

INTERACTIONS BETWEEN MACROPARASITES AND MICROPARASITES DRIVE INFECTION PATTERNS IN FREE-RANGING AFRICAN BUFFALO

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Abstract. Epidemiological studies typically focus on single-parasite systems, although most hosts harbor multiple parasite species; thus, the potential impacts of co-infection on disease dynamics are only beginning to be recognized. Interactions between macroparasites, such as gastrointestinal nematodes, and microparasites causing diseases like TB, AIDS, and malaria are particularly interesting because co-infection may favor transmission and progression of these important diseases. Here we present evidence for strong interactions between gastrointestinal worms and bovine tuberculosis (TB) in free-ranging African buffalo (*Syncerus caffer*). TB and worms are negatively associated at the population, among-herd, and within-herd scales, and this association is not solely the result of demographic heterogeneities in infection. Combining data from 1362 buffalo with simple mechanistic models, we find that both accelerated mortality of co-infected individuals and TB transmission heterogeneity caused by trade-offs in immunity to the two types of parasites likely contribute to observed infection patterns. This study is one of the first to examine the relevance of within-host immunological trade-offs for understanding parasite distribution patterns in natural populations.

Key words: African buffalo; bovine tuberculosis; co-infection; gastrointestinal nematode; immunological trade-off; *Mycobacterium bovis*; parasite interaction; *Syncerus caffer*; synergistic mortality.

INTRODUCTION

Emerging infections are attracting increasing public attention as major threats to human, animal, and ecosystem health (Daszak et al. 2000, Corvalan et al. 2005). Parasite distributions are shifting at an unprecedented rate as a result of processes such as climate change, biodiversity loss, land use change, and the environmental impacts of globalized economies (Patz et al. 2000, Weiss and McMichael 2004, Eisenberg et al. 2006, Parmesan 2006). As host populations become exposed to novel combinations of parasites, it will be critical to understand how interactions between parasites may affect disease dynamics. Parasites can interact competitively or facilitatively, by modifying each other's transmission efficiencies (Lietman et al. 1997, Rodriguez et al. 1999) and virulence (Harries et al. 2001, Graham et al. 2005), or by removing hosts from a shared susceptible pool (Rohani et al. 2003). Immunological mechanisms for macroparasite–microparasite interactions, in particular, are well documented. The mammalian adaptive immune response mobilizes distinct pathways to control intracellular microparasites (e.g., viruses, most bacteria) vs. extracellular macroparasites (e.g., helminths). The

immune response to typical intracellular parasites involves T helper 1 (Th1) cells, while defenses against extracellular parasites are primarily mediated by T helper 2 (Th2) cells. The two pathways are cross-regulated; cytokines (messenger molecules) produced by Th1 cells suppress Th2 immune function and vice versa (Abbas et al. 1996, Yazdanbakhsh et al. 2002). Laboratory-based studies have shown that it may be difficult for a host to mount effective Th1 and Th2 immune responses simultaneously (Morel and Oriss 1998). The potential importance of cross-regulated immune defenses for disease control and public health has been recognized (Bentwich et al. 1999, Elias et al. 2006, Hotez et al. 2006), but their relevance to the dynamics of infectious diseases in wildlife populations has yet to be demonstrated.

Here we present evidence for interactions between gastrointestinal worms and bovine tuberculosis (TB) in free-ranging African buffalo (*Syncerus caffer*). We describe a strong negative correlation between the two types of parasite, and use a combination of empirical data and disease dynamic models to explore the mechanistic basis underlying this pattern. This is one of the first studies to link immune-mediated macroparasite–microparasite interactions to population-level infection patterns in a wild mammalian host (but see Cattadori et al. 2007).

African buffalo are gregarious bovids known to host a diverse assemblage of macroparasites and micropara-

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sites (Penzhorn 1996). Most nematodes found in buffalo are relative generalists in their host choice and have also been recorded in livestock or other wild bovid species (Penzhorn 1996, Ezenwa 2003). Bovine tuberculosis (caused by *Mycobacterium bovis*) is an exotic and emerging wildlife disease in sub-Saharan Africa (Michel et al. 2006) that chronically infects a wide range of mammalian hosts (Bengis 1999). In African savanna ecosystems, buffalo appear to be the primary maintenance hosts of the disease (DeVos et al. 2001), and TB-infected buffalo can suffer declines in body condition (Caron et al. 2003), adult survival, and fecundity (Jolles et al. 2005). To understand the consequences of interactions between TB and worms for buffalo hosts and parasite dynamics, we collected data on both types of infection and host age, sex, and herd affiliation for 1362 individual buffalo captured between 2001 and 2006 in Hluhluwe-iMfolozi Park, South Africa. We found negative association between infection with worms and TB and investigated three nonexclusive hypotheses that could contribute to this pattern: (1) demographic (age-sex) heterogeneity in infection; (2) increased mortality of co-infected individuals; and (3) trade-offs between immune defenses against microparasites and macroparasites.

METHODS

Study site and population

Hluhluwe-iMfolozi Park (HIP) is located in the province of KwaZulu-Natal (KZN), South Africa, and comprises almost 900 km². The buffalo population in HIP consists of ~3000 individuals in fairly stable herds of 70–180 animals. Herds occupy well-defined, largely nonoverlapping home ranges (Dora 2004). TB was first diagnosed in a buffalo at HIP in 1986 (Jolles et al. 2005). Buffalo were captured as part of the HIP TB control program conducted by KwaZulu-Natal Wildlife, the organization managing HIP. Buffalo herds were chased into a capture corral using a helicopter. All captured animals were marked with brands to allow identification of recaptured animals. Buffalo were tested for TB using a tuberculin skin test, with sensitivity 80.9% and specificity 90.2% (J. P. Raath, unpublished data). TB-negative animals were released; TB-positive animals were slaughtered. The data presented in this study were collected between April and November 2001–2002 and 2004–2006.

Age and body condition determination

In juveniles up to two years (no permanent incisors yet) we estimated age according to body size and horn development. In animals 2–5 years old, we determined age from incisor emergence patterns (Grimsdell 1973), and for buffalo aged 6+ years we used tooth wear of incisor one to estimate age (Jolles 2007). Body condition was measured by visually inspecting and palpating the animals, focusing on coat condition and areas where body fat is typically stored in buffalo (ribs, spine, hips,

and the base of the tail). Each area was scored on a scale of 1 (very poor) to 5 (excellent), and a body condition index calculated as the average of all five areas. This index is strongly correlated with the kidney fat index (Ezenwa et al., *in press*), and similar body condition indices have been used by Prins (1996) and Caron et al. (2003).

