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RESEARCH ARTICLE

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Long-term effects of antibiotic treatments on honeybee colony fitness: A modelling approach

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Abstract

1. Gut microbiome disequilibrium is increasingly implicated in host fitness reductions, including for the economically important and disease-challenged western honey bee *Apis mellifera*. In laboratory experiments, the antibiotic tetracycline, which is used to prevent American Foulbrood Disease in countries including the US, elevates honey bee mortality by disturbing the microbiome. It is unclear, however, how elevated individual mortality affects colony-level fitness.
2. We used an agent-based model (BEEHAVE) and empirical data to assess colony-level effects of antibiotic-induced worker bee mortality, by measuring colony size. We investigated the relationship between the duration that the antibiotic-induced mortality probability is imposed for and colony size.
3. We found that when simulating antibiotic-induced mortality of worker bees from just 60 days per year, up to a permanent effect, the colony is reduced such that tetracycline treatment would not meet the European Food Safety Authority's (EFSA) honey bee protection goals. When antibiotic mortality was imposed for the hypothetical minimal exposure time, which assumes that antibiotics only impact the bee's fitness during the recommended treatment period of 15 days in both spring and autumn, the colony fitness reduction was only marginally under the EFSA's threshold.
4. *Synthesis and Applications*. Modelling colony-level impacts of antibiotic treatment shows that individual honey bee worker mortality can lead to colony mortality. To assess the full impact, the persistence of antibiotic-induced mortality in honey bees must be determined experimentally, in vivo. We caution that as the domestication of new insect species increases, maintaining healthy gut microbiomes is of paramount importance to insect health and commercial productivity. The recommendation from this work is to limit prophylactic use of antibiotics and to not exceed recommended treatment strategies for domesticated insects. This is especially important for highly social insects as excess antibiotic use will likely decrease colony growth and an increase in colony mortality.

KEYWORDS

American foulbrood disease, antibiotics, *Apis mellifera*, BEEHAVE, colony mortality, dysbiosis, gut microbiome, honey bee

1 | INTRODUCTION

Managed Western honey bees *Apis mellifera*, dominate the commercial pollination of monocultures (Rader et al., 2009), increasing yields of the most nutritionally rich fruit, vegetable and nut crops by up to 90% (Southwick & Southwick, 1992). Since the turn of the century, managed *A. mellifera* colonies have experienced health declines worldwide and apiarists are losing up to 50% of colonies per annum in Europe and North America (Jacques et al., 2017; VanEngelsdorp et al., 2012, 2017). Habitat loss, climate change, pesticide exposure and the global spread and emergence of bee parasites and pathogens are all implicated in these losses (e.g. Potts et al., 2016).

American Foulbrood Diseases (AFB), caused by *Paenibacillus larvae*, is the most virulent bacterial disease of honey bee larvae (Krongdang et al., 2017). This disease is ubiquitous globally, and despite comprehensive understanding of its epidemiology and pathogenicity, it still often results in colony death. In Europe, hives with AFB infections must be burnt and buried (Anjum et al., 2015). Treatment with antibiotics in the tetracyclines class, including tetracycline and the related compound oxytetracycline, has been the preferred method to treat AFB in the United States for the last 50 years. Although *P. larvae* only infects larvae, non-target adult bees also ingest the antibiotic during prophylactic treatment. Raymann et al. (2017) found that experimental exposure to a therapeutic dose of tetracycline considerably altered worker bees' gut microbiome abundance and community composition and increased the bees' mortality. By treating germ-free workers with tetracycline, they determined that the fitness reduction was not due to direct toxicity of tetracycline on the workers. Instead, immune-challenge experiments attributed the fitness decrease to a reduction in microbiome-derived immune capacity (Raymann et al., 2017). Moreover, prophylactic treatment of bees with tetracycline has been implicated in the presence of antibiotic resistance genes (ARGs) within the honey bee holobiont, including within *P. larvae* (Levy & Marshall, 2013; Tian et al., 2012). Not only does this reduce the efficacy of tetracycline in treating American Foulbrood disease in honey bees but it also increases the global reservoir of ARGs available to be acquired by human pathogens, thus contributing to the antibiotic resistance crisis (Allen et al., 2010; Hu et al., 2017).

