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

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Running Title: Hemoparasite infections in wild and domestic ruminants

26 **HIGHLIGHTS**

- 27 • 99% of wild Grant's gazelle, 66% of domestic sheep were PCR-positive for *Theileria*
- 28 • 32% of Grant's gazelle, 47% of sheep were PCR-positive for *Anaplasma*
- 29 • *Theileria* sequences sorted into 2 genotypes; each was specific to the host
- 30 • *Anaplasma* sequences sorted into 5 genotypes; 1 contained samples from both hosts

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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.

Keywords: *Anaplasma*; *Theileria*; hemoparasite; tick-borne diseases; parasite sharing; sheep; gazelle

73 **1. INTRODUCTION**

74 Pathogen transmission occurring between wild and domestic animals is gaining attention, in part
75 because pathogen spillover can be devastating to both wildlife and livestock (Siembieda et al., 2011;
76 Wiethoelter et al., 2015). For example, wildlife-endemic diseases like foot-and-mouth disease and
77 heartwater that naturally occur in African buffalo (*Syncerus caffer*) are now considered among the most
78 serious livestock diseases on the African continent, particularly in cattle (Bengis et al., 2002). On the
79 other hand, livestock diseases like rinderpest and brucellosis have contributed to precipitous wild
80 ruminant declines in Africa and North America, (Grootenhuys, 2000; Miller et al., 2013; Nishi et al.,
81 2002). Given that livestock and wildlife are estimated to share three quarters of their pathogens
82 (Cleaveland et al., 2001; Wiethoelter et al., 2015), spillover events between the two groups may be
83 inevitable. Nevertheless, understanding the ease with which various pathogen taxa transmit across the
84 wildlife-livestock interface may help narrow the scope of efforts seeking to prevent future outbreaks.

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

96 to livestock in Mexico and Japan, respectively (Cantu et al., 2007; Kawahara et al., 2006). Livestock
97 TBHs may also impact wildlife (Miller et al., 2013), although TBH spillover from livestock to wildlife
98 populations has received less attention and clear examples are absent from the literature.

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

119 and Pedersen, 2008). For example, a recent study of 16 TBH species circulating among 18 wild and
120 domestic ungulates in South Africa found that TBHs tended to cluster by the phylogenetic history of
121 hosts (Berggoetz et al., 2014).

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123 On the African continent, TBHs of greatest concern include protozoan piroplasms of the genera *Babesia*
124 and *Theileria*, and rickettsial bacteria of the genera *Anaplasma* and *Ehrlichia*. These species are
125 common in cattle, sheep, and goats (Njiiri et al., 2015; Uilenberg, 1995), and are an increasingly
126 reported in wildlife (Criado-Fornelio et al., 2004; Eygelaar et al., 2015; Heyman et al., 2010). However,
127 descriptions of TBH sharing between wildlife and livestock are infrequent. Here, we examine the extent
128 to which these TBHs are shared between a wild (Grant's gazelle) and domestic (sheep) ruminant in
129 central Kenya. In this region, livestock densities are increasing, and there is extensive overlap between
130 livestock and wildlife populations (Georgiadis et al., 2007; Kinnaird and O'Brien, 2012). Interestingly, a
131 close relative of *Theileria ovis* (a common TBH of sheep; Altay et al. (2005)), was recently isolated
132 from Grant's gazelle (*Nanger granti*) (Hooge et al. 2015). This raises the question of whether this new
133 genotype is transmissible to domestic animals, which extensively overlap with wildlife in the study
134 region. Grant's gazelle occur across East Africa, can persist at high livestock density (Georgiadis et al.,
135 2007), and also host a number of tick species known to infest livestock (Walker et al., 2003). Using
136 molecular detection by PCR and sequencing, we screened Grant's gazelle samples collected in three
137 separate years for the piroplasms *Babesia* and *Theileria*, and the rickettsiae *Anaplasma* and *Ehrlichia*.
138 We compared these TBH profiles to those from sheep inhabiting high wildlife density (considerable
139 wildlife-livestock overlap) and low wildlife density (little wildlife-livestock overlap) areas. This
140 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 17-112 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

163 were kept on ice in the field until transport to the laboratory where they were stored at -20°C until
164 processing.

