Downstream Effects: Impact of Antibiotic Pollution on Aquatic Host-Parasite Interactions

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Abstract

The global increase in antibiotic use has led to contamination of freshwater environments occupied by parasites and their hosts. Despite the identified impacts of antibiotics on humans and wildlife, the effect of antibiotics on host-parasite life cycles is relatively unexplored. We utilize the trematode parasite *Schistosoma mansoni*, and its snail intermediate host *Biomphalaria glabrata* to explore the influence of an ecologically relevant antibiotic concentrations on the life history characteristics of both parasite and host. Our results demonstrate that antibiotics not only accelerate parasite development and have a positive effect on parasite reproduction, but also increase the likelihood of host egg laying, and delay parasite-induced host castration. Using a mathematical model, we suggest that these life history alterations associated with antibiotics are likely to increase parasite transmission and disease burden.

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Introduction

Antibiotic usage is increasing worldwide in association with growing demand in livestock production, industry, and human healthcare (Daghrir et al., 2013). As a result, antibiotic contamination from wastewater treatment plants and sewers often end up in freshwaters (Kraemer et al., 2019). Presence of antibiotics in aquatic environments affects the life forms within them, extending beyond microscopic organisms to other non-target species (Danner et al., 2019, Sundberg et al., 2018). Although antibiotic concentrations in freshwater are not typically lethal to non-target 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 et al., 2018). Antibiotics can act as environmental stressors to alter bacterial communities through direct or indirect mechanisms (Grenni et al., 2018). They are designed to decrease pathogenic bacteria within the organism; however, they also impact overall bacterial community diversity within the microbiome and alter community function (Yoon et al., 2018, Morley, 2010, Akbar et al., 2020). Research has shown that the microbiome plays a third-party role in host-parasite interactions (Knutie et al., 2017), however, there is little evidence supporting the effects of sublethal antibiotic exposure on host-parasite interactions (Morley, 2009). Antibiotics likely have widespread impacts on many host-parasite systems either directly (by influencing the organisms involved) or indirectly (by influencing predators/prey/parasites of the organisms involved) (Pravdová et al., 2020). Tetracycline is a common broad-spectrum antibiotic that contaminates freshwater environments due to its water solubility and widespread use in agriculture (Lin et al., 2013). Connections have

also been drawn between high rates of tetracycline consumption with prevalence of disease in high-risk areas (Faleye et al., 2018).

Schistosoma mansoni is a parasitic blood-fluke that causes the human disease schistosomiasis in tropical regions and accounts for as many as 200,000 annual deaths (Adenowo et al., 2015, Verjee, 2019). Eggs of *S. mansoni* from infected humans hatch into miracidia, a larval stage of the parasite, when they contact freshwater. These miracidia can penetrate the snail intermediate host, *Biomphalaria glabrata*. Maturation of the parasite occurs within the snail gonads leading to castration of the host. The snails release free-swimming larval stages called cercariae which directly infect humans as the definitive host (CDC, 2018).

Given the human toll of schistosomiasis and the use of antibiotics in medicine and agriculture in tropical regions (Faleye et al., 2018), we designed an experiment to analyze the effects of antibiotic contamination on *S. mansoni* development rate and reproductive output, as well as host fecundity. The antibiotic chosen for this experiment was tetracycline due to its water solubility, easy accessibility, and widespread use in agriculture and human medicine (Daghrir et al., 2013). Based on previous evidence of increased growth and reproduction in some species through antibiotic exposure (Flaherty et al., 2005, Gaskins et al., 2002), we hypothesized that tetracycline would accelerate parasite development time, increase reproductive output in parasites, and enhance host reproduction. We further use our results to parameterize an epidemiological model and demonstrate potential long-term consequences of freshwater antibiotic contamination on human disease burden.

Methods

One hundred sixty lab-reared *B. glabrata* snails were used in a full factorial experiment combining parasitic infection and antibiotic exposure. Forty snails per treatment group were size-

matched ranging from 8-13mm in shell diameter and placed in 120ml individual jars. Well water and antibiotic treatments were further categorized into unexposed or exposed to *S. mansoni* for a total of four treatments (antibiotic + parasite, parasite only, antibiotic only, control; see Table 1). 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 vary from region to region, but 50µg/L is a common concentration in areas near waste treatment plants and was used for this experiment (Daghrir et al., 2013). A 50µg/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 8 miracidia of *S. mansoni* for 24 hours. Unexposed snails were sham exposed for the same period of time. 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 snail housing jars weekly for 9 weeks. To determine the impact of antibiotics on the rate of development of the parasite and infection prevalence in snails, cercarial output was assessed in *B. glabrata* beginning week 4 post exposure weekly until the end of the experiment at week 9. This window coincides with the patent period of parasite development which normally involves 4 weeks of parasite development where no parasite release occurs, followed by release of cercariae (termed shedding) beginning week 4 or later.