Dysbiosis (i.e. gut microbiome imbalance) has until recently largely been omitted from the investigation into reduced colony fitness (Hamdi et al., 2011). Honey bees have a highly conserved mutualistic relationship with their gut microbiome (Kwong & Moran, 2016; Schwarz et al., 2016), which is acquired socially and passed from nurse bees to newly emerged worker bees (Kwong, Medina, et al., 2017). The microbiome aids in nutrient acquisition and detoxification, development and immune defence (Kwong, Mancenido, et al., 2017; Zheng et al., 2017).

While the short-term fitness effect of antibiotic exposure on individual workers has been documented, the long-term impact at the colony level for these highly eusocial insects remains unknown. To assess the potential risks of long-term antibiotic exposure for honeybee colonies, we take an in-silico approach in this study. We use the agent-based honeybee model BEEHAVE (Becher et al., 2014), which links colony processes with resource availability in a heterogeneous and dynamic landscape. The model runs for a number of years, allowing stressors to potentially accumulate over time. This enables investigations into the long-term effects of different factors on colony fitness and dynamics. BEEHAVE is a mechanistic, mathematical representation of a honey bee colony based on empirical data and accepted theory. It has been positively evaluated by the European Food Safety Authority (EFSA) for its suitability as a tool in regulatory risk assessment (EFSA, 2015; Rortais et al., 2017) and confirmed to correctly implement the most important in-hive dynamics (Agatz et al., 2019). Moreover, BEEHAVE has been used in numerous studies to simulate the impact of viruses and pesticides on colony dynamics (e.g. McMahon et al., 2016; Prado et al., 2019; Rumkee et al., 2015; Thorbek, Campbell, & Thompson, 2017).

We implemented the effects of a disturbed gut microbiome in the model by increasing the daily mortality risk of bees during a defined period of time after treatment with antibiotics, parameterised based on Raymann et al. (2017). As it is unclear how long bees are affected by dysbiosis after exposure to antibiotics, we simulated a variety of possible effect durations. This ranged from two short periods to represent a hypothetical minimum effect scenario in which the antibiotics only cause increased worker mortality during the recommended application period, up to a permanent effect. To evaluate our results, we compare the simulated reduction of the colony size with honey bee protection goals defined by EFSA.

2 | MATERIALS AND METHODS

2.1 | The BEEHAVE model

Simulations were run in the BEEHAVE model V. 2013 (Becher et al., 2014; www.beehave-model.net), and run in Netlogo v. 5.3.1 (Wilensky, 1999). BEEHAVE simulates the development of daily bee cohorts from eggs, to larvae, pupae and adult workers and drones. Younger workers act as in-hive bees, caring for the brood until they develop into foragers and collect nectar and pollen from food sources in the landscape. Daily mortality probabilities are specific to the developmental stage and additional mortalities may apply due to other stressors, for example, foraging risks. To implement the impact of microbiome disturbance on the bees, we modified the daily mortality probabilities of adult workers for a specific period. Details of this and all other changes to BEEHAVE are shown in the Supporting Information and Table S1. For a full documentation of the model, including sensitivity analysis and ODD protocol ('Overview, Design concepts, Details', Grimm et al., 2006, 2010), see supplementary material of Becher et al. (2014). Evaluations of the BEEHAVE model have been done by the European Food Safety Authority (2015) and Agatz et al. (2019).

2.2 | Calculation of mortality rates from empirical data

The antibiotic-induced mortality probability was derived from Raymann et al. (2017), in which five experimental treatment groups consisting of ~30 age-controlled *A. mellifera* workers were fed 450 μg/ml tetracycline in sugar syrup for 5 days. The survival of the bees was then monitored daily for 10 days following the exposure.

2.3 | Imposed worker mortality

A binomial model was fitted to the available mortality data, where x_i is the number of bees that died each day (for days $i = 1, ..., T$). We therefore model $x_i \sim \text{Bin}(n_i, p)$, where p is the daily probability of mortality and n_i is the number of bees that are alive each day. This is equivalent to assuming a constant mortality rate (e.g. Becher et al., 2014), but follows the structure of the empirical mortality data (Raymann et al., 2017) where the number of deaths in a series of discrete time periods is reported, and the length of the time periods are the same throughout. We estimate p using maximum likelihood, and used a 'resample with replacement' bootstrap approach ($n = 1{,}000$) on the empirical data to derive consistent estimates of uncertainty at the level of the mortality probability parameter values. The distribution of the derived mortality probabilities is shown in Figure S2. These bootstrapped samples are then propagated through the BEEHAVE model as the daily worker mortality probabilities. This is in contrast to earlier papers that used a single estimated value with no parameter uncertainty (e.g. Becher et al., 2014).