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166 2.2 Parasitological analyses

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

176

177 To screen for *Anaplasma/Ehrlichia*, we amplified a ~450 bp fragment of the V1 hypervariable region of
178 16S ribosomal DNA using previously published primers EHR-F (5'-GGA ATT CAG AGT TGG ATC
179 MTG GYT CAG-3') and EHR-R (5'-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3')
180 (Bekker et al., 2002). This reaction was carried out in 25 µL volumes. The reaction mixture contained 3
181 µL of template DNA, 0.4 µM of each forward and reverse primer, 200 µM of dNTP, 2.5 µL 10X PCR
182 buffer, 2.0 mM of MgCl₂, 5 µL 360 GC Enhancer, and 0.25 µL (1.25 units) of AmpliTaq Gold 360 DNA
183 Polymerase (all reagents from Thermo Fisher Scientific, NY, CA, USA). Reactions were cycled with the
184 following thermal profile: 95°C for 10 min., 35 cycles of 95°C for 30 sec., 55.5°C for 30 sec., and 72°C

185 for 60 sec., and a final 10 min. extension at 72°C. All gazelle samples (2009, 2011 and 2015), and all
186 sheep samples (2015 MRC and Lekiji) were screened by this method.

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188 All PCR products were electrophoresed on a 1% agarose gel stained with GelRed (Biotium Inc., CA,
189 USA) and visualized under UV light to determine positivity for *Babesia/Theileria* and
190 *Anaplasma/Ehrlichia*. A subset of samples with the most brightly banded PCR products were selected
191 for sequencing. A total of 38 gazelle samples (n = 18 from 2015, 20 from 2011) and 36 sheep samples (n
192 = 18 from MRC, 18 from Lekiji) were sequenced for *Babesia/Theileria*. In addition, 21 gazelle samples
193 (n = 1 from 2015, 13 from 2011, 7 from 2009) and 34 sheep samples (n = 7 from MRC, 27 from Lekiji)
194 were sequenced for *Anaplasma/Ehrlichia*. Selected samples were purified using the QIAquick PCR
195 Purification Kit (Qiagen, CA, USA) and Sanger sequenced on an ABI3730 Genetic Analyzer (Applied
196 Biosystems, CA, USA) using both forward and reverse primers. Sequencing results were edited using
197 Geneious v. 8.1 (Biomatters, Auckland, New Zealand).

198

199 2.3 Statistical Analyses

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

206

207 2.4 Phylogenetic Analyses

208 *Theileria* and *Anaplasma* sequences were separately aligned in ClustalX v. 2.1. *Theileria* sequences
209 isolated from gazelle and sheep in this study were aligned with 76 representative *Theileria* sequences
210 (including the GG1, GG2 and GG3 genotypes identified by Hooge et al. [2015] from 2009 gazelle) and
211 a *Toxoplasma gondii* outgroup sequence. *Anaplasma* sequences isolated in this work were aligned with
212 29 representative *Anaplasma* sequences and an *Ehrlichia ruminatum* outgroup sequence. All
213 representative sequences used were available from Genbank (see S4), and were required to fit two of
214 three criteria for inclusion: 1) belonging to scientific papers published in peer-reviewed literature; 2)
215 isolated from African mammals; 3) isolated from ungulates (both Artiodactyla and Perrisodactyla). For
216 both alignments, poorly aligned regions were manually adjusted and regions of ambiguous alignment
217 were identified and removed in Gblocks v. 0.91 using the least stringent parameter settings (Castresana,
218 2000; Talavera and Castresana, 2007). Alignments were trimmed to identical lengths and models of
219 sequence evolution were selected using the MrModelTest v. 2.3 executable in PAUP* v. 4 (Nylander,
220 2004; Swofford, 2003).