Nematode infection

We used fecal egg counts (FEC) as a nonlethal method to estimate gastrointestinal (GI) nematode prevalence and intensity (Coltman et al. 2001, Ezenwa 2004, Gillespie and Chapman 2006). GI nematode infections in buffalo at HIP are caused by genera such as *Haemonchus*, *Cooperia*, and a newly described genus, *Africanastrongylus* (Hoberg et al. 2008, A. E. Jolles and V. O. Ezenwa, unpublished data). Although the relationship between FEC and actual parasite intensity can vary, this measure has been shown to be a reliable estimate of relative GI parasite infection rates across hosts (Stear et al. 1995, Cabaret et al. 1998, Seiwright et al. 2004). Fecal samples were collected rectally while the buffalo were immobilized for TB testing. Egg counts were performed using a modified McMaster method (MAFF 1980). From 2001 to 2005, FECs were performed by the KZN State Veterinary Laboratory (Allerton, South Africa); thereafter all egg counts were performed in our field laboratory at HIP. We investigated patterns of co-infection with worms and TB by scoring individuals as positive or negative for worm infection, according to the presence or absence of worm eggs in their feces.

Immunology

Blood samples for immunology measurements were collected from 280 buffalo aged three years and older in 2005–2006. We focused on this age group to avoid variability due to maturation of the immune system in younger animals. Eosinophil counts were used to assess Th2 responsiveness (Scott and Koski 2000, Claerebout et al. 2005). White blood cell counts and differentials were performed by the pathology laboratory of Dr. Bouwer and Partners (Durban, South Africa) on an Advia 120 automated analyzer (Bayer, Leverkusen, Germany). To assess Th1 responsiveness, we measured the IFN γ response (IFN γ is interferon-gamma, a key cytokine involved in Th1 immune responses; Grobler et al. 2002, Claerebout et al. 2005) to *in vitro* challenge with *M. bovis* antigen (bovine tuberculin), following standard protocols developed for IFN γ -based TB-testing in buffalo (Grobler et al. 2002). Briefly, blood samples were partitioned into two aliquots. Bovine tuberculin (*M. bovis* purified protein derivative) was added to one sample, no addition was made to the control sample, and both samples were incubated at 37°C for 28 hours. Following incubation, the plasma was pipetted off and plasma IFN γ titers were assessed using ELISA (enzyme-linked immunosorbent assay). We quantified Th1 response as the proportionate change

in IFN γ titers, $\ln([\text{IFN}\gamma \text{ (bovine)}]/[\text{IFN}\gamma \text{ (control)}])$, where IFN γ (bovine) is the concentration of IFN γ measured in samples stimulated with bovine tuberculin, and IFN γ (control) is the concentration of IFN γ measured in control samples, representing circulating IFN γ levels.

Statistical analyses

TB and worm infection data were collected from 1362 individual buffalo in 24 herds, and 192 recaptured animals were excluded from population-level analyses. Similarly, to avoid pseudoreplication, we excluded six recaptured herds from comparisons among herds, leaving a final sample size of 18 herds in among-herd level analyses. To examine infection patterns within herds we included all 24 herds because it is individuals within herds that are being compared to one another at this level of analysis.

We computed the expected number of co-infected hosts, if worms and TB operate independently from one another, as $E_{\text{TB}\wedge\text{W}} = Np_{\text{TB}}p_{\text{W}}$, where N is the number of hosts and p_{TB} and p_{W} are the prevalences of TB and (subscript \wedge) worms among the host population. The expected number of single infections with worms or (subscript \vee) TB was $E_{\text{TB}\vee\text{W}} = (Np_{\text{TB}} - E_{\text{TB}\wedge\text{W}}) + (Np_{\text{W}} - E_{\text{TB}\wedge\text{W}})$, and we used a χ^2 test to determine whether the observed distribution of infections differed from this null expectation. We used correlation tests to examine the relationship between TB and worm infection status across the whole population, and the relationship between TB prevalence and worm prevalence among herds. We used a paired t test to assess whether TB prevalence differed among worm-positive and worm-negative hosts within the same herds.

In all analyses subsequent to the initial description of infection patterns, we treated TB status as the response and worm status as the independent variable. To perform multivariate analyses on possible effects of demographic factors on infection, along with effects of one infection on the other, we assigned directionality. We chose to assign TB as the response for two reasons. First, worms usually precede TB infection temporally, based on the age-prevalence curves for both infections (Fig. 1a). Second, we found no evidence for an immunologic effect of TB infection on Th2 immunity, which is the main pathway involved in defenses against gut helminths (comparison of eosinophil counts in TB-positive vs. TB-negative hosts: t test, $df = 559$, $t = 0.419$, $P = 0.676$). By contrast, we found evidence for an effect of worms on susceptibility to TB (see *Results: Immunological trade-offs*).

We used multivariate logistic regressions to ask to what extent host age, sex, and herd affiliation explained the observed association between host worm infection status and TB status. To assess the significance of the regression coefficient for each of the variables, we tested its Wald statistic against the χ^2 distribution. The Wald statistic is based on the asymptotic normality property

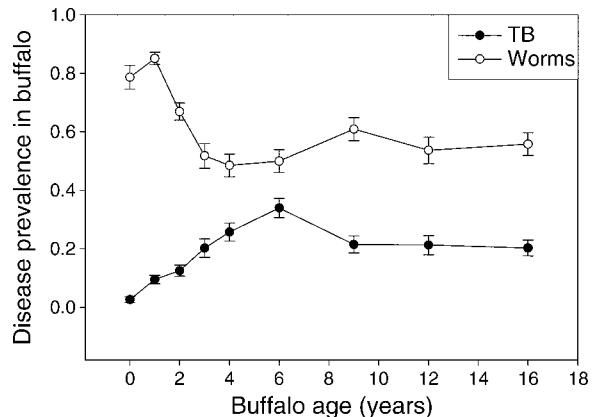


FIG. 1. Age-prevalence plots for TB and worms. Age is a significant predictor of TB status in buffalo up to five years old, and of worm status in animals up to two years old; beyond these ages, age has no effect on infection status. Values are means \pm SE.

of maximum likelihood estimates, and is computed as $W = v^2/\text{var}(v)$, where v is the maximum likelihood parameter estimate, and $\text{var}(v)$ stands for the asymptotic variance of the parameter estimate.

We used linear regression to examine whether herd age composition and sex ratio might drive the association between herd worm prevalence and TB prevalence. We described herd age composition simply as the proportion of juveniles 0–2 years of age, because worm prevalence changes little when buffalo are older than three years (Fig. 1a).

