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# Effects of heterogeneity in infection-exposure history and immunity on the dynamics of a protozoan parasite

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Infection systems where traits of the host, such as acquired immunity, interact with the infection process can show complex dynamic behaviour with counter-intuitive results. In this study, we consider the traits 'immune status' and 'exposure history', and our aim is to assess the influence of acquired individual heterogeneity in these traits. We have built an individual-based model of *Eimeria acervulina* infections, a protozoan parasite with an environmental stage that causes coccidiosis in chickens. With the model, we simulate outbreaks of the disease under varying initial contaminations. Heterogeneity in the traits arises stochastically through differences in the dose and frequency of parasites that individuals pick up from the environment. We find that the relationship between the initial contamination and the severity of an outbreak has a non-monotonous 'wave-like' pattern. This pattern can be explained by an increased heterogeneity in the host population caused by the infection process at the most severe outbreaks. We conclude that when dealing with these types of infection systems, models that are used to develop or evaluate control measures cannot neglect acquired heterogeneity in the host population traits that interact with the infection process.

**Keywords: epidemiology; population dynamics; individual-based model; structured population; acquired heterogeneity**

## 1. INTRODUCTION

Understanding the dynamics of infectious diseases in populations is important for the development and improvement of intervention methods. Much understanding has been obtained by use of mathematical and simulation models. In the most recent decades, such models have increasingly been able to incorporate population structure, interpreted as differences between individuals in traits that influence transmission (Diekmann & Heesterbeek 2000). Where the infection process is, in some way, influenced by all relevant traits, many of these traits will not in turn be influenced by the infection dynamics (think of age and sex), or at least this is usually implicitly assumed (e.g. spatial location might change, but decision to move or movement itself is usually taken independent of infection dynamics).

There are important situations where the interaction between trait and infection dynamics does work both ways. A class of systems where this happens are those systems where the heterogeneity between individuals lies in the somewhat implicit trait, 'infection history', and the more explicit trait, 'immune status'. Examples of such systems are most host–parasite systems with protozoan parasites or worm parasites, where the heterogeneity in infection history and immune status (however defined) exists due to stochastic differences in exposure. The circle of interaction consists of the exposure of an individual host shaping its infection history and immune status (acquired immunity), which, in turn, will influence the infectiousness of that individual to others. It is this type of *acquired heterogeneity* which is not well understood in the context of pathogen transmission, and notably the effects that the interaction has on the dynamics and the effectiveness of control measures. In a very caricatural model of an unspecific parasite–host system, Roberts & Heesterbeek (1998) showed that even including the two-way interaction between immunity and transmission (acquired immunity) alone can have substantial and counter-intuitive effects. In that model, all individuals responded similarly; there was acquired immunity but not yet acquired heterogeneity.

In this paper, we present an initial study of the effects of including acquired heterogeneity in exposure into an epidemiological model. For this, we model the host dynamics of a protozoan parasite, including acquired immunity and repeated infection through a spatially structured environment. We have chosen to develop our ideas using the specific parasite–host system of *Eimeria acervulina* in chickens for which a

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large body of experimental results is available and for which we have gained previous insight combining data and (within- and between-) host models (Klinkenberg & Heesterbeek 2005, in press). We use an individual-based model to allow for stochastically emerging heterogeneity. The dynamics are modelled with only a limited number of infection and immunity levels, which allows us to easily track the immune status, stochastic exposure history and pathogen excretion of each individual host through time.

We describe the biological system, and give the model description and assumptions in §2. The simulations that we have performed with the system to gauge the influence of various sources of heterogeneity are shown in §3. Notably, we study variation in the immune response, transmission and movement of individuals. We discuss our results in §4.

The system is studied by relating initial levels of oocysts in the environment to the average cumulative excretion of the chickens in the weeks thereafter as a measure of unprotected infection. Experiments have shown a non-monotonous relationship between the initial oocyst levels, disease and production loss, with intermediate oocyst levels giving optimal results. We also observe this in our model analysis, and we can explain this pattern from the variation in exposure history and immunity between individuals.

## 2. BIOLOGICAL SYSTEM AND MODEL DESCRIPTION

### 2.1. E. acervulina *life cycle*

We model the dynamics of *E. acervulina* infections, one of the milder and most common species of the coccidium *Eimeria*, in a commercial broiler chicken setting. The disease that these *Eimeria* parasites cause is called coccidiosis, and is economically very important owing to reduced growth of the animals. We first briefly describe the life cycle. Chickens continuously peck on the stable floor and can thereby pick up oocysts, the infectious stage of the parasite. Within the cells of the gut epithelium, the parasite undergoes a series of replications resulting in the formation of new oocysts that are excreted in the faeces (Allen & Fetterer 2002); after excretion, oocysts have to sporulate to become infectious. Excretion of oocysts starts 4 days after infection and may last for 10 days, whereas sporulation takes another 2 days to complete the cycle (Graat *et al.* 1994; *The Merck Veterinary Manual* 2006). Under favourable circumstances, oocysts may survive a long time (Reyna *et al.* 1983); however, in chicken manure, they have a half-life of approximately 2 days (Reyna *et al.* 1983; Williams 1995).

