that LpxF is dispensable in the absence of inflammation, suggesting that the broad conservation of this enzyme across commensal Bacteroidetes reflects selective pressures imposed by periodic inflammatory events. A delicate balance between microbial resilience and host tolerance thus allows for commensal persistence throughout a diverse range of perturbations while preventing commensal overgrowth or depletion, either of which could have deleterious effects on the host.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S8
Tables S1 to S9
References (*24*–*53*)

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EPIDEMIOLOGY

Opposite effects of anthelmintic treatment on microbial infection at individual versus population scales

Vanessa O. Ezenwa[1]* and Anna E. Jolles[2]

Parasitic worms modulate host immune responses in ways that affect microbial co-infections. For this reason, anthelmintic therapy may be a potent tool for indirectly controlling microbial pathogens. However, the population-level consequences of this type of intervention on co-infecting microbes are unknown. We evaluated the effects of anthelmintic treatment on bovine tuberculosis (BTB) acquisition, mortality after infection, and pathogen fitness in free-ranging African buffalo. We found that treatment had no effect on the probability of BTB infection, but buffalo survival after infection was ninefold higher among treated individuals. These contrasting effects translated into an approximately eightfold increase in the reproductive number of BTB for anthelmintic-treated compared with untreated buffalo. Our results indicate that anthelmintic treatment can enhance the spread of microbial pathogens in some real-world situations.

Helminths are among the most ubiquitous parasites on earth, infecting more than 1 billion people (*1*) and causing substantial production losses in livestock (*2*). Because chronic helminth infection can modulate host immune responses, there is considerable interest in the role helminth infection may play in the progression of co-infecting microbial diseases (*3*, *4*). In the laboratory, mouse and nonhuman primate studies show that helminths can skew host immunity in ways that alter the outcomes of viral and bacterial infections (*5*–*7*). Specifically, T helper cell 2 (T_H2) responses triggered by helminths can bias the mammalian immune responses away from antiviral or antibacterial T_H1 responses, increasing host vulnerability to certain intracellular pathogens. Some human studies have also linked helminth coinfection to enhanced morbidity for other infectious diseases, such as tuberculosis and HIV (*8*–*11*). Although individual studies suggest that the specific outcomes of helminth coinfection can vary by pathogen system, important trends have emerged linking concurrent helminth infection to changes in host responses to microbial infections (*4*). This general observation has triggered calls for integrating anthelmintic treatment into control efforts for some microbial diseases of humans as a means of improving disease outcomes (*12*–*14*). However, few data exist to show how such individual-level interventions might affect population-level disease patterns of target microbes.

We used a wild population of African buffalo (*Syncerus caffer*), naturally infected with gastrointestinal helminths (strongyle nematodes of various species) and exposed to bovine tuberculosis (BTB, *Mycobacterium bovis*), to investigate the consequences of anthelmintic treatment on BTB dynamics (*15*). Patterns of BTB and nematode infection in buffalo indicate strong immune-mediated interactions between the two parasites (*16*). Moreover, short-term anthelmintic treatment of buffalo has been shown to increase T_H1 immunity, demonstrating that helminth-mediated immune suppression occurs in this species (*17*). By monitoring a cohort of more than 200 anthelmintic-treated and control animals, we tested for the effects of treatment on immunity, BTB infection probability, and BTB-associated mortality and then explored the implications of treatment for the fitness of *M. bovis*, as measured by this pathogen's reproductive number (*15*). We found that treatment improved the survival of individual hosts infected with BTB but also enhanced pathogen fitness.

We captured 216 female African buffalo in Kruger National Park, South Africa, approximately every 182 days over a 4-year period. At capture, animals in the experimental group (n = 108) received a long-lasting anthelmintic bolus (Panacur, Intervet, UK), whereas controls (n = 108) were left untreated. At the beginning of the experiment, all of these animals were BTB-free. Before anthelmintic treatment, the treated and control groups did not differ in their likelihood of being infected with worms (control = 57 of 103; treated = 55 of 107; Pearson's χ^2 test, $\chi^2 = 0.33$, $P = 0.57$) nor in the number of worm eggs they were shedding in feces (Wilcoxon rank sum test, $Z = 1.16$, $P = 0.25$). After treatment, treated individuals were less likely to be infected with worms [generalized linear mixed model (GLMM), n = 214 individuals, 1134 observations, β estimate ± SE (control) = 1.69 ± 0.27, $P < 0.0001$ (table S1)] and were shedding significantly fewer worm eggs than were

