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Helminth–microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits

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Summary

Co-infection is now recognized as the natural state of affairs in most hosts and co-infecting parasites interact in a variety of ways that can impact host health and parasite fitness. Interactions between helminths and microparasites have captured particular attention in this regard owing to the ubiquity of helminth infections in many host populations. The mechanistic underpinnings and health implications of co-infection are typically studied in laboratory and clinical settings, but recently studies of wild species have begun to tackle similar issues. Case studies from three wild mammal groups—ruminants, rodents and rabbits—serve to highlight how wild studies are contributing to the broader co-infection literature. This work suggests that wildlife research can generate new and unique insights about helminth–microparasite co-infection that are fostered in part by studying parasite interactions in a natural context. For this reason, increased integration of wild studies with research in human, laboratory and veterinary animal populations can help pave the way towards a more complete understanding of the issue of co-infection.

KEYWORDS

Co-infection, Disease, Helminth

1 | INTRODUCTION

Co-infection, or the simultaneous infection of hosts by multiple pathogens, is the norm in the real world; however, until recently most infectious disease research focused on one-host one-pathogen systems. Co-infection between helminths (worms) and microparasites, including viruses, bacteria and protozoa, have garnered particular attention, due in part to the ubiquity of worm infections in human and animal populations,[1–3] and the strong immunomodulatory effects these parasites are known to have on their hosts.[4,5] Indeed, these two features of worm infections set the stage for potentially strong interactions with co-occurring microparasites, and evidence that worms can have profound effects on susceptibility to microparasites, the course of microparasitic infections and the efficacy of disease control strategies is mounting.[6–8]

Until recently overlap between human and laboratory animal studies of co-infection and studies of parasite interactions occurring in natural populations has been almost nonexistent.[9,10] However, researchers are beginning to bridge this divide as ecologists studying wild animals start to consider the mechanistic basis of parasite interactions,[10] and biomedically oriented scientists become more interested in immunological variation.[11] In the context of co-infection, the practical relevance of biomedical studies has always been clear. Mouse models have advanced our understanding of immunological and genetic mechanisms that underlie interactions between parasites[12–14]; and clinical studies in humans indicate that co-infections can have adverse impacts on public health.[15,16] Studies of co-infection in wild populations are also relevant, but for different reasons. Wildlife studies, for instance, help clarify whether and when mechanistic interactions between parasites might "scale up" under natural conditions to shape the distribution patterns of parasites and the consequences of infection for hosts. Importantly, this type of information can be highly complementary to insights gained from the laboratory. Motivated by a surge in wild animal studies focused on co-infection, in this paper I discuss recent findings from studies that have examined interactions between helminths and microparasites in the wild. I also explore how the integration of wild

studies with work on humans, laboratory animals and domestic species can help advance the study of co-infection more generally. I begin by reviewing three well-studied "wild" systems that serve as case studies for how wildlife research is addressing the issue of co-infection. Next, I discuss the value of integrating wild studies with ongoing human, laboratory animal and veterinary research. I conclude by outlining some practical steps that might facilitate such integration.

2 | CASE STUDIES: WHAT CAN WILD RUMINANTS, RODENTS AND RABBITS TELL US ABOUT CO-INFECTION?

The description of association patterns between parasite species occurring in wild vertebrate populations has a rich tradition in ecology.[9,10] More recently, this descriptive work has given rise to a new generation of studies that attempt to uncover the processes that account for observed patterns of parasite associations and to understand the consequences of these patterns. This new line of research spans taxonomic groups, from amphibians to birds and mammals (e.g. 17–19). Due in large part to a more sophisticated understanding of the immune system of mammals compared with other vertebrate groups, wildlife studies of worm–microparasite co-infection have tended to focus on mammals. Therefore, here I use three mammal groups—ruminants, rodents and rabbits—as case studies to highlight how wild organisms are contributing to the larger body of co-infection literature. These case studies show how wild species are being used to tackle issues that lie at the forefront of co-infection research, including: (i) examining how immune-mediated interactions between parasites affect disease outcomes, (ii) understanding which pairs of parasites are most likely to interact and (iii) identifying the mechanisms accounting for these interactions.

