Infected host competence overshadows heterogeneity in susceptibility in shaping experimental epizootics

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Abstract

The accelerated rate of disease emergence in recent decades underscores the need to understand conditions that promote or dampen epidemics. Theoretical models consistently show that epidemics are smaller in populations with higher among-individual heterogeneity in susceptibility. Experimental tests of these predictions are rare but critical for understanding how heterogeneity in susceptibility shapes epidemics in natural systems. We directly link data-parameterized models from previous dose response experiments in the house finch and *Mycoplasma gallisepticum* system to experimental epidemics in replicated aviary mesocosm flocks. We manipulated flock-level heterogeneity in susceptibility by seeding epidemics in flocks composed of either pathogen-naïve or previously exposed birds, which prior work showed have higher heterogeneity in susceptibility relative to pathogen-naïve populations. We tracked epidemics for over two months, combining empirical data and stochastic compartmental models to address how heterogeneity in susceptibility changes epidemic severity. Consistent with previous work, heterogeneity in susceptibility was higher in flocks given prior pathogen exposure. However, even pathogen-naïve flocks, which had relatively homogeneous susceptibility in previous experiments, were best described by heterogeneous, rather than homogenous, models of susceptibility. This suggests flock-level epidemics captured sources of heterogeneity absent in controlled experiments, such as transmission heterogeneity leading to variable host competence. Finally, although prior exposure conferred protection from disease at the individual level, prior exposure and its associated flock-level heterogeneity did not dampen epidemics as predicted by deterministic models. Further, our stochastic simulations generated a range of outcomes for small epidemics over the timescales examined, underscoring the challenges of measuring transmission dynamics in naturalistic settings.

Introduction

The accelerated emergence and spread of diseases in both human and wildlife populations[1-3] underscores the urgent need to better characterize the conditions that promote or dampen epidemics and their severity. A growing body of work suggests that key traits of hosts in a population are often highly heterogenous in nature, and this complexity can have considerable epidemiological influence[4-7]. Some sources of host heterogeneity have been well-studied, such as transmission-relevant traits[8,9], including heterogeneity in infectiousness[10-12] and host

competence[13,14], or contact rates and exposure[15-17]. This body of work has consistently highlighted the importance of incorporating certain types of heterogeneity into disease models to accurately predict outbreaks and apply realistic control measures[11,15,16]. While less well-studied, how heterogeneous a population is in its susceptibility to a given pathogen (defined here as the probability of infection given exposure) can have far-reaching downstream epidemiological[18-22] and evolutionary[23,24] consequences. However, to date, there have been no opportunities to experimentally test how alterations in the degree of heterogeneity in susceptibility in a population alter epidemic outcomes.

Heterogeneity in susceptibility among individuals can stem from intrinsic factors [e.g., genetics, age class] but can also be induced or augmented by prior pathogen exposure, whether that be through vaccination or natural infection[25]. Prior pathogen exposure reduces the mean susceptibility of a population while also increasing the variance by generating incomplete or variable immune protection across individuals. Even where exposure dose can be controlled (i.e., vaccines), the degree and duration of protection generated can vary substantially across individuals, further augmenting the heterogeneity that prior exposure induces[26]. While the degree of heterogeneity in susceptibility can be challenging to measure empirically because it is inherently a population-level metric, dose-response experiments that measure infection probability over a series of controlled challenge doses[25,27,28] allow direct quantification of population-level heterogeneity in susceptibility that are not confounded by other sources of host variation. Using these approaches, two studies to date demonstrated that vaccination against or prior exposure to a pathogen augment heterogeneity in susceptibility: rainbow trout vaccinated for infectious hematopoietic necrosis virus (IHNV) show significantly higher heterogeneity in susceptibility than unvaccinated trout[21], and house finches (*Haemorhous mexicanus*) with prior exposure to either or a low or high dose of their natural pathogen *Mycoplasma gallisepticum* show higher heterogeneity in susceptibility relative to pathogen-naïve birds[20].

Population-level heterogeneity generated by prior pathogen exposure is particularly important because theoretical models consistently show that heterogeneous susceptibility dampens the size of epidemics[5,21,22,25,28,29], in large part because the most susceptible individuals become infected first ("cohort selection") while the most resistant individuals remain[20,21,25,29]. For instance, in an SIR model of vaccinated versus unvaccinated rainbow trout, Langwig et al. (2017) found that when controlling for the expected mean reduction in susceptibility from

vaccination, transmission models indicated that heterogeneous populations would still result in a 35% reduction in outbreak size relative to homogeneous – or non-vaccinated – populations. Further, using experimental data on previously exposed house finches to parameterize SIR models, epidemics were substantially reduced in heterogeneous populations relative to homogenous ones (> 55%), even when controlling for changes in mean susceptibility[20]. Importantly however, the way that effects of heterogeneity in susceptibility shape epidemics in natural settings, where additional sources of heterogeneity are present, has not been empirically tested. Here we use the house finch – *Mycoplasma gallisepticum* system to directly link experimentally-derived models to experimental epidemics in aviary mesocosm flocks.

The house finch is a common backyard North American songbird that experiences seasonal epidemics of a bacterial pathogen (*Mycoplasma gallisepticum*; MG), which causes severe conjunctivitis[30]. Mortality in this system is largely indirect, likely due to increased depredation of infected birds in the wild[31], but birds in captivity (in the absence of predators) typically recover. Birds that recover from prior infection have incomplete acquired protection[32] which also wanes over time[33] – characteristics that augment heterogeneity in susceptibility to reinfection[20]. But, importantly, and as in any natural disease system, multiple other sources of transmission heterogeneity exist, each with a differential degree of influence on epidemiological outcomes[8]. For instance, in this system, characteristics such as the degree and duration of infectiousness (as measured by pathogen loads) can vary substantially among infected house finches[34,35]. Contact rates or exposure risk can also be highly heterogenous. Because MG-transmission is primarily linked to the use of supplemental feeders which act as fomites[36], foraging behaviors (e.g., time spent at bird feeders) are correlated with pathogen transmission and acquisition[37], which covary with other factors such as social dominance and ambient temperature[38-40]. In this way, some birds may act as “super-spreaders” and others as “super-receivers”[37].

