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Original article

# Tick-borne pathogens in the blood of wild and domestic ungulates in South Africa: Interplay of game and livestock

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ABSTRACT

We screened for tick-borne pathogens blood samples from 181 wild and domestic ungulates belonging to 18 host species in 4 South African Provinces. Polymerase chain reaction followed by reverse line blotting and sequencing allowed detecting 16 tick-borne pathogen species belonging to the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia*. Ten pathogen species were involved in 29 new host–pathogen combinations. Most infections (77.9%) involved more than one pathogen species. Principal component analysis (PCA) assigned the 163 infections, identified to species level, to 4 groups. Three groups were associated with sheep, cattle, and horse and their respective wild counterparts. Each group was characterised by high homogeneity in pathogen assemblage and host phylogenetic status. These groups characterised the most privileged transmission routes between and among wild and domestic ungulates. The 4th group showed high heterogeneity in pathogen assemblage and host phylogenetic status. This group seems to indicate frequent spill over events in impala of pathogens that usually circulate among cattle- or sheep-related species. Within 6 localities, we sampled an equal number of wild and domestic animals ($n = 128$). On this dataset once having controlled for the significant variation among localities, the infection prevalence and intensity of infection did not differ significantly between wild and domestic hosts. This suggests that both animal types, domestic and wild hosts, could act as evenly efficient sources of infection for themselves and for each other. Overall, this study shed new light on the pathogen circulation naturally achieved at the interplay between wild and domestic ungulates.



## Introduction

The knowledge on the epidemiological relations between wild and domestic ungulates in the context of tick-borne pathogens of the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* in southern Africa remains incomplete. Some of these pathogens evolved together with wild ungulates over many million years resulting, usually, in a state of equilibrium due to reciprocal adaptations (Jongejan and Uilenberg, 2004). Livestock animals arrived on the current South African territory about 2000 years ago represented by fat-tailed sheep followed by Sanga-type cattle approximately 1500 years ago (Du Toit, 2008). European livestock breeds introduced since 1706 were more susceptible to tick-borne pathogens, which challenged their establishment in South Africa (Bigalke, 1994).

In South Africa, the main indigenous tick-borne diseases affecting cattle are corridor disease, heartwater, African redwater, and gallsickness which are caused by *Theileria parva*, *Ehrlichia ruminantium*, *Babesia bigemina*, and *Anaplasma marginale*, respectively (Bigalke, 1994; Uilenberg, 1995). Less pathogenic organisms like *T. mutans* and *T. velifera* also affect cattle. All these pathogen species were first described in cattle. However, the ancestral host of *T. parva*, *T. mutans*, and *T. velifera* is the African buffalo (*Syncerus caffer*) (Bigalke, 1994; Mans et al., 2011). Moreover, it seems likely *that E. ruminantium*, *B. bigemina*, and *A. marginale* first circulated among wild African ruminants (Bigalke, 1994). According to Yin et al. (2007), *T. separata* that is genetically related to the sable antelope *parasite Theileria* sp. (sable) probably arose from a spillover from African sable antelope (*Hippotragus niger*) to sheep. Zebras looked also as the most likely ancestral hosts of *T. equi*, the agent

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of equine piroplasmosis, since a unique genotype seems to occur in these animals (Bhoora et al., 2009). Moreover, the introduction of exotic cattle breeds may also have promoted the introduction of new pathogens species in South Africa. For instance, the causative agent of Asiatic redwater, *B. bovis* is generally thought to be an Asian parasite and has probably been introduced into South Africa at the beginning of the 19th century (Theiler, 1962). Similarly, the cattle parasite *T. buffeli* is most likely an Asiatic water buffalo (*Bubalus bubalis*)-derived parasite (Gubbels et al., 2000).

