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Downstream effects: Impact of antibiotic pollution on an aquatic host–parasite interaction

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Abstract

The global increase in antibiotic use has led to contamination of freshwater environments. Despite the identified impacts of antibiotics on humans and wildlife, the effect of antibiotics on host–parasite life cycles in freshwater is relatively unexplored. In the current study, we utilize the trematode parasite *Schistosoma mansoni*, and its snail intermediate host, *Biomphalaria glabrata*, to investigate the influence of an ecologically relevant antibiotic concentration on the life history characteristics of both parasite and host. Our results demonstrate that antibiotics not only accelerate parasite development time, but also increase host reproduction and delay parasite-induced host castration. Using a mathematical model, we suggest that life history alterations associated with antibiotics are likely to increase parasite transmission and disease burden. Our study suggests that antibiotic pollution could impact freshwater ecosystems by influencing host–parasite dynamics and potentially increase the burden of schistosomiasis in endemic regions.

KEYWORDS
disease burden, fecundity, life history, public health, *Schistosoma*, tetracycline

INTRODUCTION

Antibiotic usage is increasing worldwide in association with growing demand in livestock production, industry, and human healthcare (Daghrir & Droghui, 2013). As a result, antibiotic contamination from wastewater treatment plants and sewers is often deposited in freshwaters (Kraemer et al., 2019). Antibiotics in aquatic environments affect the life forms within them, extending beyond microscopic organisms to other nontarget species (Danner et al., 2019; Sundberg & Karvonen, 2018). Although antibiotic concentrations in freshwater are not typically lethal to nontarget organisms, the sublethal impact of runoff on biotic interactions is largely unknown (Cairns et al., 2018; Kim et al., 2014).

The magnitude and severity of antibiotic contamination can have diverse effects depending on the nature of the biotic system (Sundberg & Karvonen, 2018). Antibiotics can act as environmental stressors due to their toxicity and ability to alter bacterial communities through direct or indirect mechanisms (Akbar et al., 2020; Grenni et al., 2018; Pravodá et al., 2020). These alterations arise because antibiotics are designed to decrease pathogenic bacteria within an organism; however, they also impact overall bacterial diversity (Akbar et al., 2020; Morley, 2010; Yoon & Yoon, 2018). The

relationship between antibiotics and biotic communities, particularly host–parasite interactions, is an emerging topic in ecology (Akbar et al., 2020).

Antibiotic contamination can have varying interactions and influences on host–parasite systems. Research has shown that the microbiome plays a third-party role in host–parasite interactions (Abraham et al., 2017; Gall et al., 2016; Knutie et al., 2017, 2018). For instance, reducing microbiome diversity through exposing hosts to antibiotics can result in an increased susceptibility to parasite infection (Viera & Moraes-Santos, 1987). Antibiotics may also impact host susceptibility through a reduced immune capacity following antibiotic exposure by creating a more hospitable environment for parasites to invade and reproduce within the host (Evering & Weiss, 2006; Gust et al., 2013). In addition to altering the host–parasite dynamics, antibiotic exposure may have direct negative effects on both organisms individually. Within parasites, antibiotics have been shown to reduce motility, interfere with metabolism, and alter cell permeability (Campbell & Soman-Faulkner, 2021; Mahajan et al., 2010). Within hosts, reduced movement levels, modified growth, and disrupted immune system regulation have been observed following antibiotic exposure (Allan & Blouin, 2017; Gust et al., 2013). Understanding the consequences of sublethal antibiotic exposure on host and parasite life histories is critical to assessing the impact of these pollutants on disease dynamics (Morley, 2009) and could have implications for many human pathogens. *Schistosoma mansoni* is a parasitic blood-fluke that causes the human disease schistosomiasis, which accounts for as many as 200,000 annual deaths (World Health Organization, 2020). Eggs of *S. mansoni* from infected humans hatch into miracidia, a larval stage of the parasite, when they contact freshwater. These miracidia then penetrate a snail intermediate host, *Biomphalaria glabrata*. Maturation of the parasite occurs within the snail gonads, leading to castration of the host. The snails then release free-swimming larval stages called cercariae, which directly infect humans as the definitive host (Centers for Disease Control and Prevention, 2018).

Given the human toll of schistosomiasis and the use of antibiotics in medicine and agriculture in tropical, schistosome endemic regions (Faleye et al., 2018), we designed an experiment to analyze the effects of tetracycline, a common broad-spectrum antibiotic, on the life history of *S. mansoni* and its snail host. Tetracycline was utilized due to its water solubility, easy accessibility, and widespread use in agriculture and human medicine (Daghrir & Droghui, 2013). The impact of tetracycline antibiotic exposure on host growth and reproduction is debated. Some studies show inhibitory effects (Chernin, 1957; Chernin & Schork, 1960), while more recent findings show an antibiotic-induced increase in growth and reproduction (Flaherty & Dodson, 2005; Gaskins et al., 2002). Previous work that demonstrated growth inhibition used higher antibiotic doses than more recent studies. Our study focuses on the impact of a low dose, ecologically relevant concentration of antibiotics on both host and parasite. We predicted that tetracycline would accelerate the development of the parasite, increase parasite reproductive output, and enhance host reproduction possibly driven by alterations to the host–parasite microbiome, and/or lowered host immune capacity. We then used our results to parameterize a mathematical model and demonstrate potential long-term consequences of freshwater antibiotic contamination on human disease burden. Although mechanisms underpinning enhanced host and parasite production due to antibiotic exposure have yet to be fully explored, our results suggest that antibiotic contamination may play a significant role in this host–parasite system.

