PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2005) **272**, 593–600
doi:10.1098/rspb.2004.2987
Published online 22 March 2005

A simple model for the within-host dynamics of a protozoan parasite

D. Klinkenberg* and J. A. P. Heesterbeek

Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

The dynamics of parasite–host systems can be complicated if the parasite life cycle contains an obligatory environmental stage and if the hosts' immunity increases upon re-infection. The dynamics then greatly depend on the relation between infection history and parasite uptake and excretion of individual hosts. In an effort to better understand such systems, we study *Eimeria* spp. in chickens as our model. In this paper we take a first step and study the within-host dynamics of *Eimeria* spp., transmitted through oocysts in the environment, with a mathematical model for the parasite life cycle in discrete time, interacting with a single variable describing the immune response. The model can explain various types of oocyst input–output behaviour as described in previous experiments, in particular the characteristic crowding effect, which causes a decreasing oocyst production with increasing single dose oocyst uptake. Oocyst excretion during constant oocyst uptake (trickle infection) and the immunizing effect of single and trickle infections also appears in accordance with published experiments. The model seems a good description of oocyst input–output behaviour in individual hosts; it provides a solid basis for the study of between-host dynamics, where individuals interact in a common environment, thereby affecting their own and each other's infection pattern.

Keywords: within-host dynamics; immune system; mathematical modelling; epidemiology; protozoa

1. INTRODUCTION

A large class of infectious disease systems is characterized by essential environmental stages in the parasite life cycle, which causes heterogeneity in exposure to infection in time and space and in parasite load and infectivity of hosts (e.g. Roberts *et al.* 1995). Complexity can even increase, as many of these parasites induce an acquired immune response, which becomes stronger upon re-infection and reduces the excretion of infectious material (e.g. Anderson 1998). As a consequence, these parasites both give rise to and experience a very heterogeneous host population with respect to immune response capability. In previous work, a model was studied for such a host–parasite system, where a very simple but non-trivial description of within-host dynamics was combined with between-host spread (Roberts & Heesterbeek 1998). This work showed that the dynamics of the system as a whole become complex and the outcome of control measures unpredictable. Obtaining insight into the dynamics of such host–parasite systems is essential for planning and evaluating control strategies. To this end we started a project in which experiments are combined with mathematical modelling to investigate the full cycle of within- and between-host parasite dynamics of a protozoan parasite. Here we report on a first step in this larger study. We address here the relation between infection history, uptake of parasites and excretion of parasites at the individual host level.

As a model system, we consider infection of chickens by protozoans of the genus *Eimeria*, causing coccidiosis. The disease is endemic to the poultry industry worldwide and it mainly reduces production, although it can cause severe intestinal damage (Allen & Fetterer 2002). In chickens, seven species of *Eimeria* are known that differ in their location in the gut and that do not seem to induce cross-immunity. *Eimeria* is capable of eliciting an effective acquired immune response in chickens, which can reduce epithelial lesions, growth reduction and oocyst production upon challenge infections (Lillehoj & Lillehoj 2000; Yun *et al.* 2000; Allen & Fetterer 2002).

In this paper, we study oocyst uptake and excretion in relation to the acquired immune response, by means of a mathematical model. A specific feature of protozoan infections is that parasite load increases both by multiplication within the host and by reinfection, whereas microparasites mainly use multiplication and macroparasites re-infection. Therefore, studying the dynamics of protozoan infections requires an approach that combines both mechanisms. In the model, the parasite life cycle within the chicken is described with both mechanisms separately interacting with a simple immune response variable. Since many experiments have been conducted in which known quantities of oocysts were fed in one or more doses and oocyst excretion was measured, we use those to validate the model qualitatively and to estimate some of the parameters.

2. THE MODEL

(a) *The* Eimeria *cycle*

Figure 1 shows the development of *Eimeria* spp. inside the host: a process with distinct, successive developmental stages (Allen & Fetterer 2002). After uptake of oocysts by the bird, sporozoites are released, which enter the gut epithelial cells and form first-generation schizonts

* Author for correspondence (d.klinkenberg@vet.uu.nl).

Received 12 May 2004
Accepted 22 October 2004

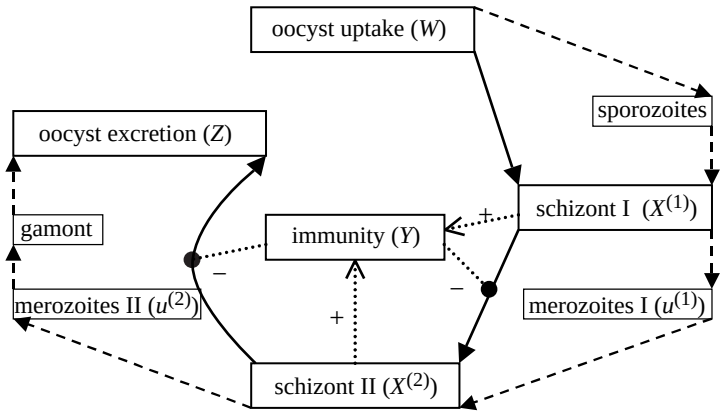

Figure 1. Schematic of the *Eimeria* cycle and the interaction with immunity. The outer ring of boxes and dashed arrows is the complete cycle as described in the text, and includes the model equation (2.1). The inner ring of large boxes and bold arrows is the reduced cycle of model equation (2.2), interacting with immunity through stimulation (dotted lines with open arrows) and inhibition (dotted lines with large dots).

the second day after infection. In the schizonts, asexual proliferation takes place, resulting in the release of first-generation merozoites into the intestinal lumen on the third day. The merozoites infect new epithelial cells, thereby forming second-generation schizonts the fourth day after infection, which may be followed by third or fourth generation schizonts depending on the *Eimeria* species. Cells infected by the last generation merozoites will harbour gamonts, in which sexual reproduction cells are produced. From male gamonts, microgametocytes are released to fertilize the female macrogametes. The resulting zygote turns into an unsporulated oocyst, which is shed by the chicken on the eighth day after infection. In the outside environment, the oocyst sporulates in 2 days and can be ingested by a bird to complete the cycle. All times are approximations and may be different for each *Eimeria* species, for example, oocyst excretion after a single infection may be observed 4–15 days after infection (Williams 2001; Allen & Fetterer 2002).

