} \frac{(W_i(t) - W_\mathrm{MT})\theta_i(t)}{N_\mathrm{V}}}{\sum_{t=0}^{T_\mathrm{F}} \sum_{j=1}^{N_\mathrm{U}} \frac{(W_i(t) - W_\mathrm{MT})\theta_j(t)}{N_\mathrm{U}}}. \tag{4.3}$$

It can be interpreted as a measure of reduction in the 'intensity of morbidity'.

4.3. Comparison of measures of vaccine efficacy

To investigate the relationship between the vaccine efficacy defined on the basis of a reduction in mean worm burden and a reduction in morbidity, we define two measures of symmetry (S_MP and S_MI) between two vaccine efficacy patterns P_i and P_j, which are given as follows:

$$S_\mathrm{MP}(P_i, P_j) = \left(\frac{\mathrm{VE_{MWB}}(P_i) - \mathrm{VE_{MWB}}(P_j)}{\mathrm{VE_{MWB}}(P_i) + \mathrm{VE_{MWB}}(P_j)}\right) \times \left(\frac{\mathrm{VE_{MP}}(P_i) - \mathrm{VE_{MP}}(P_j)}{\mathrm{VE_{MP}}(P_i) + \mathrm{VE_{MP}}(P_j)}\right), \tag{4.4}$$

$$S_\mathrm{MI}(P_i, P_j) = \left(\frac{\mathrm{VE_{MWB}}(P_i) - \mathrm{VE_{MWB}}(P_j)}{\mathrm{VE_{MWB}}(P_i) + \mathrm{VE_{MWB}}(P_j)}\right) \times \left(\frac{\mathrm{VE_{MI}}(P_i) - \mathrm{VE_{MI}}(P_j)}{\mathrm{VE_{MI}}(P_i) + \mathrm{VE_{MI}}(P_j)}\right). \tag{4.5}$$

It is important to stress that neither S_MP nor S_MI are measurable quantities, but are simple conceptual tools that allow us to display our results in an efficient manner. In particular, we are interested in their sign rather than their exact numerical value. A positive symmetry value means that, comparing vaccine efficacy patterns P_i and P_j, the pattern associated with the highest reduction in the intensity of infection (i.e. a higher $\mathrm{VE_{MWB}}$) is also the pattern associated with the highest reduction in morbidity (i.e. a higher $\mathrm{VE_{MP}}$ or $\mathrm{VE_{MI}}$). Conversely, a negative symmetry means that the vaccine efficacy pattern associated with the highest reduction in the intensity of infection is associated with the lowest reduction in morbidity. The symmetry between the two patterns will depend not only on vaccine efficacy and coverage (c), but also on the parasite–host dynamics and therefore on parameters such as R_0, W and k. In particular, if the symmetry between the two patterns remains positive for all plausible values of the parameters, then we are able to use the vaccine efficacy defined on the basis of a reduction in mean worm burden ($\mathrm{VE_{MWB}}$) to compare the impact of those patterns. However, if negative symmetries are observed, then relying on $\mathrm{VE_{MWB}}$ as a measure of the population impact of a vaccine would lead to a selection of a pattern that is not optimal in terms of reducing morbidity in the community.

4.4. Variation in vaccine efficacy by worm burden

As indicated, a key characteristic of helminth epidemiology is heterogeneity in worm burden within host populations. To explore the role of this feature on post-vaccination transmission dynamics, we stratify the population into three groups, according to an arbitrarily defined scale of worm burden: low (below 28 worms/person); medium (28–65 worms/person); and high (above 65 worms/person) and observe the impact of interventions on these three subpopulations. This is one of many possible stratifications and it has been chosen for convenience. The conclusions of this study do not depend on the specific choice of the population strata. In the presence of predisposition to infection, a higher intensity of infection is associated with a higher risk of infection. An intensity-of-infection stratification can therefore be used as a proxy for a risk-of-infection stratification of the population and therefore defines the susceptibility $h = \{\text{low, medium, high}\}$. For the sake of simplicity, here we consider only three possible levels of vaccine efficacy, for each subpopulation $\mathrm{VE}(h) = 0.25, 0.5, 0.75$. We define a vaccine efficacy pattern as the set of vaccine efficacies in each of the three susceptibility groups. Figure 1 displays seven representative patterns of efficacy, obtained by allowing different levels of efficacy within the three transmission intensity subpopulations under study. Although entirely arbitrary, these patterns

