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Research paper

Limited sharing of tick-borne hemoparasites between sympatric wild and domestic ungulates

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ABSTRACT

Tick-borne hemoparasites (TBHs) are a group of pathogens of concern in animal management because they are associated with a diversity of hosts, including both wild and domestic species. However, little is known about how frequently TBHs are shared across the wildlife-livestock interface in natural settings. Here, we compared the TBHs of wild Grant's gazelle (*Nanger granti*) and domestic sheep (*Ovis aries*) in a region of Kenya where these species extensively overlap. Blood samples collected from each species were screened for piroplasm and rickettsial TBHs by PCR-based amplification of 18S/16S ribosomal DNA, respectively. Overall, 99% of gazelle and 66% of sheep were positive for *Babesia*/*Theileria*, and 32% of gazelle and 47% sheep were positive for *Anaplasma*/*Ehrlichia*. Sequencing a subset of positive samples revealed infections of *Theileria* and *Anaplasma*. Sequences sorted into seven phylogenetically distinct genotypes—two *Theileria*, and five *Anaplasma*. With the exception of a putatively novel *Anaplasma* lineage from Grant's gazelle, these genotypes appeared to be divergent forms of previously described species, including *T. ovis*, *A. ovis*, *A. bovis*, and *A. platys*. Only one genotype, which clustered within the *A. platys* clade, contained sequences from both gazelle and sheep. This suggests that despite niche, habitat, and phylogenetic overlap, the majority of circulating tick-borne diseases may not be shared between these two focal species.

1. Introduction

Pathogen transmission occurring between wild and domestic animals is gaining attention, in part because pathogen spillover can be devastating to both wildlife and livestock (Siembieda et al., 2011; Wiethoelter et al., 2015). For example, wildlife-endemic diseases like foot-and-mouth disease and heartwater that naturally occur in African buffalo (*Syncerus caffer*) are now considered among the most serious livestock diseases on the African continent, particularly in cattle (Bengis et al., 2002). On the other hand, livestock diseases like rinderpest and brucellosis have contributed to precipitous wild ruminant declines in Africa and North America (Grootenhuis, 2000; Miller et al., 2013; Nishi et al., 2002). Given that livestock and wildlife are estimated to share three quarters of their pathogens (Cleaveland et al., 2001; Wiethoelter et al., 2015), spillover events between the two groups may be inevitable. Nevertheless, understanding the ease with which various pathogen taxa transmit across the wildlife-livestock interface may help narrow the scope of efforts seeking to prevent future outbreaks.

Tick-borne hemoparasites (TBHs) are one group of pathogens that occur in both livestock and wildlife, and may be commonly transmitted between wild and domestic species (Dantas-Torres et al., 2012; Gortazar et al., 2007). Not only do TBHs cause significant morbidity and mortality in livestock (Uilenberg, 1995), but wildlife can contribute to livestock TBHs by acting as both sources and maintenance hosts for disease (Kock, 2005). For example, East Coast Fever, caused by the protozoan parasite *Theileria parva*, originates from African buffalo which harbor "silent" infections, and now circulates in cattle, which often succumb to the disease (Bengis et al., 2002; Olwoch et al., 2008). Similarly, free-ranging white-tailed deer (*Odocoileus virginianus*) and sika deer (*Cervus nippon*) have been identified as reservoir hosts that maintain transmission of piroplasm (*Babesia bigemina*, *B. ovis*) and rickettsial (*Anaplasma bovis*, *A. centrale*, *A. phagocytophilum*, and *Ehrlichia* spp.) TBHs endemic to livestock in Mexico and Japan, respectively (Cantu et al., 2007; Kawahara et al., 2006). Livestock TBHs may also impact wildlife (Miller et al., 2013), although TBH spillover from livestock to

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wildlife populations has received less attention and clear examples are absent from the literature.

One way to understand the likelihood of pathogen spillover is to quantify the frequency with which wildlife and livestock share pathogens under natural conditions. Increased TBH sharing between wildlife and livestock may occur for at least three reasons. First, interactions between wildlife and livestock are expanding due to human-induced changes (Miller et al., 2013; Wiethoelter et al., 2015). As such, increases in direct or indirect contact (e.g. habitat overlap) may facilitate exposure and sharing of previously isolated pathogens (Daszak et al., 2001; Maxwell et al., 2013). Human translocation of wildlife or livestock to new areas is one possible means of increasing contact between wild and domestic species. Translocations have resulted in outbreaks and mortality among naïve inhabitants—as was the case in cattle when *Theileria parva*-infected buffalo were translocated to the Highveld of Zimbabwe (Latif et al., 2002). Second, many TBHs are vectored by ticks that have broad host ranges and require multiple blood meals to complete their lifecycles (Jongejan and Uilenberg, 2004). Pathogens vectored by ticks with catholic feeding habits are more likely to be shared between species, because these vectors can facilitate exposure to new hosts with each feeding event (McCoy et al., 2013; Shaw et al., 2001). *Theileria parva* is a striking example of this, being vectored primarily by the brown ear tick (*Rhipicephalus appendiculatus*), which requires three separate hosts to complete development. Interestingly, this tick has been collected from over a hundred host species (Cumming, 1998; Jongejan and Uilenberg, 2004), which may contribute to *T. parva*'s propensity for transmission across the wildlife-livestock interface. Finally, hosts that are phylogenetically closely related are often more likely to share pathogens than distantly related hosts, due to physiological and ecological similarity (Davies and Pedersen, 2008). For example, a recent study of 16 TBH species circulating among 18 wild and domestic ungulates in South Africa found that TBHs tended to cluster by the phylogenetic history of hosts (Berggoetz et al., 2014).