MATERIALS AND METHODS

One hundred sixty lab-reared *B. glabrata* snails were used in a full factorial experiment, combining parasitic infection and antibiotic exposure for a total of four treatments (antibiotic + parasite, parasite only, antibiotic only, and control; Appendix S1: Table S1). Forty snails per treatment were size-matched ranging from 8 to 13 mm in shell diameter and housed individually in 120 mL jars. Prior to parasite exposure, snails underwent a 4-day acclimation period in well water or well water with the set concentration of antibiotic. Ecologically relevant concentrations of tetracycline range from 2 ng/L to more than 50 μg/L depending on the location, but 50 μg/L was chosen for this experiment as it is a common concentration near waste treatment plants found in Africa, China, and the United States (Daghrir & Droghui, 2013; Islam & Gilbride, 2019; Xu et al., 2021). For instance, the United States has recorded influent wastewater concentrations ranging from 0.32 to 48 μg/L, and China has recorded concentrations of up to 129.3 μg/L found in pharmaceutical wastewater (Xu et al., 2021). A 50 μg/L solution of antibiotic was replaced every 7 days to maintain efficacy and minimize the impact of antibiotic degradation (Schmidt et al., 2007). Antibiotic solutions were prepared by dissolving 1 mg of tetracycline (Research Products International, Tetracycline HCl, Lot #36063-101361) in 20 L of well water a few hours prior to use.

Each snail in the parasite-only treatment and antibiotic + parasite treatment was exposed to eight miracidia of *S. mansoni* in 10 mL of well water. Exposures were done in six-well plates for 24 h. Unexposed snails were sham exposed for the same period

of time using the same procedure as the experimental group in six-well plates and 10 mL of well water with no parasites. Miracidia were harvested from livers of infected mice (in accordance with Purdue Animal Care and Use Committee protocol #1111000225) by blending in saline and filtering according to standard protocols (Tucker et al., 2013). To quantify host reproductive output, egg masses were counted and removed from all individual snail housing jars weekly for 9 total weeks. *Biomphalaria* snails are hermaphroditic and can self-fertilize but may also store sperm from previous encounters. In our experiment, isolation does not ensure self-fertilization as snails were old enough to have had breeding encounters prior to isolation. To determine the impact of antibiotics on the rate of development of the parasite and infection prevalence in snails, parasite production (count of parasite larvae [cercariae]) was assessed weekly in *B. glabrata* beginning week 4 postexposure until the end of the experiment at week 9. This window coincides with a 4-week prepatent period of parasite development where no parasite release occurs, followed by release of cercariae beginning at approximately week 4.

To measure parasite production, snails were placed in well plates with 10 mL of well water and positioned under fluorescent light to allow parasite emergence. After 1 h, snails were returned to their respective jars and the presence or absence of cercariae was recorded. If cercariae were detected, a 1 mL aliquot of well water was taken and all cercariae within the aliquot were counted (Gleichsner et al., 2016). Lastly, the survival of snails was checked weekly.

Statistical analysis

Data on parasite and host reproduction had considerable zero inflation with data overdispersion. As such, we constructed mixed-effects hurdle models to account for data overdispersion using the glmmTMB package in R (Brooks et al., 2017). Hurdle models model the probability of obtaining a zero value, similar to logistic regression. Then, if the value is nonzero, hurdle models use a specific error distribution to further predict host/parasite reproduction. As such, all hurdle model outputs contain coefficients for a zero-inflated model, predicting the probability of a zero, and a conditional model, predicting nonzero measurements based on a specific error distribution. Models for host and parasite reproduction were fit with treatment, week-of-experiment, and treatment × week-of-experiment interaction terms as fixed effects predictors, except where inclusion of the interaction term was uninformative. Host individual was used as a random intercept within each model to account for repeated measures on individual host snails. However, no random slope (week of experiment) was incorporated as supplemental analyses showed that inclusion of random slopes did not change the significance of individual model components, led to overfit models, and added model complexity prevented convergence when included in our original zero-inflation model design (supplemental analyses can be found within our deposited code files: Melchiorre.stats.R). Additionally, we generated these hurdle models using both a Poisson and negative binomial error distribution for nonzero values and used Akaike information criterion (AIC) to determine which model best fit the data. In order to reduce the probability of false-positive results, some pairwise comparisons were omitted. For example, antibiotic-only snails were not compared with parasite-only snails as no biologically feasible scenario would lead to uninfected, antibiotic-exposed snails living alongside parasite-exposed, nonantibiotic-exposed snails, and these comparisons would not inform our hypotheses. Statistical models were visualized using the interactions package in R 3.6.3 (R Core Team, 2020).