In contrast to standard toxicological assays where the toxicant-induced mortality is the difference between mortality rates in treated minus rates in control groups, we modelled the control and treatment mortality probabilities separately so that we could qualitatively compare them. This also allowed us to determine the appropriateness of using the empirical control data (Raymann et al., 2017) rather than the BEEHAVE mortality value to represent a healthy colony.

Under default settings in BEEHAVE, a baseline daily mortality probability of 0.004 is imposed on all in-hive adult bees. This was derived by Becher et al. (2014) from a survival curve of healthy winter worker bees from Martin (2001). Honey bees kept separate from their colony under experimental conditions experience high background mortality (Williams et al., 2013). Indeed, the empirical control mortality probability from the Raymann et al. (2017) antibiotic experimental data derived mortality probability was ~10 times greater than the standard mortality probability in BEEHAVE (Figure S1). To adjust for the experimentally inflated background mortality, we transformed the data so that the pre-bootstrapped control mortality probability derived from the empirical data (0.029) was divided by the BEEHAVE baseline control for worker adults. This provided a transformation factor (7.25) which we could then use to transform all of the treatment and control mortality probabilities after the bootstrapping stage to more realistically represent natural colony development in the absence of individual-level mortality data from the field.

2.4 | Simulations

Every simulation was run with 1,000 bootstrapped mortality values giving 1,000 colony repeats per scenario, each with a unique random seed, and therefore resulting in a distribution of outputs. Simulations started on January 1st, with 10 000 initial bees and ran for a period of 10 years. We added 10 deformed wing virus infected and 10 uninfected varroa mites at the start of simulations alongside the varroa treatment, as is standard in the EFSA risk assessment simulations (Becher et al., 2014; EFSA, 2013). All other parameters were set to the BEEHAVE defaults.

To address the uncertainties regarding the duration of microbiome disturbance and to understand the costs of antibiotic treatment, we ran several scenarios spanning a gradient of effect durations of imposed antibiotic-induced mortality probabilities per annum with increments of 30 days per treatment duration scenario (Table 1). During the treatment days, the daily probability of mortality experienced by the worker bees changed to one of the bootstrapped treatment mortality probabilities. For non-effect days, it was set to one of the bootstrapped control mortality probabilities.

The 30-day effect duration is the minimum effect scenario and it assumes that antibiotics only increase the bee's probability of mortality during the treatment period, after which the gut microbiome recovers. The current guidelines for US apiarists state that to protect against AFB, hives should be treated with three applications of powdered oxytetracycline, 4–5 days apart in the spring and the autumn (Lafrenière & Ostermann, 2017). Hence, we imposed a minimum antibiotic-induced mortality probability for 15 days in spring and

TABLE 1 Description of simulated antibiotic effect scenarios and corresponding conditions

Scenario: days of antibiotic-induced mortality	Days of imposed mortality
0	No imposed mortality
30	Days 60–75 + days 260–275
60	Days 60–90 + days 260–290
90	Days 60–105 + days 260–305
120	Days 60–120 + days 260–320
150	Days 60–135 + days 260–335
180	Days 60–150 + days 260–350
210	Days 60–165 + days 260–365
240	Days 1–15 + days 60–180 + days 260–365
270	Days 1–30 + days 60–195 + days 260–365
300	Days 1–45 + days 60–210 + days 260–365
330	Days 1–60 + days 60–225 + days 260–365
365	Continuous imposed mortality

autumn of each year. In the maximum effect scenario, we assume that gut microbiome depletion after antibiotic exposure is irreversible and results in a permanently increased probability of mortality. This is plausible because the only route by which *A. mellifera* acquire and replenish their core gut microbiome, is through social transfer (Powell et al., 2014); if the whole colony has antibiotic-induced dysbiosis, then all future generations could inherit a maladapted microbial community. In this scenario, the daily probability of mortality experienced by the worker bees is always one of the treatment antibiotic-induced mortality probabilities. All simulations were run under the assumption that every worker will ingest antibiotics at a therapeutic dose.

2.5 | Data manipulation and visualisation

Data were assembled in R version 3.4.1 (R Core Team, 2016), using the DPLYR (Wickham et al., 2017) package. The survival models were fitted using the SURVIVAL package (Therneau, 2015). Bootstrapping was performed using the BOOT package (Canty & Ripley, 2017) and visualised using ggplot2 (Wickham, 2009).