We use a discrete-time model with a time unit of 1 day for the description of *Eimeria* development. The variable w_t is the number of oocysts ingested (at day t), $x_t^{(1)}$ and $x_t^{(2)}$ are the numbers of first- and second-generation schizonts, $u_t^{(1)}$ and $u_t^{(2)}$ are the numbers of first- and second-generation merozoites, and z_t is the number of shed oocysts. The parameters a_1–a_5 are the coefficients of the assumed linear relations between the subsequent stages of the *Eimeria* cycle; $\lambda_1 = a_2 a_3$ and $\lambda_2 = a_4 a_5$, so λ_1 and λ_2 are the number of second-generation schizonts per first-generation schizont, and the number of shed oocysts per second-generation schizont, respectively. Without interaction with the immune system, the model reads:

$$\begin{aligned} x_{t+2}^{(1)} &= a_1 w_t \\ u_{t+3}^{(1)} &= a_2 x_{t+2}^{(1)} = a_1 a_2 w_t \\ x_{t+4}^{(2)} &= a_3 u_{t+3}^{(1)} = a_1 a_2 a_3 w_t = a_1 \lambda_1 w_t \\ u_{t+5}^{(2)} &= a_4 x_{t+4}^{(2)} \\ z_{t+8} &= a_5 u_{t+5}^{(2)} = a_4 a_5 x_{t+4}^{(2)} = \lambda_2 x_{t+4}^{(2)} \end{aligned} \qquad (2.1)$$

The linearity of the model allows us to omit the merozoite stages $u^{(1)}$ and $u^{(2)}$ and use the model as depicted in figure 1 in the rest of the paper.

The fecundity of *Eimeria*, in the model equal to $a_1\lambda_1\lambda_2$, is in the range of 10^3–10^6 oocysts excreted per oocyst (Williams 1973; Joyner & Norton 1976; Norton & Joyner 1986; Williams 2001), whereas a_1 is maximally eight, because each oocyst contains up to eight sporozoites. For the only two *Eimeria* species for which there is some evidence, *Eimeria tenella* and *Eimeria necatrix*, it has been reported that the numbers of merozoites are approximately equal in the first two schizont generations, and smaller in the third generation (McDonald & Elaine Rose 1987); therefore, in the model we will assume $\lambda_1 = \lambda_2$.

(b) *The immune response*

Eimeria infections cause species-specific immune responses (Lillehoj & Lillehoj 2000; Allen & Fetterer 2002). Although humoral as well as cellular immune responses have been observed, the major actors of the immune system in the control of *Eimeria* infections seem to be the CD8+ T cells. These cytotoxic T lymphocytes (CTLs) are often observed around infected epithelial cells and proliferate upon the encounter of antigen *in vitro*. CTLs act against the intracellular stages of *Eimeria* by lysis of infected cells, and thus reduce the production of merozoites or gametes (Lillehoj & Lillehoj 2000; Allen & Fetterer 2002). Therefore, immunity is incorporated into the model by reducing the numbers of merozoites per schizont, depending on the force of an immunity variable y_t.

The model with immune response becomes (figure 1):

$$\begin{aligned} x_{t+2}^{(1)} &= a_1 w_t \\ x_{t+4}^{(2)} &= a_1 \lambda_1 w_t f(y_{t+2}) \\ z_{t+8} &= \lambda_2 x_{t+4}^{(2)} f(y_{t+4}) \\ y_{t+4} &= g(y_{t+2}, x_{t+2}^{(1)} + x_{t+2}^{(2)}) \end{aligned} \qquad (2.2)$$

The function $f(y_t)$ is the fraction of successful schizonts, and $g(y_t, x_t^{(1)} + x_t^{(2)})$ describes the development of the immune response. Because an increase of the numbers of T cells in the gut was observed 4 days after infection (Vervelde 1995), we chose the immune variable y_t to react to schizonts with a delay of 2 days to have a similar timing to the primary response.

The behaviour of the model can be studied by only regarding $x_t^{(2)}$ and y_t in relation to w_t. The excretion variable z_t does not determine the model's behaviour, but

is interesting to study because of the relation to experimental data. Changing the time unit to $t' = 2t$ (time is now measured in units of 2 days), dropping the accent and the superscript (2) yields the model:

$$\begin{aligned} x_{t+2} &= a_1\lambda_1 w_t f(y_{t+1}) \\ y_{t+2} &= g(y_{t+1}, a_1 w_t + x_{t+1}) \\ z_{t+2} &= \lambda_2 x_t f(y_t) \end{aligned} \tag{2.3}$$

(c) *The functions f and g*

The inhibition function $f(y)$ describes the relation between the amount of immunity y and the fraction of schizonts developing into merozoites. Because absence of immunity should result in uninhibited schizont development and increasing immunity should lead to decreasing schizont development, two conditions for $f(y)$ are: $f(0) = 1$ and $f'(y) \leq 0 \,\forall\, \{y | y \geq 0\}$. We considered two simple functions, a negative exponential and a hyperbolic, and let the experimental data decide, resulting in the hyperbolic relation (see §3a):

$$f(y) = 1/(1 + y^m). \tag{2.4}$$

If $m = 1$, $f(y)$ describes an inverse linear relation between y and the development of schizonts. The case $m > 1$ reflects a situation in which immune cells enhance each other's effectiveness, for instance by releasing cytokines to attract more T cells to infected sites in the gut. If $m < 1$, the immune cells would be inhibited in the presence of many cells.