Early investigations of tick-borne pathogens in wild ruminants mainly aimed at evaluating their role in the epidemiology of livestock diseases (Neitz and Du Toit, 1932; Neitz, 1935; Löhr and Meyer, 1973; Löhr et al., 1974; Carmichael and Hobday, 1975). Wild ruminants can nevertheless suffer from tick-borne pathogens. For instance, natural infection by *T. taurotragi* can be fatal in the common eland (*Tragelaphus oryx*) (Lawrence et al., 1994b). Nevertheless, most of the fatal cases in game animals are associated with translocations, when naive animals or pathogens are introduced into new regions (Oosthuizen et al., 2009). For instance, death of translocated black rhinoceroses (*Diceros bicornis*) due to *Babesia bicornis* was reported after translocation (Nijhof et al., 2003). Similarly, fatal infection cases after translocation were reported for infection by *Theileria* sp. (sable) in roan antelope (*H. equinus*) (Nijhof et al., 2005), a parasite known to cause mortality in roan and sable antelopes (Steyl et al., 2012), threatening these declining host species (http://www.iucnredlist.org). All this suggests that relations between wild and domestic ungulates are tight and that pathogens of domestic animals may threaten health of wild animals and vice versa.

Recent studies, based on large-scale molecular detection methods, allowed identifying a broader host range in game and livestock animals for several tick-borne pathogen species than suspected (Pfitzer et al., 2011; Tonetti et al., 2009; Muhanguzi et al., 2010; Yusufmia et al., 2010; among others). We used the advantages of reverse line blot (RLB) hybridisation to screen blood samples from wild and domestic animals for tick-borne pathogens. In this study, we aimed at (i) evaluating the tick-borne pathogen load in wild and domestic ungulates living in close vicinity, (ii) studying associations between host and pathogen species, (iii) examining coinfection patterns of pathogen species, and (iv) highlighting aspects of the nature of the interplay existing between the pathogens and their wild and domestic hosts.

## Materials and methods

### *Study areas and datasets*

Sampling was undertaken during 3 fieldwork sessions (May–July 2009, January–May 2010, and April–June 2011) in 4 South African provinces (Fig. 1). A first dataset of 128 animals corresponds to a paired-sampling of wild and domestic hosts within each of 6 localities (Fig. 1). Wild animal samples were collected at Tüssen-Die-Riviere, Willem Pretorius, and Sandveld in the Free State Province (3 provincial reserves), near Bethal in the Mpumalanga Province (one private game farm) and in the Lephalale and Thabazimbi areas in the Limpopo Province (5 private game farms). Domestic animal samples were collected within the same week as their wild counterparts from several commercial livestock farms located in the close vicinity of these 6 sites (common border or within a range of 40 km). This first dataset was extended by the addition of 53 animals. These animals originated from Tüssen-Die-Riviere, Willem Pretorius, and Sandveld and were sampled from 3 additional sites at the same time as animals from the first sampling: at Sterkfontein, Seekoeivlei in the Free State Province (2 nature reserves) and Pretoria in the Gauteng Province (2 livestock farms).

### *Blood sampling*

Blood was collected from game animals during game capture, culling operations, and hunts. Cattle were driven into kraals where holding facilities were available. Sheep were brought into smaller camps and immobilised by hand. Blood was withdrawn from the jugular or the coccygeal vein from live animals or collected from the throat of hunted animals, and 200 μl were placed on FTA Classic Cards (Whatman®, Buckinghamshire, UK). Once dried, the cards were stored as indicated by the manufacturer. All sampled animals appeared in good health conditions, except one cow (see below).