To examine parasite development time, we conducted a time-to-event analysis to determine how long postexposure-infected snails would begin producing parasites using treatment group as the predictor. Additionally, a survival analysis was run on all individuals within a treatment, irrespective of infection status, to look for differences in survival using treatment group as the predictor. Examining all individuals within each treatment was necessary as absence of infection cannot be definitively confirmed until week 6, meaning infected individuals who died prior to shedding would be unknown. Both survival analyses were run in R 3.6.3 using the survival (Therneau & Grambsch, 2000) and survminer (Kassambara et al., 2019) packages using the G-rho family with log-rank comparisons of Kaplan–Meier survival estimates. Analysis was conducted over the course of the 9-week experiment and used to parameterize daily mortality rates within our mathematical model.

Mathematical model

In order to further understand how antibiotic contamination may alter disease dynamics, we adapted the differential equation model of Hoover et al. (2020). We used a base model without predation or agrochemical pollution and added snail reproduction associated with fecundity compensation (Minchella & LoVerde, 1981; see Table 1 for parameter values) and delayed castration. While previous models assume complete castration of infected individuals and no reproductive alterations in exposed individuals, our model explicitly incorporates these biologically relevant values. Appendix S1: Figures S1–S3

TABLE 1 Model parameters and estimates from Hoover et al. (2020) and antibiotic-altered parameters.

Variable	Description	Estimate	Antibiotic-altered estimate
f_N	Per capita snail reproduction	0.60 (a)	0.81
φ_N	Density-dependent snail population growth parameter	10^4 (a)	
χ	Reproduction of E (exposed snails) relative to S (susceptible snails: due to fecundity compensation)	1.332; this study	1.174
ρ	Reproduction of I (infected snails) relative to S from incomplete castration	0.007; this study	0.115
μ_N	Natural snail mortality rate	0.017 (a)	0.015
β	Human to snail infection probability	6.67×10^{-7} (b)	8.66×10^{-7}
σ	Conversion rate from exposed to infected	0.025 (c)	
μ_I	Additional mortality rate of infected snails	0.083 (c)	0.107
λ	Snail to human infection probability	7.5×10^{-8} (d)	
μ_H	Mortality rate of adult worms associated with human mortality	4.57×10^{-5} (b)	
μ_W	Mortality rate of adult worms	8.3×10^{-4} (e)	
m	Eggs produced per day	432 (d)	
v	Miracidial viability	0.084 (f)	
π_m	Miracidia-hours	6.22 (g)	
θ	Relative cercarial shedding rate	15.6 (d)	22.2
π_C	Cercariae-hours	14.22 (h)	

Note: Letters in parentheses correspond to sources: (a) Woolhouse and Chandiwana (1990); (b) Sokolow et al. (2015); (c) Anderson and May (1991); (d) Hoover et al. (2020); (e) Goddard and Jordan (1980); (f) Halstead et al. (2018); (g) Tchounwou et al. (1991); (h) Tchounwou et al. (1992).

shows our analysis of fecundity compensation, which generates the parameters χ and ρ. We additionally assumed all female *S. mansoni* worms are paired. These alterations result in the set of differential equations and dynamic variables below. Here, S, E, and I represent susceptible, exposed, and infected snails, respectively. W represents the mean worm burden in the human population (influenced both by human mortality [μ_H] and worm mortality [μ_W]), with M representing the number of female worms, C representing infective parasite cercariae, and N representing the total snail population:

$$\frac{dS}{dt} = f_N\left(1 - \frac{N}{\varphi_N}\right)(S + \chi E + \rho I) - \mu_N S - \beta M S,$$

$$\frac{dE}{dt} = \beta M S - \mu_N E - \sigma E,$$

$$\frac{dI}{dt} = \sigma E - (\mu_N + \mu_I) I,$$

$$\frac{dW}{dt} = \lambda C - (\mu_H + \mu_W) W,$$

$$M = 0.5 W H m v \pi_m,$$

$$C = \theta I \pi_C,$$

$$N = S + E + I.$$

The above model assumes human population size is constant across the relevant timescale, that the snail population undergoes logistic growth with a carrying capacity set by the parameter φ_N, and a background snail mortality rate (μ_N) that is enhanced when snails become infected (μ_I). Further details on the base model and parameterization of parasite larval viability (M and C) can be found in Hoover et al. (2020). We used our experimental data to calculate the percent change in snail and parasite life history characteristics in response to antibiotic exposure and altered model parameters accordingly (Table 1). Two model scenarios were run: an antibiotic scenario and an antibiotic-free (control) scenario as these are the only two biological feasible scenarios that would exist in nature. This exercise is intended only to make qualitative predictions on

how antibiotic exposure may alter disease dynamics. Analysis was run using the deSolve package in R 3.6.3 (Soetaert et al., 2010).