2.6 | Data analysis

To assess differences in colony fitness outcomes, we used the number of overwintering adults and percentage decrease in colony size; these parameters are used in EFSA's plant protection product risk assessment (EFSA, 2013). In BEEHAVE, the census date for overwintering colony size is the 31st of December; if the colony size is below 4,000 adults on this census date, overwinter colony mortality is applied (Becher et al., 2014). As is standard plant protection product risk assessments, we present the change in colony size as the difference in overwintering colony size between the treatment and control groups, divided by the number of adults in the control group (EFSA, 2013). Modelled colony losses were then compared with two recommended protection goals. First, a maximum reduction of 7% in colony size which is deemed as a 'negligible effect' cut-off by the EFSA, and second a maximum reduction of 20% in colony size which is an 'economic viability' cut-off suggested by Thorbek, Campbell, and Thompson (2017) and Thorbek, Campbell, Sweeney, et al. (2017).

3 | RESULTS

3.1 | Effects of antibiotic-induced mortality on colony growth and colony size

The model outputs show a negative relationship between the increase in the number of days of imposed antibiotic-induced mortality (ABM) and the percentage change in overwintering colony size (Figures 1 and 2). This negative relationship was amplified with each additional year due to the cumulative reduction of colony size affecting the potential capacity of the colonies each year.

3.2 | Protection goals

Colony size in the simulated treatments with 60–365 days of annual imposed ABM per year were all reduced by more than 7% by the end of the first year and for all consecutive years, compared to the 0-day ABM control colony size in the same year, therefore surpassing the EFSA 'negligible threshold' (Figure 1; Table S2; EFSA, 2013). Despite the minimum effect scenario (30-day ABM) not breaching the EFSA protection goal cut-off, it still causes a noticeable fitness cost across all years (Figures 1 and 2; Table S2). The more permissive 20% economic viability cut-off (Thorbek, Campbell, & Thompson, 2017) was breached for the scenarios with 120–365 days of ABM (Figure 1; Table S2).

3.3 | Colony losses

The model outputs displayed a positive relationship between the duration of imposed antibiotic-induced mortality and the percentage of colony failure (Figure 3). Once a colony has failed (conditional on them having fewer than 4,000 adults on the 31st December), the

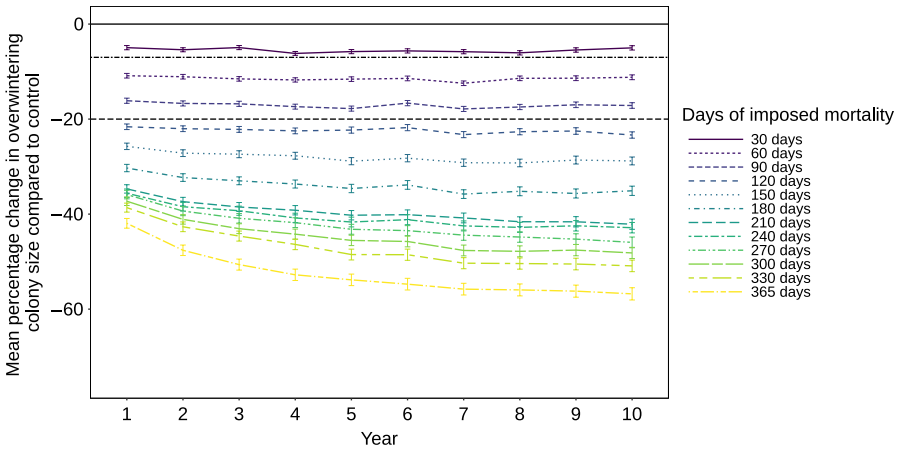

FIGURE 1 Mean percentage change in the colony size on the 31st December of each year ± *SE*compared to the 0-day antibiotic-induced mortality control on the 31st December in the same year ($n = 1{,}000$). The black horizontal solid line corresponds to a zero per cent fitness difference. The black horizontal long dash represents the 7% negligible fitness affect cut-off set by the EFSA (2016). The black horizontal short dash line represents the 20% economic viability threshold (Thorbek, Campbell, & Thompson, 2017)