The well-characterized MG house finch system is poised to examine heterogeneity in susceptibility and its epidemiological influence under semi-natural conditions. Here, we experimentally test whether prior-exposure induced population heterogeneity in susceptibility dampens the size of experimental epidemics in free-flying aviaries, which naturally harbor other sources of transmission heterogeneity. Unlike past experimental dose-response studies, our use of a mesocosm experimental design examines heterogeneity in susceptibility in a context where

it is, by definition, broader: because exposure dose and transmission cannot be controlled for under natural conditions, in this context, heterogeneity in susceptibility is better defined as heterogeneity in the likelihood of hosts to become infected. We do this by initiating epidemics in mesocosm flocks comprised of house finches with experimentally manipulated exposure histories: either no prior exposure (pathogen-naïve) or prior exposure to a low dose of MG, which past work showed significantly augments population-level heterogeneity in susceptibility[20]. We longitudinally track epidemics over more than two months and combine empirical data and compartmental models to address how prior exposure-induced heterogeneity in susceptibility (i.e., host risk of infection) influences epidemic severity.

Methods

Experimental design

To test how prior exposure to MG alters epidemic dynamics, we set up experimental flocks comprised of birds that varied in their exposure history and seeded experimental epidemics of MG using three "index" birds per flock (Figure 1). Index birds were all pathogen-naïve (see Supplement) to control for potential effects of prior exposure on infectiousness. A total of 84 "flockmates" were randomly assigned to either the prior exposure (n = 42) treatment or no prior exposure (hereafter, referred to as "naïve"; n = 42), which received a sham dose. Sex ratios were made as even as possible within treatment groups and flocks, although some incidental mortality unrelated to infection (see sample sizes in Table S1) and males with female-typical plumage before molting resulted in a skewed sex ratio for one flock [Table 2; note that this had no statistical effect, see Supplementary - Results]. Birds were kept in individual cages during the controlled prior exposure treatments and then allowed to recover from this initial pathogen exposure for 38 days. Then, birds were placed into one of six flocks (three per treatment) for experimental epidemics, with 17 birds per flock (14 flockmates from the same treatment group [prior exposure or naïve] plus three index birds). Flocks were established in six large replicate aviary units, which are partially exposed to the environment so that animals experience ambient conditions. The experimental epidemics began when these index birds were inoculated and re-released back into their flocks.

Experimental timeline and sample collection

On day 0 of the experiment (i.e., the day primary inoculations took place; September 2nd, 2022) flockmates in the prior exposure group received a bilateral ocular inoculation of 70 µl (35 µl per eye) of 7.5 x 10^2 color-changing units (CCU)/mL of MG suspended in Frey's media. The MG isolate used was the original index isolate, VA1994[30]. The birds in the naïve group received a sham inoculation of the same amount of sterile Frey's media. Thirty-eight days post inoculation (DPI), flocks were established by moving birds into each of the six aviary units (Figure 1). All prior exposure birds were confirmed to be recovered – i.e., absence of clinical signs – before flock establishment. To seed epidemics, four days after establishing the flocks at the aviaries (October 14th; DPI 42), the three index birds in each flock received a high inoculation dose (3.0 x 10^6 CCU/mL) of 50 µl (25 µl per eye) of the same MG isolate the prior exposure flockmates had received. All index birds were successfully infected, presented clinical signs in one or both eyes by the first sampling occasion six days later, and had comparable pathogen loads (Figure S1). On sampling days, birds were caught with butterfly nets and epidemic progression was monitored using eye swab (pathogen loads; [see Supplement]) and eye score (pathology) data collected longitudinally throughout the experiment for each individual bird (Table S2).

Disease severity was measured using a categorical scoring system where the severity of mycoplasmal conjunctivitis is measured from 0 - 3 in 0.5 increments for each eye, where 0 indicates there is no pathology and 3 is the most severe, for a total score of 0 - 6 per bird at a given time point[33]. Eyes were scored while blind to flock treatment group. To collect pathogen load data, palpebral conjunctiva were swabbed with small, sterile cotton swabs that were then vigorously dipped and swirled in 300 µl tryptose phosphate broth (TPB). The swabs were discarded and the TPB sample solution was frozen at -20°C until nucleic acid extraction. The same two researchers scored and swabbed the birds throughout the experiment to minimize observer biases.

Data Analysis

Statistical analysis

To understand the effects of prior exposure and associated heterogeneity in susceptibility on flock level prevalence of infection, we fit a generalized linear mixed effects model (GLMM; *glmmTMB*[41]; RStudio v. 4.3.1[42]) with a binomial distribution and a logit link. The response

variable was individual presence/absence of infection (with infection defined as >15 copies of MG detected via qPCR; see Supplement) each day. Mean duration of index bird infection per flock and flock type were interacting fixed effects. Random effects were individual bird nested within flock to account for repeated measures (the sampling of the same birds and flocks over time). Model selection was performed using Akaike's Information Criterion values adjusted for small sample sizes[43] (AIC_C). Because we expected prior exposure to confer some degree of protection to re-infection and the development of conjunctivitis, we also modeled prevalence as defined by the presence/absence of pathology (> 0 eyescore) in the same manner. To account for the one flock with a skewed sex ratio, we asked whether an individual's infection probability differed by sex in a GLMM with a binomial distribution and logit link where the response variable was 0|1 ever infected.

To capture differences in infection severity, we fit a GLMM with a negative binomial distribution (used instead of the Poisson to account for overdispersion) and a log link function where the variance was allowed to increase quadratically with the mean ("nbinom2" in *glmmTMB*) where the response variable was flockmate maximum pathogen load (count variable). Mean duration of index bird infection per flock and flock type were interacting fixed effects with a random intercept for each flock. In models that included effects of flock type and mean infection duration of the index bird, we calculated and accounted for variance inflation factors[44] (VIF; see Supplement).

To ask whether prior exposure reduced infection or disease severity, we compared maximum pathogen loads and pathology by flock type for infected flockmates only. Pathogen loads and pathology of infected birds did not follow a Gaussian or other described distribution, so we used nonparametric Wilcoxon rank sum tests. Finally, for birds in the prior exposure treatment only, we asked whether becoming infected during the primary exposure portion was independent from reinfection or developing pathology during the epidemics. We used a Fisher's Exact Test to compare the number of individuals that were successfully infected or uninfected during their primary exposure with the number of those that were infected or uninfected during the epidemics. A second test was used to compare the number of individuals that were successfully infected or uninfected during their primary exposure with the number of those that developed pathology or not during the epidemics (Supplementary – Results; Figure S2).

Population model fitting

To determine whether prior exposure induced heterogeneity in susceptibility in the experimental epidemics, and whether, under these more natural conditions, the observed heterogeneity in infection risk follows previous patterns of experimental dose-response data where exposure was controlled for each individual, we modeled epidemics within flocks using a continuous-time Markov chain SIR model.

