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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.

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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

gazelle samples (n = 18 from 2015, 20 from 2011) and 36 sheep samples (n = 18 from MRC, 18 from Lekiji) were sequenced for *Babesia/Theileria*. In addition, 21 gazelle samples (n = 1 from 2015, 13 from 2011, 7 from 2009) and 34 sheep samples (n = 7 from MRC, 27 from Lekiji) were sequenced for *Anaplasma/Ehrlichia*. Selected samples were purified using the QIAquick PCR Purification Kit (Qiagen, CA, USA) and Sanger sequenced on an ABI3730 Genetic Analyzer (Applied Biosystems, CA, USA) using both forward and reverse primers. Sequencing results were edited using Geneious v. 8.1 (Biomatters, Auckland, New Zealand).

2.3. Statistical analyses

To test for effects of acaricide treatment on TBH infection status in sheep, we used generalized linear mixed models (GLMMs) with a binomial error distribution. Two separate models were run with either *Theileria* or *Anaplasma* infection status as the response variable. Infection status was classified as a binary variable (either uninfected or infected). The main predictor variable was time since last acaricide treatment; age, sex, and location (MRC or Lekiji) were included as covariates. Herd membership was also included as a random effect. Models were run in R v. 3.2.0 (R-Development-Team, 2015).

2.4. Phylogenetic analyses

Theileria and *Anaplasma* sequences were separately aligned in ClustalX v. 2.1. *Theileria* sequences isolated from gazelle and sheep in this study were aligned with 76 representative *Theileria* sequences (including the GG1, GG2 and GG3 genotypes identified by Hooge et al. (2015) from 2009 gazelle) and a *Toxoplasma gondii* outgroup sequence. *Anaplasma* sequences isolated in this work were aligned with 29 representative *Anaplasma* sequences and an *Ehrlichia ruminatum* outgroup sequence. All representative sequences used were available from Genbank (see S4), and were required to fit two of three criteria for inclusion: (1) belonging to scientific papers published in peer-reviewed literature; (2) isolated from African mammals; (3) isolated from ungulates (both Artiodactyla and Perrisodactyla). For both alignments, poorly aligned regions were manually adjusted and regions of ambiguous alignment were identified and removed in Gblocks v. 0.91 using the least stringent parameter settings (Castresana, 2000; Talavera and Castresana, 2007). Alignments were trimmed to identical lengths and models of sequence evolution were selected using the MrModelTest v. 2.3 executable in PAUP* v. 4 (Nylander, 2004; Swofford, 2003).

Phylogenetic relationships were reconstructed using maximum likelihood methods in PhyML v. 3.0 (Guindon et al., 2010; Guindon and Gascuel, 2003). Specifically, the Tamura-Nei model with gamma shape parameter and fixed (invariant) sites was selected for *Theileria* (Tamura and Nei, 1993), while the Kimura 2-parameter model with a gamma shape parameter and fixed sites (Kimura, 1980) was selected for *Anaplasma*. Phylogeny support was assessed using 1000 bootstrap iterations. Percentage nucleotide similarity within and between clades was calculated for both *Theileria* and *Anaplasma* using 1000 bootstrap replicates of the uncorrected pairwise proportion of divergent nucleotides (*p*-distance) in MEGA v. 6.0 (Tamura et al., 2013). Sequence data reported in this paper are available in GenBank under accession numbers KU569699 (*Theileria*) and KU569700-4 (*Anaplasma*).

3. Results

3.1. Patterns of infection

3.1.1. Babesia/Theileria

A total of 120 Grant's gazelle samples from 2011 (n = 62) and 2015 (n = 58), and 134 sheep samples from MRC (n = 50) and Lekiji (n = 84) were screened for *Babesisa/Theileria* (Table 1). In gazelle, a 98.4 and 100% prevalence of *Babesia/Theileria* was detected in 2011 and 2015, respectively, which is similar to the 100% prevalence documented by Hooge et al. (2015) for animals sampled in 2009 (n = 65). In sheep, 58% of animals sampled at MRC and 71.4% of animals sampled at Lekiji were *Babesia/Theileria* positive (Table 1). There was no association between time since the last acaricide treatment and *Babesia/Theileria* infection status (Number days since acaricide treatment: $Z = -0.52$, $P = 0.603$; Age: $Z = 1.60$, $P = 0.111$; Location (MRC): $Z = -2.44$, $P = 0.015$; Sex (Male): $Z = 1.97$, $P = 0.049$), so it seems unlikely that treatment affected our ability to detect this parasite in sheep. All sequenced samples from both gazelle and sheep were identified as *Theileria* spp., which suggests that infections by *Babesia* were absent.