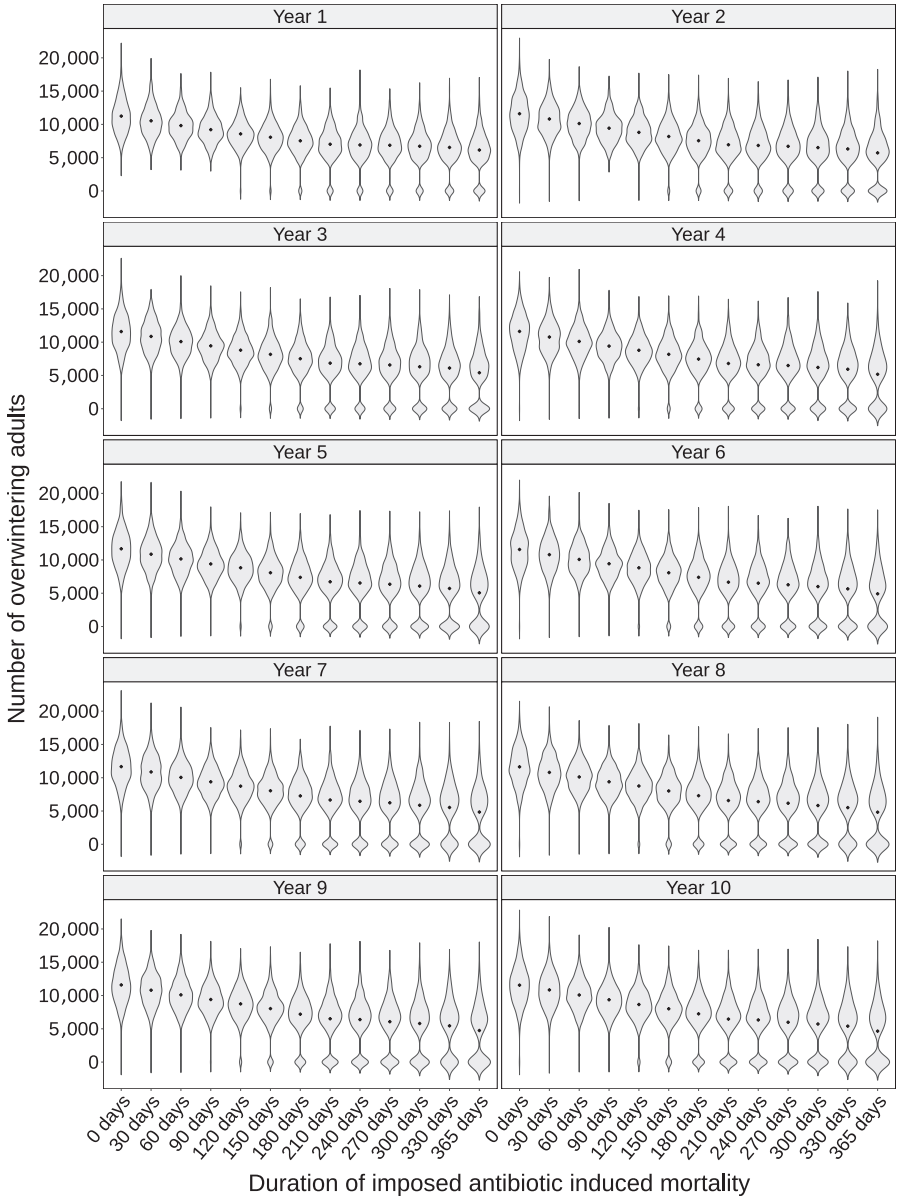

FIGURE 2 Effect of the duration of imposed antibiotic-induced mortality on the number of overwintering adults (mortality probabilities derived from Raymann et al., 2017); central diamond represents the mean; width represents the kernel density and vertical length represent the range of the data. Each treatment (n = 1,000 bootstrap repeats) represents a different effect duration simulation. Once a colony has failed, the total number of adults is recorded as 0 in the following years. The cumulative number of failed colonies can be seen in the bottom mode of the violin plot