221

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

231

232 3. RESULTS

233 3.1. Patterns of Infection

234 3.1.1. *Babesia/Theileria*

235 A total of 120 Grant's gazelle samples from 2011 (n = 62) and 2015 (n = 58), and 134 sheep samples
236 from MRC (n = 50) and Lekiji (n = 84) were screened for *Babesia/Theileria* (Table 1). In gazelle, a
237 98.4 and 100% prevalence of *Babesia/Theileria* was detected in 2011 and 2015, respectively, which is
238 similar to the 100% prevalence documented by Hooge et al (2015) for animals sampled in 2009 (n = 65).
239 In sheep, 58% of animals sampled at MRC and 71.4% of animals sampled at Lekiji were
240 *Babesia/Theileria* positive (Table 1). There was no association between time since the last acaricide
241 treatment and *Babesia/Theileria* infection status (Number days since acaricide treatment: $Z = -0.52$, $P =$
242 0.603 ; Age: $Z = 1.60$, $P = 0.111$; Location (MRC): $Z = -2.44$, $P = 0.015$; Sex (Male): $Z = 1.97$, $P =$
243 0.049), so it seems unlikely that treatment affected our ability to detect this parasite in sheep. All
244 sequenced samples from both gazelle and sheep were identified as *Theileria* spp., which suggests that
245 infections by *Babesia* were absent.

246

247 3.1.2. *Anaplasma/Ehrlichia*

248 A total of 179 Grant's gazelle samples from 2009 (n = 59), 2011 and 2015 (n as above) and 134 sheep
249 samples from MRC and Lekiji (n as above) were screened for *Anaplasma/Ehrlichia*. Grant's gazelle had
250 a prevalence of 52.5, 41.9, and 1.7% in 2009, 2011, and 2015, respectively. In sheep, 14.0% of animals
251 sampled at MRC and 66.7% sampled at Lekiji were positive for *Anaplasma/Ehrlichia*. As with
252 *Theileria*, there was no relationship between time since last acaricide treatment and
253 *Anaplasma/Ehrlichia* prevalence in sheep (Number days since acaricide treatment: $Z = 1.001$, $P = 0.314$;

254 Age: $Z = 1.95$, $P = 0.051$; Location (MRC): $Z = -3.73$, $P = <0.001$; Sex (Male): $Z = -1.61$, $P = 0.108$).

255 All sequenced samples from both gazelle and sheep were identified as *Anaplasma* spp., suggesting that
256 infections by *Ehrlichia* were absent.

257

258 3.2. Phylogenetic Relationships

259 3.2.1. *Theileria*

260 From 38 Grant's gazelle and 36 sheep samples sequenced, 14 gazelle and seven sheep sequences had
261 conspicuous overlapping chromatogram peaks that were suggestive of co-infection, as has been found
262 previously in Grant's gazelle (Hooge et al., 2015). Another five sheep sequences were of poor quality.
263 These 26 sequences were omitted from phylogenetic analyses. The remaining 48 sequences from gazelle
264 (2011: $n = 11$ & 2015 $n = 13$) and sheep (MRC: $n = 11$ & Lekiji: $n = 13$) were used for phylogenetic
265 reconstruction. These analyses revealed two distinct *Theileria* genotypes that were specific to either
266 sheep or gazelle (Fig. 1). Within these genotypes, all sequences were 100% identical. The sheep
267 genotype, designated ThSh1 (for *Theileria* Sheep Genotype 1), contained all 24 sheep sequences, and
268 was 99.9% identical to representative *T. ovis* sequences. The gazelle genotype contained all 24 gazelle
269 sequences and was 100% identical to GG2, a genotype previously described by Hooge et al. (2015) (Fig.
270 2). Interestingly, GG2 was the only genotype isolated from 2011 and 2015 gazelle samples, although
271 Hooge et al. (2015) isolated GG1 and GG3 in addition to GG2 from 2009 samples. The GG2 genotype
272 differed from GG1 by a single nucleotide substitution, and shared 94.5% nucleotide identity with GG3.
273 GG2 shared 99.4% sequence identity with *T. ovis*, and appears to be sister to the clade containing both
274 *T. ovis* and the newly identified sheep genotype ThSh1.