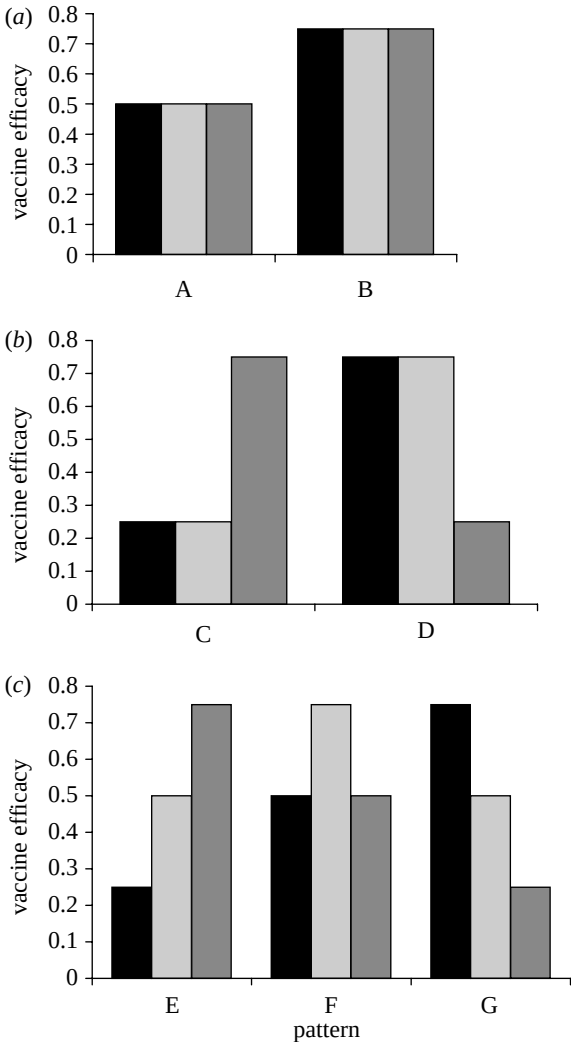

Figure 1. Patterns of efficacy. Seven possible patterns of vaccine efficacy, assuming three possible levels of efficacy for each population group (0.25, 0.50 and 0.75). The population is stratified into three groups according to the (pre-treatment) intensity of infection: low (filled black bars, below 28 worms/person), medium (filled light grey bars, 28–65 worms/person) or high (filled dark grey bars, above 65 worms/person). Each bin represents the level of vaccine efficacy (VE_h) associated with a specific group for a specific pattern. (*a*) Patterns A and B assume homogeneity of vaccine efficacy within the population. (*b*) Patterns C and D and (*c*) patterns E–G assume that there are either positive or negative correlations between pre-treatment intensity of infection and vaccine efficacy while pattern F implies that the highest vaccine efficacy is experienced by the population group with medium intensity of infection.

are useful to explain the different impact that a vaccine may have on intensity and morbidity as a consequence of a risk-of-infection-correlated level of protection.

5. RESULTS

5.1. Assessing the impact of chemotherapy and vaccination

Figure 2 shows an example of the modelled reinfection dynamics for high-, medium- and low-risk groups with either chemotherapy alone (figure 2*a*) or for chemotherapy

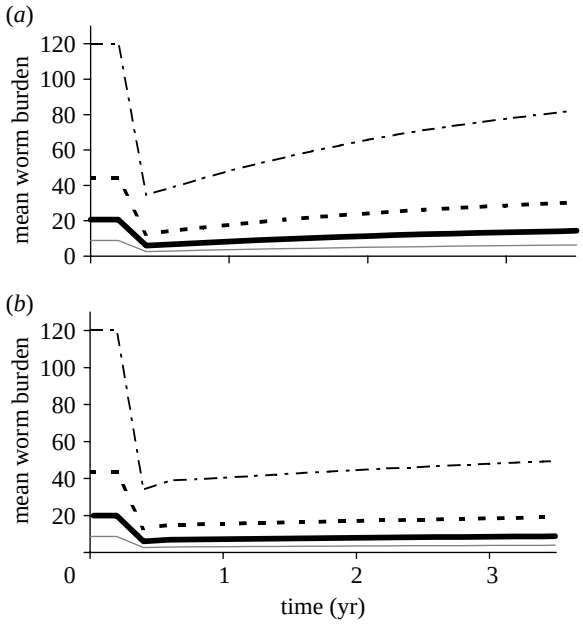

Figure 2. Dynamics of the mean worm burden for an intensity-of-infection-stratified population. The time evolution of the mean worm burden when (*a*) chemotherapy alone and (*b*) chemotherapy and vaccination with $VE_h = 50\%$ are compared. Parameters are the same as given in table 1. Solid black lines, MWB; solid grey lines, low intensity (below 28 worms); dashed lines, medium intensity (28–65 worms); dot-dashed lines, high intensity (above 65 worms).