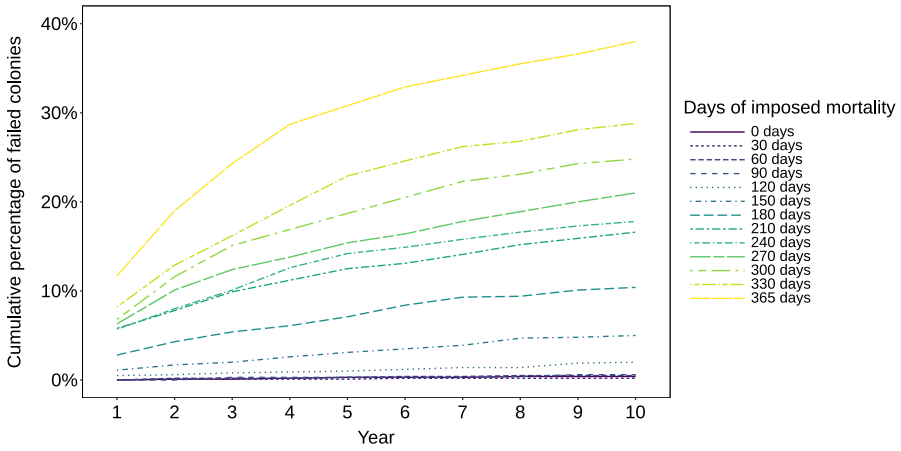

FIGURE 3 Cumulative percentage of failed colonies per treatment on the 31st December of each year (n = 1,000). The colony losses across time are cumulative and the pool of active colonies is progressively smaller with each year

total number of adults will be recorded as 0 in the following years and thus the colony losses across time are cumulative. The extent of cumulative colony losses is visualised in Figures 2 and 3 by the increasing degree of bimodality in the violin plots with both year and ABM treatment duration, where the bottom mode represents the number of colonies that have failed (see Table S3). The only

treatments with no colony loss were the 0-day control and the 30-, 60- and 90-day ABM treatments in year 1 (Table S3). The minimum time of imposed antibiotic mortality required to cause over 1% colony failure was 120-day ABM from the fifth year. 5% colony failure was reached first in the 180-day ABM treatment by the third year, while in the ninth year colony failure was over 10% for this treatment (Figure 3; Table S3). Predictably, the highest percentage of colony failure was seen in the 365-day antibiotic treatment in the tenth year, where 38% of the colonies failed (Table S3).

4 | DISCUSSION

Here, we present an in-silico approach to determine the possible effects of antibiotic treatment of honey bees at the colony level. Using BEEHAVE, we found a consistent negative relationship between the duration that antibiotic-induced mortality is imposed and the colony size over a 10-year period. Colony size was decreased by more than 7%, therefore breaching the EFSA honey bee protection threshold for all but the 0 day ABM control and the 30 days of annual ABM treatments across years, while the economic viability threshold of 20% decrease in colony size was breached for the 120–365 days of annual ABM treatments, again across all years (EFSA, 2013; Thorbek, Campbell, Sweeney, et al., 2017). We simulated scenarios that differ in how long these effects last across a year, ranging from a scenario where antibiotic ingestion causes dysbiosis and fitness reduction only while the antibiotic is being applied under the recommended minimum treatment regime, to a permanent effect scenario, which assumes that antibiotic ingestion causes permanent dysbiosis and therefore decreased fitness.

The European Food Safety Authority states that 'The effect on the colony (of plant protection products) should not exceed 7% compared with control colonies' (EFSA, 2013). The simulation experiments performed here suggest that if antibiotic treatment causes dysbiosis-induced mortality for 60–365 days or for 120–365 days per year then the 7% and 20% colony size reduction target, respectively, would be surpassed after just 1 year (Thorbek, Campbell, & Thompson, 2017). Moreover, the most recently published Bee Informed Survey 2015–2016 found that commercial beekeepers consider a 16.5% loss rate to be acceptable (Kulhanek et al., 2017); this was surpassed in the first year of the 90-day ABM treatment and all following treatment durations.

It is challenging to accurately compare the colony-level fitness effect of antibiotic treatment with that of an untreated AFB infection due to the complexity of *P. larvae* infection dynamics. *P. larvae* spores can survive for 35 years and less than 10 spores are required to cause a fatal infection in larvae, yet in some cases, colonies may contain low levels of *P. larvae* spores and never show clinical symptoms (Genersch, 2010; Haseman, 1961). For example, *P. larvae* was found in 9.7% of surveyed Danish honey bee colonies, but only 3.7% of colonies presented clinical symptoms (Hansen & Brødsgaard, 1999). In addition, there is a scarcity of published case studies where an infection was left unchecked until the death of the colony and so the average time from presentation of clinical symptoms, to colony death is not known to us. However, we can assume that a colony will be destroyed by beekeepers shortly after the development of a visible infection (Hansen & Brødsgaard, 1999). We have shown here that the minimum antibiotic effect scenario to cause colony failure after just 1 year was 120-day ABM, where 0.5% of the colonies failed while the maximum, permanent effect scenario resulted in failure of 11.7% of the colonies. The relative benefit of prophylactic antibiotic treatment of this disease then depends on the prevalence and virulence of the AFB infection and on the severity of the dysbiotic effect on the gut microbiome caused by antibiotic treatment. Although the results obtained here should not be viewed as quantitative predictions of effect sizes, this highlights the need for an experimental investigation into the long-term impact of antibiotic treatment on honey bee gut microbiomes.