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controls [GLMM, n = 214 individuals, 1134 observations, β estimate ± SE (control) = 2.49 ± 0.491, $P < 0.0001$ (table S2)]. The probablity of worm infection and egg count were also correlated with the time since last drug administration (capture interval). Infection was more likely and egg count higher as the capture interval lengthened [GLMM, infection probability, β estimate ± SE = 0.008 ± 0.002, $P < 0.0001$ (table S1); egg count, β estimate ± SE = 0.002 ± 6.3×10^{-5}, $P < 0.0001$ (table S2)]. The capture interval effect was most likely the result of reinfection in treated individuals over time. The treatment significantly affected host T_H1 immunity, measured by interferon-γ (IFN-γ) secretion in response to a pokeweed mitogen challenge. IFN-γ plays a central role in the host's defense against tuberculosis (*18*), and treated animals mounted stronger IFN-γ responses than did controls, suggesting that anthelmintic treatment increased T_H1 immunity (Fig. 1A and table S3).

With the loss of worms and increase of T_H1 immunity, we expected disease parameters to change. Of 201 animals with BTB test histories, 69 acquired BTB infection during the study period (control, 36 of 101; treated, 33 of 100). Accounting for herd, the relative risk of BTB conversion for control animals compared with treated animals was approximately one. Anthelmintic treatment was not a significant predictor of BTB infection risk [hazard ratio (HR) = 0.988, 95% confidence interval (CI) 0.615 to 1.586, $P = 0.959$ (Fig. 2B and table S4)]. However, anthelmintic treatment status was a strong predictor of mortality risk after BTB infection. Of 58 BTB positive animals with known fates, 13 died during the study period (control, 11 of 30; treated, 2 of 28). Mortality risk was approximately ninefold higher among controls than among treated animals after accounting for age [HR = 9.28, 95% CI 1.93 to 44.6, $P = 0.0054$ (Fig. 2C and table S5)]. Thus, although anthelmintic treatment had no observable effect on BTB infection probability, the mortality of BTB-positive individuals was significantly reduced by treatment.

IFN-γ is known to limit tuberculosis severity in cattle, mice, and humans (*18*, *19*); thus, the enhanced survival of anthelmintic-treated BTB-positive buffalo may be a direct consequence of improved T_H1 immunity. The role of IFN-γ in BTB protection in wildlife species is unknown, but our survival result corroborates correlational data from another buffalo population, suggesting that BTB-infected buffalo with worm infections suffer higher mortality than that of individuals infected with either BTB or worms alone (*16*).

The lack of a treatment effect on BTB incidence could arise for at least two reasons. First, in herd animals such as buffalo, exposure to BTB may be frequent because of high contact rates among individuals. In this situation, a modest reduction in susceptibility by improved T_H1 immunity may not be sufficient to cause a detectable reduction in BTB infection risk. Second, initial protection from tuberculosis infection involves several innate immune defenses, and successful elimination of *Mycobacteria* can occur before the onset of T cell–mediated adaptive immunity in humans and laboratory animals (*20*). Therefore, enhancement of T_H1 cell immunity alone may be insufficient to confer effective protection from BTB infection. In a recent study, human subjects with lower peripheral blood neutrophil counts were found to be at higher risk of *M. tuberculosis* infection, and neutrophil depletion impaired the ability of whole blood to restrict growth of *M. tuberculosis* and *M. bovis* bacille Calmette Guérin (*21*). However, we found no difference in neutrophil numbers between control and treated buffalo (Fig. 1B and table S3).

Our observation that anthelmintic treatment had asymmetrical effects on BTB infection probability and mortality has implications for BTB dynamics. The population-level consequences can be visualized by considering the impact of treatment on the basic reproductive number (R_0) of BTB. In a fully susceptible buffalo population, the R_0 of BTB can be calculated as the rate at which new infections arise multiplied by the infectious period: $\beta N \times 1/(\mu + \alpha)$, where β is the transmission rate, N is the population size, μ is the background host mortality rate, and α is the disease-induced mortality rate. Because buffalo develop life-long BTB infections (*22*), we do not consider pathogen clearance in our calculations. Anthelmintic treatment may decrease R_0 by reducing the susceptibility of buffalo to BTB infection or by decreasing pathogen shedding among infected hosts. Alternatively, treatment could increase R_0 by reducing disease-induced host mortality. We estimated R_0 for treated and control subsets of our buffalo population, accounting for the negligible effects of treatment on BTB susceptibility and strong effects on disease-induced mortality that we observed. We found that anthelmintic treatment resulted in an almost eightfold increase in the relative magnitude of R_0 for BTB in the treated subpopulation (7.73, 95% CI 1.71 to 34.9). Translating this into absolute values, we estimated R_0 for control individuals to be 2, as compared with 15 for treated individuals (Fig. 2D). Because the spread of a pathogen through a host population is generally more rapid with increasing R_0 (*23*), if BTB were introduced into a fully naïve population, such a difference in the value of R_0 would result in a higher probability of an outbreak occurring and higher overall disease prevalence.