In order to measure parasite reproduction, snails were placed in well plates with 10mL of well water and positioned under fluorescent light to allow parasite emergence. After 60 minutes, snails were returned to their respective jars and the presence or absence of cercariae was recorded. If parasites were detected, a 1 mL aliquot of well water was taken and all cercariae within the aliquot counted (Gleichsner et al., 2016). Finally, the survival of snails was checked weekly.

Statistical analysis

Data on parasite and host reproduction had considerable zero-inflation. 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 first model the probability of obtaining a zero-value, similar to logistic regression. Then, if the value is non-zero, 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 non-zero measurements based on a specific error distribution. Models were fit with treatment, week, and treatment * week interactions terms as fixed effects except where inclusion of the interaction term was uninformative. Additionally, we generated hurdle models using both a Poisson and negative binomial error distribution for non-zero values and used AIC to determine which model best fit the data. In our models, individual host ID was treated as a random factor. To compare overall prevalence and the proportion of hosts releasing parasites in week 4, we used a two-sample test of equal proportions. Survival analysis was run on all individuals within a treatment group, irrespective of infection status. This was necessary as infection cannot be definitively confirmed until week 6, meaning infected individuals who died prior to shedding would be unknown. A Cox proportional hazard regression model was run in R

3.6.3 using the survival and survminer packages. Analysis was conducted over the course of the 9-week experiment and used to parameterize daily mortality rates within our mathematical model.

Lastly, we adapted an existing schistosomiasis model (Hoover et al., 2020) and altered parameters in proportion to our data on control versus antibiotic conditions. This analysis was done to further examine how antibiotic contamination may impact human schistosomiasis. Extended discussion of the model, equations, and parameter values can be found in the supplement (Table S1).

Results

Parasite Reproduction

Antibiotics accelerated parasite development. A significantly higher proportion of antibiotic + parasite snails were releasing parasites in week 4 (56%) compared to parasite only snails (5%; χ^2= 10.317, p = 0.001, Figure 1). Although a significantly greater proportion of shedding 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 (χ^2= 1.696, p = 0.193). Additionally, antibiotics had a positive effect on parasite reproduction over time such that both parasite treatments have similar parasite output in early weeks, with the antibiotic + parasite treatment releasing more cercariae as the infection matures (Figure 2 and Table 2).

Host Reproduction

Snails in the antibiotic treatment were more likely to lay eggs relative to snails in the control treatment (Table 3, $p = 0.005$, Figure 3a). The antibiotic + parasite treatment had a higher probability of laying eggs than the parasite only treatment throughout the entire experiment (Table 4, $p = 0.006$). Additionally, comparison of the parasite treatment and antibiotic + parasite treatment suggests an initially similar reproductive output (Figure S1), yet as the infection matured, snails within the antibiotic + parasite treatment have a higher probability of laying eggs showing delayed castration compared to the parasite only treatment (Figure 3b). The observed decrease in eggs laid over time in infected snails is due to parasitic castration.

The antibiotic only treatment showed the highest survival, followed sequentially by the control treatment, then parasite only treatment, and antibiotic + parasite treatment (Figure 4). The pairwise, Cox proportional hazard comparisons including coefficient, z ratio, and p value are as follows: Control vs antibiotic: 0.419, $z = 0.967$, $p = 0.333$; Control vs parasite: -0.121, $z = -0.314$, $p = 0.753$; Antibiotic vs antibiotic + parasite: -0.872, $z = -2.187$, $p = 0.029$; Antibiotic + parasite vs parasite: 0.332, $z = 0.961$, $p = 0.337$. Observed differences were only significant when comparing the antibiotic + parasite and antibiotic only treatment ($z = -2.187$, $p = 0.029$).

Based on our adaptation of a published *S. mansoni* model, we expect areas with antibiotic contamination to have increased snail exposure to parasites and more rapid snail population growth, leading to higher infection prevalence in snails and greater worm burdens in humans (Figure 5). Additionally, snail exposure and human worm burdens increase more rapidly in the antibiotic scenario than would otherwise occur.