### 2.2. The model

*2.2.1. The model environment.* We model the complex chicken–environment interaction with an individual-based simulation model within the modelling environment of NETLOGO (Wilensky 1999) because it allows for stochastically emerging heterogeneity and studying the effect of space. The model algorithm consists of an initial step followed by a set of rules that together determine 1 day of the chickens, and change the environment; these rules are repeated until the simulation stops. The set consists of model rules for moving, taking up oocysts, excretion of oocysts and the development of immunity:

(i) *Set-up.* Initiate all model variables,
  (a) create a regular grid of square patches,
  (b) initiate a percentage of patches with a certain oocyst contamination level and specify the initial spatial distribution of these contaminated patches,
  (c) create a number of chickens with no uptake history or immunity,
  (d) randomly place the chickens onto the patches with no more than one chicken per patch allowed, and
  (e) set $day=1$.

(ii) *Chicken movement.* Let each chicken displace to a random patch (again only one chicken per patch), and let the chickens pick on the patch. Repeat 24 times, this makes up 1 day of movement and exposure.

(iii) *Oocyst uptake.* Let every chicken add the highest encountered level of contamination during 1 day to its uptake history.

(iv) *Oocyst excretion.* Adjust the contamination level of the patch on which a chicken ended in step (ii), according to the excretion corresponding to the uptake history and immune level of the chicken.

(v) *Immunity.* Adjust the immune level of each chicken according to its uptake history.

(vi) If $day=49$, then stop, otherwise $day=day+1$ and go to step (ii).

(The model is available at http://www-binf.bio.uu.nl/maite.) Below we will explain all steps in detail and discuss the biological fundaments of these rules and which assumptions and simplifications were made.

*2.2.2. Chicken movement.* We simulate a $4\times4$ m section of a broiler shed represented by a regular grid of square patches which we define to be $10\times10$ cm. During the set-up rule, chickens are randomly placed onto the patches, with only one chicken per patch allowed. The movement rule lets chickens move 24 times, i.e. once per hour. Chickens move through the shed section by randomly changing their position to another patch. Random mixing between the chickens and the patches is supported by observations in broiler sheds (Preston & Murphy 1989; Lewis & Hurnik 1990), where chickens show no sign of territorial behaviour and have an average displacement of 8 m $h^{-1}$, which is large compared with our model settings.

*2.2.3. Floor contamination.* Each patch is characterized by its oocyst contamination level, either empty, low, medium or high, reflecting oocyst numbers of the order 0, $10^3$, $10^5$ and $10^7$. During the set-up, the initial contamination level of choice is implemented as a percentage of contaminated patches. The initially contaminated patches can be randomly distributed over the field or concentrated in a single position in the field. Patch contamination levels increase when a chicken on a patch excretes a higher level of oocysts on that patch than

already present. Patch contamination levels decrease if the last excretion on that patch was 14 days ago resulting from the 2 days half-life of oocysts (Reyna *et al.* 1983; Williams 1995). We neglect the effect of decreasing oocyst levels due to uptake because it is very small relative to the natural environmental decay of oocysts.

*2.2.4. Oocyst uptake.* The movement rule lets chickens 'remember' the highest level of contamination on a patch they have been exposed to while moving through the shed during the day. The oocyst uptake rule determines that the dose of oocysts that chickens pick up from the patches is 1% of this highest contamination level that they have been exposed to yielding low, medium and high ingestion doses, equivalent to 10, $10^3$ and $10^5$ ingested oocysts per day. This implies that, within 1 day, lower levels of contamination and repeated encounters with the same contamination are neglected, justified owing to the 100-fold difference between categories. Thus, after the movement rule, 1% of the highest level of contamination encountered is saved by the uptake rule in the chickens' individual uptake record. This record is then later used by the oocyst excretion rule and the immunity rule to determine the oocyst excretion of the chicken and the chicken's immunity level, respectively.

*2.2.5. Immunity.* Various experiments have shown that past infections with *E. acervulina* induce protection against disease and oocyst production resulting from later infections (Chapman 1999; Lillehoj & Lillehoj 2000), but no single immune variable has yet been identified as a measure of protection. As we use immunity in our model to affect the dynamics of the parasite, we only regard immunity in relation to a reduction in oocyst excretion. Three immune levels are distinguished: no, partial and full immunity. A new level of immunity is reached when a certain dose and/or frequency of oocysts is ingested. In addition to the dose and frequency conditions, it takes a fixed time before a new level is reached reflecting that it takes time to build an immune response. Every day the immune rule verifies if chickens have satisfied the conditions required to reach a new level of immunity, and assigns a new immunity level to those chickens that meet the conditions.