275

276 The sheep (ThSh1) and gazelle (GG2) genotypes identified in this study were 99.4% identical. These
277 genotypes differed by five nucleotides that were consistent based on host isolation source (gazelle or
278 sheep). ThSh1 shared 99.4 and 93.7% nucleotide similarity with GG1 and GG3, the two gazelle
279 genotypes reported from Hooze et al (2009). Both sheep and gazelle genotypes were most divergent
280 from *T. equi* (87% sequence similarity), and shared 76% similarity with the outgroup *Toxoplasma gondii*
281 (see S1 for all pairwise distance calculations).

282

283 3.2.2. *Anaplasma*

284 Out of 21 Grant's gazelle and 34 sheep samples sequenced, 19 gazelle and 25 sheep samples returned
285 usable sequence data. Two gazelle and six sheep samples had conspicuous overlapping chromatogram
286 peaks suggestive of co-infection. This included the only gazelle sample positive for *Anaplasma* in 2015.
287 An additional three sheep sequences were of poor quality. All 11 of these sequences were omitted from
288 further analyses. The remaining 44 sequences from gazelle (2009: n = 7 & 2011: n = 12) and sheep
289 (MRC: n = 3 & Lekiji: n = 22) were used for phylogenetic reconstruction. These analyses revealed that
290 the *Anaplasma* genotypes detected in this study sorted into five distinct clades. These clades were
291 designated AnSh1, AnGa2, AnSh3, AnGa4, and AnGa+Sh5 to represent the genus (An for
292 "*Anaplasma*"), host species the sample was isolated from (Ga for "gazelle" or Sh for "sheep"), and an
293 arbitrary numeric value of 1-5 to represent the five clades discovered here. Genotypes 1 and 3 were only
294 isolated from sheep, and genotypes 2 and 4 were only isolated from gazelle. Genotype 5 was isolated
295 from both sheep and gazelle. The first genotype, AnSh1, comprised 56% of all successfully sequenced
296 sheep samples. AnSh1 formed a monophyletic clade that was sister to AnGa2 and AnSh3, and 99.7%
297 sequence similarity, respectively. AnGa2 comprised 16% of sequenced gazelle samples, and shared
298 99.5% similarity with AnSh3, which comprised 36% of all sequenced sheep samples. These three

299 genotypes all differed from one another by three nucleotide substitutions, and formed a monophyletic
300 sister group to representative *A. ovis* sequences. AnSh1, AnGa2 and AnSh3 shared 99.5, 99.0, and
301 99.3% sequence similarity with the *A. ovis* clade, respectively. AnGa4 comprised 63% of all sequenced
302 gazelle samples. AnGa4 clustered with the *A. bovis* clade and shared 99.1% sequence identity with other
303 *A. bovis* sequences. The AnGa4 and *A. bovis* clade was sister to the clade containing *A. centrale*, *A.*
304 *marginale*, *A. ovis*, and AnSh1, AnGa2, and AnSh3. The gazelle genotype AnGa4 shared greater
305 sequence similarity (96.7%) with the gazelle genotype AnGa2, than with the sheep genotypes AnSh1
306 and AnSh3 (96.1%). Finally, AnGa+Sh5 sequences were isolated from both Grant's gazelle and sheep.
307 25% of successfully sequenced gazelle samples in 2009 and 14% in 2011 were infected with the
308 AnGa+Sh5 genotype, compared with 9% of sheep from Lekiji and none from MRC. This genotype was
309 the most divergent of those isolated in this study, sharing between 95.2 (AnSh1, AnGa2, AnSh3) and
310 97.2% (AnGa4) sequence similarity with the other genotypes isolated here. AnGa+Sh5 sequences fell
311 within the *A. platys* species complex and shared 99.3% sequence identity with other *A. platys* sequences
312 (see S2 for all pairwise distance calculations).