RESULTS

Parasite production

Antibiotics accelerated parasite development. Antibiotic + parasite snails released parasites on average one week earlier than parasite-only snails (survival analysis, $\chi^2 = 8.9$, $p = 0.003$; Figure 1). Although significantly earlier parasite release occurred in the antibiotic + parasite treatment, the number of cercariae released was low. As such, the impact of early maturation may be limited. Infection prevalence for the antibiotic + parasite and parasite-only treatments were not significantly different at 77% and 59%, respectively (two-sample equality of proportion test, $\chi^2 = 1.696$, $p = 0.193$). Additionally, antibiotics had a dynamic effect on parasite production over time, such that the antibiotic + parasite treatment had lower initial parasite production but produced more parasites on average over the course of the experiment (Figure 2; Appendix S1: Table S2; see Appendix S1: Figure S4 in the supplement for raw data visualization).

Host reproduction and survival

Snails in the antibiotic treatment were more likely to lay eggs relative to snails in the control treatment (zero-inflation component of Appendix S1: Table S3, $p = 0.005$; Figure 3; Appendix S1: Figure S5). The antibiotic + parasite treatment had a higher probability of laying eggs than the parasite-only treatment throughout the entire experiment (zero-inflation component of Appendix S1: Table S4; $p = 0.006$). Additionally, comparison of the parasite treatment with the antibiotic + parasite treatment suggests an initially similar reproductive output (Figure 4), yet as the infection matured, snails within the antibiotic + parasite treatment developed a higher probability of laying eggs. These snails evinced delayed castration compared with the parasite-only treatment snails (visible as wide confidence intervals for the parasite treatment in Figure 4; also see the zero-inflation component of Appendix S1: Table S4). The observed decrease in eggs laid over time from infected snails in both treatments is due to parasitic castration (Minchella & LoVerde, 1981).

The antibiotic-only treatment showed the highest survival, followed sequentially by the control treatment, then parasite-only treatment, and antibiotic + parasite treatment (see Appendix S1: Figure S6). However, only the antibiotic + parasite versus the parasite-only survival

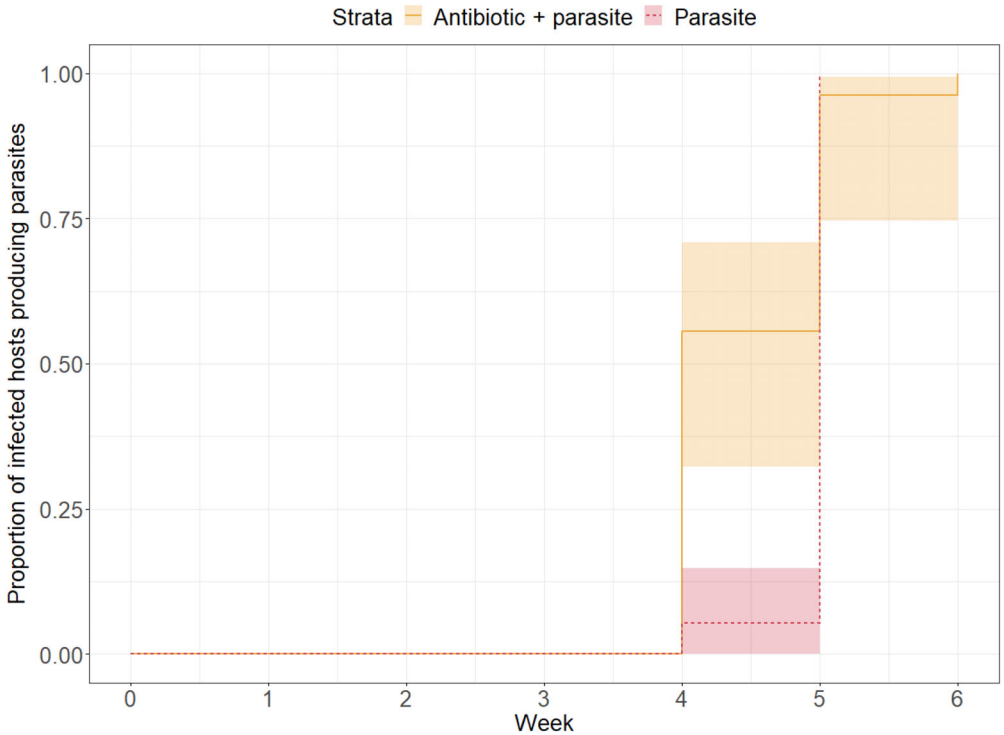

FIGURE 1 Time from initial exposure until snail hosts began producing parasites. The proportion of *Schistosoma mansoni*-infected snails that released parasite cercariae in the parasite-only treatment and the antibiotic + parasite treatment was significantly different ($\chi^2 = 8.9$, $p = 0.0029$) with antibiotic + parasite snails releasing parasites earlier than parasite-only snails.