Continuous heterogeneity in susceptibility was described using a gamma distribution that was truncated and discretized for simulations (described below; see Supplement equations S1-S3 for the equivalent deterministic model). Incorporating heterogeneity in susceptibility meant that the force of infection depended on an individual's susceptibility, denoted by x, as well as on the transmission rate β (Table 1). The use of a stochastic rather than a deterministic model allowed us to account for stochastic population processes that are relevant at small experimental population sizes (i.e., n = 17 birds per flock). Infection, recovery, and mortality were all treated as Markov processes which depend on the transition probabilities at time t but not on the history of previous events (Table 1). Transmission rate β and recovery rate γ were set at values derived from previous work in this system[45]. Mortality rate α was set at 0 for model fitting, under the assumption that disease-induced mortality is zero in captivity[46].

Stochastic models were simulated using the Gillespie algorithm[47] in R, which approximates the time to the next event τ as an exponential, and the probabilities of each event based on the states at time t and parameter values (Table 1). At the start of the epidemic, individuals were assigned to a susceptibility value according to a multinomial distribution (see details in Supplement), where the probability of each susceptibility bin was set by the gamma probability distribution function in R (*dgamma*). Therefore, heterogeneity in susceptibility became an additional source of stochasticity in the model: as the heterogeneity described by the gamma distribution increased, the probability of choosing fourteen individuals with high variance in susceptibility increased.

Model fitting

To ask how heterogeneity in susceptibility changed with prior exposure, we compared model fits to the experimental epidemic dataset using two candidate models fit to the data separately:

1) The heterogeneous SIR model described above, with a gamma distribution of susceptibilities within each flock.
2) A homogeneous SIR model assuming all individuals in a flock have equal susceptibility to infection.

For each of these models, we also compared two sets of parameter values for susceptibility:

1) Estimated from a previous study using experimental dose response data[20].
2) Fitted directly to the experimental epidemic dataset using a Bayesian approach (outlined below).

By comparing these parameters sets, we were able to determine whether prior exposure-induced heterogeneity in host risk of infection (i.e., susceptibility) in a more naturalistic setting followed patterns of heterogeneity in susceptibility in experimentally controlled laboratory infections, where susceptibility was defined more narrowly as the likelihood of infection to a controlled exposure. For the heterogenous model, we estimated the shape and scale parameters for the gamma distribution of susceptibilities directly from the epidemic dataset for prior exposure and naïve flocks using an Approximate Bayesian Criteria (ABC) approach[48] (see Supplement for details). Models were fit to the prior exposure group and naïve groups separately (Table 3). The priors were vague lognormal distributions ($\sigma^2 = 2$) centered on the gamma shape and scale values estimated from the previous dose response experiment, in which naïve and previously exposed birds were infected with the same MG strain as the current study (e.g., see Results, Table 3).

We used the same general ABC method to fit the homogeneous stochastic model to the prior exposure and naïve flock datasets. In the homogenous model, only a single parameter was fit (the mean susceptibility value), and there was only one S class (in other words, a traditional stochastic SIR model; see Supplement equation S4 – S6 for equivalent deterministic model equations). Finally, to compare goodness of fits of the parameters estimated from dose response data versus experimental epidemic data, we simulated the stochastic models 1000 x 3 times for both sets of parameter values (previous estimates from the dose response model and fitted estimates) for the heterogeneous and homogenous models (four total parameter sets). We then saved the median and minimum sums of squares discrepancy out of the 1000 realizations for each parameter set. Lower values of sums of squares discrepancy between the data and the model indicate a better fit to the data[49].

Results

Contrary to predictions generated by previous work in this system[20], experimental flocks of birds that had prior pathogen exposure (but had recovered prior to experimental epidemics) showed greater mean infection prevalence (35.7% ± 14.6 [21.4 – 71.4]) in experimental epidemics relative to flocks made up of MG-naïve birds at the epidemic start (7.1% ± 5.8 [0 – 21.4]; Figure 2; Table 2). Although immunologically-naïve index birds (three index birds per flock) that seeded the flock epidemics were treated identically, we found unexpected variation in index bird competency (defined as pathogen transmission ability[13,14]) across flocks and treatment groups, in both the average pathogen loads and lengths of infection for index birds. By chance, the longest index bird infections occurred in two prior exposure flocks (b and d) which each contained an index bird that did not resolve their infection during the period they were sampled (Figure 3; Table 2). Therefore, we accounted for this random variation among index birds in infection duration (6-35 days; Figure 3) by including mean index bird duration in statistical models of prevalence.

The most informative model (generalized linear mixed model with binomially distributed data and a nested random effect of bird/flock) of infection prevalence (presence/absence infection) included an interaction between flock type and mean index bird infection duration, although the model with a fixed effect of mean index bird infection duration ranked similarly ($\Delta AIC_C < 2$; Table S3). After adjusting for multicollinearity by centering predictors and rechecking VIF (variance inflation factors) values, the most informative model had a significant interaction between flock type and mean index bird infection duration (coefficient ± 1 SE: 1.23 ± 0.544, $p = 0.024$). The second ranked model indicated that index mean infection duration was statistically significant (coefficient ± 1 SE: 0.323 ± 0.156, $p = 0.0382$).

Likewise, the top model of flockmate maximum pathogen load (Table S3) included an interaction term between flock type and mean index bird infection duration (generalized linear mixed model with binomially distributed data and a random effect of flock). After adjusting for multicollinearity, the results showed a significant interaction between flock type and mean index bird infection duration (coefficient ± 1 SE = 5.37 ± 1.04, $p < 0.001$), as well as significant main effects (flock type: coefficient ± 1 SE = 6.97 ± 2.38, $p = 0.003$; mean index bird infection duration: coefficient ± 1 SE = -3.51 ± 0.83, $p < 0.001$). These results indicate that, as mean duration of infection of the index birds increased, the epidemics and maximum pathogen load

copies were larger among flockmates in the prior exposure flocks than in the naïve flocks. However, when prevalence was defined by the presence/absence of pathology, the top model was the null model (generalized linear mixed model with binomially distributed data and a nested random effect of bird/flock, including only the intercept; $\beta_0 = -7.99 \pm 1.05$, $p < 0.001$), indicating that flock type did not improve model explanatory power of the prevalence of pathology. Models with single fixed effects (mean index infection duration: coefficient ± 1 SE = 0.074 ± 0.233, p = 0.751; flock type: coefficient ± 1 SE = 0.167 ± 1.044, p = 0.873) ranked similarly to the top model ($\Delta AIC_C < 2$; Table S3).