2.1 | African buffalo: evaluating the consequences of worm–microparasite co-infection

The effect of concomitant worm infection on host susceptibility to and the progression of *Mycobacterium tuberculosis* (the causative agent of human TB) in people is a topic of considerable interest.[20,21] Laboratory animal work has implicated mechanisms such as T-helper cell (Th2)-induced suppression of Th1 immunity and induction of arginase-1-expressing macrophages in the dampening of the host immune response to *M. tuberculosis*.[22,23] Cattle studies on *Mycobacterium bovis* (the causative bovine tuberculosis and close relative of *M. tuberculosis*; [24]) also show that immune responses to *M. bovis* can be attenuated during liver fluke (*Fasciola hepatica*) co-infection.[25,26] Indeed, accumulating evidence now supports the existence of immune-mediated interactions between worms and *Mycobacteria*; nevertheless, important gaps remain in our understanding of how immunological changes due to worm infection impact the course of TB disease and TB epidemiology. These gaps arise, in part, because the studies required to understand how immunological effects of worms on TB translate to real-world disease outcomes are challenging to perform in humans or laboratory animals.[21] However, research on free-ranging animals is showing that insights into the effects of worms on TB infection can come from unexpected places.

Recent studies of free-ranging African buffalo (*Syncerus caffer*) have focused on understanding the consequences of interactions between gastrointestinal nematodes and *M. bovis* for both individual level outcomes of TB infection and epidemiological patterns of TB (Fig. 1a–c). Buffalo are important reservoir hosts for bovine tuberculosis in Southern Africa, and initial co-infection studies in this species documented key effects of worms on immune responses relevant to TB defence. A cross-sectional analysis of animals in Hluhluwe-iMfolozi Park, South Africa, showed that circulating levels of the cytokine interferon γ (IFNγ) were negatively correlated with eosinophil counts [27] and positively correlated with worm faecal egg counts.[28] Both of these patterns were suggestive of immune trade-offs whereby hosts that mount stronger Th2 responses against worms have lower Th1 responses. This observation was confirmed with an anthelmintic treatment experiment which showed that perturbing worm infections in buffalo increased IFNγ secretion.[28] In combination, these results suggested that just as in humans, livestock and laboratory animals, worm infections can lead to suppression of key anti-TB immune defences in buffalo, an observation that set the stage for using this wild species as a new model for exploring the real-world consequences of co-infection.

Although worms can clearly impact host immunity to TB, it is difficult to estimate how these immunological effects alter parameters that determine how fast the bacteria might spread in a host population (e.g. the transmission rate from infected to susceptible hosts, the disease-induced mortality rate, the duration of infectiousness), or which combination of these parameters is most affected by observed immunological changes.[29] In laboratory animals and people, quantifying these effects is often impossible for logistical (e.g. natural transmission is required) and ethical (e.g. interventions must be provided for infected individuals) reasons. However, such studies are feasible in some wildlife populations. Ezenwa and Jolles[30] did this in buffalo by capturing and monitoring anthelmintic-treated and untreated animals for four years in Kruger National Park, South Africa, to quantify the effects of worm treatment on host immunity, the likelihood of TB infection and TB-induced mortality. Intriguingly, study results showed that while anthelmintic treatment enhanced IFNγ production in buffalo, this change in immunity was not associated with reduced incidence of TB disease—both treated and control individuals had similar TB infection rates. However, anthelmintic-treated buffalo survived TB infection much better than untreated individuals, suggesting that, in buffalo, worm-induced immune suppression translates into much stronger effects on TB mortality than TB susceptibility. Because treated buffalo were no less likely to acquire TB infection, yet survived longer with TB, the surprising net effect of anthelmintic treatment on TB epidemiology is that it can enhance *M. bovis* spread. In fact, given the magnitude of the survival benefit of treatment, an eight-fold increase in the basic reproductive number (R_0) of *M. bovis* with treatment was estimated for the buffalo system.[30] These results reveal that asymmetrical effects of worms on different aspects of microparasite infection