coupled with a 50% efficacious vaccine (figure 2*b*). The overall mean worm burden is dominated by the high-intensity group while the vaccine's effect in the low-intensity group has relatively little impact on the overall change in mean worm burden. It therefore follows that the greatest impact of vaccination in terms of worm burden will be attained when vaccine efficacy is high among those individuals who are predisposed to being heavily infected. As population coverage (for both chemotherapy alone and chemotherapy combined with vaccination) increases, the relative impact of the vaccine on the mean worm burden decreases, although the absolute impact on the mean worm burden and the relative impact of vaccination on the high-intensity group increase. At very high coverage (above 70%), the relative impact on the high-intensity group declines, due to the mass effect of chemotherapy on reinfection (results not displayed).

5.2. Risk-of-infection-correlated vaccine efficacies

If we consider only the reduction in the mean worm burden by applying definition (4.1) of vaccine efficacy, we find that vaccine efficacy depends crucially on the population patterns of efficacy (as shown in figure 1). Figure 3 illustrates this point for two efficacy patterns, C and D. If calculated in terms of mean worm burden, vaccine efficacy is 32% for pattern C and 30% for pattern D (figure 3). However, if vaccine efficacy is defined on the basis of reduction in morbidity (i.e. either according to definition (4.2) or (4.3)), the reduction in morbidity is 63% for pattern C and 36% for pattern D (figure 3).

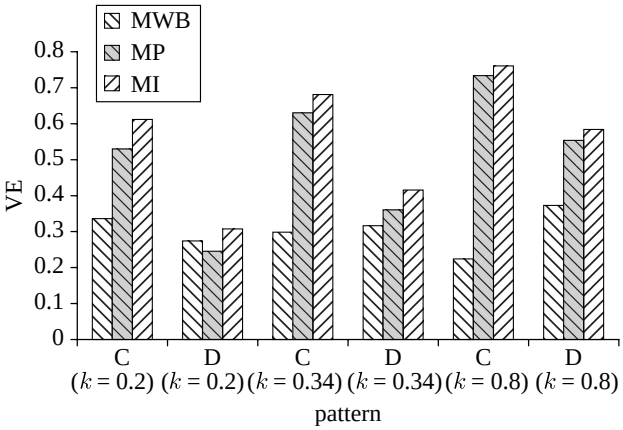

Figure 3. Reduction in the intensity of infection (mean worm burden), prevalence of morbidity and intensity of morbidity as functions of k. The bars show the three estimated vaccine efficacies VE_{MWB}, VE_{MP} and VE_{MI} for pairs of patterns {C; D} for k=0.2, 0.34 and 0.8. All other parameters are the same as given in table 1.

In general, independently of the stratification, if levels of protection are correlated with the risk of infection, a higher reduction in the intensity of infection may not necessarily translate to a higher reduction in morbidity. For example, in the presence of either a positive or no correlation between risk of infection and levels of protection (patterns A, B, C and E in figure 1), the relative impact of the vaccine on the mean worm burden decreases, while the impact on morbidity increases, as the degree of overdispersion in worm burden in the population is decreased (i.e. parameter k is increased; figure 3). In contrast, if the vaccine efficacy is negatively correlated with risk (such as for patterns D and G in figure 1), we observe an increase in the relative impact on the mean worm burden as well as on the morbidity as the degree of overdispersion is decreased. These patterns are consistently observed across a range of values for R_0 and vaccine duration (T; results not shown).

As the mean worm burden in the population, W, increases, so does prevalence and intensity of morbidity and thus increasingly higher vaccine efficacy is needed in the high- and medium-risk groups to keep morbidity under control. At high coverage (above 50–70% in our examples), the mass effect of deworming boosts the reduction in mean worm burden achieved by chemotherapy and the effect of the vaccine's indirect protection tends to counterbalance the heterogeneity in direct protection. At low levels of coverage (e.g. below 20%), herd immunity and chemotherapy mass effect are negligible and therefore only the direct impact of the vaccine contributes to determining the extent to which the two vaccine efficacy measures result in similar interpretations.