The function g describes the new amount of specific immunity y as a function of the previous y and the number of schizonts present. Conditions for g are that naive immune cells should be stimulated by antigen, that specific immune cells should proliferate upon the encounter of antigen, and that immunity should decrease in absence of schizonts. The most simple function fulfilling these three conditions has linear growth and decline and has interaction based on a law of mass action encounter rate between host immune cells and schizonts:

$$g(y, a_1 w + x) = \alpha y + \beta(a_1 w + x) + \gamma y(a_1 w + x) \tag{2.5}$$

That is, we let y increase linearly with the number of schizonts reflecting stimulation of naïve cells, determined by β ($\beta \geq 0$), and with additional growth driven by an interaction of present immunity and schizonts reflecting proliferation of specific cells, determined by γ ($\gamma \geq 0$). Finally, a fraction α ($0 \leq \alpha \leq 1$) of y will survive to the next time step.

3. MODEL ANALYSIS

We analysed the model by studying three types of model experiments that were related to published empirical experiments. In the first type of experiment, the relation between a single oocyst uptake w_0 and oocyst excretion z_4 was studied assuming $x_0 = y_0 = 0$. The results were compared with cumulative oocyst excretion data for naive chickens that were given different single oocyst doses (Brackett & Bliznick 1952; Williams 1973, 2001; Johnston *et al.* 2001). The experiments indicated a so-called crowding effect, where high doses give rise to lower excretion than intermediate doses (see figure 2, data from Williams (2001)).

In the second type of experiment, a constant oocyst uptake $\bar{w}$ per time unit was assumed, and the resulting model behaviour was analysed. The results should agree with experiments on chickens that were given small oocyst doses at regular time-intervals (Joyner & Norton 1976; Norton & Joyner 1986; Galmes *et al.* 1991; Parry *et al.* 1992; Stiff & Bafundo 1993; Graat 1996; Graat *et al.* 1997). All data showed that these so-called trickle infections lead to cessation of oocyst excretion, with doses ranging from 1 oocyst per day to 28 000 twice per week.

In the third type of experiment, administration of a single oocyst dose w_s or 10 consecutive doses $w_s/10$ were compared with respect to the resulting duration of protection against challenge infection. The results should agree with experiments on chickens that were immunized with a single dose or with the same amount of oocysts in multiple small doses at regular time-intervals (trickle immunization; Joyner & Norton 1976; Norton & Joyner 1986; Galmes *et al.* 1991; Nakai *et al.* 1992). The experiments showed that trickle immunization reduces oocyst excretion upon a challenge infection much better than single immunization.

(a) *Single oocyst doses*

Oocyst excretion after a single oocyst dose is described by

$$z_4(w_0) = \frac{a_1\lambda_1\lambda_2 w_0}{1 + (\beta a_1 w_0)^m} \tag{3.1}$$

if $x_0 = y_0 = 0$, for primary infections. On log–log scale, the oocyst input–output relation

$$\log z_4 = \log w_0 + p_1 - \log(1 + 10^{m(p_2 + \log w_0)}), \tag{3.2}$$

with $p_1 = \log(a_1\lambda_1\lambda_2)$ and $p_2 = \log(\beta a_1)$, is depicted in figure 3*a* for the parameters $a_1 = 4$; $\lambda_1 = 100$; $\lambda_2 = 100$; $\beta = 0.001$; and $m = 1.2$. The graph starts with a linear increase, then saturates to reach a maximum oocyst output, and from that decreases linearly.

In the first linear part, $\beta a_1 w_0 \approx 0$, resulting in the relation:

$$\log z_4 = \log w_0 + p_1. \tag{3.3}$$

The slope of the line is 1, because a small w_0 results in an excretion of $a_1\lambda_1\lambda_2$ oocysts per ingested oocyst.

In the second linear part, $\beta a_1 w_0 \gg 0$, resulting in the relation:

$$\log z_4 = (1 - m)\log w_0 + p_1 + mp_2. \tag{3.4}$$

The slope of the line is $1 - m$, which indicates that a decreasing oocyst production with high w_0, as observed in many experiments, requires a nonlinear inhibition function f, with $m > 1$. If the immune effector function f were a negative exponential instead of a hyperbolic function, the graph would decrease much faster, which is not supported by experimental data (Brackett & Bliznick 1952; Johnston *et al.* 2001; Williams 2001).

The data of Williams (2001) were used to estimate the parameters p_1, p_2, and m by least-squares on the log scale. The resulting curves are shown in figure 2, with the original data. By bootstrapping the residuals ($B = 10\,000$ replications), 95% percentile intervals were obtained (Efron & Tibshirani 1993). From table 1, showing the