*2.2.6. Oocyst excretion.* Essential for the dynamics is the relationship between uptake and excretion of oocysts. This relationship is mainly determined by the dose and the frequency with which the oocysts are ingested, the immune status, age and, to a lesser extent, the breed of the chicken. In our model, we take into account dose, frequency and immune status, neglecting breed and age due to limitation of data; most experiments we used to fit the excretion patterns were done with chickens within the age range of the model chickens. We do not explicitly model the within-host dynamics, but instead define excretion templates as a function of the uptake history, derived from experiments in the literature (table 1). We recognize nine templates, one for each combination of the three immune levels and the three doses that can be ingested. The dose of oocysts excreted by a chicken always reflects its current immunity level and the dose it has taken up 4 days earlier, i.e. the first template of a chicken is started 4 days after its first ingested dose of oocysts. Chickens follow their excretion template in the days thereafter, until it finishes or is interrupted by the uptake of an equal or higher dose, or when a higher immune level is reached. In these cases, the template is restarted or a new template starts.

Single-dose experiments were used to translate the ingested and excreted numbers of oocysts to the categories in our model, i.e. low, medium and high. The excretion patterns thus obtained were roughly

Table 1. Templates showing daily excretion dose of naive, partial and full immune birds after ingestion of a low, middle or high dose of oocysts starting from the fourth day after ingestion. (In the templates, 2, 3 and 4, respectively, represent low, middle and high doses of oocyst excretion.)

| immune state | ingested dose | template |
|---|---|---|
| naive | low | [2 2 2] |
| | middle | [3 3 3 3 3 3 3 2 2] |
| | high | [4 4 4 4 4 4 4 3 3 2] |
| partial | low | [ ] |
| | middle | [2 3 2 2 2] |
| | high | [3 3 3 2 2 2] |
| full | low | [ ] |
| | middle | [ ] |
| | high | [0 2] |

Table 2. Experiments from the literature and model simulations, where birds are inoculated with one dose of oocysts and the number of daily shed oocysts are recorded. (The first column shows the inoculation dose; the experimental inoculation dose is grouped into the model categories for low (until 100 oocysts), middle (more than 100 until $10^4$ oocysts) and high (more than $10^4$ until $10^6$ oocysts) uptake. The second column shows the excreted oocysts in the days subsequent to the inoculation. The experimental excretion data are translated into the model categories for low (until $10^4$ oocysts, depicted as 2), middle (more than $10^4$ oocysts until $10^6$ oocysts, depicted by 3) and high (more than $10^6$ oocysts until $10^8$ oocysts, depicted by 4) doses of excretion. *Less than 500 000 oocysts.)

| inoculation dose (oocysts) | excreted | origin |
|---|---|---|
| low | 0 0 0 0 2 2 2 | model |
| middle | 0 0 0 0 3 3 3 3 3 3 3 2 2 | model |
| 150 | 0 0 0 0 2 3 3 3 3 3 3 3 | Joyner & Norton (1976) |
| 1250 | 0 0 0 0 3 4 4 4 3 3 3 | Hein (1968*a*) |
| 5000 | 0 0 0 0 3 4 4 4 4 4 3 | Hein (1968*a*) |
| high | 0 0 0 0 4 4 4 4 4 4 4 3 3 2 | model |
| 20 000 | 0 0 0 0 4 4 4 3 3 3 3 3 2 2 2 | Galmes *et al.* (1991) |
| 1 000 000 | 0 0 0 0 4 4 4 4 4 4 3 3 2 2 | Galmes *et al.* (1991) |
| 20 000 | 0 0 0 0 4 4 4 4 4 4 4 3 | Hein (1976) |
| 20 000 | 0 0 0 0 3 4 4 4 4 4* | Hein (1968*a*) |
| 80 000 | 0 0 0 0 3 4 4 4 4 4* | Hein (1968*a*) |
| 230 000 | 0 0 0 0 3 4 4 4 4 4 4** | Hein (1968*a*) |
| 80 000 | 0 0 0 0 4 4 4 4 4 4**** | Hein (1968*b*) |
| 320 000 | 0 0 0 0 4 4 4 4 4 4*** | Hein (1968*b*) |
| 50 000 | 0 0 0 0 3 4 4 3 3 2 | Vermeulen *et al.* (2004) |

Table 3. Trickle infection experiments from the literature and model simulations. (The first column shows the inoculation dose the birds receive per day and for how many days. The second column shows the excreted oocysts in the days subsequent to the inoculation. The experimental excretion data are translated into the model categories for low (until $10^4$ oocysts, depicted as 2), middle (more than $10^4$ oocysts until $10^6$ oocysts, depicted by 3) and high (more than $10^6$ oocysts until $10^8$ oocysts, depicted by 4) doses of excretion.)

| trickle infection (days×oocysts) | excreted | origin |
|---|---|---|
| 25×low | 0 0 0 0 3 3 3 3 3 3 3 3 2 2 2 2 2 2 | model |
| 25×25 | 0 0 0 0 2 3 3 3 3 3 3 3 3 3 3 3 3 3 | Joyner & Norton (1976) |
| 20×middle | 0 0 0 0 4 4 4 4 4 4 4 4 2 2 2 2 2 | model |
| 20×1000 | 0 0 0 0 4 4 4 4 4 4 4 3 3 3 2 2 | Galmes *et al.* (1991) |

averaged (table 2) to become the excretion templates as shown in table 1. Single-dose experiments performed at our group by Velkers *et al.* (in preparation *b*) were also used (data not shown), in particular for the low-dose template.