Discussion

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*. We assessed host reproduction and survival as well as parasite reproduction and development time in the presence and absence of the antibiotic, tetracycline. Our results suggest that antibiotics are likely to impact snail and parasite reproduction with potentially significant ecological ramifications. We show that tetracycline facilitated earlier cercarial release by infected hosts and increased cercarial output as the infection matured (Figure 2). Additionally, the presence of antibiotics increased egg laying in uninfected snails when compared to uninfected, well water controls (Table 3). Lastly, parasitic castration is delayed in the antibiotic + parasite snails, and they have a significantly higher egg output throughout the experiment compared to the parasite only treatment (Table 4). To the best of our knowledge, this is the first study to document the impact of antibiotic contamination on the host and parasite life cycle parameters of this freshwater snail and its medically relevant parasite.

These modifications in host-parasite interactions by antibiotic contamination are likely associated with changing microbiome dynamics (Hernández-Gómez et al., 2020, Knutie et al., 2017). Antibiotics specifically have been found to disturb microbiomes by decreasing useful or increasing harmful bacteria (Akbar et al., 2020). The alterations observed in parasite development time, parasite reproduction, and host reproduction from addition of tetracycline are congruent with research findings of microbiome-related impaired immune function following parasitic infection (Portet et al., 2018). Portet et al. (2018) suggested that following infection of *S. mansoni*, the bacterial microbiome of *B. glabrata* changed its composition, which could account for the altered immune function. Decreased immune function weakens defense mechanisms within hosts (Akbar et al., 2020), which can increase susceptibility to parasites and

corresponds with our findings of an accelerated parasite development time in the antibiotic + parasite treatment (Pravdová et al., 2020). A potential alternative explanation is that the parasites were more aggressively exploiting their host, indicating increased parasite pathogenicity (Anzia et al., 2018, Schlüter-Vorberg et al., 2019).

Another finding consistent with an altered microbiome was the significant increase of parasite reproduction in the antibiotic + parasite treatment compared to the parasite only treatment. A study conducted by Schlüter-Vorberg et al. (2019) found similar results when studying the effects of pharmaceuticals on pathogen virulence in a host-parasite system. They demonstrated that chemically induced suppression of immune systems in *Daphnia* weakens disease resistance by enhancing the virulence of the parasite and increases the proportion of infected hosts (Schlüter-Vorberg et al., 2019). However, they additionally observe an increased speed of host sterilization, which contrasts our findings of a delayed castration period in hosts. Our initial survival data suggested that the observed increase in reproductive output may also be associated with increased mortality within infected snails in the antibiotic treatment which could result in a net decrease in parasite fitness. However, our model proposes 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 in humans are likely to increase in regions with sufficient antibiotic concentrations.

Finally, hosts exposed to antibiotics were more likely to lay eggs. Increased reproduction due to antibiotic exposure has also been shown in other studies of invertebrates (Flaherty et al., 2005). A similar study investigating the effect of pharmaceuticals on *Daphnia* reproduction found that chronic exposure to certain types of antibiotics induced significantly greater development and reproduction measurements (Flaherty et al., 2005). These influences on typical

development patterns varied depending on both the duration of exposure, and number of pharmaceuticals to which they were exposed. The strongest and most complex effects between fecundity and antibiotics were observed with long term (30-day) exposure and a combination of multiple pharmaceuticals mixtures, respectively. This potential alteration to snail population dynamics could have cascading impacts within food webs as snails are important herbivores and prey within many systems (Johnson, 2009). The capacity to change food web dynamics could also manifest as an increased antibiotic sensitivity in primary producers such as algae, affirming the potential risks of contamination in non-target species that could influence snail-host populations (Isidori et al., 2005).

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 cercarial release and increasing cercarial output as the infection matures. Infected hosts affected by antibiotic contamination demonstrated increased egg laying and egg output throughout the experiment when compared to 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 results in 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 merits 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.

Conflict of Interest

The authors declare that they have no conflict of interest.

Statement of Human and Animal Rights:

All applicable institutional and/or national guidelines for the care and use of animals were followed in accordance with Purdue Animal Care and Use Committee protocol #1111000225.

Informed Consent:

This study did not involve human subjects.

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Table 1. Treatment groups designated by presence or absence of antibiotic and/or parasite, N = sample size after acclimation and with active infections for parasite (+) treatments.