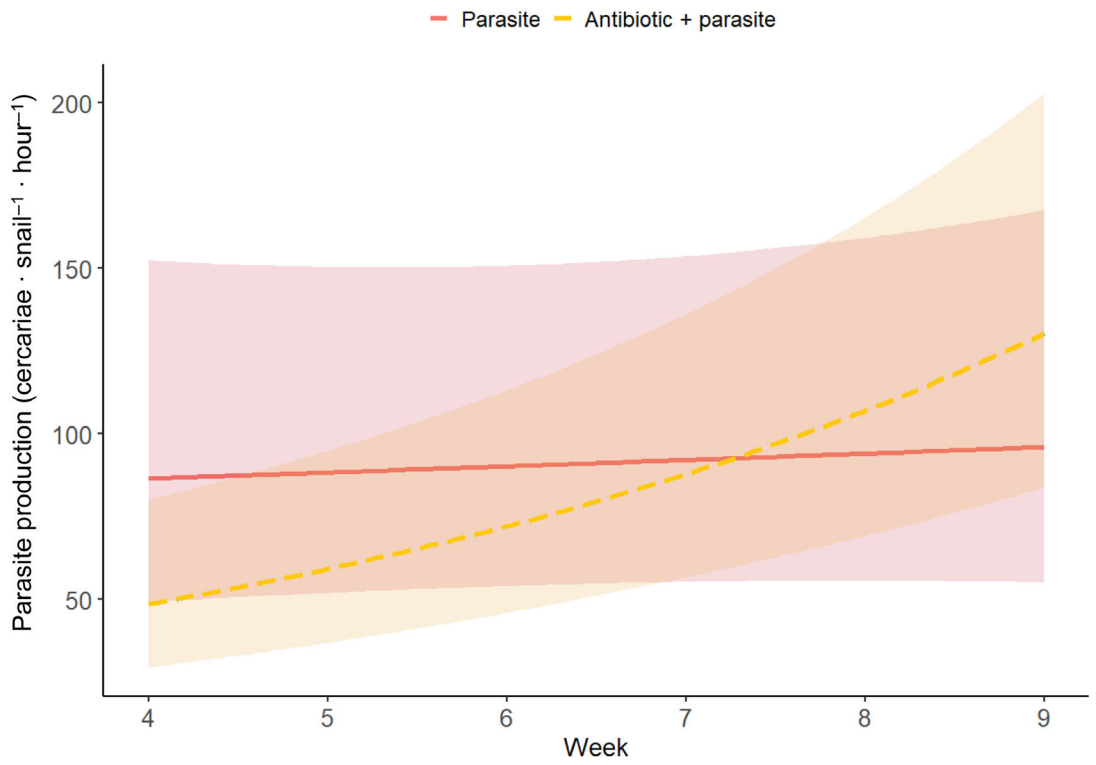

FIGURE 2 Parasite production during the patent period of infection in the experiment. Antibiotic + parasite snails had initially low parasite production that increased as the infection progressed compared with the parasite-only treatment. The *y*-axis is on a log scale to account for negative binomially distributed data. See Appendix S1: Table S2 for summary statistics.