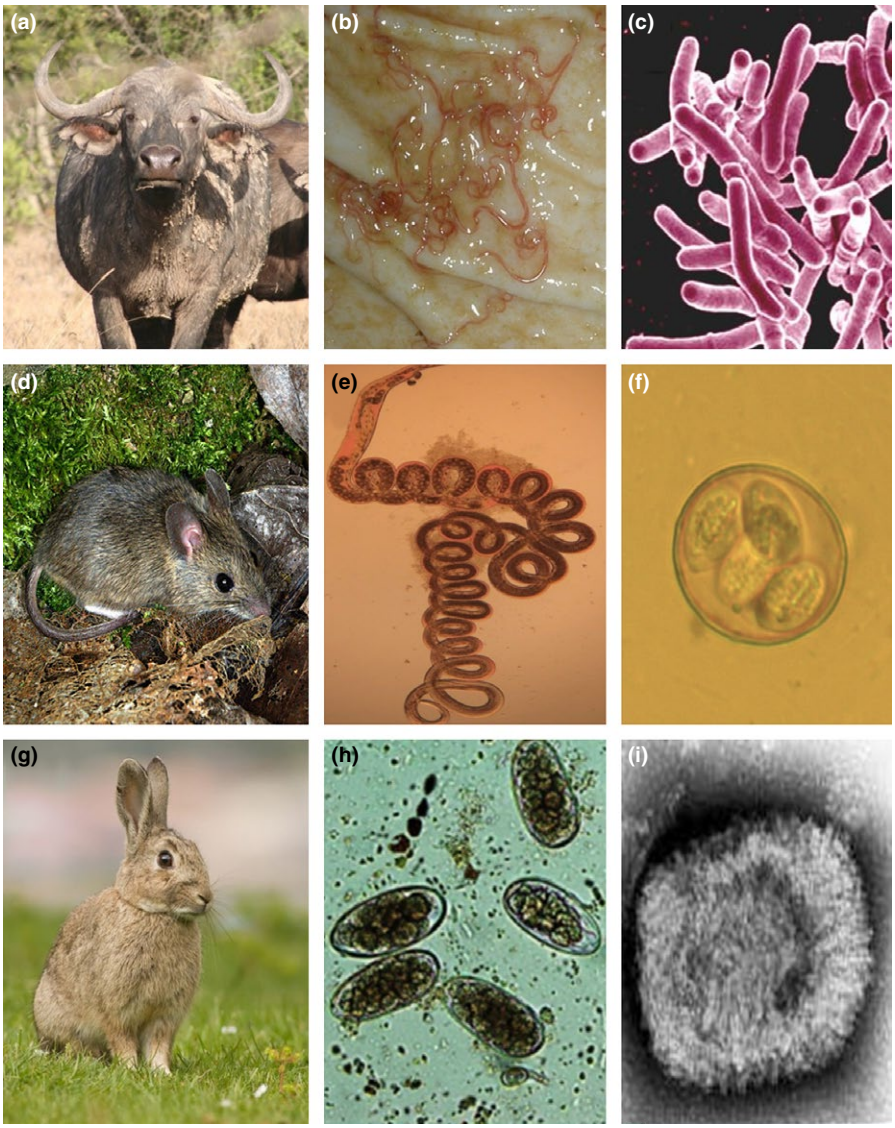

FIGURE 1 (a–c) African buffalo (a) were used to study the consequences of interactions between gastrointestinal worms (b) and the causative agent of bovine tuberculosis (c). Experimentally dewormed buffalo had lower worm burdens and stronger T-helper cell (Th1) immune responses to mitogen challenge. This effect translated into no difference in TB infection probability for treated animals, but a ninefold improvement in survival upon TB infection, an outcome that could enhance the spread of TB at the population level. (d–f) Wood mice (d) studies showed that worm co-infection had the strongest effect microparasites sharing a common location within the host. Mice relieved of their gastrointestinal worm infections, dominated by *Heligmosomoides polygyrus* (e), were shedding 15 times fewer *Eimeria* oocysts than control individuals. The effect of worm removal was particularly strong on *E. hungaryensis* (f), which shares habitat with *H. polygyrus*. (G-I) A combination of field and laboratory studies on European rabbits (g) pieced together the probable mechanisms underlying asymmetrical effects of a virus on two worm species. Laboratory studies showed that rabbits respond immunologically very differently to infection by *Trichostrongylus retortaeformis* (h) vs *Graphidium strigosum*. In a natural host population where both worms co-occur with myxoma virus (i), viral suppression of the more effective host immune response against *Trichostrongylus retortaeformis* helped explain observed patterns of worm infection. Image credits: (a) V. Ezenwa, (b) R. Kaplan, (c) NIAID, (d)D. Perez (Wikimedia commons), (e) D. Davesne (Wikimedia commons), (f) M. Clerc, (g) J. Harrison (Wikimedia commons), (h) K. Szkucik et al. (Open i), (i) ICTV.