313

314 **4. DISCUSSION**

315 This study investigated the potential for TBH sharing between one wild and one domestic ruminant in
316 central Kenya. Based on habitat overlap, shared tick vectors, and family-level phylogenetic relatedness,
317 we expected that Grant's gazelle and sheep would share some of their TBHs. We discovered seven
318 distinct TBH genotypes (two *Theileria* spp., and five *Anaplasma* spp.), and only one (*Anaplasma*:
319 AnGa+Sh5) was identical in both gazelle and sheep. Interestingly, this genotype was only detected in
320 sheep at the high-intensity livestock site where overlap with wildlife (including gazelle) is limited

321 (Georgiadis et al., 2007). These results suggest that despite an opportune system for pathogen sharing,
322 the majority of TBH infections are not shared across the wildlife-livestock interface in this system.

323

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

337

338 The majority of genotypes isolated in this study differed between gazelle and sheep by five or fewer
339 nucleotides (*Theileria*: ThSh1 and GG2; *Anaplasma*: AnSh1, AnGa2, AnSh3; see S3 for alignments).
340 While these substitutions alone may not preclude transmissibility between hosts, the conservation of
341 these nucleotide differences between hosts suggests that Grant's gazelle genotypes are not necessarily
342 readily transmissible to sheep, or vice versa. This notion of minor genetic host specificity corroborates
343 results of previous research by de la Fuente et al. (2007), which found that genotypes of *A. ovis* from

344 wild mule deer (*Odocoileus hemionus*) and bighorn sheep (*Ovis canadensis*) were distinct by between
345 one and three nucleotides from previously characterized genotypes of domestic sheep. Similarly,
346 research in central Spain found that while overlapping populations of Iberian red deer (*Cervus elaphus*
347 *hispanicus*) and domestic cattle both harbored *Anaplasma marginale* and *A. phagocytophilum*, each
348 species possessed distinct genotypes that were >95% genetically similar to one another (De La Fuente et
349 al., 2005). Taken together, it seems possible that these host-specific genotypes are less readily
350 transmissible across the wildlife-livestock interface. Nevertheless, wildlife and livestock co-habitation
351 may still influence TBD dynamics by contributing to the maintenance of generalist tick populations.

352

353 Aside from the examples above, studies that compare the TBHs of wild and domestic species occurring
354 in the same geographic area are rare. One notable exception characterized the infections of 18 wild and
355 domestic hosts, which harbored 16 TBHs of the genera *Babesia*, *Theileria*, *Anaplasma* and *Ehrlichia*
356 (Berggoetz et al., 2014). The study described broader host ranges than previously reported for several
357 TBHs, including multiple *Theileria* (*T. bicornis*, *T. buffeli*, *T. separata*, *T. sp*) and *Anaplasma* (*A.*
358 *centrale*, *marginale*) species (Berggoetz et al., 2014). This appears contrary to our results of subtle
359 nucleotide specificity between host species. However, Berggoetz and colleagues used reverse line blot
360 (RLB) hybridization to detect species or genus-level TBH infection. The RLB approach is a widely used
361 method for TBH detection (Bekker et al., 2002; Gubbels et al., 1999; Schnittger et al., 2003), and has the
362 advantage of being able to detect multiple species even among co-infected samples. Indeed, inability to
363 use co-infected samples is a limitation of this study. However, PCR-based amplification and sequencing
364 in this study allowed the detection of fine-scale genetic differences that may not be distinguishable with
365 RLB. Future research attempting to quantify the actual cross-species transmission potential of closely
366 related TBH genotypes is needed to better understand the natural host range of these pathogens.

367

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

380

381 5. CONCLUSIONS

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

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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415 **TABLES**

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

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	Grant’s Gazelle – MRC*				Sheep – 2015		
	2015	2011	2009	TOTAL	MRC	Lekiji	TOTAL
	(n = 58)	(n = 62)	(n = 59)	(n = 179)	(n = 50)	(n = 84)	(n = 134)
<i>Theileria</i>	100.0	98.4	100.0	99.4	58.0	71.4	66.4
<i>Anaplasma</i>	1.7	41.9	52.5	32.4	14.0	66.7	47.0

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*MRC = Mpala Research Center

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FIGURES

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.

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.

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