We explicitly distinguish between excretion after uptake of an isolated single dose and the so-called trickle infections, where chickens repeatedly take up doses on consecutive days causing accumulation of excreted oocysts. It has been shown experimentally and in mechanistic models that immune reaction and excretion patterns are different for the same total dose of oocysts, depending on uptake as either a single or a trickle dose (Williams 1973; Klinkenberg & Heesterbeek 2005; Swinkels *et al.* 2006). The same averaging of excretion patterns was done for trickle infection experiments (table 3), from which it was concluded that the same templates could be used, rather than defining separate excretion templates for trickle infections. We let excretion induced by a low-dose trickle infection follow the medium-dose template, and in chickens with no immunity, we let a medium-dose trickle infection result in excretion according to the high-dose template. Since high-dose trickle infections have not been described and are not likely to cause a large accumulation of excreted oocysts due to the so-called crowding effect (Williams 2001), in the model, chickens that undergo such a high-dose trickle infection are assigned with the corresponding single-dose template. A separate set of excretion templates for partially and fully immune chickens were constructed according to immunization experiments shown in table 4.

### 2.3. Model output

Simulations start with an initial situation of only naive chickens and a user-given percentage of low-level contaminated patches in the shed. We study the model by simulating the dynamics for 49 days, a common rearing time for broiler chickens and which proved sufficiently long to capture the dynamics of the epidemic under all initial conditions studied. During the simulations, we monitor the numbers of chickens ingesting low, medium or high oocyst levels, excreting low, medium or high oocyst levels and becoming partial or fully immune. In our analysis, we will focus on the average number of high-level excretions during one simulation, i.e. the mean high excretion per chicken during its time in the shed. This output variable gives an indication of the severity of an outbreak, since the excretion of high amounts of oocysts is more likely to be related to problems caused by clinical coccidiosis, such as intestinal damage and individual-level growth reduction (Chapman *et al.* 2005).

### 2.4. Default settings for simulation

The default setting of the model for simulation is a grid of 1681 patches of 10×10 cm on which we let 353 chickens move, yielding a chicken density of 21 chickens $m^{-2}$. During set-up, initial patch contamination is set to low levels of oocysts and concentrated in a single position in the field. An ingested dose of oocysts is defined to be part of a trickle infection when, within 4 days after uptake, a chicken picks up at least two more doses of equal or higher level. Chickens reach partial immunity 9 days after the second of two low-dose or medium-dose ingestions, or 9 days after only one high-dose ingestion. Full immunity is reached after 13 doses with a cumulative oocyst exposure of 33 (low doses counting for two, medium for three and high for four), but no earlier than 6 days after becoming partially immune. To assess the stochastic variation for one set of initial conditions, we vary the random seed of the model.

## 3. RESULTS

### 3.1. Default result

The average number of high-level excretions per chicken is shown for a range of initial contaminations in figure 1*a*. At each initial contamination depicted, simulations were executed in threefold with varying random seeds, which proved sufficient to capture a large part of the stochastic variation. The graph shows a non-monotonous 'wave-like' relationship with alternating peaks and troughs between the percentage of patches initially contaminated and the average number of high excretions per individual. The effect of field size was studied by increasing the field to 14×14 m, which also allowed us to simulate with even lower initial levels and observe that the wave-like pattern continued. The time course of the infection with respect to exposure and immunity at the peaks in excretion (initial contamination of 0.01 and 0.8%) and the trough (initial contamination of 0.1%) are shown in figure 1*b–d*.

The fraction of chickens that pick up a low-dose trickle infection during a simulation is shown in figure 1*b* for one random seed. Middle-dose trickle ingestion and high-dose ingestion are the ones that lead to high-dose excretions in non-immune chickens. We show the fraction of chickens that pick up either one of these in figure 1*c* for the same simulation as in figure 1*b*. Both partial and full immunity preserve chickens from high-dose excretions. The fraction of chickens that are partially or fully immune is shown in figure 1*d*.

Table 4. Immunization experiments from the literature and model simulations. (The first column shows the immunizing dose(s) the birds receive per day and for how many days. The challenge dose is shown in the second column. The third column shows the excreted oocysts in the days subsequent to the challenge. The experimental excretion data are translated into the model categories for low (until $10^4$ oocysts, depicted as 2), middle (more than $10^4$ oocysts until $10^6$ oocysts, depicted by 3) and high (more than $10^6$ oocysts until $10^8$ oocysts, depicted by 4) doses of excretion.)