(e.g. susceptibility or infection probability vs infection-induced morbidity or mortality), a phenomenon that may common under natural conditions, can have profound implications. For example, when it comes to helminth intervention and control strategies, deworming programs in humans, livestock or other animals could improve individual responses to microbial co-infection on the one hand, but simultaneously exacerbate the population-level spread of these microbes on the other.

2.2 | Wild rodents: identifying parasite interactions in a natural context

Our current understanding of worm–microparasite interactions is shaped in large part by laboratory animal studies where experimental infections are used to understand how specific species of worms affect various microparasites.[31] Often, as in the case for worms and

TB for example, the particular parasite pairings examined are motivated by clinical data from humans suggesting a role of worm infections in shaping disease outcomes, or from observations of poor microparasite control or diagnostic efficacy in human or livestock populations that carry heavy worm burdens. While these studies provide important information about targeted parasite pairs, we lack a more general understanding of when and under what circumstances to expect strong interactions between different combinations of worms and microparasites. However, recent experimental studies in wild rodents are tackling this broader issue of which co-occurring worms and microbes are likely to interact most strongly. By perturbing parasitic nematodes in wild rodents with anthelmintic drugs and tracking the responses of different co-occurring microparasites, these studies are revealing that in natural parasite communities not all interactions are created equal and that some microparasites respond more strongly to worms than others (Fig. 1d–f).

Initial studies on *Peromyscus* (white-footed mice [*P. leucopus*] and deer mice [*P. maniculatus*]) in the eastern United States examined how natural communities of nematodes in these mice interact with gastrointestinal protozoa (*Eimeria* spp.). This was done by treating wild mice with an anthelmintic drug and then examining the effect of treatment on both nematodes and nontarget parasites.[32] *Peromyscus* in the study region are commonly infected by intestinal worm species and the anthelmintic treatment successfully reduced the proportion of mice that were infected with these parasites.[32] The focal nontarget microparasite group (three species of *Eimeria*) collectively increased in prevalence in parallel with the decrease in nematode prevalence, suggesting that worms interact negatively with some *Eimeria* species. A study performed several years later at the same site found highly consistent results, showing that *Eimeria* intensity in *Peromyscus* increased in parallel with a decrease in nematode prevalence after anthelmintic treatment.[33] In combination, these findings point to consistent and robust interactions between intestinal nematodes and *Eimeria*.

Using similar methodology, a follow-up study on wild wood mice (*Apodemus sylvaticus*) in the UK expanded upon the results from *Peromyscus* by broadening the complement of microparasites examined. In this study, Knowles et al.[34] tested for interactions between gastrointestinal nematodes and three different groups of microparasites, including *Eimeria*, *Bartonella* (bacteria) and *Trypanosoma* (protozoa). Wood mice in the study were infected with five different species of nematodes, but the vast majority of infections were caused by a single species, *Heligmosomoides polygyrus*. Treatment effectively reduced nematode infection probability in the mice, and strikingly, the reduction in worms was once again accompanied by an almost mirror-image increase in *Eimeria*. The effect of treatment was such that treated mice were shedding approximately 15 times more *Eimeria* oocysts in their faeces than were control mice,[34] a figure that highlights the strength of the negative interaction between worms and *Eimeria*. Interestingly, the *Eimeria* effect appeared to be species specific—by examining the effect of anthelmintic treatment separately for the two most prevalent *Eimeria* species, Knowles and colleagues showed that interactions with worms were much stronger for one species (*E. hungaryensis*) than the other (*E. apionodes*). The authors suggest that the stronger effect on *E. hungaryensis* may arise because this species shares a common infection site in the GI tract with the dominant worm, *H. polygyrus*. Moreover, as no effect of worm treatment was detected for any other microparasite in the study, including *Bartonella* species, which reside in vascular endothelial cells and erythrocytes, and *Trypanosoma grosi*, which circulates in the blood, the results suggest that worms interact most strongly with other parasites that share a common location. At a minimum, this study indicates that removing worms from natural parasite assemblages can facilitate the establishment of some *Eimeria* species. More broadly, this work raises the intriguing question—relevant to both human and animal health—of whether unanticipated effects of anthelmintic treatments are most likely among microparasites that share the same compartment as the worm species that are most impacted by these treatments.