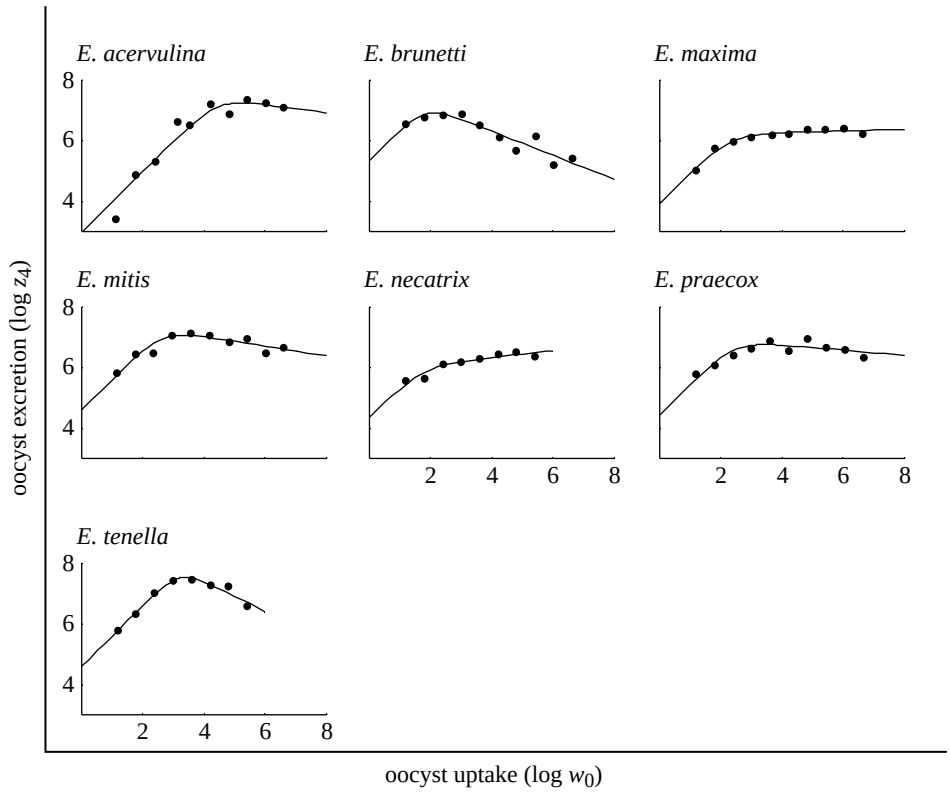

Figure 2. Relation between oocyst uptake and excretion for all seven *Eimeria* species. The points are the data from Williams (2001); the lines are the model fits.

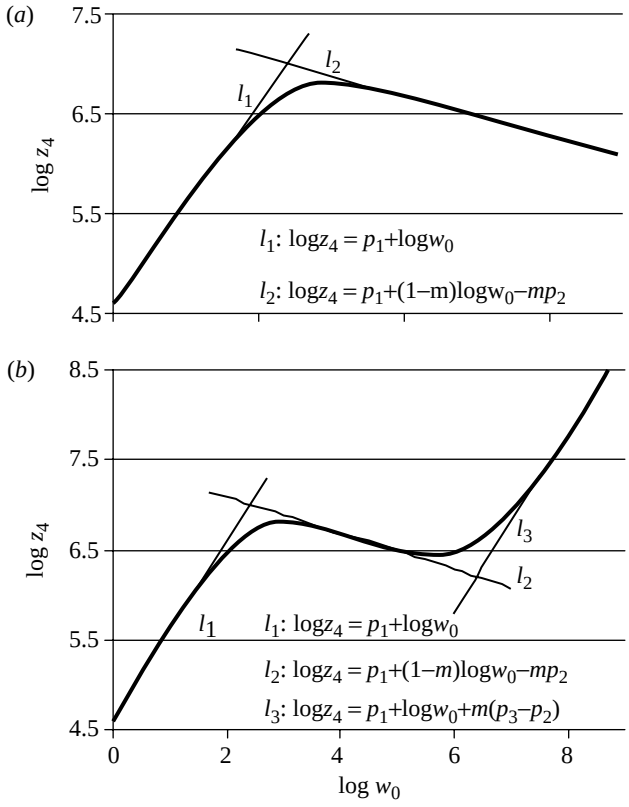

Figure 3. Relation between oocyst uptake and excretion as predicted by the model: (*a*) the relation according to the model (2.3)–(2.5), with two linear phases l_1 and l_2; (*b*) the relation with an alternative function g_s (equation (4.1)), with a third linear phase l_3. Parameter values: $a_1=4$, $\lambda_1=\lambda_2=100$, $\beta=0.001$, $m=1.2$, $\zeta=10^{-6}$.

parameter estimates for all seven *Eimeria* species, it appears that the fecundity ($=a_1\lambda_1\lambda_2$) of most *Eimeria* species is around $10^{4.5}$, ranging from $\sim 10^3$ to $\sim 10^{5.3}$. When assuming that $a_1=4$, half of its maximum, β ranges from $\sim 10^{-5.0}$ to $\sim 10^{-2.3}$.

(b) *Model behaviour during trickle infections*

In the second type of experiment, model behaviour resulting from a constant oocyst uptake $\bar{w}$ was analysed. Possible equilibria $\bar{y}$ are solutions to the equation

$$\bar{y}^m(\bar{y}(1-c)-b)+\bar{y}(1-c-c\lambda_1)-b-b\lambda_1=0, \qquad (3.5)$$

with

$$b=\frac{\beta a_1\bar{w}}{1-\alpha}; \quad c=\frac{\gamma a_1\bar{w}}{1-\alpha}$$

The model (equations (2.3)–(2.5)) determines the corresponding equilibria $\bar{x}$ and $\bar{z}$.

The quantity b can be interpreted as the amount of immunity owing to all previous first generations of schizonts and produced independently of present immunity. The quantity c has a similar interpretation, only that it is the production per unit of immunity in the equilibrium. Hence the equilibrium amount of immunity owing to all previous first generations of schizonts is $b+c\bar{y}$. The dynamic behaviour depends on the value of the quantity c.

For $c\geq 1$, it is easy to see that there exists no equilibrium $\bar{y}$, because the total amount of immunity in the equilibrium $\bar{y}$ should be larger than or equal to the immunity owing to the first generations of schizonts. If $c\geq 1$, then $g(y, a_1\bar{w}+x)>y$ for all y, which means that the decay of immunity $(1-\alpha)y$ is smaller than the induction of immunity by the

Table 1. Least-squares estimates (LSEs) and 95% bootstrap percentile intervals (PIs) for the three parameters of the single dose input–output relation (3.2), for all seven *Eimeria* species.
(Data from Williams (2001).)