| immunization dose (days×oocysts) | challenge (oocysts) | excreted | origin |
|---|---|---|---|
| 20×middle | high (day 21) | 0 0 0 0 0 2 | model |
| 20×1000 | 1 000 000 (day 21) | 0 0 0 0 3 2 | Galmes *et al.* (1991) |
| 1×high | high (day 15) | 0 0 0 0 3 3 3 2 2 2 | model |
| 1×80 000 | 160 000 | 0 0 0 0 3 3 3 | Hein (1968*b*) |
| high (days 1 and 15) | high (day 28) | 0 0 0 0 3 3 3 2 2 2 | model |
| 80 000 (day 1) 160 000 (day 15) | 80 000 (day 28) | 0 0 0 0 3 3 3 3 3 3 3 3 3 3 | Hein (1968*b*) |
| 80 000 (day 1) 160 000 (day 15) | 5 120 000 (day 28) | 0 0 0 0 0 3 3 3 3 3 3 3 | Hein (1968*b*) |

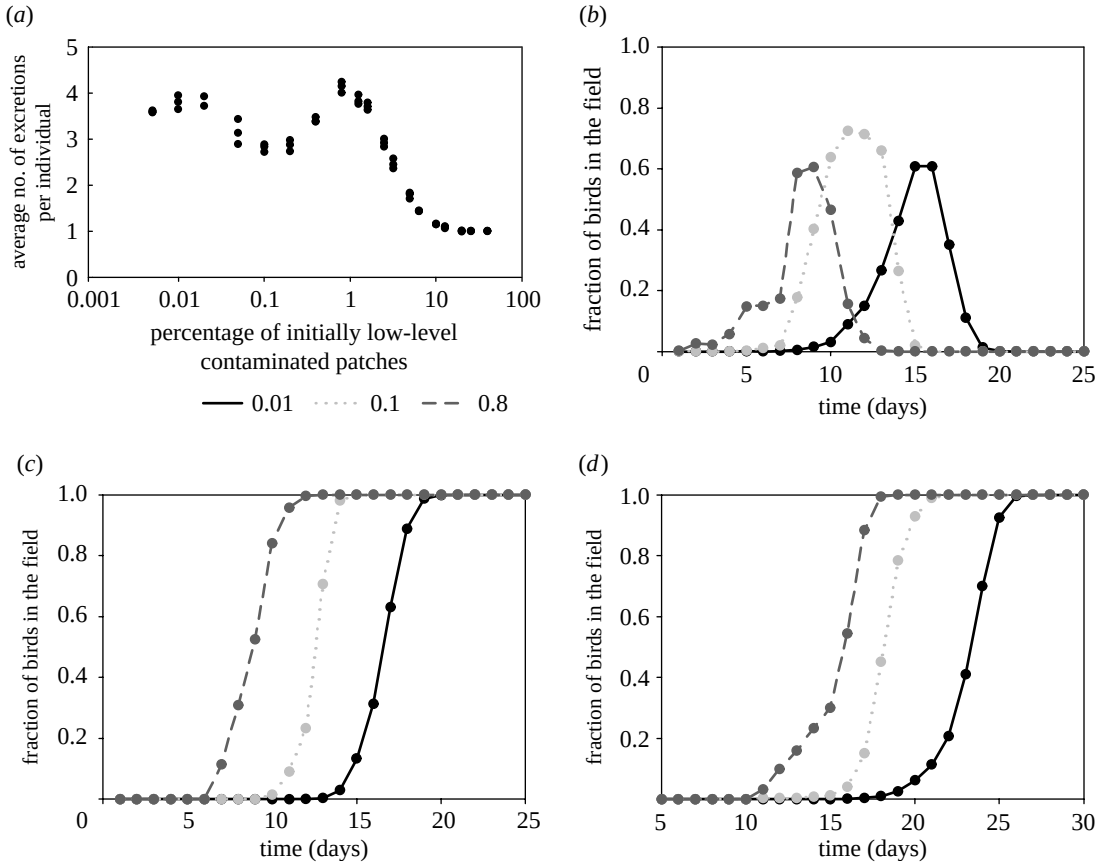


Figure 1. (*a*) The average number of high-dose excretions per individual for the default model settings plotted for a range of initial percentage of contaminated patches with a low level of oocysts. For each initial contamination, three simulations with different random seeds are plotted. The time course of the fraction of individuals that have (*b*) ingested a low-dose trickle infection, (*c*) ingested either middle-dose trickle or high-dose infection and (*d*) reached either partial or full immunity is plotted for the two peaks in high-dose excretion (of (*a*)) at 0.01 and 0.8% initial contamination and the trough in excretion at 0.1% initial contamination. Note the difference in the time range in (*b–d*).

### *3.2. Robustness and sensitivity*

We carried out a sensitivity analysis with respect to the model settings to assess the robustness of the wave-like pattern and investigate the underlying mechanism. These analyses were performed for the initial contaminations of 0.05 up until 40%, which we tested to be sufficient to capture the sensitivity of the parameters.

*3.2.1. Density.* The maximal density of broilers for conventional broiler farms is 23 birds $m^{-2}$ (Anonymous 2000). To assess the effect of broiler density on the results, we have studied densities of 6, 10, 13 and 23 birds $m^{-2}$ additional to the default settings. No or negligible difference with the default result was found.