To assess worm aggregation in TB-positive and TB-negative buffalo, we computed the $\log(\text{variance}/\text{mean})$ of FEC. We included only hosts that were infected with worms, so that our measure of worm aggregation depends only on intensity of infection in TB-positive and TB-negative buffalo and is not skewed by differences in worm infection prevalence. We used a bootstrap test to evaluate whether differences in worm aggregation between TB-positive and TB-negative buffalo were caused by different sample sizes for the two groups: we randomized TB status among all observed FEC, then compared the difference in $\log(\text{variance}/\text{mean})$ of FEC with the distribution of differences from 10 000 bootstrap runs to assess the likelihood of observing our results if there was no difference in aggregation between TB-positive and TB-negative buffalo. We used linear regression to examine worm aggregation patterns among herds; and a paired t test to test for differences in worm aggregation between TB-negative and TB-positive buffalo residing in the same herds. Our within-herd analysis of aggregation was based on 16 rather than 18 herds, because two herds lacked co-infected buffalo. We tested the hypothesis that worm infection was associated with poor body condition using one-tailed t tests.

We used factorial ANOVA to examine the effects of worm infection status and season (October, following

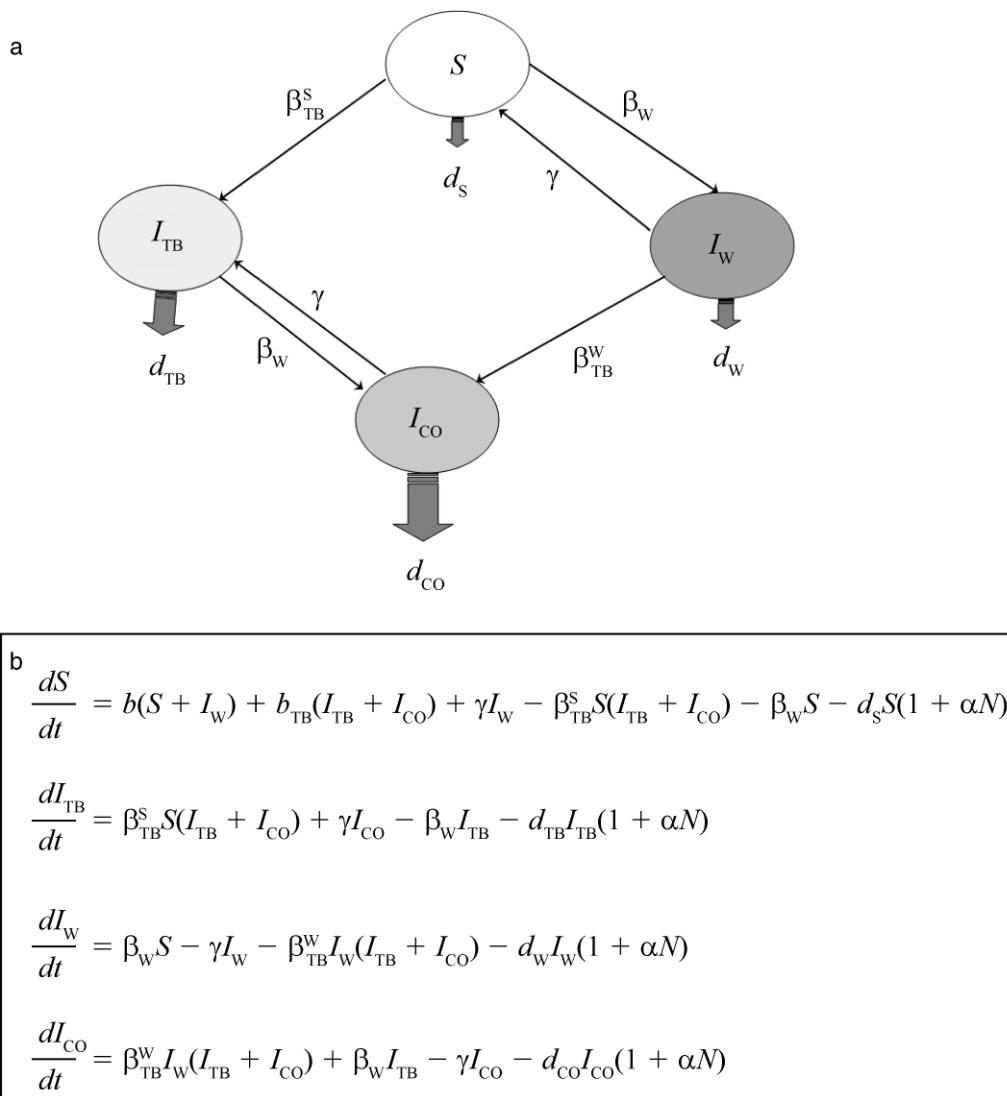


FIG. 2. Disease dynamic model. (a) Schematic representation of the model; (b) model equations. We partitioned hosts into four groups: susceptibles (S), hosts infected with TB only (I_{TB}), hosts infected with worms only (I_W), and co-infected hosts (I_{CO}). The total host population is $N = S + I_{TB} + I_W + I_{CO}$. Hosts are born to TB-negative hosts at rate b and to TB-positive hosts at rate b_{TB} . They die at rates specific to each group, d_S , d_{TB} , d_W , and d_{CO} . For models examining effects of d_{CO} only, $\beta_{TB}^S = \beta_{TB}^W$; for models examining effects of d_{CO} in combination with transmission heterogeneity, $\beta_{TB}^S = 3 \times \beta_{TB}^W$. Parameter values: $\alpha = 0.02$; $b = 0.37$; $b_{TB} = 0.27$; $d_S = 0.05$; $d_{TB} = 0.15$ (Jolles et al. 2005); $d_W = d_S$; $\beta_{TB} = 0.04$ (Cross and Getz 2006); $\gamma = 0.5$; $0.1 \leq \beta_W \leq 0.9$. Low $d_{CO} = d_{TB} = 0.15$, intermediate $d_{CO} = 0.3$, and high $d_{CO} = 0.6$.

the dry season; or May, at the end of the wet season) on Th2 immune function, and linear regression of IFN γ response ($\ln([\text{IFN}\gamma \text{ (bovine)}]/[\text{IFN}\gamma \text{ (control)}])$, dependent variable) on eosinophil counts (independent variable) to evaluate the evidence for immunological cross regulation.