On an individual level, however, prior exposure appeared to induce protection in birds that became detectably infected (qPCR+) during the epidemics, particularly protection from disease (Figure 4). In Wilcoxon rank sum tests, infected bird maximum pathogen load did not differ by flock type (W = 39, p = 0.0564). However, maximum pathology was greater in naïve flockmates (W = 41.5, p = 0.0184; [median] prior exposure flockmates = eyescore 0, [median] naïve flockmates = eyescore 3), suggesting that, prior pathogen exposure protects birds from severe disease upon reinfection, even if pathogen loads do not vary as strongly. Whereas all infected naïve birds also had detectable conjunctivitis, in prior exposure flocks, most infected birds were aclinical. This resulted in discrepancies between the prevalence of infection versus pathology, where the total number of infected birds (n = 15) exceeded that with conjunctivitis (n = 9; Figure 2 left versus right panels; Table 1) in prior exposure flocks.

Population model fitting

When stochastic models were fit to the data from each treatment (Figure 5), heterogeneity in susceptibility was greater for prior exposed flocks relative to naïve flocks when modeled with gamma-distributed heterogeneity in susceptibility (Table 3). However, naïve flocks also showed increased heterogeneity relative to estimates acquired from dose response experimental data. Using the epidemic data from naïve flocks, models with heterogeneity in susceptibility were preferred to homogenous models (Table 3). While prior exposure flocks showed a similar qualitative pattern of increased heterogeneity compared to dose response models[20], all four models considered (i.e., homogeneous and heterogeneous with parameter values from dose response versus fit to the flock data) produced similar fits using the residual sums of squares values (median or minimum across 100 model realizations) to compare predicted versus observed numbers of infected birds in prior exposure flocks (Table 3). While the median

discrepancy across 100 realizations was slightly lower for the homogeneous model than the heterogeneous model, the opposite was true for the minimum discrepancy across those same realizations. This indicates that, while more of the simulated trajectories from the homogeneous model were slightly closer to the observed data in the prior exposure flocks, the best matching individual realization for a given parameter set was slightly better for the heterogeneous model. The heterogeneity model introduced substantial additional variability, particularly in the initial conditions (i.e., susceptibility values of each of the initial 14 susceptible flockmates), resulting in a flatter likelihood and more difficulty distinguishing between parameter values.

Discussion

Infectious disease outbreaks and their characteristics are fundamentally driven by pathogen and host traits[5,8,11] which can vary substantially in natural populations[9]. Although continuous distributions of host traits are increasingly included in epidemiological models[5,50,51], experimental transmission studies that integrate theoretical predictions and empirical data remain rare. Here we asked whether experimental epidemics in flocks of birds that differ in pathogen exposure history match theoretical predictions generated for populations with prior-exposure induced heterogeneity. Importantly, we were able to compare heterogeneity in susceptibility estimates from a previous dose response experiment performed in the same house finch – *M. gallisepticum* system[20] to those generated from the experimental epidemics. Consistent with our previous work, heterogeneity in susceptibility was higher in flocks with prior pathogen exposure, though even pathogen-naïve flocks, previously considered relatively homogeneous, were better described by heterogeneous models. This suggests that the epidemics captured additional sources of heterogeneity absent in more controlled experiments such as variable host competence. While heterogeneity did not reduce epidemic size as predicted by deterministic models, stochastic simulations revealed variable outcomes for small epidemics, highlighting the challenges of capturing transmission dynamics in naturalistic settings.

Our study allowed for the examination of heterogeneity in susceptibility in a natural epidemic. In our prior work[20] with individually-housed birds we showed that prior pathogen exposure reduced mean host susceptibility and, notably, increased population-level heterogeneity in susceptibility. Consistent with these past results, our models estimated higher heterogeneity in susceptibility (CV = 3.53) in flocks with prior pathogen exposure relative to flocks composed of

pathogen-naïve birds, which had lower heterogeneity (CV = 1.79), although model fits were too similar for the prior exposed group to fully distinguish a "best fit" model for that group. However, we also detected key differences with past work: while pathogen-naïve birds in the individual-cage study were best described by models assuming homogenous susceptibility, the pathogen-naïve flocks in our experimental epidemics were best described by models that incorporated gamma-distributed heterogeneity rather than homogenous susceptibility. This result indicates that in a more "natural" mesocosm setting, infection risk can be heterogenous, even for a population with no previous pathogen exposure. This was further supported by the fact that irrespective of prior exposure treatment, our models estimated lower mean susceptibility and greater heterogeneity in our experimental epidemics relative to birds that received the same exposure treatment in the dose response experiments done in controlled conditions. Finally, there was no indication that, under the more natural conditions of a mesocosm, where more potential sources of transmission variability are at play, that heterogeneity in susceptibility dampened epidemics as predicted when other variability is controlled for. However, it is important to note a key distinction between this study and previous ones: while earlier studies[20,21] were able to directly measure heterogeneity in susceptibility by controlling for exposure dose within populations, we were unable to do so here, a limitation discussed further below.

The substantial differences in our estimates for mean susceptibility and heterogeneity in susceptibility (Table 3) between our flock mesocosm study and prior work on individually-housed birds directly exposed to pathogen are important but not surprising. The probability of infection, and thus mean susceptibility, is likely to be lower in a setting where transmission is natural (i.e., from other birds or fomites versus direct inoculation), the exposure dose is uncontrolled, animals can freely move and interact/avoid one another, and hosts and pathogen are exposed to ambient conditions. The discrepancy in the conclusions generated by controlled experiments (which estimate that a naïve population's response is relatively homogenous for the two studies done to date[20,21]) versus mesocosm epidemics, highlights the importance of quantifying heterogeneity in infection risk in more naturalistic settings. These results also give even greater credence to the necessity of incorporating heterogeneity in susceptibility in epidemiological models when fitting real-world data[5]. Not only does the incorporation provide more realistic epidemiological estimates, it offers opportunities to deconstruct the factors (e.g.,

behavioral, environmental, endogenous traits) with the greatest contributions to the observed dynamics.