	Antibiotic (+)	Antibiotic (-)
Parasite (+)	Antibiotic + parasite (N=27)	Parasite only (N=19)
Parasite (-)	Antibiotic only (N=33)	Control(N=34)

Table 2. Model results for parasite reproduction comparing the antibiotic + parasite treatment relative to the parasite treatment (intercept). Coefficients of the zero-inflation model show the probability of obtaining a zero value.

Zero Inflation Model	**Coefficient**	**Standard error**	**Z value**	**P value**
Intercept	3.841	1.180	3.255	0.001 *
Antibiotic + parasite	1.765	2.267	0.778	0.436
Week	-0.867	0.218	-3.982	<0.001 *
Antibiotic + parasite: Week	-0.519	0.471	-1.102	0.270
Conditional Model				
Intercept	4.362	0.334	13.069	< 0.001 *
Antibiotic + parasite	-1.285	0.417	-3.083	0.002 *
Week	0.021	0.047	0.441	0.660
Antibiotic + parasite: Week	0.177	0.058	3.058	0.002 *

Table 3. Model results for host reproduction comparing the antibiotic treatment relative to the control treatment (intercept). Coefficients of the zero-inflation model show the probability of obtaining a zero value.

Zero Inflation Model	**Coefficient**	**Standard Error**	**Z Value**	**P Value**
Intercept	-0.812	0.540	-1.503	0.132
Antibiotic	-2.166	0.700	-2.813	0.005 *
Week	-0.121	0.0587	-2.062	0.039 *
Conditional Model				
Intercept	1.107	0.095	11.676	<0.001 *
Antibiotic	0.190	0.105	1.813	0.070
Week	0.030	0.010	2.890	0.004 *

Table 4. Model results for host reproduction comparing the antibiotic + parasite treatment relative to the parasite treatment (intercept). Coefficients of the zero-inflation model show the probability of obtaining a zero value.

Zero Inflation Model	**Coefficient**	**Standard Error**	**Z Value**	**P Value**
Intercept	-7.756	1.688	-4.596	<0.001 *
Antibiotic + parasite	4.667	1.702	2.742	0.006 *
Week	2.609	0.523	4.989	<0.001 *
Antibiotic + parasite: Week	-1.820	0.505	-3.603	<0.001 *
Conditional Model				
Intercept	1.705	0.220	7.733	<0.001 *

Antibiotic + parasite	0.337	0.252	1.338	0.181
Week	-0.201	0.111	-1.812	0.070
Antibiotic + parasite: Week	-0.061	0.121	-0.502	0.616

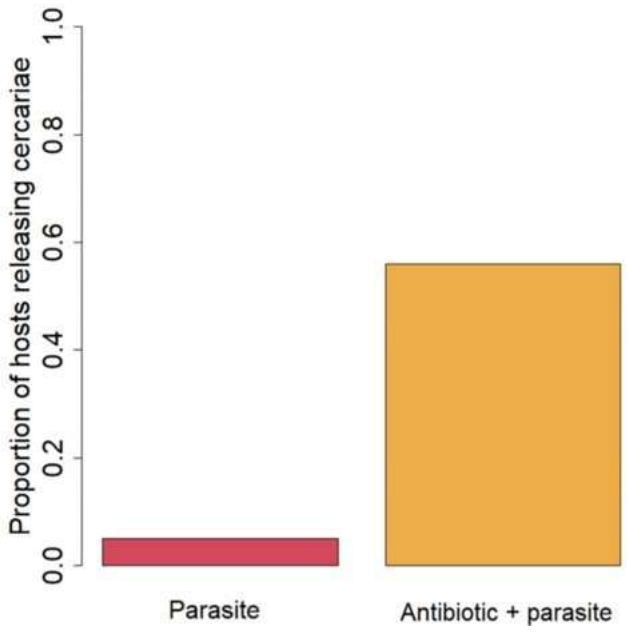

Figure 1. Proportion of exposed *Schistosoma mansoni* infected snails that released cercariae on week 4.