### *Detection and identification of pathogen species*

To obtain template DNA for PCR, 1.2 mm diameter discs were punched into FTA card blood spot with the use of the Harris Micro-Punch® (Whatman®, Buckinghamshire, UK). Before each sample, the tip of the Harris Micro-Punch® was rinsed in bleach and 70% alcohol and dried with sterile wipe. Discs were washed 3 times in 200 μl FTA purification reagent (Whatman®, Buckinghamshire, UK) and twice in 200 μl house-made TE-1 buffer (10 mM Tris–HCL, 0.1 mM EDTA, pH 8.0) in 300 μl tubes as recommended by the manufacturer. Discs were dried at 56 °C for 20 min in a stove, followed by PCR amplification. Primers 16S8FE (5′-GGAATTCAGAGTTGGATCMTGGYTCAG-3′) and B-GA1B-new (5′-Biotin-CGGGATCCCGAGTTTGCCGGGACTTYTTCT-3′) (Schouls et al., 1999, modified by Bekker et al., 2002) were used to amplify an approximately 500-base-pair (bp) fragment of the 16S ribosomal RNA (rRNA) gene spanning the hypervariable V1 region of the genera *Anaplasma* and *Ehrlichia*. To detect the genera *Babesia* and *Theileria*, an approximately 400-bp fragment of the 18S rRNA gene spanning the V4 hypervariable region was amplified with the use of the primer set RLB-F2 (5′-GACACAGGGAGGTAGTGACAAG-3′) and RLB-R2 (5′-Biotin-CTAAGAATTTCACCTCTGACAGT-3′) (Georges et al., 2001). Primers were obtained from Microsynth AG (Balgach, Switzerland). Amplification reactions were performed in a Master Mix volume of 25 μl (Bekker et al., 2002). PCR reactions took place in a Whatman Biometra® Tprofessional Basic Gradient (Göttingen, Germany) by using a touchdown PCR programme (Bekker et al., 2002), with an annealing temperature lowered by 1 °C instead of 2 °C (Tonetti et al., 2009). Positive controls consisting in FTA cards with cattle blood from the Ivory Coast infected by *A. centrale* or *A. marginale* and *B. bovis* (kindly provided by C. Rühle, University of Neuchâtel) and negative control consisting in a clean FTA card were included in each PCR run. To identify pathogens at species level, the obtained PCR products were analysed by RLB hybridisation (Gubbels et al., 1999, modified by Tonetti et al., 2009) using 41 oligonucleotide probes, including 3 genus-specific probes Babesia/Theileria, Anaplasma/Ehrlichia, and Theileria (Supplementary Butler et al., 2008; Hea et al., 2011; Matjila et al., 2004; Nagore et al., 2004; Oosthuizen et al., 2008; Oura et al., 2004; Pfitzer, 2009; Ros-Garcia et al., 2011; Table S1). Samples reacting only with the Babesia/Theileria probe were considered as *Babesia* spp. since a genus-specific probe was included for the genus *Theileria*. In order to test for theoretical specificity, the oligonucleotide probes used in this study were aligned with various sequences of targeted species available from the National Centre for Biotechnology Information (NCBI) using a software package: CLC Sequence Viewer 6 (CLC bio, Aarhus, Denmark).

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.ttbdis.2013.10.007.