Eimeria species	$p_1=\log(a_1\lambda_1\lambda_2)$		$p_2=\log(\beta a_1)$		m	
	LSE	95% PI	LSE	95% PI	LSE	95% PI
E. acervulina	2.97	(2.63; 3.36)	−4.43	(−5.24; −3.00)	1.14	(0.65; 2.00)
E. brunetti	5.34	(4.91; 6.18)	−1.82	(−2.29; −1.01)	1.40	(1.27; 1.53)
E. maxima	3.93	(3.79; 4.12)	−2.28	(−2.56; −1.96)	0.97	(0.91; 1.04)
E. mitis	4.60	(4.35; 4.78)	−2.63	(−2.99; −2.21)	1.16	(1.05; 1.29)
E. necatrix	4.38	(4.04; 4.96)	−1.69	(−2.28; −0.59)	0.89	(0.78; 1.02)
E. praecox	4.43	(4.20; 4.76)	−2.43	(−2.85; −1.90)	1.08	(0.98; 1.20)
E. tenella	4.59	(4.47; 4.75)	−3.21	(−3.46; −2.96)	1.51	(1.31; 1.72)

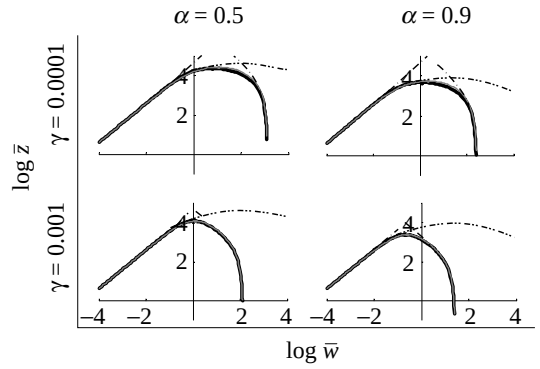

Figure 4. Relations between constant oocyst uptake $\bar{w}$ and excretion $\bar{z}$ for the full model (thick black curves) and three reduced models: $\gamma=0$ and $m=1$ (dash–dot–dot curves), $\beta=0$ and $m=1$ (dashed curves), $m=1$ (grey curves). Each of the panels shows a different combination of (α, γ), except when $\gamma=0$. Parameter values: $a_1=4$, $\lambda_1=\lambda_2=100$, $\beta=0.001$, $m=1.2$.

first-generation schizonts $(\beta+\gamma\bar{y})a_1\bar{w}$. As a result, y grows infinitely large, and x as well as z decrease to 0, so trickle immunization leads to a complete cessation of oocyst excretion, as in the experiments.

If $c<1$, it can be proven that there exists a single equilibrium $\bar{y}$ (Electronic Appendix A), which is always stable if $m(1-\alpha)<1$ (Electronic Appendix B). If $m(1-\alpha)>1$, oscillations of x, y, and z can occur for certain $\bar{w}$, depending on the values of the other parameters (e.g. with the values $a_1=4$, $\lambda_1=\lambda_2=100$, $\beta=\gamma=0.001$, $\alpha=0.05$, $m=1.5$, $\bar{w}=50$). Hence instability can occur with a highly nonlinear inhibition of schizont development by immunity (high m) or with a high immunity turnover (low α).

To determine how the parameters of the immune response affect the equilibrium excretion if $c<1$, we study the equilibrium excretion in three reduced models: one with $\gamma=0$, one with $\beta=0$, and all three with $m=1$. For these models, we could explicitly derive relations between $\bar{w}$ and the resulting equilibria $\bar{x}$, $\bar{y}$ and $\bar{z}$. Figure 4 shows the $\log(\bar{z})$ as a function of $\log(\bar{w})$ for the reduced models and the full model, with default parameters within the range of the estimates of table 1: $a_1=4$; $\lambda_1=100$; $\lambda_2=100$; $\beta=0.001$; $m=1.2$, and with two values for γ (0.0001; 0.001) and two values for α (0.5; 0.9), as no estimates were available.

(i) Case 1: $\gamma=0$, $m=1$, so immunity grows proportionally only to the number of schizonts. Since $c<1$ and $m(1-\alpha)<1$, the equilibrium $\bar{y}=(b-1+\sqrt{(b+1)^2+4b\lambda_1})/2$ always exists and is stable. If $\bar{w}\to\infty$, then $\bar{y}\to b$, and $\bar{z}\to a_1\lambda_1\lambda_2\bar{w}/b^2\propto 1/\bar{w}$, so with high $\bar{w}$, the oocyst production will be minimized. Figure 4, however, shows that with intermediate $\bar{w}$ ($\sim 10^3$), oocyst excretion is still rather high ($\bar{z}\sim 10^3$–10^5, depending on α). Apparently, an immunity-dependent growth term ($\gamma>0$) is needed in g for realistic excretion levels with trickle infections.

(ii) Case 2: $\beta=0$, $m=1$, so immunity grows proportionally to the product of the number of schizonts and the amount of present immunity only. This model has two equilibria, viz. the trivial equilibrium $\bar{y}=0$, and $\bar{y}=(c\lambda_1+c-1)/(1-c)$. If $c<1/(1+\lambda_1)$, the trivial equilibrium $\bar{y}=0$ is stable, because then the maximum number of schizonts $a_1\bar{w}(1+\lambda_1)$ cannot induce enough immunity to compensate for the decay of $(1-\alpha)y$ each time step. The positive equilibrium $\bar{y}$ only exists if $1/(1+\lambda_1)<c<1$, and is always stable since $m(1-\alpha)<1$. A high oocyst excretion is only observed with low values of $\bar{w}$ and a low value of γ and/or α (figure 4). These results suggest that $\alpha>0.9$ and $\gamma>0.001$.