3.1.2. Anaplasma/Ehrlichia

A total of 179 Grant's gazelle samples from 2009 (n = 59), 2011 and 2015 (n as above) and 134 sheep samples from MRC and Lekiji (n as above) were screened for *Anaplasma/Ehrlichia*. Grant's gazelle had a prevalence of 52.5, 41.9, and 1.7% in 2009, 2011, and 2015, respectively. In sheep, 14.0% of animals sampled at MRC and 66.7% sampled at Lekiji were positive for *Anaplasma/Ehrlichia*. As with *Theileria*, there was no relationship between time since last acaricide treatment and *Anaplasma/Ehrlichia* prevalence in sheep (Number days since acaricide treatment: $Z = 1.001$, $P = 0.314$; Age: $Z = 1.95$, $P = 0.051$; Location (MRC): $Z = -3.73$, $P = < 0.001$; Sex (Male): $Z = -1.61$, $P = 0.108$). All sequenced samples from both gazelle and sheep were identified as *Anaplasma* spp., suggesting that infections by *Ehrlichia* were absent.

3.2. Phylogenetic relationships

3.2.1. Theileria

From 38 Grant's gazelle and 36 sheep samples sequenced, 14 gazelle and seven sheep sequences had conspicuous overlapping chromatogram peaks that were suggestive of co-infection, as has been found previously in Grant's gazelle (Hooge et al., 2015). Another five sheep sequences were of poor quality. These 26 sequences were omitted from phylogenetic analyses. The remaining 48 sequences from gazelle (2011: n = 11 & 2015 n = 13) and sheep (MRC: n = 11 & Lekiji: n = 13) were used for phylogenetic reconstruction. These analyses revealed two distinct *Theileria* genotypes that were specific to either sheep or gazelle (Fig. 1). Within these genotypes, all sequences were 100% identical. The sheep genotype, designated ThSh1 (for *Theileria* Sheep Genotype 1), contained all 24 sheep sequences, and was 99.9% identical to representative *T. ovis* sequences. The gazelle genotype contained all 24 gazelle sequences and was 100% identical to GG2, a genotype previously described by Hooge et al. (2015) (Fig. 2). Interestingly, GG2 was the only genotype isolated from 2011 and 2015 gazelle samples, although Hooge et al. (2015) isolated GG1 and GG3 in addition to GG2 from 2009 samples. The GG2 genotype differed from GG1 by a single nucleotide substitution, and shared 94.5% nucleotide identity with GG3. GG2 shared 99.4% sequence identity with *T. ovis*, and appears to be sister to the clade containing both *T. ovis* and the newly identified sheep genotype ThSh1.

The sheep (ThSh1) and gazelle (GG2) genotypes identified in this study were 99.4% identical. These genotypes differed by five nucleotides that were consistent based on host isolation source

Table 1
Prevalence of TBHs *Theileria* and *Anaplasma* based on PCR and sequencing. "n" indicates the total number of samples collected.

	Grant's Gazelle – MRC[a]				Sheep – 2015		
	2015 (n = 58)	2011 (n = 62)	2009 (n = 59)	TOTAL (n = 179)	MRC (n = 50)	Lekiji (n = 84)	Total (n = 134)
Theileria	100.0	98.4	100.0	99.4	58.0	71.4	66.4
Anaplasma	1.7	41.9	52.5	32.4	14.0	66.7	47.0

[a] MRC = Mpala Research Center.