2.3 | European rabbits: linking real-world patterns to mechanisms

Microparasites can also have profound effects on worm infections.[35–37] The consequences of HIV infection for worms have captured particular attention in this regard owing to the known immunosuppressive effects of HIV.[38] As one example, reductions in *Schistosoma mansoni* and *S. haematobium* egg excretion have been linked to HIV-positive status in people, likely due to HIV-induced CD4 T-cell depletion.[39] Moreover, T cell-deficient mice infected with *S. mansoni* suffer liver damage because of their inability to form granulomas that wall off parasite eggs, suggesting that co-infection with HIV can increase the severity of *Schistosoma* infections.[39] Interestingly, few effects of HIV co-infection on *S. mansoni* pathology have been detected in people, possibly because the degree of immunodeficiency is magnified in T cell-depleted laboratory mice compared with typical levels of HIV immunodeficiency observed in human patients.[39] This observation underscores the point that there can often be a mismatch between co-infection outcomes detected in laboratory studies and real-world impacts. However, wildlife species with close laboratory animal relatives may provide a creative way to reconstruct the outcomes of co-infection in natural settings and then tie these patterns back to underlying mechanisms in the laboratory. A similar concept is used in many aspects of biomedical research where human and laboratory animal studies are frequently paired. The potential to do this in wild animals is highlighted by studies of another immunosuppressive virus (myxoma virus) and its effects on gastrointestinal worm infections in free-ranging European rabbits (*Oryctolagus cuniculus;* Fig. 1g–i).

In Scotland, wild rabbits harbour a diverse community of gastrointestinal tract helminths[40] and are also exposed to myxoma virus, the causative agent of myxomatosis. Using 26 years of field data on the parasites infecting these rabbits, Cattadori and colleagues[41] explored possible immunoregulatory effects of myxoma on two worm species: *Trichostrongylus retortaeformis* and *Graphidium strigosum*, with the goal of understanding whether the virus affects both the susceptibility of rabbits to these worms and the distribution of worm

infections in rabbit populations. This analysis revealed distinct effects of virus co-infection on the two worm species. For *T. retortaeformis*, the relationship between host age and infection intensity was altered by myxoma co-infection; in single *T. retortaeformis* infection, the age–intensity pattern was convex with worm intensity initially increasing with age and then declining in older animals; however, myxoma co-infection changed this profile by increasing worm intensities in older animals. For *G. strigosum*, on the other hand, worm intensity increased exponentially with age in single infection, and myxoma co-infection had no effect on this pattern. Because convex age–intensity profiles can indicate acquired immunity to helminth infections,[42] these field results suggest that rabbits acquire immunity to *T. retortaeformis* over time and that myxoma co-infection interferes with the acquisition of this immunity.[41] The age–intensity profile for *G. strigosum* suggests that rabbits do not immune-regulate this parasite, which might explain the lack of an effect of myxoma on the age–intensity profile. These field patterns highlight key differences in how a common virus interacts with different worm species and generate testable hypotheses about the mechanisms that might account for the divergent patterns.

As rabbits are common laboratory animals, the field-based results outlined by Cattadori et al.[41] were complemented with laboratory studies examining differences in the rabbit immune response to *T. retortaeformis* and *G. strigosum*. In this work, New Zealand White rabbits were infected with either *T. retortaeformis* or *G. strigosum* and worm intensities and immune responses were monitored destructively over time.[43] In *T. retortaeformis*-infected rabbits, adult worm burdens consistently decreased with time since infection, whereas worm burdens were consistent through time for *G. strigosum*-infected animals. Interestingly, infected hosts responded immunologically to both worm species. However, while a combination of local and systemic responses were effective against *T. retortaeformis*, the immune response to *G. strigosum*, characterized by robust systemic responses but low mucosal antibody secretion, was ineffective. These nuanced differences in the host immune response to the two worm species clarify the distinct age–intensity patterns observed in the field. The details of the host immune response to *T. retortaeformis* also help explain why the immunosuppressive effects of myxoma virus led to strong interactions with this worm under natural conditions. Importantly, other work showed that the interaction between myxoma and *T. retortaeformis* likely has consequences for both individual host susceptibility to *T. retortaeformis* and transmission dynamics of this worm species. This conclusion was drawn by comparing observed field patterns to the predictions of a mathematical model, which included an immune-mediated interaction between the two parasites.[44] Ultimately, these rabbit studies remind us that microparasites also have the capacity to affect worms and that fully understanding the epidemiology of some worm infections may require information about co-occurring microbes. This work also shows that integrating laboratory and field studies can be a powerful approach for connecting real-world epidemiological patterns to underlying mechanisms, a goal that is difficult to achieve in either the laboratory or field alone.