(iii) Case 3: $m=1$. This model has the positive equilibrium $\bar{y}=(b+c+c\lambda_1-1+\sqrt{(b+1-c-c\lambda_1)^2+4b\lambda_1})/(2-2c)$, which exists if $c<1$, and is stable since $m(1-\alpha)<1$. Figure 4 shows a reduced maximum equilibrium excretion compared with the second case ($\beta=0$), especially if $\gamma=0.0001$. When comparing the third case with the full model, hardly any difference is seen, which suggests that the precise value of m (within the range of the estimates in table 1) is of minor importance for the equilibrium value. With experimental results showing that oocyst excretion also ceases with low values of $\bar{w}$, γ and α should not be too small, that is, $\gamma>0.001$ and $\alpha>0.9$ with the default parameter values used for figure 4.

Because the reduced models indicate that $\alpha>0.9$, and because m is not larger than 2 (table 1), the stability condition $m(1-\alpha)<1$ will certainly be met, so unstable equilibria will not occur with realistic parameter values.

(c) *Trickle and single immunization*

Trickle and single immunization were compared by simulation with the full model, with the default parameters of §3b, and various combinations of α and γ. Single

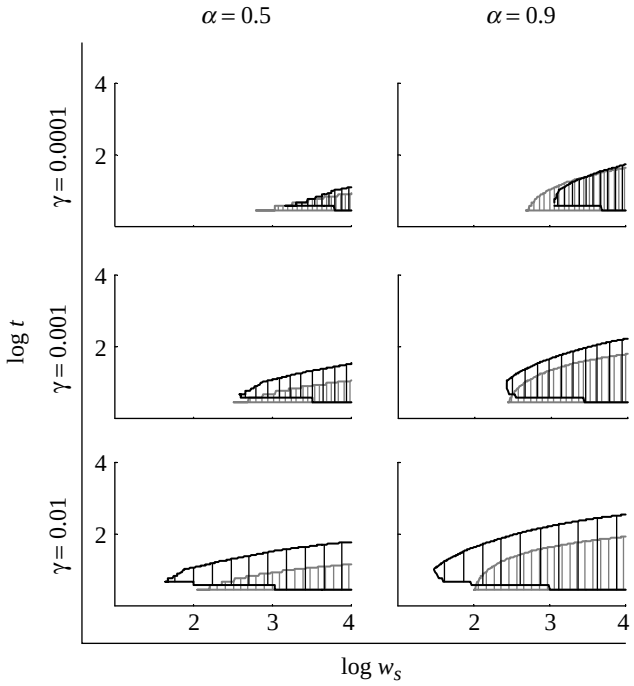

Figure 5. Relations between immunization dose w_s and protection period in time t after start of immunization. Black areas denote trickle immunization with 10 doses $w_s/10$; grey areas denote single immunization with dose w_s. Each of the panels shows a different combination of (α, γ). Parameter values: $a_1=4$, $\lambda_1=\lambda_2=100$, $\beta=0.001$, $m=1.2$.

immunization was simulated with $w_0=w_s$, $w_t=0$ ($t>0$); trickle immunization was simulated with $w_0=w_1=\cdots=w_9=w_s/10$, $w_t=0$ ($t>9$). Subsequently it was determined during which time-interval the value of y was large enough to give an oocyst excretion $z_{t+4}<1$ after an oocyst uptake $w_t=10$. These 'protection intervals' are depicted in figure 5 as a function of w_s.

Figure 5 shows that trickle immunization is better than single immunization for most combinations of α and γ and for most immunization doses. Single immunization is only better if $\gamma=0.0001$ and the infection dose is relatively small. Increasing α improves both immunization methods by making the protection intervals longer, and is more beneficial to single than to trickle immunization. Increasing γ improves both immunization methods by reducing the minimum dose required for protection, and is more beneficial to trickle than to single immunization. The effects of changing α and γ could be explained by the following different mechanisms.

(i) Increase of α gives the immunity y a longer half-life, extending the protection interval. This mechanism equally improves both single and trickle immunization.
(ii) Increase of α leads to a larger immunity y during trickle infection. As a result, less second generation schizonts will develop, which will reduce a further increase of y. This mechanism only reduces the effectiveness of trickle immunization, but figure 5 shows that the positive effect of mechanism (i) is larger than this negative effect.
(iii) Increase of γ enhances the immunity growth stimulated by existing immunity, which is more beneficial to trickle infections. However, single immunization also improves, as immunity induced by the first generation of schizonts enhances immunity growth owing to the second generation of schizonts, even within one *Eimeria* cycle.
(iv) Consider the simulations with small γ ($\gamma=0.0001$), in which single immunization is sometimes better than trickle immunization. If γ is small, the maximum amount of immunity that is produced is approximately $y_{\max}=\beta(a_1w_s+a_1\lambda_1w_s)$. Upon single immunization, most of $y_{\max}$ is produced at once, owing to the second-generation schizonts $x_2=a_1\lambda_1w_s$. With trickle immunization, however, production is divided over 10 time-points. Because immunity decays during the trickle infection, and because the developing immunity reduces development of second-generation schizonts, the immunity level at the end of the trickle infection will be smaller than $y_{\max}$. Therefore, if γ is small, single immunization can be more effective than trickle immunization, which is seen for some values of w_s in figure 5. However, if γy becomes large enough owing to larger w_s, trickle immunization will again lead to higher immunity levels and longer protection against challenge infections.

4. DISCUSSION

We have presented a model for the dynamics of *Eimeria* spp. within the chicken. The model, with a linear description of the parasite life cycle and a nonlinear interaction with a single immunity variable, was able to explain various types of oocyst input–output behaviour as observed in published experiments. All aspects of the immunity model appeared essential in showing this behaviour: $\beta>0$ and $m\neq1$, that is, growth of immunity independent of immunity present (naive growth) and nonlinear effectiveness of the immune response (e.g. cytokines), were needed for the oocyst input–output relation of equation (3.2), and $\gamma>0$, that is, growth of immunity dependent on immunity present (booster response), was necessary for the equilibrium oocyst output and the effectiveness of trickle immunization.