Although we estimated higher heterogeneity in susceptibility in our prior exposure flocks relative to pathogen-naïve flocks, this difference did not result in smaller epidemics, as predicted by our prior work[20]. In fact, we saw higher epidemic prevalence in our flocks with prior exposure and greater estimated heterogeneity in susceptibility, despite the strong theoretical basis predicting otherwise[5,20-22,25,28,29]. There are several possible reasons why this might be. First, the relative effects of heterogeneity in susceptibility from prior exposure and transmission are largely inseparable, despite our attempts to standardize index bird infectiousness by using all naïve index birds (regardless of flockmate treatment) in our experimental design. Despite being treated identically and given high inoculation doses, the index birds used to seed epidemics showed high transmission heterogeneity, varying substantially in their time to pathogen clearance, which, conservatively, ranged from 6 to 35 days (Figure 3; Figure S1). Moreover, the random placement of the two index birds with the longest infections in two of the three prior exposure flocks appeared to be the strongest driver of flockmate infection and disease prevalence in our experiment. Extreme infectiousness (i.e., “superspreaders” or “superspreading events”), where a subset of individuals produces a disproportionate number of secondary infections, is a common and important feature of disease dynamics[8-11]. Greater heterogeneity in infectiousness causes less frequent but more intense (occurring faster and reaching higher epidemic peaks) disease outbreaks[6,10,11]. While individual variability in transmission is a product of multiple underlying factors [i.e., environmental conditions, immunological status, physiology, behavior[16,52]], the index birds in this present study showed clear inter-individual variability in host competence, as measured by their infection duration, with which infectiousness, or pathogen shedding, was highly correlated (Figure 3; Figure S1). The inclusion of contact data, either through proximity indicators or some other method, would allow for a higher resolution examination of the observed transmission heterogeneities in the index birds.

Another possible, but unlikely, explanation for the larger epidemics in the prior exposure flocks is infection recrudescence. In a past aviary experiment[53], adult house finches that had completely recovered from an initial infection relapsed and successfully transmitted MG to naïve hatch-year birds. While this could have occurred in our study, it is unlikely to be a primary driver of the observed trends for several reasons: Of those birds that were successfully infected during

the priming phase (n = 9), only three were detectably reinfected during the epidemics, and these reinfections were brief (i.e., 1 day; Figures S2, S3). While one of these three reinfected birds was in flock d, which had the largest epidemic, index bird infection duration was also the longest in this flock. Likewise, flock b, which contained none of these reinfected birds but had a large epidemic, had prolonged index bird infection. The two other reinfected birds were in flock f, the only flock where it seems plausible that infection recrudescence could have played a role. This was a prior exposure flock with only two infected flockmates – both of which were infected during their primary exposure. However, these infections did not ignite additional transmission events. Instead, what was likely the primary force here was the short duration of the index birds' infections, whose brevity exactly matched that of the naïve flocks where no epidemics occurred.

An intriguing but speculative final possibility for the higher prevalence in prior exposure relative to pathogen-naïve flocks is that birds in prior exposure flocks were more likely to be aclinical (infected with no pathology) carriers. Indeed, prior exposure provided protection to birds at the individual level, in that birds infected during the epidemics showed significantly lower maximum pathology if they had prior pathogen exposure, with many infected flockmates in prior exposure flocks entirely aclinical. This raises the possibility that the lack of pathology in infected birds with prior exposure allowed for better maintenance of normal behaviors during infection, which can be positively correlated with transmission in this system[54,55]. In this way, the protection garnered by prior exposure, regardless of whether it produced an initial infection or pathology during the priming phase, may have facilitated asymptomatic pathogen spread. On the other hand, more severe conjunctivitis is also known to facilitate transmission in this system[38,56]. Thus, the lower maximum pathology of infected flockmates in prior exposure flocks likely reduced transmission relative to naïve flocks, suggesting the individual-level differences in symptom severity and pathogen load are unlikely to explain the higher prevalence in prior exposure flocks.

Overall, the mesocosm conditions and limited sample sizes used here may have resulted in such high variability that it masked our ability to detect differences associated with prior exposure. In stochastic models with small sample sizes, even where there is a difference in mean response, this result (i.e., undampened epidemics) is a possible outcome. It is also possible that the global increase in heterogeneity for both flock types in the mesocosm setting made it more difficult to detect any potential dampening effects of heterogeneity in susceptibility between the

treatments. Finally, it is possible that the experimental epidemics were simply too short to fully capture the epidemiological impacts of heterogeneity in susceptibility. Our stochastic simulated epidemics had not yet burned out by the timepoint at which they were truncated (67 days) to correspond with the experimental epidemic data. Among the many challenges of quantifying heterogeneity in susceptibility empirically is reliably measuring it during the early stages of an outbreak and at small sample sizes[50]. These challenges are difficult to feasibly remedy for systems that use wild-caught vertebrates in captivity. However, recently developed methods that harness contact tracing data could be used in the future to measure heterogeneity in susceptibility in the earlier stages of an epidemic, which, incidentally, may include the entire time span of this study.

In conclusion, we show that prior pathogen exposure induces host heterogeneity in susceptibility that was measurable in our experimental epidemics, offering further support to the importance of variable acquired immune protection (likely as a function of waning or incomplete immunity[25,26]) and its role in determining a population's disease dynamics and subsequent host-pathogen evolution[18-21,23,24,32]. Intriguingly, however, other sources of heterogeneity were present in the more naturalistic mesocosm setting, which may have masked any epidemiological consequences of the detected effects of prior exposure on host heterogeneity in susceptibility. Lloyd et al. (2020)[4] argues that future work in this field should focus on "coupled" heterogeneities – where heterogeneity of a trait such as susceptibility or transmission extends beyond a single source of variation, but rather is modeled as a function of multiple covarying and coincident sources, such as coupled heterogeneities in infectiousness, contact rates, or infection duration[9,16]. Broadly speaking, incorporating multiple types (i.e., susceptibility, transmission) of heterogeneity and multiple sources of that variation are key to understanding disease dynamics in human and wildlife populations.

Acknowledgements

This research was made possible by grant R01GM144972 from the National Institutes of Health (NIH) Ecology and Evolution of Infectious Diseases (EEID) program. All birds were handled and captured under U.S. Fish and Wildlife Service (MB154804-0) and Virginia Department of Game and Inland Fisheries (066646) permits. All housing and experimental protocols were approved by the Virginia Tech Institutional Animal Care and Use Committee (IACUC). We thank Edan Tulman and Steve Geary (University of Connecticut) for providing the pathogen inoculum and plasmids, as well as Bill Hopkins (Virginia Tech) for facilitating our use of the

Research Aviaries. We also thank Danielle Alms, Madeline Alt, Alicia Arneson, Annabel Coyle, Zhang Gao, Noelle Hodges, Sadoni James, Marissa Langager, Riley Meyers, Sara Teemer, and Caro Vela for assistance with experimental sampling.