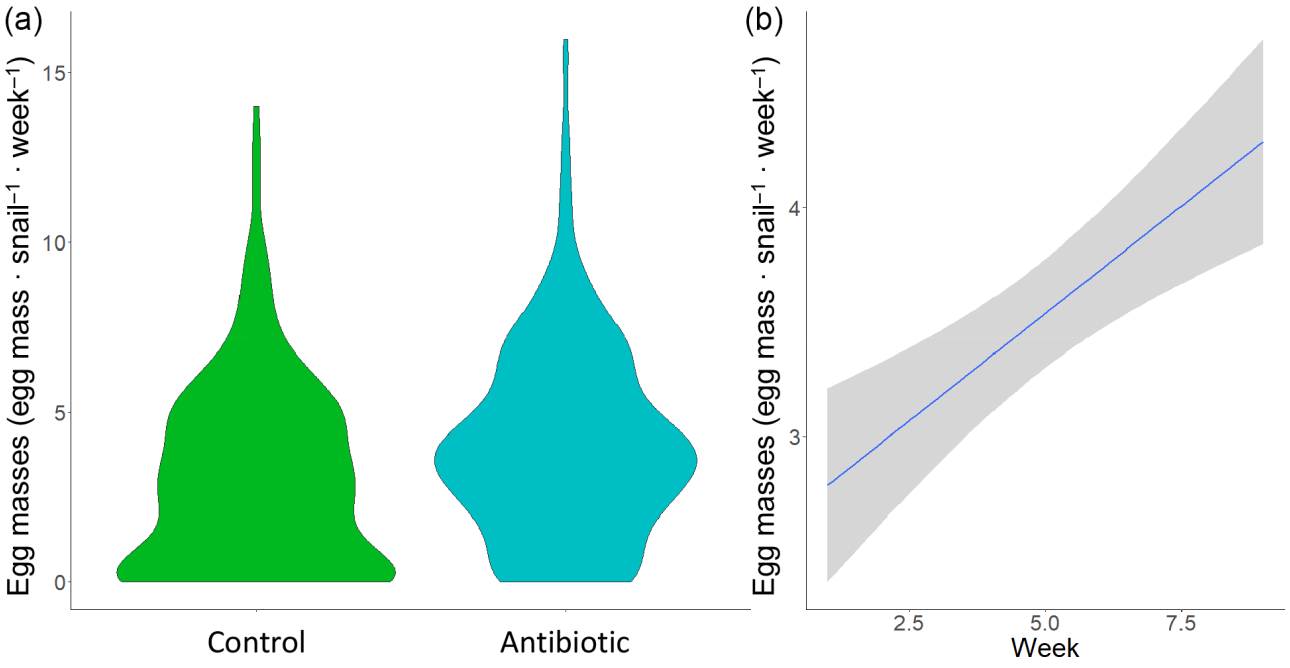

FIGURE 3 Host reproduction over the course of the experiment. Antibiotic-only snails were significantly more likely to produce offspring compared with control snails. Additionally, antibiotic-only snails were more likely to produce offspring and produced more offspring later in the experiment. The interactive effect of treatment × week of experiment was uninformative in this statistical model and was omitted. See Appendix S1: Table S3 for summary statistics.

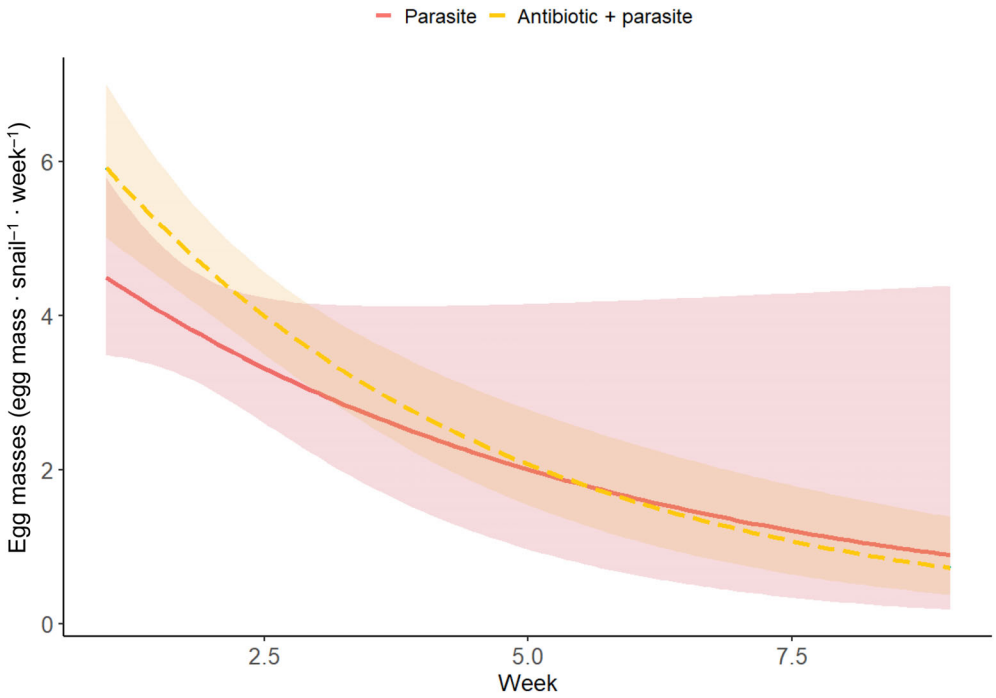

FIGURE 4 Host reproduction over the course of the experiment. Antibiotic + parasite treatment and parasite-only treatment had initially similar host reproductive patterns. However, after week 4, parasite-only snails were more likely to lay no eggs than antibiotic + parasite snails as evinced by wide confidence intervals in the parasite-only treatment. The *y*-axis is on a log scale to account for negative binomially distributed data. See Appendix S1: Table S4 for summary statistics.

curves were significantly different from one another ($\chi^2 = 5.4$, $p = 0.02$).

Antibiotics and disease dynamics

Based on our adaptation of a published *S. mansoni* differential equation model, our data suggest that snails in areas with antibiotic contamination may have increased exposure to parasites and experience more rapid population growth. These observations may potentially lead to higher infection prevalence in snails and greater worm burdens in humans (Figure 5). Additionally, the model suggests that snail exposure and human worm burdens would increase more rapidly in the antibiotic scenario than would otherwise occur (see Appendix S1: Figure S7 for proportional changes in model state variables).