(a) *Biological implications*

The most remarkable result is the striking similarity between the model-derived graph in figure 3*a* and the results as published by Williams (2001), but also shown by others (Brackett and Bliznick 1952; Johnston *et al.* 2001), who observed a crowding effect with increasing oocyst dose (figure 2). Two possible mechanisms have been suggested to cause the crowding effect: reduction in the availability of epithelial cells and acquired immunity (Williams 1998; Johnston *et al.* 2001). While Johnston *et al.* (2001) could not explain the crowding effect with their model of host cell availability, we are able to observe a crowding relation with our model of acquired immunity.

The essential ingredient of the model, responsible for the characteristic figure 3*a*, is the cross-immunity to both schizont generations, which affects the parasite development of a single oocyst dose. Cross-immunity between the first and second schizont generation has been shown experimentally, but may be limited owing to a large

antigenic variation (McDonald *et al.* 1988; Tomley 1994). However, as schizonts of the second, third, and fourth generation possibly do not differ too much, the essential cross-immunity may have its effect in those generations, resulting in a similar input–output relation.

Two immune effector functions f were considered, and the hyperbolic function follows the experimental observations better than the negative exponential. The two functions mostly differ in their tail: the hyperbolic decreases slower, thus showing incomplete protection even with high immunity. This confirms the idea of gradually increasing immunity upon re-infection which is typical of macroparasitic and protozoal infections, so our model suggests that the effectiveness rather than the induction of immunity might be responsible for this behaviour.

The data of Williams (2001) were used to estimate m, measuring nonlinear effects and two (compound) model parameters, $p_1 = \log(a_1\lambda_1\lambda_2)$ as a measure of oocyst production per ingested oocyst, $p_2 = \log(\beta a_1)$ as a measure of naive immune growth (table 1). Because both p_1 and p_2 contain the model parameter a_1, the number of released sporozoites per oocyst, a positive relation between the two might be expected if the value of a_1 differs between species. The results suggest that this is not the case, with the exception of *Eimeria acervulina*, for which both the values of p_1 and p_2 are considerably smaller than for the other six species; the value of a_1 might be 10 times as small, suggesting a low sporozoite release or an ineffective infection of epithelial cells by sporozoites.

The parameter m was estimated significantly larger than 1, indicative of mutual stimulation of immune effector cells, for example, by the release of cytokines, for only three species, *Eimeria brunetti*, *Eimeria mitis* and *E. tenella*. These three species cause the most distally located infections, close to or in the caecum. As the caecal tonsils contain most of the lymph nodes of the gut-associated lymphatic tissue (Yun *et al.* 2000), it might be that the proximity of lymph increases the nonlinearity of the immune response.

Information on the parameters α and γ could only be obtained through the equilibrium excretion and trickle immunization experiments (figures 4 and 5). Comparing the results for several parameter combinations to empirical results led to the conclusion that α and γ should exceed 0.9 and 0.001, respectively (with the other parameters at their default values). This suggests that some immunological memory is indeed present, and that the presence of immunity considerably stimulates its growth.

(b) *The model*

Unlike most models describing within-host dynamics of pathogens with simple immunity functions (e.g. Anderson 1998; Molineaux & Dietz 1999; Nowak & May 2000), our model is in discrete time. The parasite life cycle, with distinct successive developmental stages, made a description in discrete time more appropriate. This is consistent with the conclusion of Molineaux & Dietz (1999), who reviewed within-host models of malaria, also a protozoan infection with alternating intracellular and extracellular stages. They stated that a discrete-time model is probably more realistic than the usual continuous-time approach. A second argument for using a discrete-time model with successive developmental stages for protozoan infections is that it enables a clear separation of the two mechanisms of increase in parasite load, multiplication within the host and re-infection. Because of that, both multiplication in the single dose experiments and re-infection in the trickle experiments could be studied.

A conceptual problem, however, may be that the immune variable y goes to 0 without re-infections (if immunity survival fraction $\alpha < 1$, that is), and that y increases to infinity if immunity grows faster than it is broken down ($c > 1$), there is no saturation of the immune response. An alternative function g_s, with saturation, may be formulated as

$$g_s(y, a_1 w + x) = \frac{\alpha y + \beta(a_1 w + x) + \gamma y(a_1 w + x)}{1 + \vartheta y + \zeta(a_1 w + x)} \qquad (4.1)$$

In g_s, α determines the maintenance of immunity, so it can take any positive value. With g_s instead of g, absence of re-infection will cause the system to settle at the stable equilibrium $(\bar{x}, \bar{y}, \bar{z}) = (0, (\alpha - 1)/\vartheta, 0)$, if $\alpha > 1$. Moreover, the growth of immunity will decrease with many schizonts or large y, so that y will not increase to infinity.

The use of g_s, however, does change the oocyst input–output relation of equation (3.2). The new relation is shown in figure 3*b* for the default parameters and $\zeta = 10^{-6}$. Compared with figure 3*a*, a third linear part has come up, which can be described by the function $\log z_4 = \log w_0 + p_1 + m(p_3 - p_2)$, provided that $p_3 < p_2$, with p_1 and p_2 as in equations (3.3) and (3.4), and with $p_3 = \log(\zeta a_1)$. The increase in excretion with very high dose is caused by the saturation of the immune response with high schizont numbers. If the third linear part is a realistic phenomenon, the doses administered by Williams (2001) may have been too small to reveal it, or the required doses too high to keep the chickens alive. In this case, ζ is very small compared with β, so the number of schizonts will hardly saturate the increase of y.

Although the absence of saturation may be unrealistic, it will not cause practical problems for studying oocyst uptake and excretion in a natural environment, since reinfections will make loss of immunity very unlikely, and the difference between a large y and a very large y is not relevant when it concerns oocyst excretion.