(gazelle or sheep). ThSh1 shared 99.4 and 93.7% nucleotide similarity with GG1 and GG3, the two gazelle genotypes reported from Hooge et al. (2015). Both sheep and gazelle genotypes were most divergent from *T. equi* (87% sequence similarity), and shared 76% similarity with the outgroup *Toxoplasma gondii* (see S1 for all pairwise distance calculations).

3.2.2. Anaplasma

Out of 21 Grant's gazelle and 34 sheep samples sequenced, 19 gazelle and 25 sheep samples returned usable sequence data. Two gazelle and six sheep samples had conspicuous overlapping chromatogram peaks suggestive of co-infection. This included the only gazelle sample positive for *Anaplasma* in 2015. An additional three sheep sequences were of poor quality. All 11 of these sequences were omitted from further analyses. The remaining 44 sequences from gazelle (2009: n = 7 & 2011: n = 12) and sheep (MRC: n = 3 & Lekiji: n = 22) were used for phylogenetic reconstruction. These analyses revealed that the *Anaplasma* genotypes detected in this study sorted into five distinct clades. These clades were designated AnSh1, AnGa2, AnSh3, AnGa4, and AnGa + Sh5 to represent the genus (An for "*Anaplasma*"), host species the sample was isolated from (Ga for "gazelle" or Sh for "sheep"), and an arbitrary numeric value of 1–5 to represent the five clades discovered here. Genotypes 1 and 3 were only isolated from sheep, and genotypes 2 and 4 were only isolated from gazelle. Genotype 5 was isolated from both sheep and gazelle. The first genotype, AnSh1, comprised 56% of all successfully sequenced sheep samples. AnSh1 formed a monophyletic clade that was sister to AnGa2 and AnSh3, and 99.7% sequence similarity, respectively. AnGa2 comprised 16% of sequenced gazelle samples, and shared 99.5% similarity with AnSh3, which comprised 36% of all sequenced sheep samples. These three genotypes all differed from one another by three nucleotide substitutions, and formed a monophyletic sister group to representative *A. ovis* sequences. AnSh1, AnGa2 and AnSh3 shared 99.5, 99.0, and 99.3% sequence similarity with the *A. ovis* clade, respectively. AnGa4 comprised 63% of all sequenced gazelle samples. AnGa4 clustered with the *A. bovis* clade and shared 99.1% sequence identity with other *A. bovis* sequences. The AnGa4 and *A. bovis* clade was sister to the clade containing *A. centrale*, *A. marginale*, *A. ovis*, and AnSh1, AnGa2, and AnSh3. The gazelle genotype AnGa4 shared greater sequence similarity (96.7%) with the gazelle genotype AnGa2, than with the sheep genotypes AnSh1 and AnSh3 (96.1%). Finally, AnGa + Sh5 sequences were isolated from both Grant's gazelle and sheep. 25% of successfully sequenced gazelle samples in 2009 and 14% in 2011 were infected with the AnGa + Sh5 genotype, compared with 9% of sheep from Lekiji and none from MRC. This genotype was the most divergent of those isolated in this study, sharing between 95.2 (AnSh1, AnGa2, AnSh3) and 97.2% (AnGa4) sequence similarity with the other genotypes isolated here. AnGa + Sh5 sequences fell within the *A. platys* species complex and shared 99.3% sequence identity with other *A. platys* sequences (see S2 for all pairwise distance calculations).

4. Discussion

This study investigated the potential for TBH sharing between one wild and one domestic ruminant in central Kenya. Based on habitat overlap, shared tick vectors, and family-level phylogenetic relatedness, we expected that Grant's gazelle and sheep would share some of their TBHs. We discovered seven distinct TBH genotypes (two *Theileria* spp., and five *Anaplasma* spp.), and only one (*Anaplasma:* AnGa + Sh5) was identical in both gazelle and sheep. Interestingly, this genotype was only detected in sheep at the high-intensity livestock site where overlap with wildlife (including gazelle) is limited (Georgiadis et al., 2007). These results suggest that despite an opportune system for pathogen sharing, the majority of TBH infections are not shared across the wildlife-livestock interface in this system.