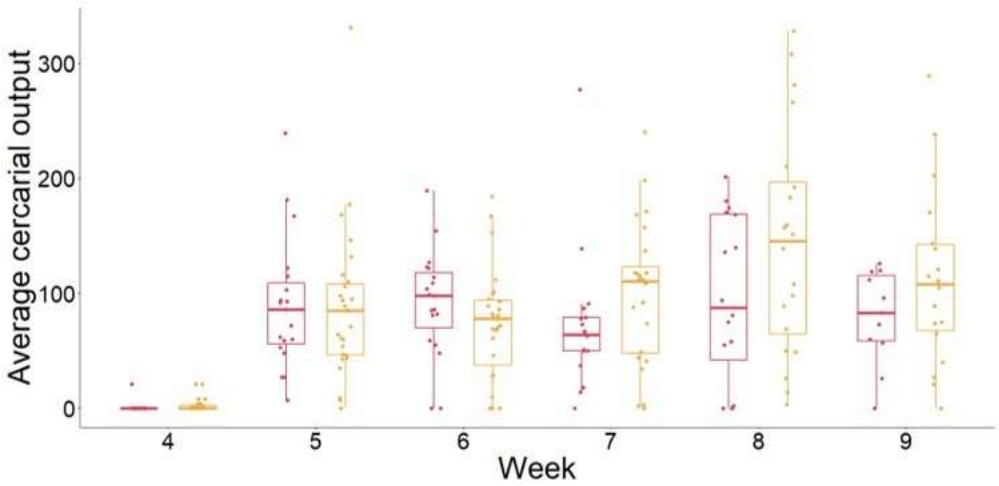

Figure 2. Boxplots of average cercarial output showing increased output as the infection progresses in the antibiotic + parasite compared to the parasite only treatments. Antibiotic + parasite treatment is shown in gold, and parasite only treatment in red. Overlaid data points are jittered for visualization.