Tetracyclines are broad spectrum antibiotics which affect gram-positive and gram-negative bacteria. Disturbing the mutualistic core microbiome of honey bees can trigger an interdependent cascade of events at a molecular and ecological level which together affect the fitness of their host and the colony as a whole. Treatment with antibiotics may prevent the core bacteria from providing vital services such as detoxification, dietary supplementation and protection against parasites and pathogens (e.g. Raymann et al., 2017; Schwarz et al., 2016). Metagenomic surveys revealed that honey bees from colonies with colony collapse disorder had different community compositions compared to healthy colonies and were lacking strains known to positively stimulate immunity and aid in nutrient acquisition (Cox-Foster et al., 2007).

Predicting the potential for colonies to recover from gut dysbiosis is problematic, and at present there is no long-term data to elucidate the possibility either way. As there are no microbiome acquisition pathways independent of social transfer within the colony, the microbiome is unlikely to recover completely after an application of antibiotics to the hive (Powell et al., 2014). The honey bee's core gut microbiome shows a high degree of spatial structure and co-dependence and the order of colonisation by different species is important for maintenance of an effective gut microbiome (Ellegaard & Engel, 2019). Raymann et al. (2017) found that even when tetracycline-treated bees were returned to a hive alongside nestmates with a healthy microbiome, the treated bees' microbiome did not return to the baseline composition of abundance after a week and they still experienced increased mortality akin to bees who were returned to xenobiotic nestmates. After dysbiosis, previously mutualistic microbes can transition into pathogenic states, or transient pathogens such as *Serratia marcescens* can fill the niche space of a core symbiont in honey bees (Schwarz et al., 2016). Tetracycline treatment resulted in an increase in the core bacteria *Gilliamella apicola* and a decrease in the core *Bifidobacterium* (Raymann et al., 2017), which is a trend seen in the microbiomes of colonies with CCD (Cox-Foster et al., 2007) and therefore is likely representative of an unhealthy bee.

It could be postulated that a colony may re-establish a healthy gut microbiome community under certain circumstances, for example if

only a small proportion of bees within the colony ingest the antibiotics at a concentration capable of causing dysbiosis. Healthy bees would then be able to re-inoculate newly hatched bees, and restore the microbiome in future generations. However, tetracycline can be detected in hives up to 3 months post-application (Al-Waili et al., 2012); therefore, it appears unlikely that any individuals could avoid exposure.

Given that *P. larvae* is also already developing resistance to tetracyclines (Alippi et al., 2014), prophylactic antibiotic treatment should be used with caution. Furthermore, there is a strong case for universally reducing our use of antibiotics where possible, to preserve their efficacy in treating human pathogens. Tian et al. (2012) found that most of the tetracycline resistance genes within the bee gut microbiome have >99% sequence identity to genes which are carried by human pathogens. This indicates that bees can potentially act as a reservoir for medically important resistance genes, subsequently increasing that gene's ecological connectivity and therefore its risk to humans and livestock.

Consequently, non-antibiotic treatments including the shook-swarm method and probiotics should be favoured where possible. Dietary probiotic lactobacilli treatment is as efficient at inhibiting *P. larvae* as the recommended antibiotic treatment (Daisley et al., 2019). In the future, probiotics may both prevent and treat American Foulbrood disease and therefore present a clear alternative. The most common current non-antibiotic AFB management technique is the shook-swarm method, whereby colonies with light AFB infections in the spring have their adults 'shaken' into a new nest and the comb is destroyed (Hansen & Brødsgaard, 2003). This method has been shown to be effective at removing AFB symptoms and is more profitable than simply destroying the whole colony and purchasing new bees (Pernal et al., 2008). It does however present a considerable cost to the 'shaken' colony, which will have to re-build its resources and rear new brood at the beginning of the foraging season.