3 | SYNTHESIS: THE VALUE OF AN INTEGRATIVE APPROACH TO CO-INFECTION RESEARCH

The case studies summarized above show that research on wild animal populations can offer interesting and unique insights on core topics related to co-infection. These insights arise for at least two reasons. First, wild animals are embedded in a natural ecological context—characterized by many forms of variability (e.g. genetic, physiological, behavioural, microbial, environmental)—which plays a key role in shaping the strength, direction, and outcome of interactions between parasites. Context dependence in species interactions, where an outcome changes depending on the context in which it is embedded, is a well-known phenomenon in ecological research.[45] By allowing for the tracking or manipulation of study subjects "in situ", wildlife studies help reveal the contexts in which parasite interactions are most important.

A second reason why wildlife studies might facilitate novel insights is that they allow for study designs that are often intractable in laboratory, clinical or veterinary settings. For example, wild animal studies are often by default "prospective" allowing parasite infections to occur naturally without the aid of artificial infections or the need for intervention once infections occur. Moreover, in cases where one parasite is perturbed by treatment, often all other differences among hosts are preserved.[30,34] Such flexibility in research design is fundamental to understanding how two parasites might interact, especially when it comes to identifying how one parasite alters host susceptibility to another. In a notable example of this, Telfer et al.[46] studied interactions between pairs of microparasites in field voles (*Microtus agrestis*) by sampling over 5000 individuals repeatedly over five years. This design allowed the authors to ask whether infection with one parasite affected natural susceptibility to other parasites—a question that is not easily addressed in the laboratory but is fundamental to understanding the consequence of co-infections for individual health and parasite population dynamics.

The benefits of studying wild animals also come with many disadvantages as has been noted by previous authors (e.g. 47). One problem is that there is often too much variability among study subjects, an issue that can frequently mask important patterns if sample sizes are inadequate. Another problem is the lack of necessary tools and reagents for examining the mechanistic (e.g. immunological, genetic) basis of patterns that are observed. Clearly, wild studies have drawbacks as do laboratory and clinical studies. However, many of the challenges that arise from these different study systems are potentially resolved by combining approaches, as illustrated, for example, by co-infection work in rabbits which paired field studies of wild rabbits with mechanistic studies in laboratory animals.[41,43] Indeed, the idea that studies of closely related laboratory and wild species can be paired to harness the power of both is gaining attention in the literature. For example, a recent review on immunology in the laboratory mouse (*Mus musculus*) highlighted the benefits of using both laboratory and wild mice to drive new advances in immunological research.[48] Others have

suggested that immunology research can benefit from considering animals along a continuum from the laboratory to wild, with domestic, captive, urban, feral and managed populations falling in between.[11] For co-infection research, one practical way to embrace this continuum concept is by adopting an integrated or "one health" perspective that seeks to merge approaches and/or insights from human, laboratory animal, veterinary and wildlife studies, where possible, to build a more complete understanding of co-infection and its consequences.

As an example, consider a "one health" perspective on worm–TB co-infection. So far, interactions between various helminth species and *Mycobacteria* (*M. tuberculosis, M. bovis, M. avium*) have been studied in humans, laboratory mice, cattle and at least two wild species: African buffalo and Eurasian wild boar (*Sus scrofa*). Mouse studies provide some of the strongest mechanistic evidence for an interaction between these two parasite types to date, although there is still much to learn. Potian et al.,[22] for example, showed that *Nippostrongylus brasiliensis* infection compromised the ability of mice to limit *M. tuberculosis*, in part because of its role in inducing the accumulation of alternatively activated macrophages in the lung. In parallel with these laboratory animal studies, work on humans, livestock and wildlife suggests that a number of different worm species can suppress the host immune response in ways that impinge on *Mycobacteria* defences[20,29,49]; this includes elegant research pairing mouse and human studies.[23] But what are the real-world consequences of this type of immune suppression? Human and livestock studies have played a crucial role in addressing this question, especially as it relates to consequences for TB control. For example, in humans, it has been shown that helminth infections may limit the efficacy of Bacillus Calmette-Guerin (BCG) vaccination against *M. tuberculosis*,[50,51] while worms like *Fasciola hepatica* have been found to reduce the efficacy of the tuberculin skin test for diagnosing *M. bovis* infection in cattle.[8] Thus, work in both human and veterinary populations helps clarify the public and veterinary health (and thus socio-economic) consequences of worm co-infection.