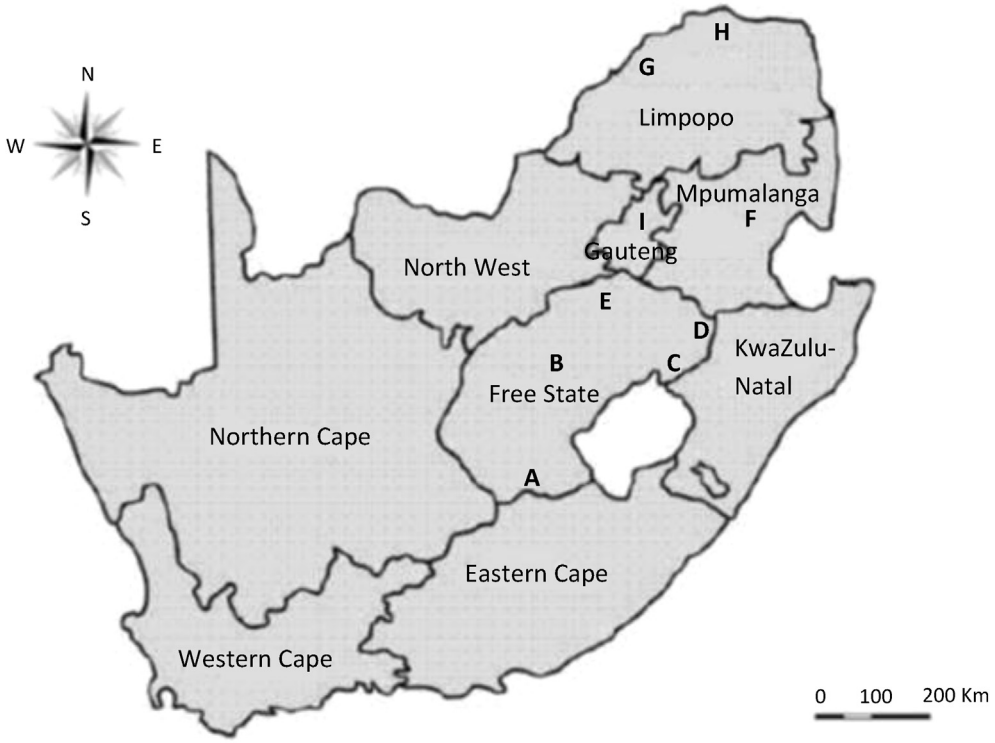


**Fig. 1.** Host sampling in study areas in South Africa. (A) Tüssen-Die-Riviere (cattle $n = 17$; sheep $n = 10$; common eland $n = 8$; springbok $n = 6$; greater kudu $n = 5$; impala $n = 5$; black wildebeest $n = 2$; African buffalo $n = 1$; additional hosts: warthog $n = 3$; common eland $n = 4$; greater kudu $n = 1$). (B) Willem Pretorius (cattle $n = 7$; African buffalo $n = 7$; additional host: African buffalo $n = 1$). (C) Sterkfontein (additional hosts: black wildebeest $n = 4$). (D) Seekoeivlei (additional hosts: black wildebeest $n = 11$; red hartebeest $n = 15$; plain zebra $n = 6$; African buffalo $n = 1$). (E) Sandveld (cattle $n = 12$; sheep $n = 4$; African buffalo $n = 7$; blue wildebeest $n = 4$; greater kudu $n = 3$; common eland $n = 1$; gemsbok $n = 1$; additional hosts: roan antelope $n = 5$). (F) Bethal (cattle $n = 4$; blesbok $n = 3$; black wildebeest $n = 1$). (G) Thabazimbi (cattle $n = 3$; impala $n = 2$; blue wildebeest $n = 1$). (H) Lephalale (cattle $n = 7$; Southern giraffe $n = 5$; sable antelope $n = 2$). (I) Pretoria (additional hosts: horse $n = 2$).
Modified from http://home.global.co.za/~mercon/map.htm

*Sequencing*

Prior sequencing, PCR products that reacted only with genus-specific probes *Babesia*/*Theileria*, *Theileria*, or *Anaplasma*/*Ehrlichia* and not with species-specific probes were purified using a commercial available kit (Wizard® SV Gel and PCR Clean-Up System, Promega, Madison, USA). The manufacturer's instructions were followed, except that we eluted in 35 μl of nuclease-free water. Sequencing was performed by Microsynth AG (Balgach, Switzerland). The obtained sequences were corrected and compared by using software packages CLC Sequence Viewer 6 (CLC bio, Aarhus, Denmark) and Bioedit (Tom Hall Ibis Biosciences, Carlsbad, USA). Sequences were compared with available sequences from the NCBI using the Basic Local Alignment Search Tool (BLAST).

*Data analysis*

Data were analysed with "R" 2.14 for Windows (R Development Core Team, 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/), using software packages (Skaug et al., 2010; Husson et al., 2012).

Two generalised linear models (GLM) were used on the pair-sample dataset involving wild and domestic animals that had been sampled within each of 6 localities. In each case, we assessed the significance of the factors LOCATION (6 localities) and HOST TYPE (wild vs. domestic) as well as the LOCATION–HOST TYPE interaction. The first GLM, with binomial errors, evaluated infection prevalence of pathogens. The second GLM, with negative binomial errors, evaluated the intensity of infection (number of infections/number infected animals).

Principal component analysis (PCA) was employed as ordination technique in order to group the host animals with regard to the pathogen species infecting them. *P* values were considered significant when below 0.05. The permutation test proposed initially by Raup and Crick (1979) and developed by Clua et al. (2010) was constrained to locations for evaluating whether the adjusted associations between pathogen species in coinfections were significant. In this later case, the *P* value was considered significant when below 0.0004 (Bonferroni corrected).

## Results

Blood samples were collected from 181 animals belonging to 18 species (Fig. 2). Among those, 128 samples were paired in order to compare the pathogen prevalence and the intensity of infection between wild and domestic animals for a given locality. Fifty-three animals were then added to evaluate whether any associations between host and pathogen species occur.