Although there was one identical TBH genotype (AnGa + Sh5) in sheep and gazelle, this was the most uncommon *Anaplasma* genotype in sheep, isolated from only 8% of sequenced samples (compared to AnSh1 [56%] and AnSh3 [36%]). AnGa + Sh5 was also rare in gazelles, isolated from 21% of sequenced gazelle samples (compared to AnGa2 [16%] and AnGa4 [63%]). Interestingly, this genotype clustered with *A. platys* sequences and may therefore be a divergent member of this clade. *A. platys* is typically considered an exclusive canine parasite that causes infectious canine cyclic thrombocytopenia, a disease associated with low platelet counts in dogs (Harvey et al., 1978; Yabsley et al., 2008). However, other recent studies have identified *A. platys*-like sequences in ruminants, including cattle and goats in China (Liu et al., 2012; Yang et al., 2015), and sheep from Sardinia and South Africa (Berggoetz et al., 2014; Zobba et al., 2014). Given our discovery of an *A. platys*-like genotype in both a wild ruminant and domestic ruminant in Kenya, future research to determine whether ruminants can act as alternative hosts for *A. platys* (as suggested by Yang et al. (2015)) or whether they host phylogenetically similar lineages with distinct host ranges seems warranted.

The majority of genotypes isolated in this study differed between gazelle and sheep by five or fewer nucleotides (*Theileria*: ThSh1 and GG2; *Anaplasma*: AnSh1, AnGa2, AnSh3; see S3 for alignments). While these substitutions alone may not preclude transmissibility between hosts, the conservation of these nucleotide differences between hosts suggests that Grant's gazelle genotypes are not necessarily readily transmissible to sheep, or vice versa. This notion of minor genetic host specificity corroborates results of previous research by de la Fuente et al. (2007), which found that genotypes of *A. ovis* from wild mule deer (*Odocoileus hemionus*) and bighorn sheep (*Ovis canadensis*) were distinct by between one and three nucleotides from previously characterized genotypes of domestic sheep. Similarly, research in central Spain found that while overlapping populations of Iberian red deer (*Cervus elaphus hispanicus*) and domestic cattle both harbored *Anaplasma marginale* and *A. phagocytophilum*, each species possessed distinct genotypes that were >95% genetically similar to one another (De La Fuente et al., 2005). Taken together, it seems possible that these host-specific genotypes are less readily transmissible across the wildlife-livestock interface. Nevertheless, wildlife and livestock co-habitation may still influence TBD dynamics by contributing to the maintenance of generalist tick populations.

Aside from the examples above, studies that compare the TBHs of wild and domestic species occurring in the same geographic area are rare. One notable exception characterized the infections of 18 wild and domestic hosts, which harbored 16 TBHs of the genera