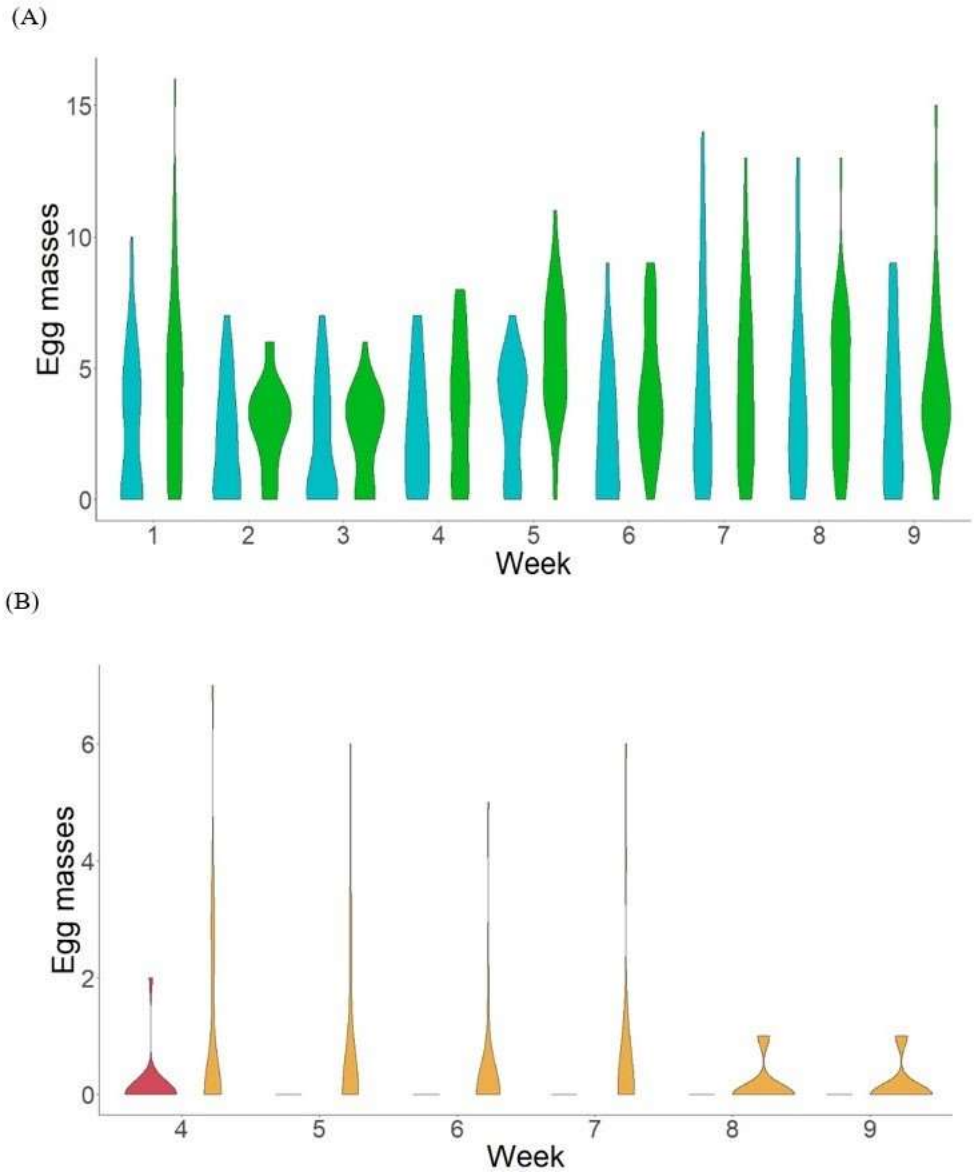

Figure 3. (A) Host reproductive output showing an increased likelihood of laying eggs for the antibiotic treatment (green) compared to the control treatment (blue) (B) Post-castration period showing a higher probability of laying eggs and delayed castration period of the antibiotic + parasite (gold) treatment compared to parasite (red) treatment during weeks 4-9 .

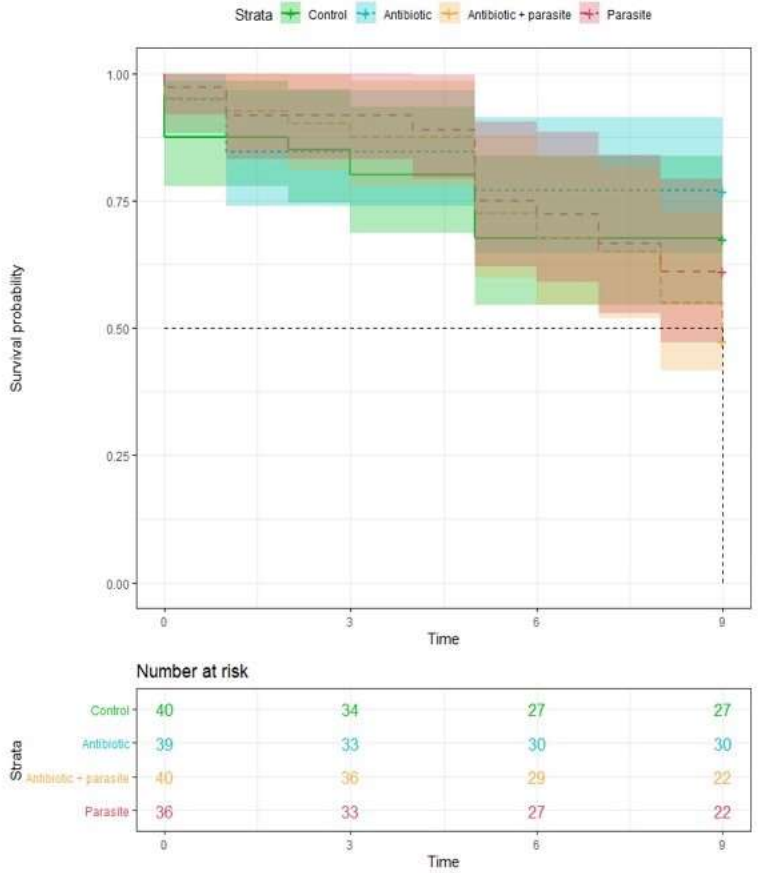

Figure 4. Survival probability of treatment snails during the 9-week experiment.