DISCUSSION

In this study, we investigated the impact of an ecologically relevant concentration of tetracycline on the life history parameters of the trematode parasite *S. mansoni* and its snail intermediate host, *B. glabrata*. Specifically, we assessed host reproduction and survival as well as parasite production and development time in the presence and absence of the antibiotic, tetracycline. Our results suggest that antibiotics are likely to impact snail and parasite production with potentially significant ecological ramifications. We show that tetracycline facilitated earlier parasite production within infected hosts and increased parasite output as the infection matured (Figures 1 and 2, respectively). Additionally, the presence of antibiotics increased egg laying in uninfected snails when compared with uninfected, well water controls (Appendix S1: Table S3). Lastly, parasitic castration was delayed in the antibiotic + parasite snails, and these snails had a significantly higher egg output throughout the experiment compared with the parasite-only treatment (Appendix S1: Table S4). To the best of our knowledge, this is the first study to document the impact of antibiotic contamination on the host and parasite life history parameters of this freshwater snail and its medically relevant parasite.

Modifications in host–parasite interactions by antibiotic contamination are likely associated with changing microbiome dynamics and/or lowered host immune capacity (Gust et al., 2013; Hernández-Gómez et al., 2020; Knutie et al., 2018). Antibiotics often disturb microbiomes by decreasing useful and/or increasing harmful bacteria (Akbar et al., 2020). As a result, disturbances to host microbiomes may create opportune environments for parasites to infect and exacerbate disease burden within their hosts (Willing et al., 2011). The alterations observed in