*3.2.2. Immune rules.* Since it is the second dose of oocysts that triggers the build-up of immunity (starting 9 days after the second dose), heterogeneity in immune status arises due to the different waiting times for chickens to encounter their second dose. If the oocyst level were constant, these waiting times would follow a hypergeometric distribution with a variance of $2r(1-r)$, $r$ being

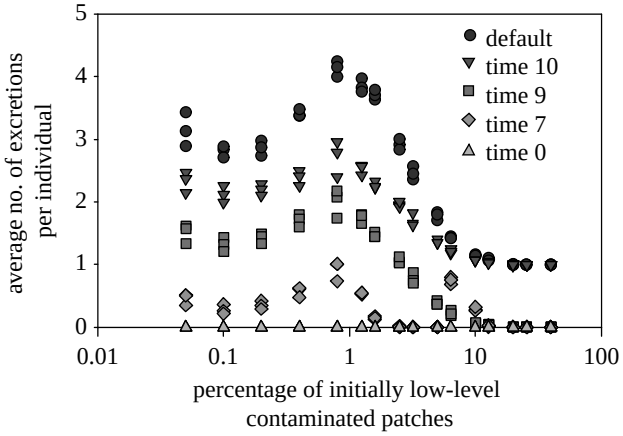


Figure 2. The average number of high-dose excretions per individual is plotted for a range of initial contaminations using different immune rules. The upper graph (full black circles) depicts simulations with the default model settings for immunity, i.e. individuals reach immunity 9 days after the second ingestion of a low or middle dose of oocysts or 9 days after the first ingestion of a high dose of oocysts. The four lower graphs denoted with time 10 to time 0 depict simulations where individuals reach immunity respectively 10, 9 and 7 days, or immediately after the first ingestion of any dose of oocyst. For each graph at each initial contamination, three simulations with different random seeds are plotted.

the daily ingestion probability. To decrease the variance (to $r(1-r)$ in a constant environment), we considered the case with immunity starting 0, 7, 9 or 10 days after the first oocyst dose (figure 2). Not surprisingly, excretion levels decreased over the whole range due to earlier immunity, but besides that the wave amplitude also decreased significantly. This suggests that heterogeneity in immunity is an important factor causing the wave-like pattern.

*3.2.3. Trickle rules.* A trickle infection occurs when ingestion of an oocyst dose is followed by two more equal or higher-level doses in the subsequent 4 days, and it results in elevated excretion levels. If the definition for a trickle infection is changed to ingestion of only one additional dose in the subsequent 4 days, it results in a faster increase of the environmental oocyst levels, and thus in an increase of high-dose excretions (figure 3). Conversely, if the definition for trickle is changed such that ingestion of three of four additional oocyst doses is required, excretion levels considerably decreased, and also the wave amplitude decreases. Since these stronger requirements for trickle infections make it less likely that there will be chickens experiencing trickle infections and those not ingesting any oocyst at the same time, this suggests that heterogeneity in exposure is crucial to the wave-like pattern.

*3.2.4. Multiple-dose initial contamination.* One can expect that at higher initial contamination levels, not only patches are low-level contaminated, but also some will be contaminated with a higher level of oocysts. These more natural conditions were simulated by letting the initially contaminated patches have a 90% probability of

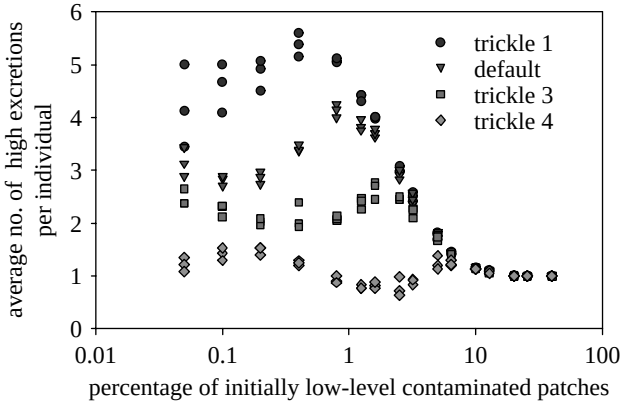


Figure 3. The average number of high-dose excretions per individual is plotted for a range of initial contaminations using different trickle definitions. The second graph (triangles) depicts simulations with the default trickle definition, i.e. within 4 days after the uptake under consideration, an individual must pick up at least two more doses of equal or higher level to acknowledge a trickle infection. The three other graphs denoted with trickle 1, trickle 3 and trickle 4 depict simulations where respectively one, three or four additional doses of equal or higher level must be ingested within 4 days after the uptake under consideration to acknowledge a trickle infection. For each graph at each initial contamination, three simulations with different random seeds are plotted.