Disease dynamic model

We used a simple deterministic model (Fig. 2) to evaluate whether immunological trade-offs and elevated mortality in co-infecteds could produce the observed infection patterns. Most TB transmission is likely to

occur among members of the same herd because within-herd contacts are far more frequent than among-herd contacts: radiotracking data indicate that herd home ranges at HIP overlap very little (Dora 2004) and dispersal among herds is limited (A. E. Jolles, *unpublished data*). We therefore focused on disease transmission within herds, setting the modeled population size at $N = 100$ individuals to reflect typical herd size at HIP, and assuming that TB transmission follows mass action. There is no evidence that animals recover from TB (Bengis 1999), so we did not include a recovered class in our model. Mortality was density dependent, with the

strength of density dependence governed by parameter α (Jolles et al. 2006). Because conditions for worm hatching success and larval survival vary across the park, we allowed the transmission coefficient for worms, β_W , to vary among herds. We assumed exposure to infective worm larvae to be independent of herd worm burden, as most of the nematodes found in buffalo are host generalists (and thus larvae originating from alternate host species are likely to mask a possible positive relationship between herd worm burden and exposure); and we did not track individual worm burdens. Worm infection was treated as a dynamic process with infection rate β_W and recovery rate γ . We set $\gamma = 0.5$ in our model, but this somewhat arbitrary choice did not affect our main conclusions (see sensitivity analysis in Appendix A). Hosts recovering from worm infection are susceptible to reinfection. Our model did not include an immune class with respect to helminth infection because, in contrast to mammalian responses to microparasites, immunity to intestinal helminths is typically partial and short-lived (Anderson and May 1991). As we do not have data to quantify effects of worms on host fecundity and mortality, we assumed no such effects in the interest of model simplicity.

To examine whether elevated mortality of co-infected hosts could produce a negative correlation between worms and TB, we varied d_{CO} between d_{TB} and $4d_{TB}$. To evaluate the effect of transmission heterogeneities, due to differences in host susceptibility, we distinguished between β_{TB}^W , (transmission rate of TB to worm-infected hosts), and β_{TB}^S , (transmission rate of TB to uninfected hosts), with $\beta_{TB}^S = \delta \times \beta_{TB}^W$, where the coefficient δ is a positive number between one and five (sensitivity analysis in the Appendix B). For the results presented in this paper, we chose the middle of this range, $\delta = 3$. We assumed that TB status does not affect susceptibility to worm infection because we found no evidence of any effect of TB status on Th2 immune function. Thus in our model the transmission rate of worms to TB-infected host equals the transmission rate of worms to uninfected hosts: $\beta_W^{TB} = \beta_W^S = \beta_W$.

RESULTS

Patterns of infection

TB prevalence varied between 2.2% and 72.5% in the 18 herds examined, averaging 21.6%. Mean herd prevalence of worm infection was 58.6%, varying between 11.9% and 87.5%. We examined patterns of co-infection at three scales: aggregated across the entire population, among herds, and within herds. Our aggregate sample of 1362 buffalo was composed of 344 individuals (25.3%) that were not infected with either parasite, 147 (10.8%) with TB only, 757 (55.6%) with worms only, and 114 (8.4%) that were co-infected. This distribution represents an excess of single infections (expected, 798; observed, 904; 13% more than expected) and a deficit of co-infections (expected, 167; observed,

114; 32% less than expected) compared to the expectation that the two parasites operate independently of one another ($\chi^2 = 57.55$, $P < 0.000001$). Across the whole population, worm infection status was thus strongly negatively correlated with TB status (Fig. 3 inset). When comparing TB and worm prevalence among herds, we found that herds with high worm prevalence tended to have low TB prevalence (Fig. 3); and within each herd, TB prevalence was higher in worm-negative than worm-positive individuals (two-tailed paired t test, $N = 24$ herds, $t = 2.72$, $P = 0.0122$). A negative association between TB and worms thus exists at the population (aggregate), among-herd, and within-herd scales.

Several different mechanisms might underlie these patterns, and different mechanisms may be important at different scales (Lafferty et al. 1994). We investigated to what extent (1) demographic (age/sex) heterogeneity in infection, (2) increased mortality of co-infected hosts, and (3) trade-offs in immunity to worms and TB, may play a role.

Demographic heterogeneity in infection.—If infection prevalence varies by age and sex, a positive or negative association between worms and TB could result without any interaction between the two types of parasite. The prevalence of TB and worm infection varied with age (Fig. 1). TB prevalence increased with age up to 5 years, but remained approximately constant thereafter; and sex did not affect TB status in either age group (logistic regression: age ≤ 5 yr, $N = 943$, Wald statistic [age] = 67.748, $P < 0.000001$, Wald statistic [sex] = 0.5625, $P = 0.3353$; age > 5 yr, $N = 419$, Wald statistic [age] = 1.2363, $P = 0.2265$, Wald statistic [sex] = 1.4626, $P = 0.2265$). Worm prevalence was high in calves and yearlings, but declined in the following two years and remained roughly constant from age 3 years onward. Sex had no effect on worm infection prevalence in either age group, but considering animals of all ages together revealed that males were slightly less likely to be infected with worms than females (logistic regression: age ≤ 2 yr, $N = 607$, Wald statistic [age] = 19.0673, $P = 0.000013$, Wald statistic [sex] = 0.9370, $P = 0.3331$; age > 2 yr, $N = 755$, Wald statistic [age] = 0.1721, $P = 0.6783$, Wald statistic [sex] = 2.9558, $P = 0.08557$; all ages, $N = 1362$, Wald statistic [age] = 50.4128, $P < 0.000001$, Wald statistic [sex] = 5.4763, $P = 0.0193$). Host age, and to a lesser degree, host sex, may thus contribute to the observed association between worms and TB in buffalo.

To ask whether demographic patterns of infection explained the observed association between worms and TB at the population level, we included host sex and age in a logistic regression analysis. This did not change the result that worm status was a strong negative predictor of TB status, but age also had a significant effect. Sex had no effect on TB status, but the interaction between sex and worms was significant (logistic regression: $N = 1362$; age, Wald statistic = 5.03, $P = 0.0248$; sex, Wald statistic = 1.62, $P = 0.2036$; worm status, Wald statistic = 50.95, $P < 0.0000001$; sex \times worm status, Wald statistic

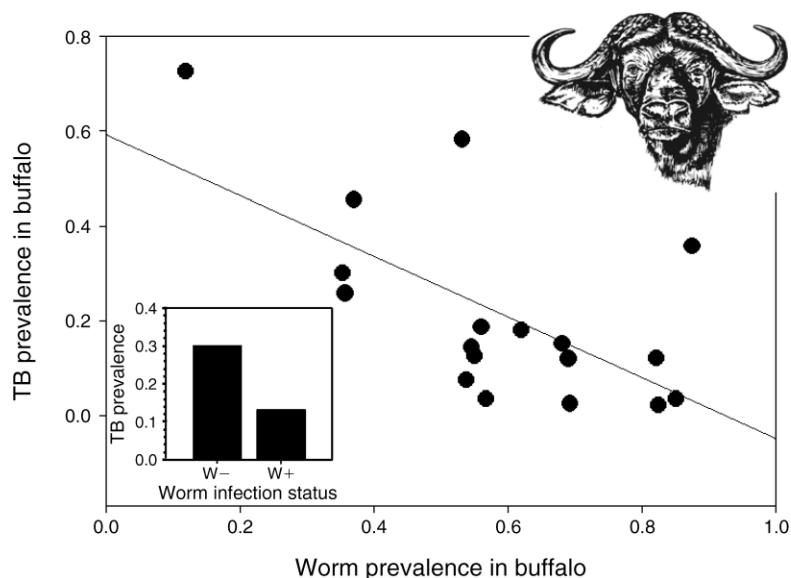


FIG. 3. Negative correlation between TB prevalence and worm infection prevalence in African buffalo at the herd level (main figure; correlation, $N = 18$ herds, $r = -0.64$, one-tailed $P < 0.0025$) and across the entire population (inset figure; correlation, $N = 1362$ buffalo, $r = -0.21$, $P < 0.000001$).