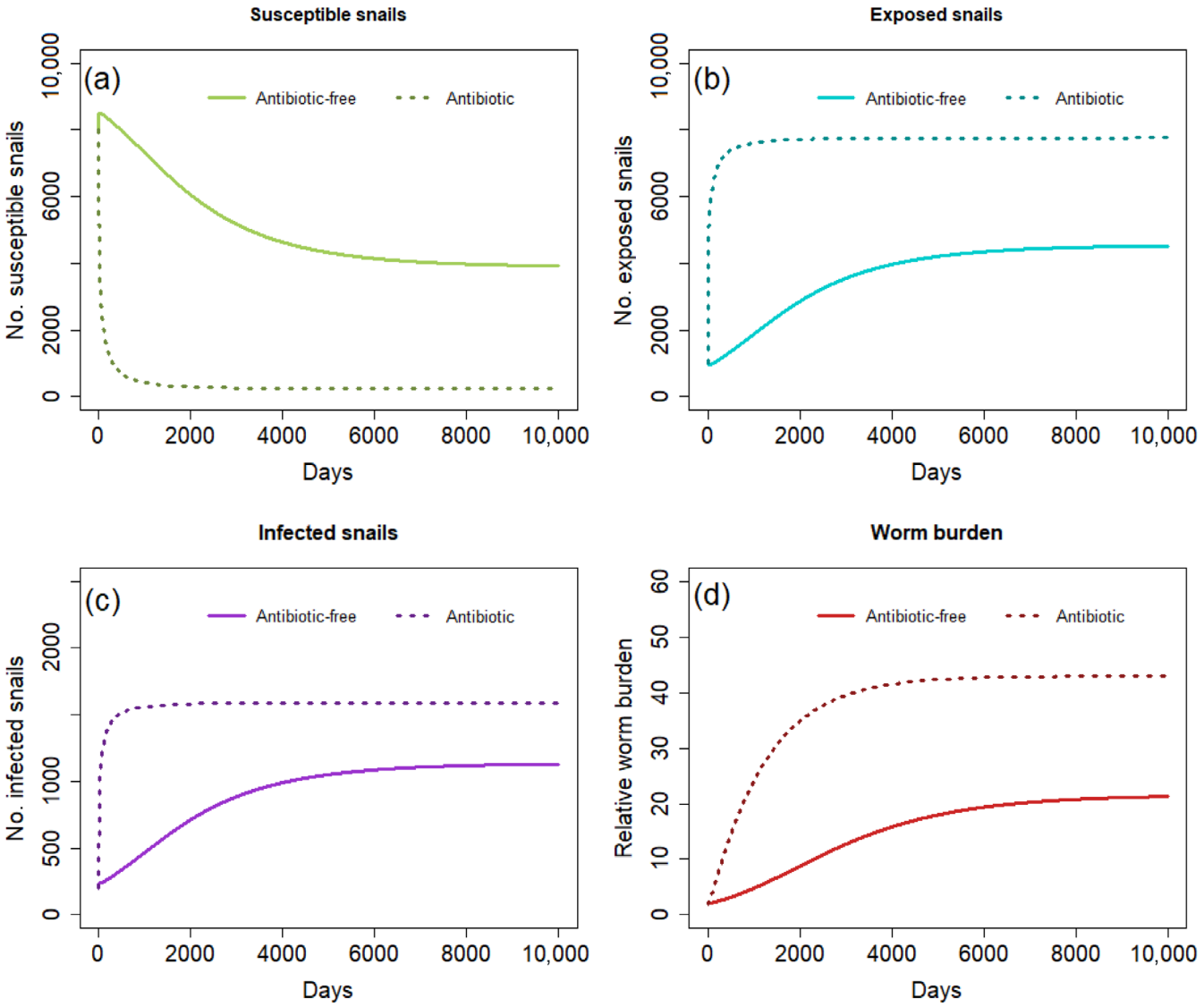

FIGURE 5 Model output showing the number of susceptible snails (a), exposed snails (b), infected snails (c), and mean worm burden (d) in the human population in the antibiotic (data from antibiotic-only and antibiotic + parasite snails) versus antibiotic-free control (data from control and parasite-only snails) scenarios. Antibiotics are likely to increase *Schistosoma* infections in snails and humans based on parameterizations generated from our experimental data.

parasite development time, parasite production, and host production from addition of tetracycline are congruent with research findings of microbiome-related impaired immune function following parasitic infection (Portet et al., 2021). Portet et al. (2021) proposed that following infection with *S. mansoni*, the bacterial microbiome of *B. glabrata* changed its composition, which could account for the altered immune function. Similar to these findings, several studies have shown that alterations of the host microbiome significantly impact parasite pathogenesis (i.e., changes in parasite burden) and host immune responses to infection (Anzia & Rabajante, 2018; Cortés et al., 2020). Our results suggest that aggressive parasite exploitation may have been occurring, as snails showed significantly lower survival in the antibiotic + parasite treatment compared with the antibiotic control.

Antibiotics may also modify host–parasite interactions by changing microbiome dynamics. Decreased immune capacity may involve several components, such as suppression of phagocytosis activity, as well as downregulation of important inflammatory response genes (Cortés et al., 2020; Gust et al., 2013). These responses to antibiotics act to weaken defense mechanisms within hosts (Akbar et al., 2020), which may increase susceptibility to parasites, influence parasite pathogenicity and proliferation within snails, and provide a more conducive environment for parasites to reproduce (Gust et al., 2013). The accelerated parasite development

time and higher parasite production throughout the course of our experiment in the antibiotic + parasite treatment could be a result of lowered immune capacity.

The significant increase of parasite production in the antibiotic + parasite treatment compared with the parasite-only treatment in the final weeks of the experiment is consistent with other findings. A study investigating the effects of pharmaceuticals on pathogen virulence demonstrated that chemically induced immune suppression in *Daphnia* weakens disease resistance by enhancing the virulence of the parasite and increasing the proportion of infected hosts (Schlüter-Vorberg & Coors, 2019). However, they additionally observed an increased speed of host sterilization, which contrasts with our findings of a delayed castration period in hosts. Our initial survival data suggest that the observed increase in reproductive output may also be associated with increased mortality within infected snails in the antibiotic + parasite treatment, which could result in a net decrease in parasite fitness. However, our mathematical model suggests that the increased mortality in infected snails is not sufficient to balance the other life history alterations associated with antibiotic exposure. Considering all factors, parasite transmission, and thus disease burden is likely to increase in regions with substantial antibiotic concentrations.

Finally, snail hosts exposed to antibiotics were more likely to lay eggs. Increased reproduction due to antibiotic exposure has been demonstrated in other invertebrates (Flaherty & Dodson, 2005). A study investigating the effect of pharmaceuticals on *Daphnia* reproduction found that chronic exposure to certain types of antibiotics induced significantly faster development and greater host reproduction (Flaherty & Dodson, 2005). These influences on typical development patterns varied depending on both the duration of exposure and number of pharmaceuticals to which they were exposed. In contrast, inhibition of growth and reproduction has been recorded in previous research on antibiotics and hosts (Chernin, 1957). These contradictory results may have arisen from the 2000-fold difference in antibiotic concentration used. The findings presented here support the idea that ecologically relevant tetracycline concentrations accelerate parasite development time, increase reproductive output in parasites, and enhance host reproduction, possibly through antibiotic-induced changes to the microbiome and/or lowered host immune capacity.

CONCLUSION

We are only beginning to understand the impacts of antibiotics on hosts, parasites, and their interactions. Here, we show that antibiotics influence parasite dynamics by facilitating earlier parasite production with increasing output as the infection matures. Infected hosts affected by antibiotic contamination demonstrated increased egg laying and egg output throughout the experiment when compared with the parasite-only treatment. In addition, parasite castration was delayed in hosts exposed to antibiotics. Our study suggests that the continued widespread use of antibiotics with improper disposal has residual consequences to freshwater ecosystems and may increase the burden of schistosomiasis in endemic regions. The largely unknown ecological and anthropogenic impacts of antibiotic contaminants including—but not limited to—trophic effects, disease risk, and ecosystem interactions therefore merit further research. As antibiotic usage increases, its role as a link between human health and host–parasite interactions emphasizes the need to further explore the consequences of human activity on all facets of global change.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Vannatta, 2023) are available from Zenodo: https://doi.org/10.5281/zenodo.7779535.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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