All samples that were identified to species level were identified by RLB except 7 samples that were identified to species level after sequencing. The first one (GenBank accession number KF414713) was isolated from sheep and showed 99% homology with *A. platys* (GenBank accession number AY040853.1). Three identical sequences (GenBank accession number KF414712) isolated from cattle and African buffalo showed 100% homology with *A. marginale* (GenBank accession number FJ155998.1). Finally, 3 identical sequences (GenBank accession number KF414721) were

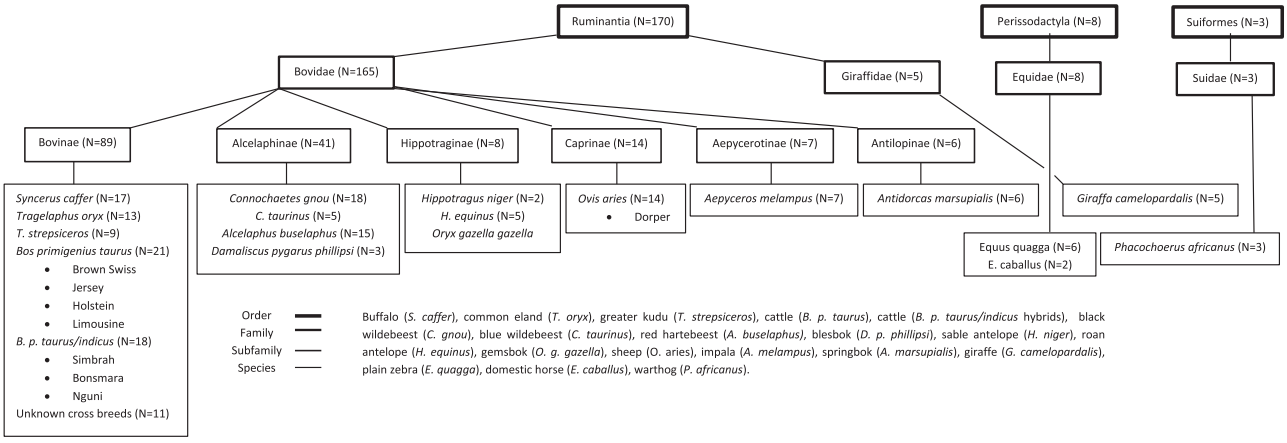


**Fig. 2.** Sampled hosts ($n = 181$) assigned to 18 species, and their taxonomic relations, according to Matthee and Davis (2001) and Fernandez and Vrba (2005).

isolated from sheep and displayed 100% homology with *A. ovis* (GenBank accession number EU191231.1).

### *Comparison between wild and domestic animals within six localities*

A total of 40 wild and 43 domestic animals (83/128, 64.8%) were found infected and 161 infections were detected in these animals (Table 1). The intensity of infection (number of infections/number of infected animals) reached 1.94 (161/83) pathogen. Thirty-three infections (20.5%) were identified at genus level only: 25 *Anaplasma* spp. and/or *Ehrlichia* spp., 5 *Theileria* spp., and 3 *Babesia* spp. (Table 1). Sequencing of these samples did not allow identification to species. The remaining 128 infections (79.5%) were identified at species level; they belonged to 15 pathogen species. *Theileria* sp. (sable) (21.9%) and *T. separata* (14.1%) were the most frequent species followed by *T. buffeli* (9.4%) and *A. marginale* (9.4%).

The infection prevalence and intensity of infection differed significantly with the LOCATION factor ($P < 0.001$ and $P = 0.021$, respectively), but not with the HOST TYPE factor ($P = 0.547$ and $P = 0.378$, respectively) or with LOCATION–HOST TYPE interaction ($P = 0.137$ and $P = 0.828$, respectively).