It is important to take note of the caveat that large complex models such as BEEHAVE are only as accurate as the data used to parameterise them, the appropriateness of mathematical models and the assumptions upon which they were created (Aldebert et al., 2018). As such, we should view these results as an attempt to explore the range of potential outcomes in the absence of gold-standard empirical studies, and to highlight the importance of future research in these areas. By bootstrapping the empirical mortality data before applying the survival model to derive mortality probabilities, we were able to incorporate uncertainty through the mortality parameters to give a predictive distribution for model outputs that accounts for uncertainties in our knowledge of this key parameter. Other parameters in the model were based on the BEEHAVE defaults, evaluated by Agatz et al. (2019). However, this means that we are likely underestimating the true variability in the outputs, and accounting for these potential additional uncertainties would be a useful area of future research.

Our study highlights the need to carefully consider how best to extract and interpret results from laboratory experiments both to explain natural systems and to parameterise models. This is especially true for social insects, where differences at an individual level or in a social context need to be taken into account when measuring physiological or behavioural responses. This is particularly important when extrapolating results from laboratory pesticide risk assessments investigating individuals, as misinterpreting these results could have great economic costs with regard to food security. Here, the control mortality derived from the empirical data did not represent mortality experienced by bees within a natural colony when input to the model, leading to unrealistic levels of colony failure (Figure S1). This discrepancy is expected because honey bees in controlled experiments, where bees are maintained outside the hive environment, are expected to show higher mortality than under normal conditions within the hive (Sonter et al., 2019). We accounted for this discrepancy by transforming our control and treatment bootstrapped data proportional with the baseline mortality in BEEHAVE.

The most recent survey of managed honey bees in the United States found that 26.3% of commercial colonies were lost in the winter of 2015 (Kulhanek et al., 2017). This degree of colony losses is first reached in our model outputs in the 8th year of the 330-day AMB treatment and in the 4th year of the 365-day ABM treatment. This highlights that we are likely missing important interacting factors which lead to greater colony loss. The reported drivers for the colony losses include viruses, pesticides, varroa and starvation; gut microbiome disequilibrium will certainly be interacting either synergistically or additively with these factors (Kwong, Mancenido, et al., 2017; Zheng et al., 2017). While BEEHAVE is able to include varroa infestations, virus infections and dynamic foraging conditions, we are unable to model the interactions between these and antibiotic-induced gut microbiome dysbiosis due to a scarcity of data. It is therefore vital that empirical studies attempt to unpick these interactions to allow appreciation of the full cost of the prophylactic use of antibiotics in honey bees.

In a broader context, our research cautions that using antibiotics to treat mass-reared animals is problematic not just for vertebrates but also for insects. Increasingly, new insect species are being domesticated for intensive pollination, for example, stingless bees, mining bees and bumble bees (Garibaldi et al., 2017). Insects' dependence on their microbiome varies depending on their life histories (Engel & Moran, 2013). Compared to social bees, solitary bees' microbiomes are more transient and environmentally determined, largely due to the lack of stable inter-generational transmission pathways (Kwong, Medina, et al., 2017; Voulgari-Kokota et al., 2019). Regardless, most pollinators rely on their microbiomes to metabolise and detoxify polysaccharides in their diet (Zheng et al., 2017). To optimise productivity, it is paramount that the gut microbiome is preserved when domesticating insects.

5 | CONCLUSIONS

The simulations presented here highlight that honey bee colonies are at risk not only from American Foulbrood disease but also

potentially from the treatment against the disease. This highlights the importance of minimising the longevity and intensity of antibiotic treatment where possible, to reduce undesirable side effects such as dysbiosis-caused host fitness reductions and evolution of resistance in commensal microbes. To validate these results, the temporal extent of the mortality effect imposed on honey bees needs to be measured, ideally by conducting long-term experiments at the colony level to validate fitness effects. If empirical studies support long-lasting effects of tetracyclines on mortality and dysbiosis, strict regulation on antibiotic use in beekeeping should be considered.

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AUTHORS' CONTRIBUTIONS

L.W., L.B., M.A.B. and T.J.M. conceived the ideas and designed the methodology; L.B. and T.J.M. modelled the data; L.B. and M.A.B. conducted the simulation study and L.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.jwstqjq7h (Bulson et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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