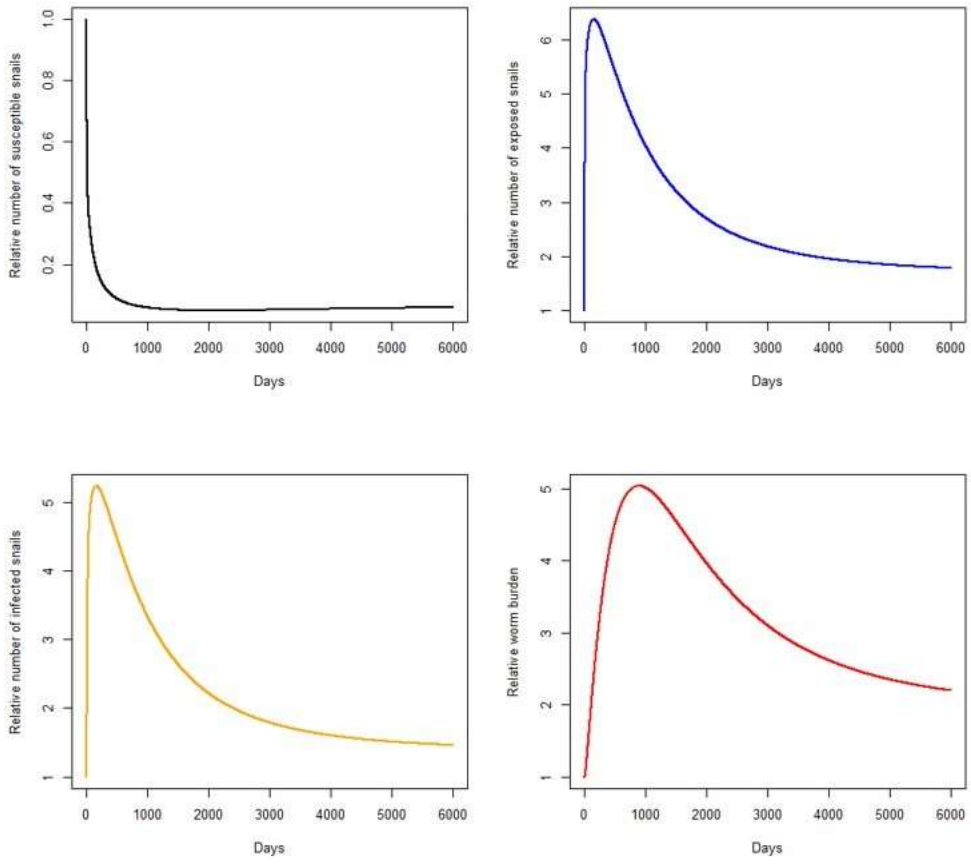

Figure 5. Model output showing the relative number of susceptible snails, exposed snails, infected snails, and mean worm burden in the human population in the antibiotic versus control scenario (y values = antibiotic scenario values / control scenario values). Y axis values greater than 1 suggest the antibiotic scenario has larger amounts of the state variable. Y axis values less than 1 suggest the antibiotic scenario has smaller amounts of the state variable.

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, including snail reproduction associated with fecundity compensation (Minchella et al., 1981) and delayed castration, and 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 with M representing the number of female worms, C representing infective parasite cercariae, and N the total snail population:

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

$$\frac{dE}{dt} = \beta MS - \mu_N E - \sigma E$$

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

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

$$M = 0.5WHmv\pi_m$$

$$C = \theta I \pi_C$$

$$N = S + E + I$$

We used our data to calculate the percent change in snail and parasite life history characteristics in response to antibiotic exposure and altered model parameters accordingly (Table S1). 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).

Table S1. Model parameters and estimates from Hoover et al. 2020 and antibiotic altered parameters.

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

[a] Anderson and May 1991, [b] Goddard and Jordan 1980, [c] Halstead et al. 2018, [d] Hoover et al. 2020, [e]Sokolow et al. 2015, [f] Tchounwou et al. 1991, [g] Tchounwou et al. 1992, [h] Woolhouse and Chandiwana 1990