### *Associations of pathogen species with host species*

To better underscore possible associations between host and pathogen species, we analysed blood samples from 53 additional hosts (Table 2). Forty-nine infections were detected in these additional hosts. Among those, 35 infections (71.4%) were identified to species level, the remaining 14 infections were identified to genus level only (8 *Anaplasma* spp. and/or *Ehrlichia* spp., 5 *Theileria* spp., and one *Babesia* spp.) (Table 2). Merging this second dataset to the previous one led to 163 infections identified at the species level, involving 16 pathogen species. This dataset (Hellinger-transformed) was used to perform PCA in order to highlight possible associations between pathogen and host species that were not due to sampling location (data not shown). Fig. 3a displays the groups of host species deriving from pathogen assemblage. Fig. 3b presents the relative contribution of a given pathogen species to the definition of host groups via pathogen assemblage. Group 1 was the largest host group. It included 9 host species (springbok, blue wildebeest, black wildebeest, red hartebeest, blesbok, sable antelope, roan antelope, gemsbok, and sheep) that showed a relatively high proportion of infections with *T. bicornis*, *T. separate*, and *Theileria* sp. (sable), and to a lesser degree with *T. ovis* and *A. ovis*. These 5 pathogen species were strongly correlated with each other, with each of them being significantly correlated with the first axis ($P$ values $< 0.001$) (Fig. 3b). *Theileria buffeli*, *A. platys*, and *A. centrale* were also correlated with the pathogens characterising Group 1, but were not significantly correlated with the first axis (Fig. 3b). In this group, *T. buffeli* ($n = 17$) was detected 11 times, *A. platys* once, and *A. centrale* 4 times out of a total of 6 detection cases (Tables 1 and 2). At the opposite side along the first axis, Group 2 consisted of buffalo, common eland, greater kudu, and cattle (Fig. 3a). *T. taurotragi*, *T. velifera*, *T. mutans*, and *A. marginale* were exclusively detected in these host species. *Babesia bigemina* and *B. bovis* were correlated with the other pathogens of Group 2 and with the first axis, although not significantly ($P = 0.45$) (Fig. 3b). *Babesia bigemina* was detected in 3 out of the 4 host species constituting Group 2 while *B. bovis* was detected only once in cattle (Table 1). Horses and zebras formed Group 3; this third group being characterised by the presence of *T. equi* (Fig. 3a). Finally, impala and giraffe formed Group 4 which is interestingly defined by a mix of the pathogen assemblages defining the Groups 1 and 2 (Fig. 3a). Impala was infected with 4 pathogen species that were abundant in Group 1 (*T. bicornis*, *T.* sp. sable, *T. buffeli*, and *A. centrale*) as well as with *B. bigemina* occurring in Group 2. The only pathogen identified to species level in giraffe was *Ehrlichia* sp. (Omatjenne) ($n = 1$), which also occurred in Groups 1 and 2.

### *Coinfections*

Among the 163 infections identified at the species level, 127 (77.9%) were coinfections involving more than one pathogen species (Supplementary Table S2). Coinfections were detected in 22.1% (40/181) individual hosts [17/64 domestic animals (26.6%) and 23/117 wild animals (20%)]. Among these coinfections, 22 (17.3%) involved 2 species, 47 (37%) were triple infections, 33 (26%) involved 4 pathogen species, and 25 (19.7%) involved 5 pathogen species. Associations among the 16 pathogen species are shown in Table 3. Significance of coinfections was computed using statistical analysis proposed by Raup and Crick (1979) and developed by Clua et al. (2010) and constrained to locations to decide whether the adjusted associations between pathogen species in coinfections were significant. Six pathogen associations, involving 5 *Theileria* species, were significant: *Theileria* sp. (sable)-*T. separata* ($n = 19$); *Theileria* sp. (sable)-*T. buffeli* ($n = 15$); *Theileria* sp. (sable)-*T. bicornis* ($n = 14$); *T. separata*-*T. bicornis* ($n = 7$); *T. separata*-*T. ovis* ($n = 6$); *T. bicornis*-*T. ovis* ($n = 4$) (Table 3). These coinfections were observed in animals belonging to Group 1 as well as in impalas from Group 4.

**Table 1**
Tick-borne pathogens belonging to the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* in wild ($n = 64$) and in domestic animals ($n = 64$) living in close vicinity (first dataset).