Our results reveal a tension between individual- and population-level consequences of anthelmintic therapy for outcomes of intracellular pathogen co-infections. At the individual level, the outcome of such an intervention is positive because it reduces BTB-induced host mortality in our case and, as recently reported in the literature, the progression or severity of other pathogens such as HIV (*10*, *24*) and *Streptococcus pneumoniae* (*25*). At the population level, however, the consequences of intervention are negative because surviving, BTB-positive, anthelmintic-treated individuals continue to spread the pathogen. This conflict arises because there is no reduction in BTB infection risk in anthelmintic-treated individuals. Even if treatment reduced bacterial shedding, which could help moderate transmission, there would have to be an almost 90% reduction in *M. bovis* shedding among treated animals to overcome the increase in pathogen fitness resulting from the extended lifespan of these hosts (Fig. 2E).

Because our drug treatment did not eliminate worms from the experimental subjects for the entire duration of the study, it is possible that we failed to observe an effect of anthelmintic treatment on BTB infection risk as a result of poor efficacy. Similarly, most large-scale deworming programs in human populations do not achieve consistent and complete parasite clearance (*26*). Hence, our results may accurately reflect the outcome of treatment programs applied in real populations.

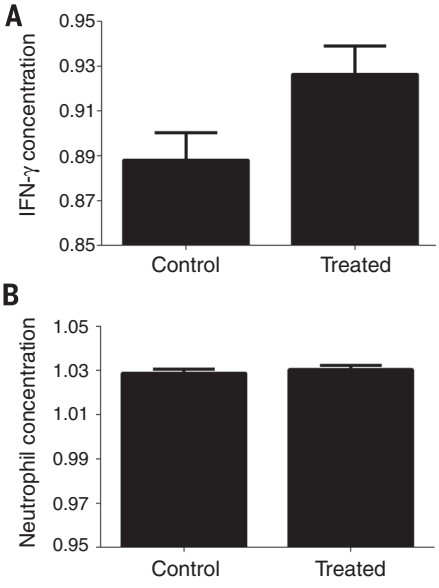

Fig. 1. Effects of anthelmintic treatment on buffalo immunity. (**A**) Treatment increased IFN-γ responses to pokeweed mitogen stimulation. Accounting for season, herd, age, and capture interval, treated individuals had significantly higher IFN-γ levels than those of controls [linear mixed model (LMM), n = 212 individuals, 1020 observations, β estimate ± SE (control) = −0.0378 ± 0.0162, $P = 0.02$ (table S3)]. (**B**) Treatment had no effect on circulating neutrophil concentrations. Accounting for the same covariates as in (A), there was no difference between treated and control individuals in neutrophils [LMM, n = 211 individuals, 941 observations, β estimate ± SE (control) = −0.0011 ± 0.0023, $P = 0.63$ (table S3)]. Both IFN-γ and neutrophil concentrations were power-transformed for analysis.