$= 4.58$, $P = 0.0323$). Worm status had a stronger effect on TB status in males than in females (TB prevalences [weighted marginal means and standard errors]: worm-free males = $29.90\% \pm 3.30\%$, worm-free females = $29.97\% \pm 2.66\%$, worm-infected males = $9.14\% \pm 1.57\%$, worm-infected females = $15.60\% \pm 1.57\%$).

To control for the potential effect of demographic factors at the herd level, we included the proportion of juveniles and sex ratio in our analysis. Because juveniles have higher worm prevalence and lower TB prevalence than adults, herds with low worm prevalence might simply be deficient of juveniles, composed mostly of adults with higher TB prevalence. Adding these factors did not change the finding that herd worm prevalence was negatively associated with herd TB prevalence; and neither the proportion of juveniles younger than three years, nor the herd sex ratio had any significant effect (linear regression, $N = 18$ herds, $F_{3,14} = 3.91$, $R^2 = 0.46$; worm prevalence, $P < 0.0043$, $\beta = -0.71$; proportion of juveniles, $P = 0.4814$, $\beta = 0.16$; sex ratio, $P = 0.3150$, $\beta = 0.24$).

To test whether age-related infection patterns accounted for the observed difference in TB prevalence between worm-infected and worm-free hosts within the same herds, we added "herd affiliation" as an independent variable to our analysis of the aggregate (population level) data while also controlling for age and sex. After thus accounting for herd affiliation, worm status was still a significant predictor of TB status (logistic regression, $N = 1362$; age, Wald statistic = 9.19, $P = 0.0024$; sex, Wald statistic = 0.0001, $P = 0.9905$; herd affiliation, Wald statistic = 224.66, $P < 0.000001$; worm status, Wald statistic = 8.90, $P = 0.0029$; sex \times worm status, Wald statistic = 7.73, $P = 0.0054$). These results

suggest that while host age, sex, and herd affiliation clearly play a role in determining infection status, the observed negative association between worms and TB is robust to controlling for these factors.

Mortality.—Co-infected individuals may suffer accelerated mortality, such that observed animals tend to be infected with one parasite or the other, but not both. To assess the evidence for this hypothesis, we examined two traits that are indicators of mortality risk: parasite aggregation and host body condition.

1. **Parasite aggregation.**—Macroparasites tend to be highly aggregated across hosts, with most hosts having low to moderate parasite burdens, and only few hosts being heavily infected (Shaw et al. 1998). If co-infection accelerates host mortality, TB positives simultaneously suffering the highest costs of worm infection should experience the greatest mortality risk. Worm infections are likely to be most costly in individuals with high fecal egg counts (FEC), because worm egg production (the product of worm burden and worm fecundity) is a direct result of resources transferred from the host to its parasites. Loss of TB-positive animals with high FEC should result in a more even distribution of worms across the remaining TB-positive hosts. The mortality hypothesis therefore predicts an absence of TB-positive animals with high FEC, and a lower degree of parasite aggregation among TB positives than TB negatives.

Consistent with the mortality hypothesis, we found the frequency distribution of FEC in the TB-positive population to be truncated compared to the distribution in TB negatives (Fig. 4a). As a result, worms were more aggregated among TB-negative buffalo than among TB positives ($\log(\text{variance}/\text{mean})$ FEC = 3.073 and 2.696, respectively; bootstrap test, $P = 0.0147$). This pattern