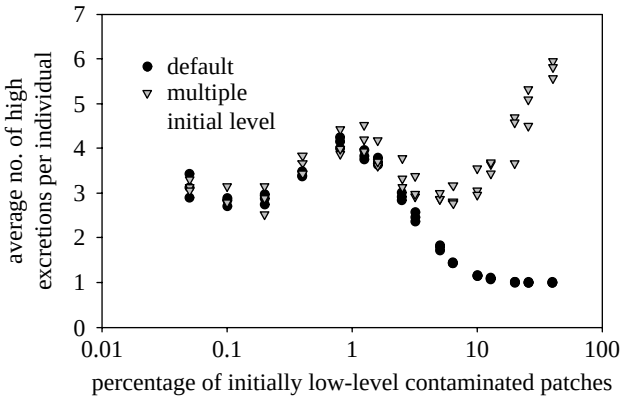


Figure 4. The average number of high-dose excretions per individual is plotted for a range of initial contaminations using different initial conditions. The graph denoted with full black circles depicts simulations where all initially contaminated patches are low-level contaminated (the default model settings). The graph denoted with triangles depicts simulations where the initially contaminated patches have a 90% probability of being low-level contaminated, 9% of being middle-level contaminated and 1% of being high-level contaminated. For each graph at each initial contamination, three simulations with different random seeds are plotted.

becoming low-level contaminated, 9% of becoming middle-level contaminated and 1% of becoming high-level contaminated (figure 4). This results in an increase in the average number of high excretions at high initial contamination but does not change the wave-like pattern.

*3.2.5. Spatial effects.* The initially contaminated patches can be randomly distributed over the field or concentrated in a single position in the field. In both cases, the results remain the same owing to the random mixing of the

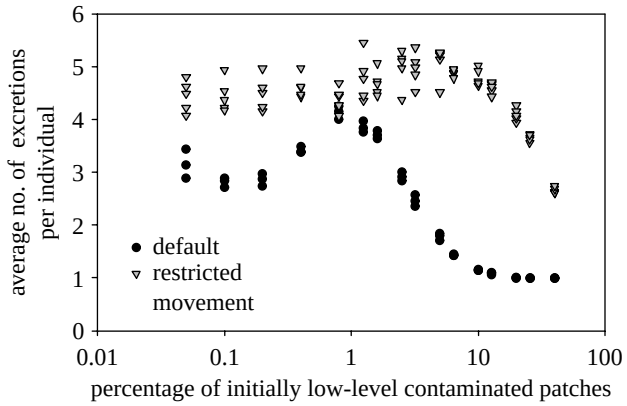


Figure 5. The average number of high-dose excretions per individual is plotted for a range of initial contaminations using different displacement rules for the individuals. The graph denoted with full black circles depicts simulations where individuals move by randomly changing the position to another empty patch in the field once every hour (the default model settings). The graph denoted with the triangles depicts simulations where the movement is restricted to only displace to one of the empty neighbouring patches every hour. If no empty patch is available, an individual will not move. At each initial contamination, three simulations with different random seeds are plotted for the default graph and five simulations with different random seeds are plotted for the restricted movement graphs.

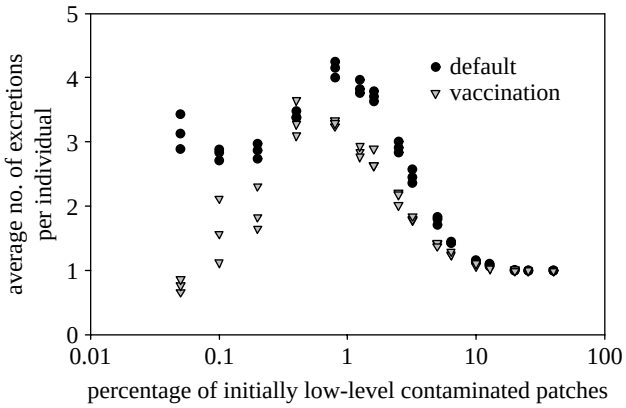


Figure 6. The average number of high-dose excretions per individual is plotted for a range of initial contaminations with and without vaccination. The graph denoted with full black circles denotes the default model settings (no vaccination). The graph denoted with triangles depicts simulations where all individuals were vaccinated by giving them a low dose of oocysts on the first day. For each graph at each initial contamination, three simulations with different random seeds are plotted.

chickens. Although biologically unrealistic, we turned off the random mixing and limited the movements of the chickens to improve our understanding about the spatial effects that could occur in this disease system under other circumstances. Restricted movement is implemented by letting chickens displace to one of their empty neighbouring patches only once every time-step (i.e. movement is restricted to 10 cm $h^{-1}$). If no empty patch is available they did not move. Restricted movement is studied with local initial contamination (figure 5), which resulted in much higher excretion and disappearance of the wave-like pattern. Since spatial effects are likely to induce much heterogeneity that was not there in the default model—by enabling trickle infections even with few oocysts as well as by keeping some birds away from oocysts even if there is quite a lot of contamination—figure 5 suggests that the spatial heterogeneity mechanism is much stronger than the one causing the waves.

*3.2.6. Vaccination.* Vaccines against coccidiosis that can be sprayed over the feathers of newly hatched chicks are available of attenuated and/or wild-type *Eimeria* oocysts causing them to ingest a low dose of oocysts while grooming. In the model, vaccination is simulated by letting all chickens ingest a low dose of oocysts on the first day (figure 6). At low initial contamination levels, broilers excrete fewer high doses of oocysts than without vaccination but at higher initial contamination levels the difference in excretion between vaccinated and non-vaccinated becomes much smaller.