Despite many breakthroughs, the broader consequences of worm co-infection for TB disease and epidemiology still remain largely conjectural. Clinical studies have been used to evaluate the impacts of helminth co-infection on TB outcomes in people, but results so far are inconsistent and sometimes hard to interpret.[52–54] For example, a randomized deworming trial of patients with pulmonary TB (*M. tuberculosis*) in Ethiopia found that albendazole treatment had no effect TB severity (as measured by chest X-ray) or mortality between treatment groups.[54] However, the subjects in this study also received TB treatment, which reduced TB severity prior to the onset of deworming. This unavoidable element of the study design may have obscured any effects of deworming on TB. In direct contrast, wildlife studies have linked helminth infections to more severe outcomes of *M. bovis* infection. In wild boar, a cross-sectional analysis showed that co-infection with pulmonary nematodes (*Metastrongylus spp.*) was strongly associated with an increase in bovine TB (*M. bovis*) severity.[55] Similarly, experimentally dewormed African buffalo were nine times more likely to survive *M. bovis* infection than untreated animals.[30] The mismatch between human and wildlife studies suggests that wildlife may fill an important gap when it comes to quantifying the effects of helminth co-infection on host morbidity and mortality to TB, and uncovering the consequences for TB epidemiology. Thus, when considered together, studies of humans, laboratory animals, veterinary animals and wildlife might paint a more complete picture of worm–TB co-infection, which can help uncover important steps for future research. This strategy could be applied to any number of co-infection problems.

4 | CONCLUSIONS

A more intense spotlight on co-infection in recent years has raised awareness of the importance of this phenomenon in both human and animal populations. Wildlife systems are emerging as new and unique models of co-infection that can contribute to our fundamental understanding of how interactions between parasites affect host and parasite fitness. These studies also have substantial applied value because they can help uncover potential unintended consequences of disease control measures. So, given the upsurge in wild co-infection studies, how can research on wild species contribute to a more integrative perspective on co-infection? A few possible ways are as follows:

4.1 | By targeting wild species with laboratory or domestic animal analogs

This approach can facilitate wildlife research by providing access to diagnostic tools, therapeutics and reagents that vastly improve field studies.[56,57] Another benefit is that shared interests in a common host may help foster collaborations with biomedical and veterinary scientists who are studying analog species—increased collaboration alone can help fuel integration. Some wild rodents (e.g. *Mus musculus*) are excellent candidates given the dominance of mice and rats as models in biomedical research.[48] Likewise, wild ungulates (e.g. wild sheep, goats, cattle and pigs), wild carnivores (e.g. canids and felids) and wild primates may be relevant analogs for livestock, companion animals and humans.

4.2 | By combining field approaches with laboratory, mesocosm or captive studies

This may not be possible for all species, but in some situations wild animals can be studied in their natural context in the field and also brought back to a laboratory or mesocosm for work in a more controlled environment. For example, a pairing of field studies with mesocosm and laboratory studies in amphibians revealed interesting consequences of macroparasite co-infection for host pathology, parasite transmission and the structure of the parasite community.[58,59]

4.3 | By linking data and theory

Although often studied as pairwise interactions between species, co-infection in natural hosts typically involves interactions among an entire community of parasites. This complexity is difficult to capture

in the lab, but may be the baseline state of affairs in human and veterinary animal populations of the most concern. Ecologists have developed a body of theory and tools for understanding multispecies interactions and this toolkit can be readily applied to co-infection research.[60] Studies of wild species can bridge data and ecological theory when it comes to advancing our understanding of multiple parasite interactions. Such research may help generate new and testable predictions to guide laboratory animal, veterinary and clinical research on this topic. This is similar to how immunological principles are now helping to guide ecological work on co-infection.

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DISCLOSURES

None.

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