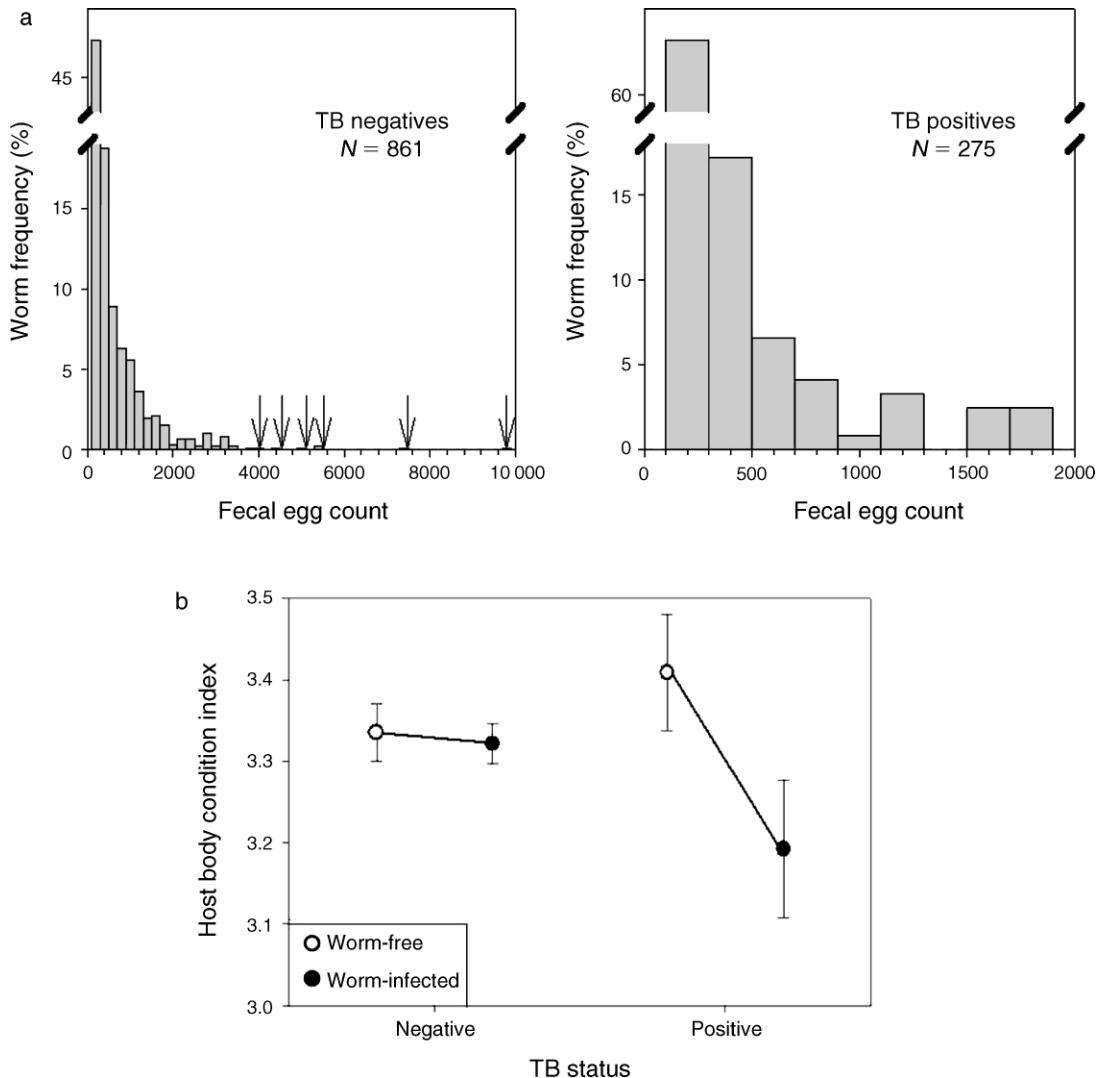


FIG. 4. Evidence for accelerated mortality in co-infected individuals. (a) Worm aggregation, showing a comparison of worm frequency distributions (as measured by a count of worm eggs in buffalo feces) in TB-negative and TB-positive hosts. Arrows point to rare observations of extreme fecal egg counts. (b) Host body condition. Worm infection is associated with poor body condition in TB-positive hosts (one-tailed t test, $N = 129$ buffalo, $t = 1.891$, $P = 0.0304$), but not in TB-negative hosts (one-tailed t test, $N = 1032$ buffalo, $t = 0.304$, $P = 0.3806$).

persisted when worm aggregation was compared across herds; herds with higher TB prevalence had more even worm distributions than herds with low TB prevalence (linear regression, $N = 18$ herds, $F_{1,16} = 15.16$, $R^2 = 0.49$, $P = 0.0013$, $\beta = -0.70$), and controlling for the proportion of hosts older than two years made no difference to this result (linear regression, $N = 18$ herds, $F_{2,15} = 6.11$, $R^2 = 0.45$; TB prevalence, $P < 0.0032$, $\beta = -0.67$; proportion > 2 yr, $P = 0.7667$, $\beta = -0.06$). At the within-herd scale, TB-positive hosts had more even worm distributions than TB-negative buffalo from the same herds (two-tailed paired t test, $N = 16$ herds, $t = 2.53$, $P = 0.0228$).

2. *Host body condition.*—Chronic infections are typically associated with declines in host body condition,

which may contribute to increased mortality risk, particularly for co-infected individuals. If co-infected hosts suffer increased mortality risk, we expected them to be in worse condition than either uninfected buffalo or buffalo with single infections. Accordingly, we found that the impact of worm infection on body condition was significantly more pronounced among individuals co-infected with TB (Fig. 4b).

To evaluate whether increased mortality of co-infected buffalo could produce the observed infection patterns, we constructed a simple disease dynamic model (Fig. 2). We partitioned hosts into four groups—susceptibles (S), hosts infected with TB only (I_{TB}), hosts infected with worms only (I_W), and hosts infected with both worms and TB (I_{CO})—and assigned death rates

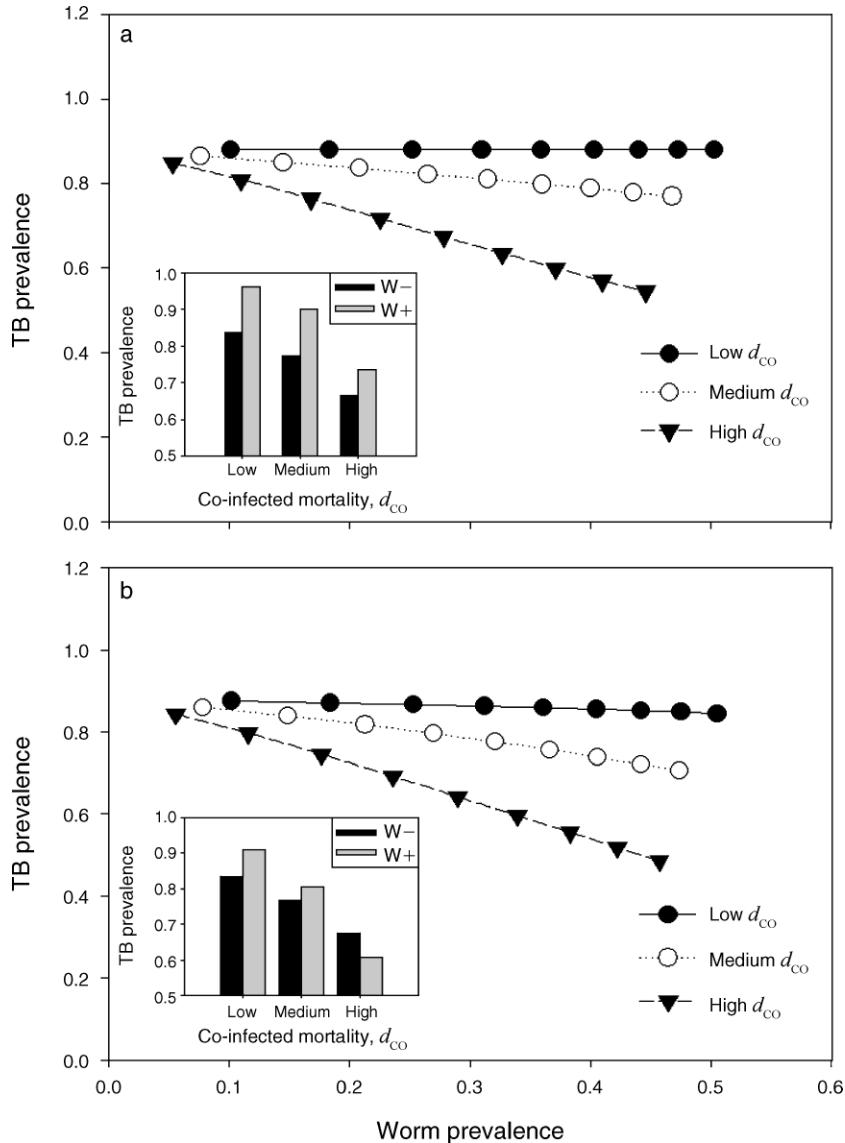


FIG. 5. Results from the dynamic model, based on: (a) elevated mortality of co-infected hosts (d_{CO}); and (b) transmission heterogeneity due to immunological interaction between worms and TB, as well as elevated d_{CO} . TB prevalence in figure insets in (a) and (b) was averaged across all levels of worm prevalence; black bars denote worm-negative hosts (W-), and gray bars denote worm-positive hosts (W+). High mortality of co-infected hosts alone can produce a negative correlation between worm prevalence and TB prevalence at the herd level (main figures a, b) but not at the individual level (inset a); transmission heterogeneity alone has little effect on infection patterns (b, low d_{CO}), but combined effects of immune interaction and high d_{CO} qualitatively reproduce observed infection patterns at both the herd (main figure b) and individual level (inset b, high d_{CO}).