## 4. DISCUSSION

Previously, a simple caricatural model for the dynamics of parasites of farmed animals including a two-way interaction between immunity and transmission (acquired immunity) showed that such a disease system can exhibit complicated dynamics (Roberts & Heesterbeek 1998). The result of the complexity is that small differences in, for example, initial contamination can lead to large differences in the severity of outbreaks. The more biologically realistic mechanistic model of Klinkenberg & Heesterbeek (in press) incorporated the interaction between immune status and infection history for a specific system, *Eimeria* infections in chicken. They show similar bifurcation behaviour as in the caricatural model. Moreover, this model showed dynamics resulting in a non-monotonous wave-like relationship between initial contamination and the severity of outbreaks. The direct consequence of this pattern in terms of control measures is counter-intuitive, better cleaning to remove more of the parasites can lead to more severe outbreaks. Both these analyses regarded all individuals to be identical and did not yet include acquired heterogeneity.

In this paper, we modelled the spread of *E. acervulina* in chickens as a parasite–host system, where traits of the host influence the infections dynamics and vice versa, making explicit differences between individuals. Our model description was based on empirical research and can also be validated empirically. We have simulated transmission experiments performed at our group by Velkers *et al.* (in preparation *a*). In these experiments, one susceptible naive 1-day-old chick and one inoculated with a dose of oocysts are reared together in a confined area. The excretions of the two chickens are recorded to study the transmission dynamics of the infection. The results of their experiments look promisingly consistent with our results.

Previous studies, both empirical and theoretical, have shown a non-monotonous wave-like relationship between initial contamination and the oocysts produced during an outbreak of coccidioses (Henken *et al.* 1994; Graat *et al.* 1996; Klinkenberg & Heesterbeek in press). With our model, we indeed also observe this wave-like

relationship between initial oocyst contamination and the numbers of oocysts excreted in the weeks thereafter. In addition, we have investigated the robustness of the wave-like relationship to various model settings and we find the pattern to be robust. In contrast to previous studies, our analysis allows us to give a possible mechanism behind the pattern. The wave-like relationship can be explained by an increased heterogeneity in the host population at the peaks, caused by the infection process. Subsequent waves arise because at lower initial contaminations, similar heterogeneity can be reached through additional oocyst generations.

Generally, the amount of high oocyst excretions is determined by a race between the chickens trying to be immune as fast as possible, and the parasite trying to induce high excretions as early as possible. When more chickens are immune earlier while oocyst accumulation remains unaltered, excretion decreases (figure 2), and when oocysts accumulate faster or slower, excretion increases or decreases, respectively (figure 3). However, the initial contamination level can also influence this race, maximizing excretion by letting a minority of chickens accumulate oocyst levels, whereas most are not yet building up immunity. This can clearly be seen in figure 1*b–d*, where for three simulations with different initial conditions, the time course of the fraction of chickens that pick up a low-dose trickle infection (which causes non-immune chickens to excrete middle doses), the fraction of chickens in the field that pick up middle-dose trickle infections or any high-dose infection (which causes non-immune chickens to excrete high doses), and the fraction of chickens that become partially or fully immune (which prevents chickens from excreting high doses) is shown.

An interesting difference occurs after an initial contamination-dependent start-up phase needed to accumulate low doses to enable trickle infections. Then, in the simulations with 0.01 and 0.8% contamination, the increase in the numbers of chickens taking up low trickle infections occurs over a much longer time span than with 0.1%, mainly due to a slow start-up phase (figure 1*b*). However, the reduced number of initially infected chickens do excrete sufficient medium doses for all birds, because the uptake of medium trickle (and subsequent high) infections is essentially the same for all three simulations (figure 1*c*). As a result, all chickens that were not infected in the slow start-up, and thus could not start to build up immunity (figure 1*d*), lose a couple of days in the 'race against the parasite' and excrete more high doses.

The importance of heterogeneity in causing problems, or conversely, of homogeneity in reducing problems is seen if a more homogeneous population is created by means of vaccination (figure 6) or when starting with a very high percentage of initial contamination (figure 1*a*). Spatial effects are likely to enhance heterogeneity, because some birds will remain near the infected patches, thus accumulating oocyst levels, whereas many other chickens will not yet be able to mount an immune response (figure 5).

In our relatively simple model with only a minimum number of oocyst and immunity levels, we show the large effect that acquired heterogeneity can have. This effect is relevant in any disease system where heterogeneity can be acquired in the traits of the host that interact with the infection process. For example, diseases where more resistant or more virulent strains arise for infectious agents, where there is some cross-immunity between strains or serotypes and where the internal dynamics are then influenced by the memory of previous exposures. In this case as well, it is the exposure history that shapes the immune status, however defined, and it is this immune status that, in turn, influences the infectiousness of that individual to others. When using models to develop or evaluate control measures for these types of disease systems, it is always crucial to incorporate the effect of acquired heterogeneity.

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