5.3. Comparison of alterative measures of vaccine efficacy

At present, we do not know which, if any, of the patterns in figure 1 is more appropriate to represent the efficacy of a hookworm vaccine. The key questions therefore are under which conditions a higher reduction in the intensity of infection corresponds to a higher reduction in morbidity and hence under which conditions our two measures of vaccine efficacy will be consistent. To study the correspondence between the reduction in worm burden and in morbidity, we use the indicators S_{MP} and S_{MI} defined in (4.4) and (4.5), which measure the degree of symmetry between our measures of vaccine efficacy. We compare the results obtained for different patterns of protection by risk groups (i.e. patterns in figure 1). Only the results obtained using S_{MP} are displayed as those obtained for S_{MI} are similar.

As an example, we display, in figure 3, the reduction in intensity and morbidity for the pair {C; D}. For k=0.2, pattern C performs better than pattern D in reducing both intensity and morbidity (i.e. $VE_{MP}(C) > VE_{MP}(D)$ and $VE_{MWB}(C) > VE_{MWB}(D)$), therefore, S_{MP} is positive; for k=0.34 and 0.8, pattern C performs better than pattern D in reducing morbidity ($VE_{MP}(C) > VE_{MP}(D)$) but pattern D performs better than pattern C in reducing intensity ($VE_{MWB}(C) < VE_{MWB}(D)$), hence S_{MP} is negative.

We consider the four pairs of patterns: (i) {A; B}, (ii) {A; G}, (iii) {E; F}, and (iv) {C; D}. Patterns A and B, in pair {A; B}, do not display any correlation with the risk of infection and provide us with a qualitative and quantitative 'scale' to gauge the symmetry values obtained for less obvious pairs of patterns. Figure 4 shows how the degree of symmetry between the vaccine measures, S_{MP}, varies as the degree of overdispersion in worm burden in the population decreases (i.e. k increases). In situations where there is no correlation between the risk of infection and the degree of vaccine protection, we expect this measure to decrease as k increases but the symmetry stays positive for all values of k (i.e. the two vaccine efficacy measures give consistent results; figure 4*a*). However, when we compare pairs of patterns displaying different degrees of correlation between the risk of infection and vaccine efficacy (figure 4*b–d*), the symmetry becomes negative as k increases. This loss of symmetry for higher values of k (i.e. lower degree of worm burden overdispersion) is due to the reduced contribution of those with worm burdens above the morbidity threshold to the overall worm burden. The degree of symmetry between the vaccine efficacy measures also depends on the endemic intensity of infection (figure 5). In the uncorrelated case (figure 5*a*), its value increases as the mean worm burden increases but, as before, it remains positive indicating that the vaccine efficacy measures give consistent results. However, when vaccine efficacy is correlated with the risk of infection (figure 5*b–d*), the symmetry is lost, this time for intermediate values of W. Similarly, the degree of symmetry is also influenced by the level of coverage although loss of symmetry in general occurs only for values of k>0.3 (results not shown).

6. DISCUSSION

We have provided a mathematical framework to evaluate the potential impact of vaccination against human hookworm. The model is used to identify the characteristics that a hookworm vaccine would require