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Figures

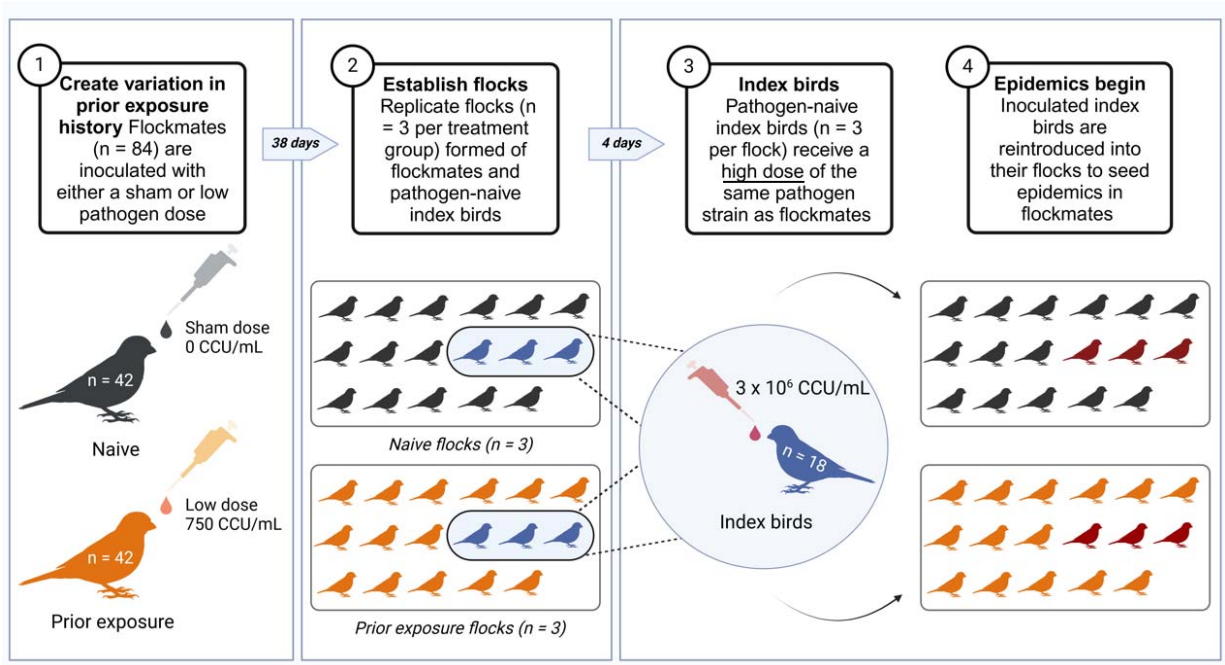

Figure 1. Experimental design. Birds (n = 84) were split into two, even treatment groups: naïve (grey) vs prior exposure (orange). Flockmates received either a sham dose of sterile media (naïve birds) or a low dose of MG (prior exposure) [panel 1]. Birds were allowed to recover from their primary exposure for more than a month. Flocks of 17 birds, comprising 14 flockmates from the same treatment group and three pathogen-naïve index birds (blue), were established in six identical aviary units [2]. Index birds received a high inoculation dose [3] to seed epidemics among the flockmates [4]. Arrows between panels indicate how many days passed between parts of the experiment. Created with BioRendr.

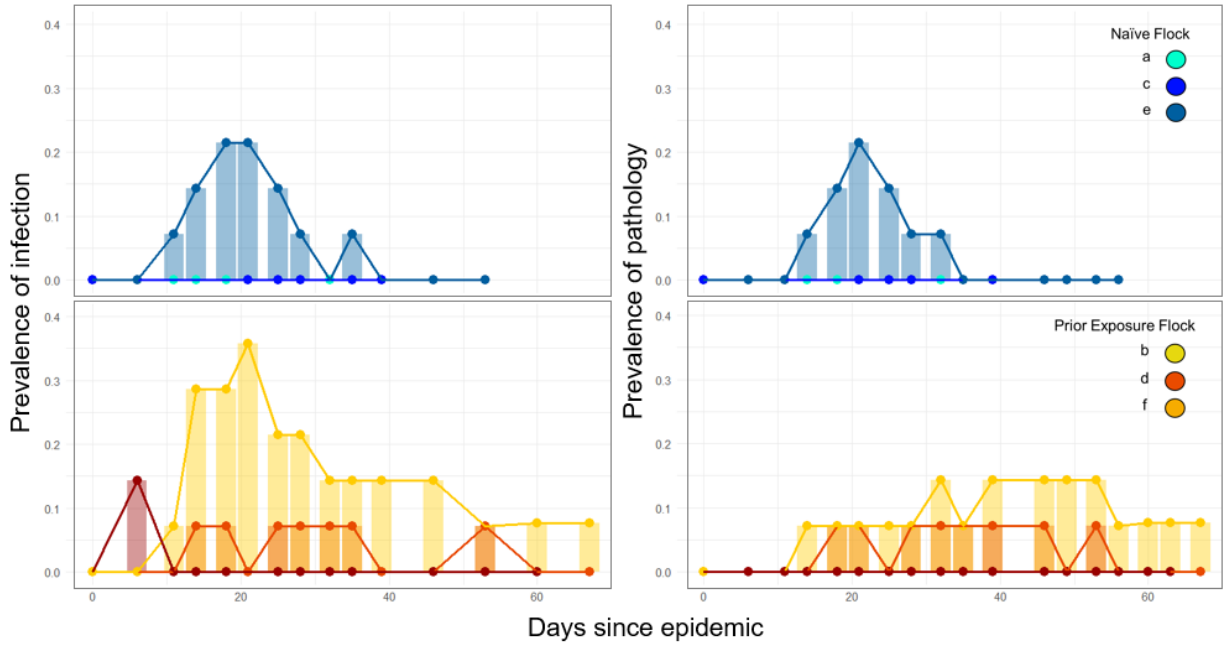

Figure 2. Prevalence of infection (left panels) and pathology (right panels) in flockmates (y-axes) over time since the inoculation of the index birds (not shown) in each flock (x-axis, t = 0 marks the start of the epidemic). Naïve (top panels) and prior exposure flocks (bottom panels) are distinguished by color.