(d_S , d_{TB} , d_W , and d_{CO}) to each group. We assumed that $d_S = d_W < d_{TB} < d_{CO}$, and varied d_{CO} between d_{TB} and $4d_{TB}$. Model results show that high mortality in co-infected hosts alone can reproduce observed patterns at the among-herd level, but not at the level of individuals within herds (Fig. 5a). Empirical and theoretical evidence thus suggest that high mortality in co-infected hosts may be important, but not sufficient to generate the observed infection patterns.

Immunological trade-offs.—The absence of worm infection may be indicative of a strong immune response

to GI nematodes, and animals with effective defenses against worms may be less capable of simultaneously mounting immune defenses against TB. This hypothesis is based on the immunological paradigm of cross-regulated immune responses to different types of parasites. To test the Th1–Th2 cross-regulation hypothesis, we measured indicators of Th1 and Th2 immunity in 280 adult buffalo. Worm-free buffalo had stronger Th2 (macroparasite) immunity than animals infected with worms (factorial ANOVA, $N = 280$; season, $F = 14.49$, $P < 0.0002$; worm status, $F = 4.39$, $P = 0.0372$;

interaction, $F = 0.43$, not significant); and there was a significant negative correlation between Th1 and Th2 immunological activity in TB-negative buffalo captured in the dry season (linear regression, $N = 97$; $F = 5.72$; $P = 0.0187$, coefficient = -0.24 , Fig. 6a), but not in the rainy season (linear regression, $N = 130$; $F = 1.64$; $P = 0.2025$, coefficient = 0.11 , Fig. 6b). These data are consistent with the hypothesis that buffalo that mount a strong immune response to worms, and are thus worm free, may be more susceptible to TB than those infected with worms. When heterogeneity in TB susceptibility due to these immunological trade-offs was included in our model (along with elevated mortality in co-infected hosts), we were able to reproduce observed patterns of infection at both the herd and individual levels (Fig. 5b). It should be noted that immunological trade-offs alone failed to reproduce the negative correlation between TB and worms (see results for low d_{CO} in Fig. 5b); thus, only the combination of two mechanisms, immunological cross-regulation and increased co-infected mortality, yielded model results that are qualitatively similar to the observed infection patterns.

DISCUSSION

Although interactions among gut helminths (Lello et al. 2004) and among viral and bacterial childhood diseases (Rohani et al. 2003) have been described for humans and other vertebrate host systems, this study is one of the first to reveal infection patterns consistent with strong interactions between macroparasites and a microparasite in a free-ranging wildlife population. Our data show that in African buffalo, TB and worm infections are negatively correlated across different scales (within and between herds and at the whole-population level). Field data on host mortality and immunity provide empirical support, and our models lend theoretical validation to the notion that both immunological and population dynamic mechanisms may underlie the disease patterns we have documented.

In our study population we found evidence for high mortality of co-infected animals, compared to healthy hosts or those with single infections. This can be understood as an interesting variant of ecological interference, as described by Rohani et al. (2003). Ecological interference occurs when infection with one pathogen removes hosts from the susceptible pool, making them unavailable to a competing pathogen. In our case, neither parasite removes susceptibles very effectively on its own, but the two reduce each others' realized infection rates by shortening the duration of infectiousness of co-infected hosts. We also presented evidence of cross-regulated immunity to worms and TB in African buffalo. This effect was detectable in the dry season but not in the wet season, suggesting that in natural populations the classical Th1/Th2 trade-off may be more pronounced under conditions of resource limitation. Our data on cross-regulated immune responses do not allow us to distinguish between inducible

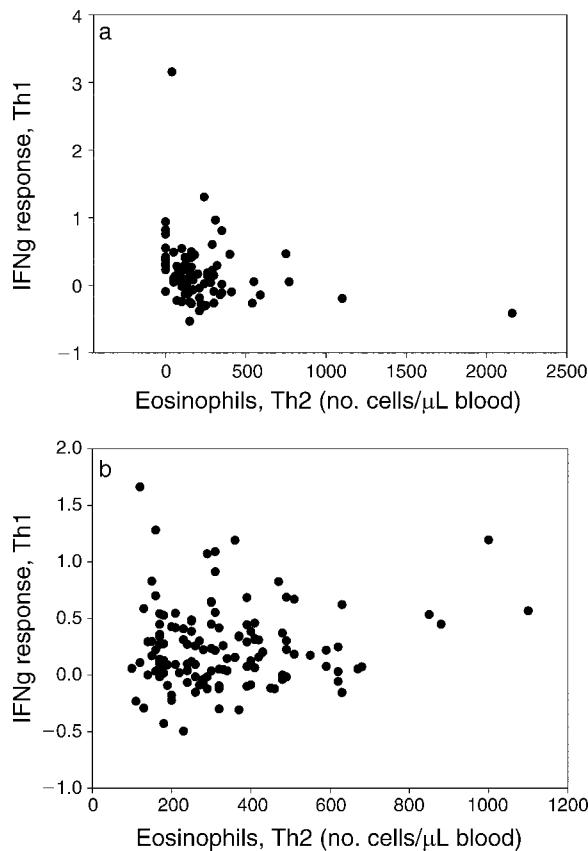


FIG. 6. Evidence for cross-regulation of Th1 and Th2 eosinophil immune responses in (a) October, but not in (b) May in adult (age ≥ 3 years) TB-negative buffalo. The immune response to typical intracellular parasites involves T helper 1 (Th1) cells, while defenses against extracellular parasites are primarily mediated by T helper 2 (Th2) cells. IFNg is interferon-gamma, a key cytokine involved in Th1 immune responses. IFNg response to in vitro challenge with *Mycobacterium bovis* antigen was measured as the log-transformed proportionate change in IFNg titers, $\ln([\text{IFNg}(\text{bovine})]/[\text{IFNg}(\text{control})])$. Note differences in scales between plots (a) and (b).

cross-regulation vs. fixed genetic predispositions of some hosts to a strong Th2 and weak Th1 response, and vice versa. This distinction does not affect our interpretation of the infection patterns; but it is relevant to the implications of this work for disease management and control. If we are seeing the effects of fixed genetic predispositions in immunity, then reducing exposure to GI nematodes will have no immediate effect on TB transmission. By contrast, if inducible cross-regulation of immune responses is involved, then measures reducing exposure to helminths may be effective in limiting the spread of TB. Genetic and immunological mechanisms are not mutually exclusive and further work is needed to validate either interpretation.