5. CONCLUSIONS

Our model of within-host dynamics of *Eimeria* spp. in chickens can explain various experimental results. An immune response with cross-immunity against two schizont generations may well be responsible for the crowding effect as observed in many experiments, without taking host cell availability into account. Nonlinear immune effectiveness ($m > 1$) is required to explain the decrease owing to the crowding effect, which points to mutual stimulation of immune cells, for instance through cytokine release. Since the model is a good description of input–output behaviour, regarding single infections, trickle infections and immunization, it will be a solid basis for studying between-host dynamics, where individuals interact in a common environment, thereby affecting their own and each other's infection pattern.

REFERENCES

Allen, P. C. & Fetterer, R. H. 2002 Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin. Microbiol. Rev.* **15**, 58–65.

Anderson, R. M. 1998 Complex dynamical behaviours in the interaction between parasite populations and the host's immune system. *Int. J. Parasitol.* **28**, 551–566.

Brackett, S. & Bliznick, A. 1952 The reproductive potential of five species of coccidia of the chicken as demonstrated by oocyst production. *J. Parasitol.* **38**, 133–139.

Efron, B. & Tibshirani, R. J. 1993 *An introduction to the bootstrap*. London: Chapman and Hall.

Galmes, M. M., Norton, C. C. & Catchpole, J. 1991 Comparison of resistance level and circulating IgG response in chickens experimentally inoculated with a multiple or a single immunizing doses of *Eimeria acervulina*. *Ann. Parasitol. Hum. Comp.* **66**, 144–148.

Graat, E. A. M. 1996 Epidemiology of *Eimeria acervulina* infections in broilers: an integrated approach. Ph.D. thesis, Wageningen University, Wageningen.

Graat, E. A. M., Ploeger, H. W., Henken, A. M. & Braunius, W. W. 1997 *Eimeria acervulina*: influence of corticosterone-induced immuno-suppression on oocyst shedding and production characteristics in broilers, and correlation with a computer simulation model. *Vet. Parasitol.* **70**, 47–59.

Johnston, W. T., Shirley, M. W., Smith, A. L. & Gravenor, M. B. 2001 Modelling host cell availability and the crowding effect in *Eimeria* infections. *Int. J. Parasitol.* **31**, 1070–1081.

Joyner, L. P. & Norton, C. C. 1976 The immunity arising from continuous low-level infection with *Eimeria maxima* and *Eimeria acervulina*. *Parasitology* **72**, 115–125.

Lillehoj, H. S. & Lillehoj, E. P. 2000 Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. *Avian Dis.* **44**, 408–425.

McDonald, V. & Elaine Rose, M. 1987 *Eimeria tenella* and *E. necatrix*: a third generation of schizogony is an obligatory part of the developmental cycle. *J. Parasitol.* **73**, 617–622.

McDonald, V., Wisher, M. H., Elaine Rose, M. & Jeffers, T. K. 1988 *Eimeria tenella*: immunological diversity between asexual generations. *Parasite Immunol.* **10**, 649–660.

Molineaux, L. & Dietz, K. 1999 Review of intra-host models of malaria. *Parassitologia* **41**, 221–231.

Nakai, Y., Uchida, T. & Kanazawa, K. 1992 Immunization of young chickens by trickle infection with *Eimeria tenella*. *Avian Dis.* **36**, 1034–1036.

Norton, C. C. & Joyner, L. P. 1986 Avian coccidiosis: the administration of encapsulated oocysts. *Parasitology* **92**, 499–510.

Nowak, M. A. & May, R. M. 2000 *Virus dynamics. Mathematical principles of immunology and virology*. Oxford University Press.

Parry, S., Barratt, M. E. J., Jones, S., McKee, S. & Murray, J. D. 1992 Modelling coccidial infection in chickens: emphasis on vaccination by in-feed delivery of oocysts. *J. Theor. Biol.* **157**, 407–425.

Roberts, M. G. & Heesterbeek, J. A. P. 1998 A simple parasite model with complicated dynamics. *J. Math. Biol.* **37**, 272–290.

Roberts, M. G., Smith, G. & Grenfell, B. T. 1995 Mathematical model for macroparasites of wildlife. In *Ecology of infectious diseases in natural populations* (ed. B. T. Grenfell & A. P. Dobson), vol. 7. Cambridge University Press.

Stiff, M. I. & Bafundo, K. W. 1993 Development of immunity in broilers continuously exposed to *Eimeria* sp. *Avian Dis.* **37**, 295–301.

Tomley, F. 1994 Antigenic diversity of the asexual developmental stages of *Eimeria tenella*. *Parasite Immunol.* **16**, 407–413.

Vervelde, L. 1995 *Eimeria tenella* infections in chickens: to recognize and to be recognized. Ph.D. thesis, Vrije Universiteit, Amsterdam.

Williams, R. B. 1973 Effects of different infection rates on the oocyst production of *Eimeria acervulina* or *Eimeria tenella* in the chicken. *Parasitology* **67**, 279–288.

Williams, R. B. 1998 Epidemiological aspects of the use of live anticoccidial vaccines for chickens. *Int. J. Parasitol.* **28**, 1089–1098.

Williams, R. B. 2001 Quantification of the crowding effect during infections with the seven *Eimeria* species of the domesticated fowl: its importance for experimental designs and the production of oocyst stocks. *Int. J. Parasitol.* **31**, 1056–1069.

Yun, C. H., Lillehoj, H. S. & Lillehoj, E. P. 2000 Intestinal immune response to coccidiosis. *Dev. Comp. Immunol.* **24**, 303–324.

The supplementary Electronic Appendix is available at http://dx.doi.org/10.1098/rspb.2004.2987 or via http://www.journals.royalsoc.ac.uk.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production cost.