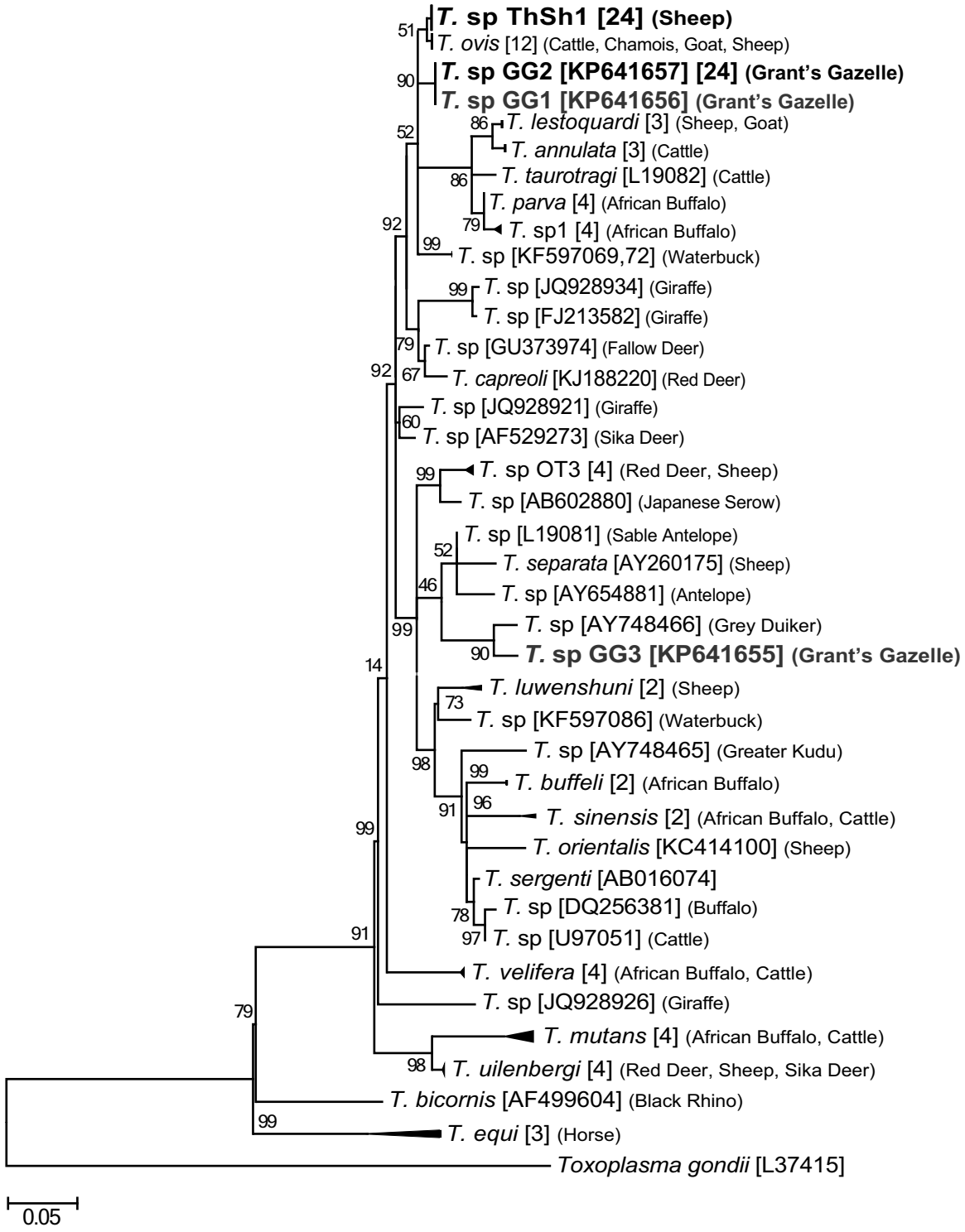

Fig. 1. Maximum likelihood (TN93 G + I) phylogenetic reconstruction of *Theileria*. Sequences isolated from this work shown in bold and black, while sequences isolated from 2009 gazelle from the same population (Hooge et al., 2015) are shown in bold and grey. Square brackets indicate either the number of sequences, or the Genbank accession number if only one or two sequences are represented. See S4 for Genbank accession numbers of all sequences used. Where possible, round brackets were also included to indicate the hosts that the sequences were isolated from. Numeric values at the nodes indicate bootstrap support established through 1000 permutations. Scale bar indicates nucleotide substitutions per site.

Babesia, *Theileria*, *Anaplasma* and *Ehrlichia* (Berggoetz et al., 2014). The study described broader host ranges than previously reported for several TBHs, including multiple *Theileria* (*T. bicornis*, *T. buffeli*, *T. separata*, *T.* sp.) and *Anaplasma* (*A. centrale, marginale*) species (Berggoetz et al., 2014). This appears contrary to our results of subtle nucleotide specificity between host species. However, Berggoetz and colleagues used reverse line blot (RLB) hybridization to detect species or genus-level TBH infection. The RLB approach is a widely used method for TBH detection (Bekker et al., 2002; Gubbels et al., 1999; Schnittger et al., 2003), and has the advantage of being able to detect multiple species even among co-infected samples. Indeed, inability to use co-infected samples is a limitation of this