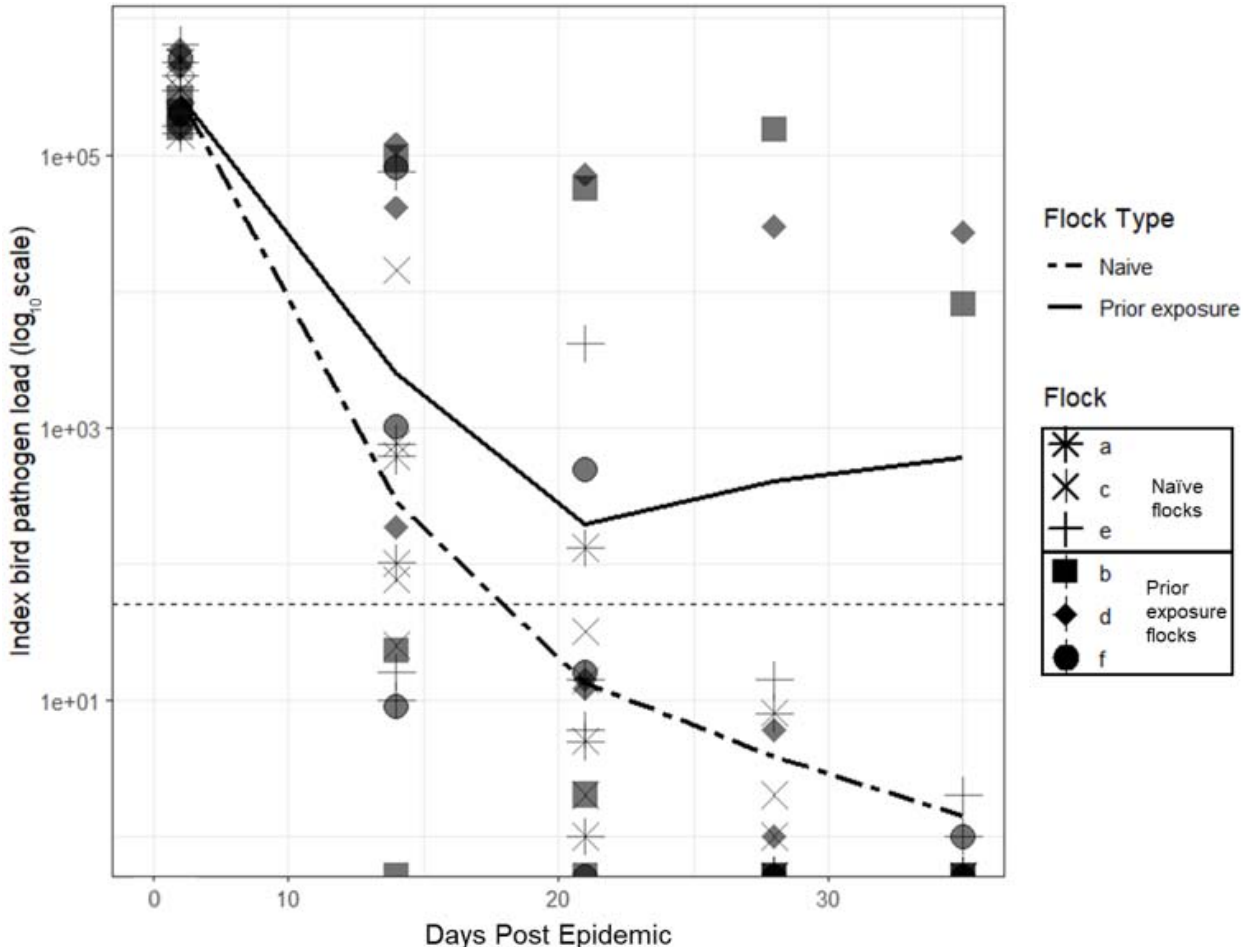

Figure 3. Pathogen loads (y-axis, $\log_{10}$ scale) per individual index bird over time since inoculation (x-axis, t = 0 marks the start of the epidemic). Flock is represented by the shape of the data points and lines show the mean per flock type (solid, prior exposure; dashed, naïve). The horizontal dashed line indicates the pathogen load cutoff used to determine infection status. Note that index birds in both treatments were pathogen-naïve when inoculated; differences between flock type are due to random chance and not to applied treatments.

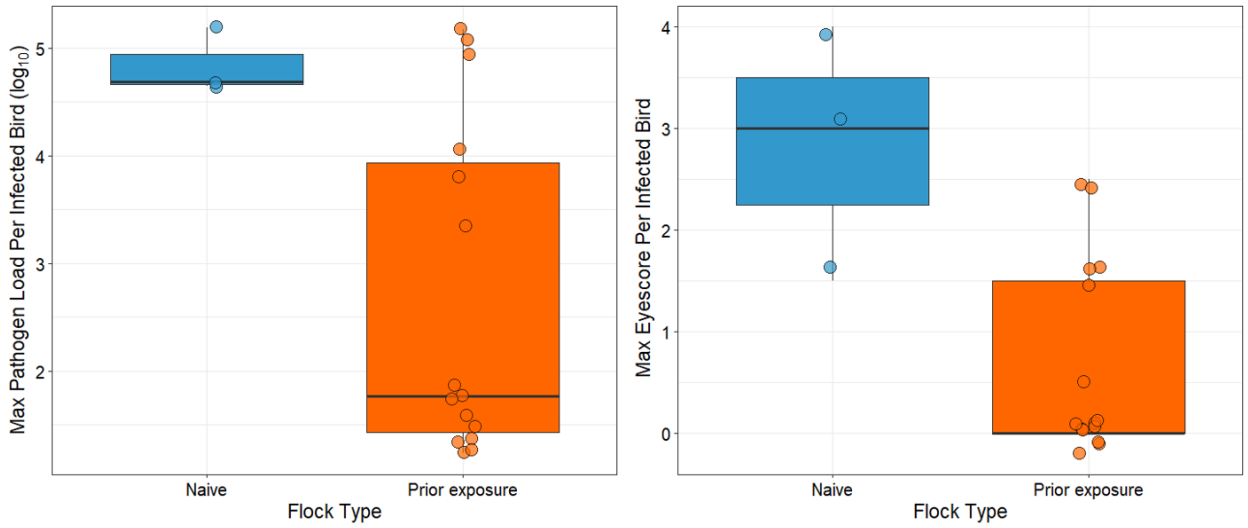

Figure 4. Flockmates with prior exposure (flock type) showed moderately, but not significantly, lower maximum pathogen loads (A), and significantly lower maximum eyescores (B) relative to flockmates that were pathogen-naïve when successfully infected during the epidemic. Boxplots overlaid by datapoints of maximum pathogen load (log_{10} scale; left) and eyescore (right) for flockmates ever infected during the epidemics (> 15 pathogen copies). Naïve flockmates are on the left of each panel (blue) and prior exposure flockmates are on the right (orange). Boxes represent the interquartile range (IQR; 25-75th percentiles) where the horizontal line is the median value, or 50th percentile. Whiskers show the maximum and minimum non-outlier points (defined as > 1.5 x IQR).