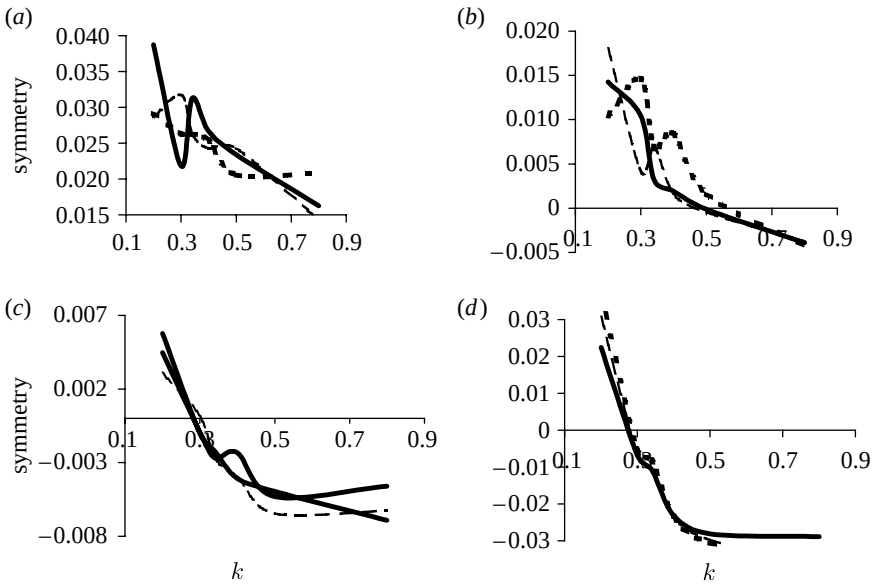

Figure 4. Symmetry in measured vaccine efficacies as a function of k. Pairs of patterns examined are: (*a*) {A; B}, (*b*) {A; G}, (*c*) {E; F }, (*d*) {C; D}. Positive symmetry values indicate that a higher vaccine efficacy measured on the basis of mean worm burden corresponds to a higher vaccine efficacy measured by the prevalence of morbidity whereas negative symmetry values indicate the opposite. In patterns A and B, the vaccine efficacy does not depend on the risk of infection. In patterns C–G, the vaccine efficacy depends on the risk of infection (figure 1). All other parameters are the same as given in table 1. Dashed lines, $R_0=2$; solid lines, $R_0=3$; dotted lines, $R_0=3.5$.

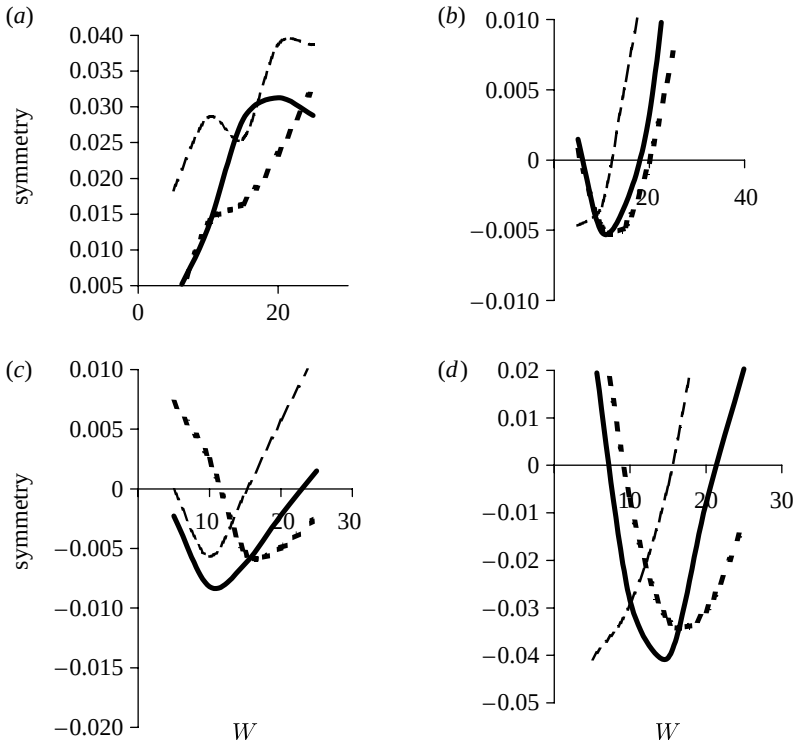

Figure 5. Symmetry in measured vaccine efficacies as a function of W. Pairs of patterns examined are: (*a*) {A; B}, (*b*) {A; G}, (*c*) {E; F}, (*d*) {C; D}. Positive symmetry values indicate that a higher vaccine efficacy measured on the basis of mean worm burden corresponds to a higher vaccine efficacy measured by the prevalence of morbidity whereas negative symmetry values indicate the opposite. In patterns A and B, the vaccine efficacy does not depend on the risk of infection. In patterns C–G, the vaccine efficacy depends on the risk of infection (figure 1). All other parameters are the same as given in table 1. Dashed lines, $k=0.2$; solid lines, $k=0.34$; dotted lines, $k=0.5$.

to be most efficacious in reducing infection rates and the morbidity associated with hookworm. In particular, we show that to have the greatest public health impact, a hookworm vaccine must have high efficacy among the proportion of the population that harbours the heaviest worm burdens (8–12% of the population, in high-transmission settings). In addition, we explore the impact of vaccination on morbidity and investigate how this differs from measures of vaccine efficacy based on worm burden alone. The developed model can readily

be used to explore the impact and cost effectiveness of vaccination in different epidemiological settings.