Synergistic effects of different parasites and pathogens on host mortality and morbidity are common (Bentwich et al. 1999, Harms and Feldmeier 2002, Joly and Messier 2005), and the results of our dynamic model show that

this can translate to negatively correlated infection patterns at the herd level. Across the whole population, however, high mortality of co-infected individuals alone appears unlikely to produce a negative association between TB and worm infection status. This is because co-infected mortality (d_{CO}) not only reduces the number of co-infecteds, but also cuts TB transmission by limiting contacts between susceptible and infected hosts. Thus, in terms of the model compartments, as d_{CO} increases, the number of co-infecteds (CO) and TB positives (TB) will tend to decrease, while the number of susceptibles (S) and worm positives (W) will increase as fewer animals are lost to TB transmission. With TB prevalence among worm positives = $CO/(CO + W)$, and TB prevalence among worm negatives = $TB/(TB + S)$, it is clear that increasing d_{CO} has similar effects on TB prevalence regardless of worm infection status, and as such cannot account for low TB prevalence in worm-infected hosts. When we add differences in TB susceptibility (resulting transmission heterogeneity) between worm-infected and worm-free animals to our model, we are able to reproduce both herd- and population-level patterns qualitatively. In this case, we are assuming TB transmission to be slower in worm-infected buffalo compared to susceptibles (S), which tends to reduce the number of co-infected individuals (CO) and increase the number of worm positives (W), reducing TB prevalence in worm-infected buffalo without affecting TB prevalence in worm-free buffalo. Both mechanisms in concert (co-infected mortality and heterogeneity in TB transmission) then generate lower TB prevalence in worm-infected compared to worm-free buffalo.

The key to reproducing the observed infection patterns thus appears to lie in the combination of the two mechanisms: neither mortality of co-infected hosts, nor immune-mediated transmission heterogeneity suffices on their own. The current model suggests that quite large differences according to worm infection are required in mortality (d_{CO} vs. d_{TB}) and TB transmission rate (β_{TB}^W vs. β_{TB}^S), to reproduce the empirical pattern of higher TB-prevalence in worm-free hosts (see Appendix A). Additional work will be needed to assess whether effects of this magnitude on mortality and TB transmission are realistic in this system, or whether additional mechanisms not captured by the current model are at work.

Several other mechanisms may play a role in generating the observed infection patterns, in addition to those examined in this study, including spatial heterogeneity in disease transmission and diagnostic biases. Buffalo herds in HIP do not range across the whole park but limit their activities to well-defined home ranges (Dora 2004). Vegetation and soil types vary across the park, and this habitat variability may affect host exposure to and transmission of TB and worms (Anderson 1993, Tanner and Michel 1999, Cunningham-Rundles et al. 2005, Smith et al. 2005). If habitats

favoring TB transmission differ from those favoring worm transmission, then differences in spatial habitat use between buffalo herds, and among individuals within a herd, may contribute to the observed negative correlation between worms and TB.

The validity of the intradermal tuberculin test we used to diagnose TB may depend on the stage of TB progression or on co-infections with helminths. Hosts in later stages of TB progression can become anergic and produce false-negative TB test results (Welsh et al. 2005). If worm infections are associated with differential TB progression, then this could introduce biases in detectability of TB according to worm infection status. Similarly, helminth infections have been shown to affect immune responses to TB vaccination in cattle (Flynn et al. 2007), where individuals exposed to helminths were less likely to mount a detectable immune response to TB vaccination than unexposed hosts. These findings beg the question whether co-infection could be systematically biasing TB tests to produce the negative correlation we observed here. In our study system we have shown that worm-free hosts have stronger Th2 immunity than hosts with worm infections. If Th2 immune responses interfered with our diagnostic tests, then we would expect to see a higher proportion of false-negative TB tests in animals with strong Th2 immunity. In our system this would lead to less detectable TB in worm-free animals, which is the opposite of the observed pattern. Thus it is possible that co-infection decreases the validity of our diagnostic test, but if anything the pattern of higher TB in worm-free animals should be even more pronounced if we could control for this putative bias. Nonetheless the possibility that co-infection may decrease TB test sensitivity is a serious concern for management of the disease in wild populations, which relies on identification and removal of TB-positive animals.

Our disease dynamic model is deliberately simplistic, designed to provide a general framework for interpreting the dynamics of mixed infections with directly transmitted microparasites and environmentally transmitted macroparasites. Several biologically relevant modifications of the model will merit further exploration. For example, our current model takes a mean-field approach to macroparasite infection, omitting differences in parasite burden among hosts. Realistically, worms are aggregated across hosts, and mortality of co-infected hosts is likely to be concentrated among heavily infected individuals. Introducing variable worm burdens into the model would set up a situation closely analogous to a host–parasite–predator interaction (Packer et al. 2003): like a predator selectively removing highly parasitized prey, a microparasitic disease might act to reduce macroparasite prevalence in host populations. Immunological differences between juvenile and adult hosts, along with seasonal variation in host susceptibility, may modify transmission dynamics of both parasites, and selective removal of hosts with high

macroparasite burdens may feed back on host exposure rates. Future empirical and theoretical work will be needed to refine model structure and parameters to deepen our understanding of the ecological consequences of co-infection.

Immunological mechanisms for macroparasite–microparasite interactions within individual hosts are well documented, but their dynamic consequences in natural host populations have not previously been explored. Our study suggests that the outcome of macroparasite–microparasite co-infection for the dynamics of the constituent parasite species results from a complex interplay of immunological and population dynamic processes. It may thus not be possible to fully understand infection patterns observed in nature by studying host–parasite species pairs in isolation, without the context of the broader parasite community supported by the host population. Our findings highlight the value of disease studies in natural populations as a complement to laboratory-based studies for understanding drivers of parasite and pathogen distributions.

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APPENDIX A

Model sensitivity to parameter γ (recovery rate from worm infection) (*Ecological Archives* E089-129-A1).

APPENDIX B

Model sensitivity to parameter δ (*Ecological Archives* E089-129-A2).