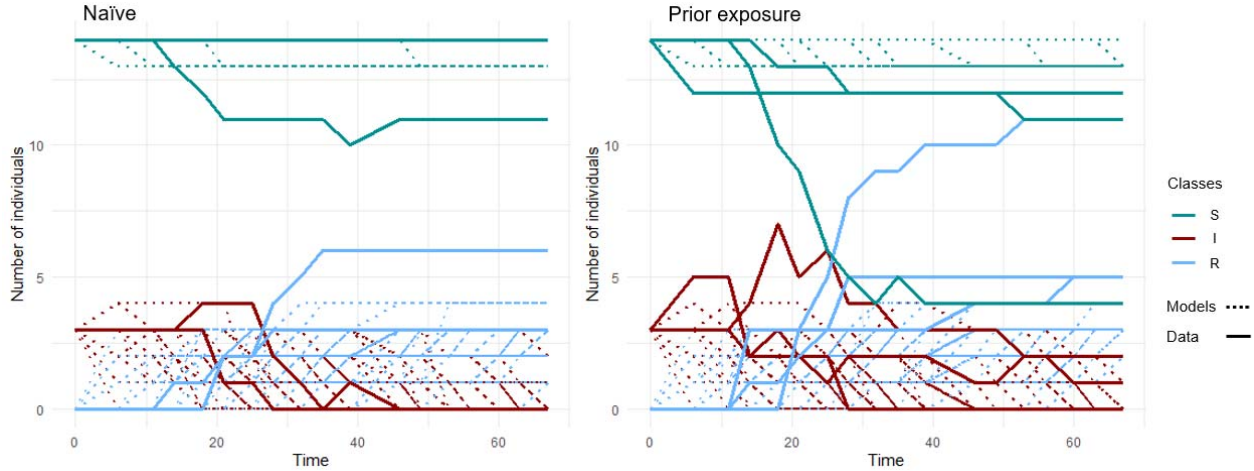

Figure 5. Stochastic model realizations (dotted lines; n = 100 realizations) and empirical data (solid lines) of an SIR model (color = SIR class) with heterogeneity in susceptibility, using best-fitting parameters for experimental flocks that were pathogen-naïve (left panel) or had prior exposure (right). Heterogeneity was described as a gamma distribution (naïve flocks: $k = 0.312$, $\theta = 0.129$; prior exposure flocks: $k = 0.08$, $\theta = 0.783$). Transmission ($\beta = 0.00275$) and recovery rates ($\gamma = 0.03$) were from prior literature[20,45]. Mortality α was set to zero because infection-induced mortality did not occur in captivity.

Table 1. Stochastic population model events and probabilities. Initial conditions: *S(x,0) ~Multinomial (n=S(0), p=Gamma(x))*

Event	Change in states	Probability
Transmission	$S(x,t+1) = S(x,t) - 1$ $I(t+1) = I(t) + 1$	$x\beta S(x,t)I(t) + o(t)$
Recovery	$I(t+1) = I(t) - 1$ $R(t+1) = R(t) + 1$	$\gamma I(t) + o(t)$
Mortality	$I(t+1) = I(t) - 1$	$\alpha I(t) + o(t)$

Table 2. Summary of results by flock. Flocks (a - f) are divided by treatment group (naïve vs prior exposure flocks). For the prior exposure flocks, the number of birds (prevalence and count) that became infected during primary inoculation are shown. The sex ratio is male (M) to female (F). Duration of infection is listed in ascending order for each flock's index birds and mean infection duration per flock is reported for infected flockmates. The mean of infected flockmates' maximum pathogen load and eyescore are the $\log_{10}$ copies and total categorical value, respectively. Prevalence of infection and pathology (conjunctivitis) is the proportion of flockmates that ever exhibited that characteristic, reported as the percentage and count. Where applicable, ranges and standard error are given with the mean value.

Treatment group	Aviary flock	Sex ratio	Primary exposure infection prevalence % (n)	Duration of infection (days)		Infected flockmates		Flockmate prevalence	
				Index birds (in ascending order)	Flockmates (infected only; mean ± SE [min-max])	Mean maximum pathogen load (log copy number; (min - max ± SE)	Mean maximum eyescore (min - max ± SE)	Infection % (n)	Pathology % (n)
Naïve	a	9M:8F	--	14, 14, 21	--	--	--	0.0 (0)	0.0 (0)
	c	9M:8F	--	14, 14, 21	--	--	--	0.0 (0)	0.0 (0)
	e	8M:9F	--	6, 14, 21	12.7 ± 4.3 (7 - 21)	4.84 (1.4 - 5.2 ± 0.1)	2.8 (1.5 - 4.0 ± 1.0)	21.4 (3)	21.4 (3)
Prior Exposure	b	9M:8F	28.6 (4)	6, 14, 35	5.0 ± 2.7 (1 – 10)	3.3 (1.7 - 5.0 ± 0.8)	2.0 (1.5 - 2.5 ± 0.4)	21.4 (3)	14.3 (2)
	d	12M:5F	14.3 (2)	14, 14, 35	10.1 ± 5.1 (1 – 53)	2.7 (1.2 - 5.2 ± 0.5)	1.5 (0.5 - 2.5 ± 0.4)	71.4 (10)	28.6 (4)
	f	8M:9F	28.6 (4)	6, 21, 21	1.0 ± 0.0 (1 - 1)	1.5 (1.4 – 1.6 ± 0.1)	--	14.3 (2)	0.0 (0)

Table 3. Model fitting for stochastic SIR. Better fitting models have a lower median and minimum discrepancy, measured as the sums of squares difference (residual sums of squares; RSS) between model realizations and the three (per treatment) empirical flock trajectories of infected birds over time. Parameters from dose response data were from a previously published study[20] and experimental epidemic data were collected in the current study. Note that a coefficient of variation (CV) for susceptibility is not reported for homogenous models because these models do not allow for any variation in susceptibility.

Treatment	Model (n parameters)	Dataset for susceptibility parameters	Mean susceptibility (rate)	CV susceptibility	Median Discrepancy (RSS) between model and flock data	Minimum Discrepancy (RSS) between model and flock data
Naïve	Homogeneous susceptibility (1)	Dose response data[20]	1.77	--	32.57	9.91
		Flock data (this study)	0.062	--	12.94	7.21
	Heterogeneous susceptibility (2)	Dose response data[20]	1.18	0.90	14.76	5.74
		Flock data (this study)	0.04	1.79	8.66	4.69
Prior exposure	Homogeneous (1)	Dose response data[20]	0.154	--	12.81	6.40
		Flock data (this study)	0.023	--	12.94	7.21
	Heterogeneous (2)	Dose response data[20]	0.45	1.63	13.19	6.00
		Flock data (this study)	0.06	3.53	13.17	6.86