The model presented here is a stochastic individual-based expansion of previous helminth models that includes the basic features of hookworm biology and epidemiology as well as the effects of vaccination. This provides a flexible framework to simulate the impact of interventions on different population groups. The biological plausibility of the model is ensured by ad hoc structural and parametric choices; in particular, the adoption of a power-law decaying density-dependence function to account for the reduction in worm fecundity induced by overcrowding and the implementation through both Markovian and non-Markovian structure. The latter process is assumed to better account for immune-mediated density-dependence mechanisms (Paterson & Viney 2002). In the absence of detailed quantitative information on the biology of hookworm larvae, time intervals are taken to be sufficiently large to be compatible with several possible dynamics and modes of transmission (namely, clumped or multiple single transmission events; Cornell *et al.* 2004), but sufficiently short to allow for a detailed description of the dynamics of infection and morbidity.

A central problem in assessing the potential impact of hookworm vaccination is that the characteristics of the final vaccine are unknown. Modelling provides useful insight by helping identify desirable vaccine characteristics. Previous economic analysis of the desirable characteristics of a schistosomiasis vaccine (Guyatt & Evans 1995; Evans & Guyatt 1997) identified vaccine price, vaccine efficacy and duration protection as critical variables in determining cost effectiveness of vaccination relative to the most commonly used alternative, annual school-based chemotherapy. This conclusion is supported by mathematical modelling of a schistosomiasis vaccine, which employs a deterministic modelling framework and shows that the impact of vaccination is highly sensitive to the duration as well as the degree of protection (Chan *et al.* 1997).

Recognizing that the impact of vaccination may be different among different subpopulations, we investigated the impact of varying vaccine efficacy in populations with different underlying pre-vaccination hookworm burdens. We show that the greatest impact of vaccination, in terms of both worm burden and morbidity, is when vaccine efficacy is high in individuals with the highest worm burden. The importance of heterogeneity in individual responses to vaccination has been demonstrated theoretically for vaccines against microparasites (Halloran *et al.* 1997) and analysed in relation to HIV vaccine trials (Boily *et al.* 1999). However, although there is evidence suggesting that risk factors, such as exposure, may influence the efficacy of some microparasite vaccines (Fine & Clarkson 1988), to our knowledge, the implications of a risk-of-infection-correlated responsiveness to a vaccine have not been investigated quantitatively. Models of vaccines against malaria have similarly emphasized the importance of individual host factors in determining the vaccine efficacy. For instance, host-dependent variations in the entomological inoculation rate, in the multiplication factors of parasites as well as in the time of onset and intensity of adaptive immune responses have been taken into account in recent models although they mainly focused on the in-host dynamics of candidate vaccines (Dietz *et al.* 2006; Smith *et al.* 2006). For helminth infections, including hookworm, the underlying biological factors and the time scales involved in the development of an immune response to infection are different. In this case, the generative mechanisms of a potential risk-of-infection-correlated heterotypic immune response are most likely to be due to a combination of host- and exposure-related factors acting over a period of years (Quinnell *et al.* 2004).

The existence of heterogeneity in the impact of vaccination has a number of important implications. First, since overall efficacy depends disproportionally on the efficacy in high-risk individuals, vaccination programmes should aim to target subgroups with the highest worm burden. Such a targeted approach to intervention was first highlighted by Anderson & May (1982) and forms the basis of current chemotherapy programmes, targeting school-based children. A second implication is that the vaccine must be efficacious in individuals with pre-existing high worm burden or who are predisposed to intense infections. Furthermore, owing to the positive relationship between worm density in an individual and the probability of mating (Churcher *et al.* 2005), a heterotypic vaccine efficacy can be expected to have important consequences on worm fecundity, and hence on transmission patterns.

The definition of vaccine efficacy is clearly important and we aimed to investigate the impact of vaccination on both worm burden and morbidity. In particular, to study the impact of the vaccine on morbidity, we introduced two new measures of efficacy (VE_{MP} and VE_{MI}). They convey specific information on the reduction in worm clustering within the human population, which is a major risk factor for severe blood losses and therefore for the hookworm-associated disease. The symmetry between measures of efficacy based on the reduction in infection (VE_{MWB}) and measures of efficacy based on the reduction in morbidity (VE_{MP} and VE_{MI}) was used to investigate the correspondence between reduction in the intensity of infection and reduction of morbidity, under different scenarios. The proposed measures of efficacy (VE_{MP} and VE_{MI}) rely upon the concept of a 'threshold for morbidity' and account for the number of morbidity cases averted and the reduction in the intensity of infection, in morbidity cases, following vaccination. Although the precise threshold level will depend on a number of host factors, including age, gender and nutrition status, the displayed results hold for any threshold equal to or greater than 35 worms/person and suggest that the benefit associated with a reduction in the mean intensity of infection may be dramatically different for different population groups and has practical consequences for the appropriate interpretation of endpoints for vaccine trials and interventions.