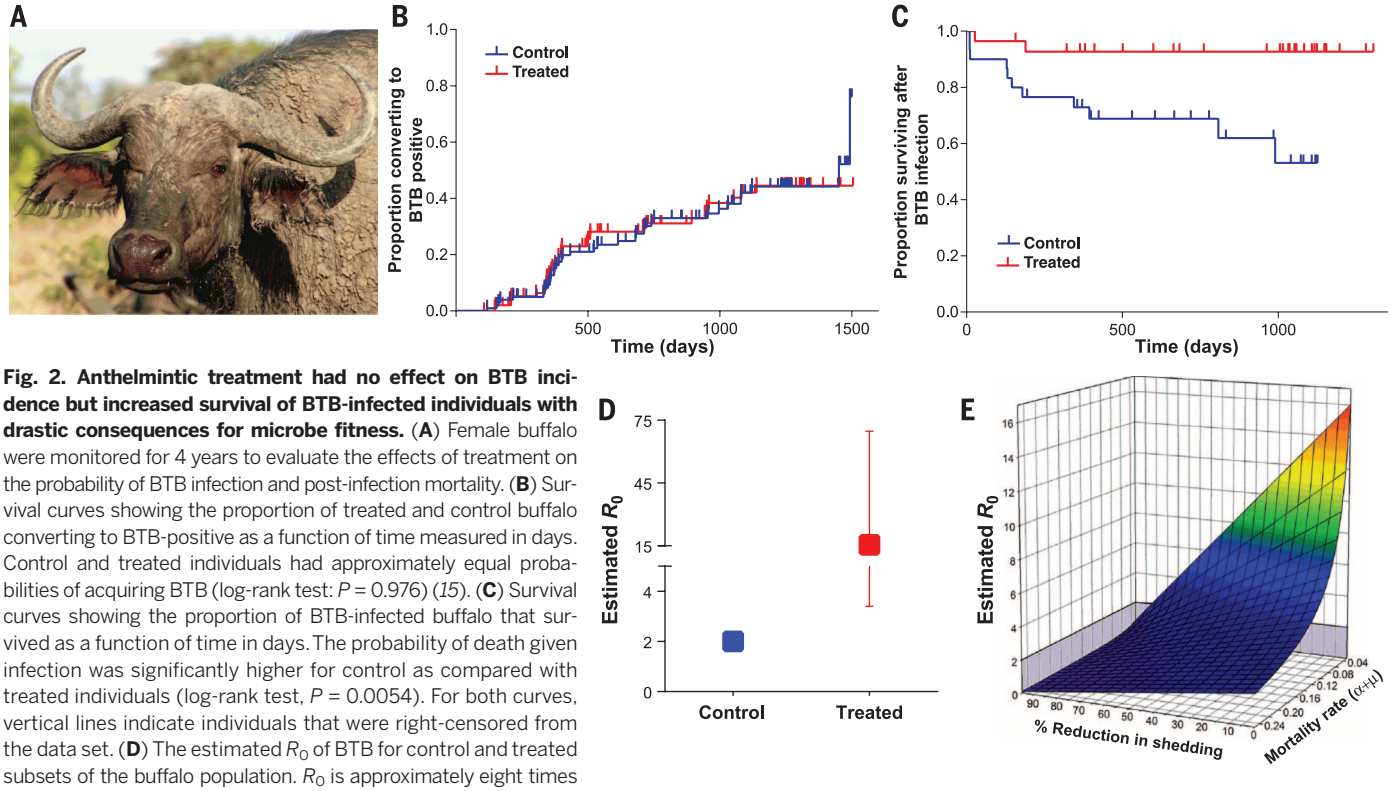

Fig. 2. Anthelmintic treatment had no effect on BTB incidence but increased survival of BTB-infected individuals with drastic consequences for microbe fitness. (**A**) Female buffalo were monitored for 4 years to evaluate the effects of treatment on the probability of BTB infection and post-infection mortality. (**B**) Survival curves showing the proportion of treated and control buffalo converting to BTB-positive as a function of time measured in days. Control and treated individuals had approximately equal probabilities of acquiring BTB (log-rank test: $P = 0.976$) (*15*). (**C**) Survival curves showing the proportion of BTB-infected buffalo that survived as a function of time in days. The probability of death given infection was significantly higher for control as compared with treated individuals (log-rank test, $P = 0.0054$). For both curves, vertical lines indicate individuals that were right-censored from the data set. (**D**) The estimated R_0 of BTB for control and treated subsets of the buffalo population. R_0 is approximately eight times higher for treated individuals (2 versus 15.5), with upper and lower estimates of 3.4 and 69.8, respectively (CIs were not calculated for controls). (**E**) Estimated R_0 of BTB across the range of mortality rates observed for treated buffalo and control buffalo (~0.03 to 0.24), accounting for possible reductions in bacteria shedding due to treatment (range, 0 to 90%). The area shaded in gray shows the baseline R_0 for control buffalo ($R_0 = 2$). At a mortality rate of ~0.03, as observed for treated buffalo, a reduction in shedding of at least 90% is needed to decrease R_0 to baseline levels.

Large-scale treatment programs that target human helminth infections are expanding around the globe (*27*). Reduced morbidity in individuals with microbial co-infections is considered a potential added benefit of such treatment programs (*27*, *28*), but based on our findings with BTB, anthelmintic treatment could improve individual morbidity or mortality and simultaneously exacerbate pathogen transmission. This is especially likely for chronic infections such as HIV/AIDS and TB, two human microbial diseases for which anthelmintic treatment strategies are being considered (*10*, *11*). Studies exploring individual- and population-level consequences of different intervention strategies, ranging from mass deworming alone to combined anthelmintic and microbial treatments (*13*), are urgently needed to establish under what conditions anthelmintic therapy is more likely to alleviate, or exacerbate, the health impacts of microbial co-infections.

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Tables S1 to S7
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