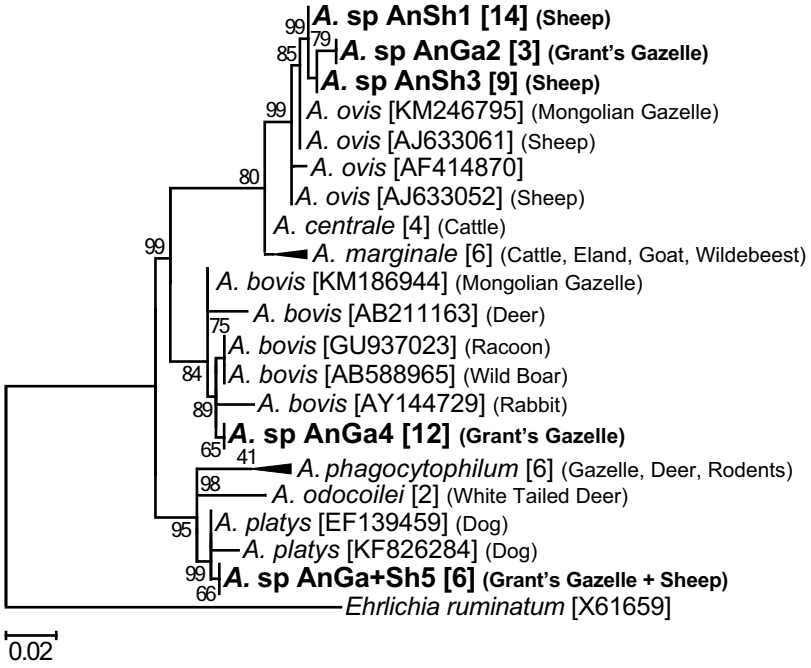

Fig. 2. Maximum likelihood (K2 G + I) phylogenetic reconstruction of *Anaplasma*, with sequences isolated from this work shown in bold. Square brackets indicate either the number of sequences, or the Genbank accession number if only one or two sequences are represented. See S4 for Genbank accession numbers of all sequences used. Where possible, round brackets were also included to indicate the hosts that the sequences were isolated from. Numeric values at the nodes indicate bootstrap support established through 1000 permutations. Scale bar indicates nucleotide substitutions per site.

study. However, PCR-based amplification and sequencing in this study allowed the detection of fine-scale genetic differences that may not be distinguishable with RLB. Future research attempting to quantify the actual cross-species transmission potential of closely related TBH genotypes is needed to better understand the natural host range of these pathogens.

In addition to determining the extent of TBH sharing occurring between wildlife and livestock, we also identified several novel TBH genotypes. For example, finding additional gazelle sequences that cluster with *Theileria* GG2 supports the conclusions of Hooge et al. (2015) that this genotype, along with GG1, form an uncharacterized clade which should be further investigated for putative species designation. In addition, no other genotype identified was 100% identical to previously characterized sequences. Discovery of novel genotypes is a recurring pattern in studies that generate TBH sequence data, especially in wildlife. For example, a study of Mongolian gazelle (*Procapra gutturosa*) in China detected four *Anaplasma* species (*A. bovis*, *A. ovis*, *A. phagocytophilum*, and an uncharacterized lineage) and several divergent forms of *Theileria luwenshuni* (Li et al., 2014). Similarly, several new genotypes of *Theileria* have been identified in waterbuck (*Kobus defassa*) and African buffalo from Kenya (Chaisi et al., 2013; Githaka et al., 2014). Taken together, these results suggest that there remains significant uncharacterized diversity within TBHs.

5. Conclusions

This study discovered new genotypes of both *Theileria* and *Anaplasma* in domestic sheep and wild Grant's gazelle, which supports a common pattern of substantial genetic diversity within TBHs. Perhaps more intriguingly, however, we discovered that despite opportune conditions for transmission across the wildlife-livestock interface, there was limited evidence of TBH sharing between this spatially overlapping pair of wild and domestic small ruminant. Although considerable attention has focused on pathogens that transmit across host species, the frequency of tick-borne hemoparasite sharing we document here suggests that transmission between livestock and wildlife may be less common than is widely expected.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2016.07.005.

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