As with all models, our framework provides only a crude representation of hookworm transmission dynamics and could be revised to capture additional features. First, the role played by immunological

processes in determining the density-dependence effects needs to be investigated, as it may have an impact on the post-vaccination dynamics of infection and the vaccine estimates. In general, immune regulatory mechanisms are fundamental features of macroparasite transmission (Anderson & May 1985; Woolhouse 1994). For instance, theoretical studies of the transmission dynamics of the sheep nematode *Teladorsagia circumcincta* suggest that much of the heterogeneity in transmission may be due to genetic variation in immune responsiveness (Stear *et al.* 2007). However, to date, there is no clear understanding of the role played by immunological processes in human hookworm infection (Anderson & May 1985; Quinnell *et al.* 2004). If acquired immunity is important in the dynamics of human hookworm infection, the model needs to be modified to account for the existence of an individual memory of infection and individual stochastic processes need to be non-Markovian. The precise structure of the model would then depend on the way immune response acts on different life stages of the parasite (i.e. on whether and how it inhibits larva penetration and migration or the establishment of adult worms or their ability to reproduce and in which proportions) and on the way immunological memory wanes.

Second, we could better incorporate spatial heterogeneity in transmission patterns and its contribution to individual predisposition to infection. Hookworm transmission is characterized by small-scale space heterogeneity (Brooker *et al.* 2006) that might reflect environmental and socio-economic differences. To date, few studies have investigated this problem, disentangling other sources of heterogeneity, including behavioural and genetic factors. Third, seasonal effects have not been included in the model since, although seasonal dynamics in transmission processes can occur, they have relatively limited impact on overall transmission dynamics and vaccination impact due to the lifespan of adults worms being greater than 1 year (Anderson & May 1982). However, seasonal patterns may have an impact on the short-term accuracy (on a time scale of months) of the quantitative estimates provided here. Better empirical information is required on a number of processes: density dependence; worm fecundity as a function of the host worm burden, immunity and genetic proximity of in-host parasites; small-scale temporal patterns of infection; and genetic determinants of predisposition.

Our simulations considered only a limited set of scenarios, including variable vaccine efficacy, duration of protection, vaccine coverage and efficacy of chemotherapy. Future work will explore the impact of vaccination under a range of epidemiological settings. Specifically, analyses will investigate the relative importance of heterogeneities in vaccine efficacy, duration of protection and coverage as well as chemotherapy efficacy in areas with varying transmission intensities. An extension of the current work will be to include the impact of vaccination on different outcome indicators, including host iron status.

Modelling outputs will also be combined with current estimates of the potential cost of hookworm vaccination in different regions to evaluate the cost effectiveness of vaccination relative to annual school-based chemotherapy. Initial cost estimates of potential vaccination programmes in Brazil and Uganda indicate that the cost per fully immunized child will be in the range US$2.57–4.49 depending on the number of doses (two or three) and delivery systems (campaigns, schools or routine immunization; M. Temperley *et al.* 2007, unpublished report). Ultimately, decisions about the provision of a human hookworm vaccine will depend on a number of other considerations in addition to effectiveness and cost effectiveness, including affordability, political commitment, delivery infrastructure, regulatory hurdles and financing.

Mathematical models can help address many questions about the use of potential vaccines. Our model provides a framework for identifying desirable characteristics of a hookworm vaccine and how to best evaluate its public health impact. Our analysis suggests that to have a great public health impact, a hookworm vaccine should be highly efficacious among individuals heavily infected. We also show that the correspondence between the average impact of an anti-hookworm vaccine on the worm population dynamics and the impact on morbidity will vary according to epidemiological setting. Further collection of empirical data to parametrize and validate the model will help in strengthening the usefulness of the model to simulate the public health impact and cost effectiveness of hookworm vaccination relative to and in combination with other helminth control strategies.

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