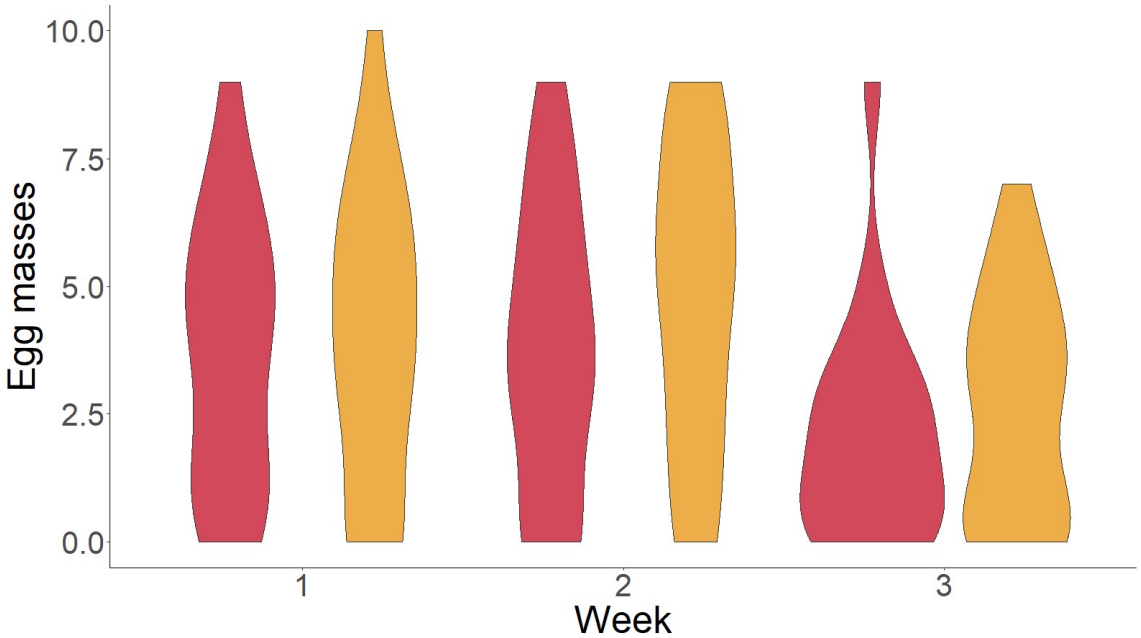

Figure S1. Pre-castration periods showing an initially similar reproductive output in the antibiotic + parasite treatment (gold) when compared to the parasite treatment (red) during weeks 1-3.

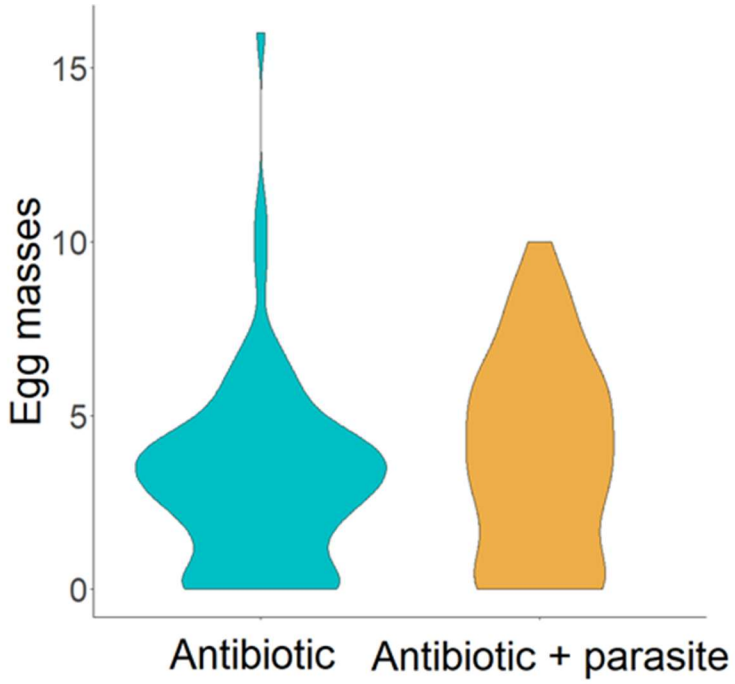

Figure S2: Host reproductive output by antibiotic + parasite treatment is significantly larger than antibiotic only treatment during the first 3 weeks of infection. Mean values over this period were used to inform the model.

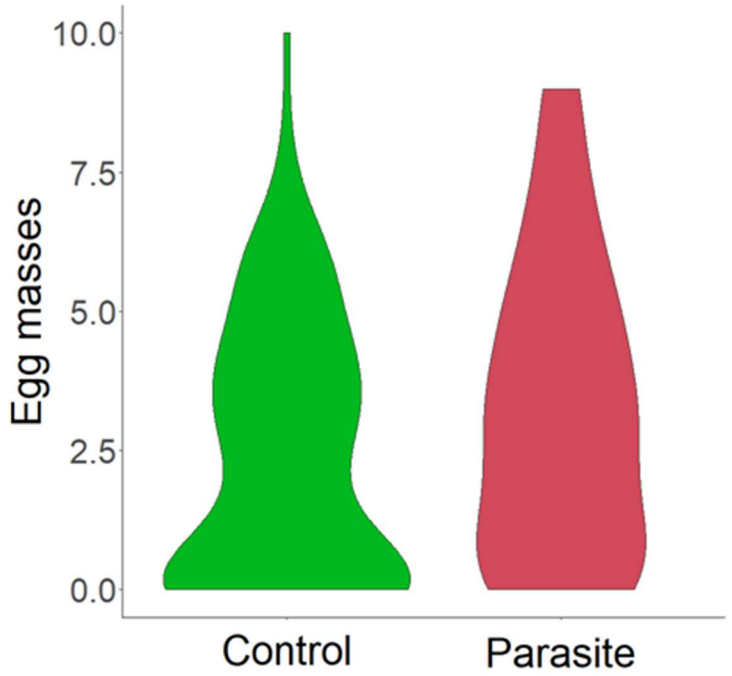

Figure S3: Host reproductive output of the control treatment compared to the parasite treatments prior to castration. Mean values over this period were used to inform the model.