On the African continent, TBHs of greatest concern include protozoan piroplasms of the genera *Babesia* and *Theileria*, and rickettsial bacteria of the genera *Anaplasma* and *Ehrlichia*. These species are common in cattle, sheep, and goats (Njiiri et al., 2015; Uilenberg, 1995), and are an increasingly reported in wildlife (Criado-Fornelio et al., 2004; Eygelaar et al., 2015; Heyman et al., 2010). However, descriptions of TBH sharing between wildlife and livestock are infrequent. Here, we examine the extent to which these TBHs are shared between a wild (Grant's gazelle) and domestic (sheep) ruminant in central Kenya. In this region, livestock densities are increasing, and there is extensive overlap between livestock and wildlife populations (Georgiadis et al., 2007; Kinnaird and O'Brien, 2012). Interestingly, a close relative of *Theileria ovis* (a common TBH of sheep; Altay et al. (2005)), was recently isolated from Grant's gazelle (*Nanger granti*) (Hooge et al., 2015). This raises the question of whether this new genotype is transmissible to domestic animals, which extensively overlap with wildlife in the study region. Grant's gazelle occur across East Africa, can persist at high livestock density (Georgiadis et al., 2007), and also host a number of tick species known to infest livestock (Walker et al., 2003). Using molecular detection by PCR and sequencing, we screened Grant's gazelle samples collected in three separate years for the piroplasms *Babesia* and *Theileria*, and the rickettsiae *Anaplasma* and *Ehrlichia*. We compared these TBH profiles to those from sheep inhabiting high wildlife density (considerable wildlife-livestock overlap) and low wildlife density (little wildlife-livestock overlap) areas. This approach allowed us to investigate: (1) whether we could detect identical parasite genotypes in sheep and gazelle, and (2) whether these genotypes were more likely to be shared under conditions of greater host overlap.

2. Methods

2.1. Animal sampling

This research was approved by the University of Georgia Animal Care and Use Committee (#A2013 08-018-Y3-A1 and #A2015 04-004-Y1-A0). All samples were collected in Laikipia County, Kenya. Grant's gazelle were sampled at the Mpala Research Center (MRC) in August 2009 (n = 62), July 2011 (n = 62), and June 2015 (n = 58). The total samples size (n = 182) represents 40–60% of the total gazelle population at MRC during the sampling period. Gazelle were captured by helicopter using a hand-held net gun fired from the aircraft. Sheep were sampled at two locations: MRC (n = 50), a private ranch with low-intensity livestock production and considerable wildlife-livestock overlap, and Lekiji (n = 84), an adjacent community-owned ranch with higher intensity livestock production and significantly less wildlife-livestock overlap (Georgiadis et al., 2007). Sheep sampling occurred between June 8 and July 1 2015. Samples from MRC were collected from a single herd of 92 animals, while samples from Lekiji were collected from eight herds that varied in size from 17 to 112 animals. Owner consent was granted prior to all sampling.

For all animals, blood was collected from the jugular vein into 10 mL heparinized vacutainer tubes as described in Ezenwa et al. (2012). In addition to blood sampling, we also collected information on the number of days since the last acaricide treatment for sheep, since we considered that regular treatment could diminish the prevalence and therefore detection rate of TBHs in this species. All blood samples were kept on ice in the field until transport to the laboratory where they were stored at −20 °C until processing.

2.2. Parasitological analyses

DNA was extracted from 100 μL of whole blood using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, CA, USA) according to the manufacturer's instructions. To screen for *Babesia/Theileria*, we followed the polymerase chain reaction (PCR) protocol (reagents and cycling parameters) used by Hooge et al. (2015). This method amplified a ~450 bp fragment of the V4 hypervariable region of 18S ribosomal DNA. Specifically, primers RLB F2 (5′-GAC ACA GGG AGG TAG TGA CAA G-3′) and RLB R2 (5′-CTA AGA ATT TCA CCT CTA ACA GT-3′) were used (Gubbels et al., 1999). The results of *Babesia/Theileria* infection from the 2009 Grant's gazelle sample set were recently published (Hooge et al., 2015); here, we screened gazelle samples from 2011 and 2015 and all sheep samples (2015, MRC and Lekiji).

To screen for *Anaplasma/Ehrlichia*, we amplified a ~450 bp fragment of the V1 hypervariable region of 16S ribosomal DNA using previously published primers EHR-F (5′-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3′) and EHR-R (5′-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3′) (Bekker et al., 2002). This reaction was carried out in 25 μL volumes. The reaction mixture contained 3 μL of template DNA, 0.4 μM of each forward and reverse primer, 200 μM of dNTP, 2.5 μL 10X PCR buffer, 2.0 mM of $MgCl_2$, 5 μL 360 GC Enhancer, and 0.25 μL (1.25 units) of AmpliTaq Gold 360 DNA Polymerase (all reagents from Thermo Fisher Scientific, NY, CA, USA). Reactions were cycled with the following thermal profile: 95 °C for 10 min, 35 cycles of 95 °C for 30 s, 55.5 °C for 30 s, and 72 °C for 60 s, and a final 10 min extension at 72 °C. All gazelle samples (2009, 2011 and 2015), and all sheep samples (2015, MRC and Lekiji) were screened by this method.

All PCR products were electrophoresed on a 1% agarose gel stained with GelRed (Biotium Inc., CA, USA) and visualized under UV light to determine positivity for *Babesia/Theileria* and *Anaplasma/Ehrlichia*. A subset of samples with the most brightly banded PCR products were selected for sequencing. A total of 38