| Hosts/pathogens | No. infected/tested | B/T genus | *B. bovis* | *B. bigemina* | *Theileria genus* | *T. mutans* | *T. taurotragi* | *T. velifera* | *T. bicornis* | *T. separata* | *T. buffeli* | *T. ovis* | *T.* sp. (sable) | *A/E* genus | *A. centrale* | *A. marginale* | *A. ovis* | *A. platys* | *E. sp.* (Omatjenne) | No. of infections |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cattle | 31/50 (62%) | 1 | 1[d] | 2[c,e] | 0 | 10[e,f] | 2[d,f] | 6[e,f] | 0 | 0 | 3[e,f,b] | 0 | 5[e,f] | 16 | 0 | 2[a,c] | 0 | 0 | 3[b,c] | 51 |
| Sheep | 12/14 (85.7%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6[a,c] | 9[a,c] | 0 | 7[a,c] | 9[a,c] | 0 | 4[a] | 0 | 3[a,c] | 1[a] | 0 | 39 |
| **Prevalence D** | **43/64 (67.2%)** | **1 (1.6%)** | **1 (1.6%)** | **2 (3.1%)** | **0** | **10 (15.6%)** | **2 (3.1%)** | **6 (9.4%)** | **6 (9.4%)** | **9 (14.1%)** | **3 (4.7%)** | **7 (10.9%)** | **14 (21.9%)** | **16 (25%)** | **4 (6.3%)** | **2 (3.1%)** | **3 (4.7%)** | **1 (1.6%)** | **3 (4.7%)** | **90** |
| Blue wildebeest | 5/5 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4[c,e] | 3[c] | 1[e] | 4[c] | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| Black wildebeest | 2/3 (66.7%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2[a,d] | 1[d] | 0 | 2[a,d] | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Blesbok | 3/3(100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1[d] | 2[d] | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Springbok | 2/6(33.1%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1[a] | 0 | 0 | 1[a] | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Sable antelope | 2/2 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2[f] | 2[f] | 2[f] | 2[f] | 2[f] | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Gemsbok | 1/1 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1[c] | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| Buffalo | 9/15 (60%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1[c] | 5[b,c] | 0 | 0 | 0 | 10 |
| Common eland | 5/9 (55.6%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2[a] | 0 | 0 | 0 | 4 |
| Greater kudu | 3/8 (37.5%) | 1 | 0 | 1[c] | 0 | 0 | 0 | 0 | 0 | 0 | 1[c] | 0 | 0 | 0 | 0 | 3[a,c] | 0 | 0 | 0 | 6 |
| Impala | 3/7 (42.9%) | 0 | 0 | 1[a] | 0 | 0 | 0 | 0 | 2[e] | 0 | 2[e] | 0 | 2[e] | 0 | 1[e] | 0 | 0 | 0 | 0 | 8 |
| Giraffe | 5/5 (100%) | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1[f] | 9 |
| **Prevalence W** | **40/64 (62.5%)** | **2 (3.1%)** | **0** | **2 (3.1%)** | **5 (7.8%)** | **0** | **0** | **0** | **4 (6.3%)** | **9 (14.1%)** | **9 (14.1%)** | **4 (6.3%)** | **14 (21.9%)** | **9 (14.1%)** | **2 (3.1%)** | **10 (15.6%)** | **0** | **0** | **1 (1.6%)** | **71** |
| Total | 83/128 (64.8%) | 3 (2.3%) | 1 (0.8%) | 4 (3.1%) | 5 (3.9%) | 10 (7.8%) | 2 (1.6%) | 6 (4.7%) | 10 (7.8%) | 18 (14.1%) | 12 (9.4%) | 11 (8.6%) | 28 (21.9%) | 25 (19.5%) | 6 (4.7%) | 12 (9.4%) | 3 (2.3%) | 1 (0.8%) | 4 (3.1%) | 161 |

*B/T*: Includes infections reacting with this probe only; considered as belonging to the genus *Babesia*.
D, domestic animals; W, wild animals. Locations of the infections identified at species level:

[a] Tüssen-Die-Riviere.
[b] Willem Pretorius.
[c] Sandveld.
[d] Bethal.
[e] Thabazimbi.
[f] Lephalale.

**Table 2**
Tick-borne pathogens belonging to the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* infecting the 53 additional hosts (second dataset).

| Host/pathogens | No. infected/tested | *B/T* genus | *Theileria* genus | *T. bicornis* | *T. separata* | *T. buffeli* | *T.* sp. (sable) | *T. equi* | *A/E* genus | *A. marginale* | *E.* sp. (Omatjenne) | No. of infections |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Black wildebeest | 7/15 (46.7%) | 0 | 0 | 1[c] | 6[b,c] | 0 | 5[b,c] | 0 | 0 | 0 | 0 | 12 |
| Red hartebeest | 1/15 (6.7%) | 0 | 0 | 0 | 1[c] | 0 | 1[c] | 0 | 0 | 0 | 0 | 2 |
| Roan antelope | 5/5 (100%) | 0 | 0 | 5[d] | 0 | 5[d] | 5[d] | 0 | 3 | 0 | 2[d] | 20 |
| Buffalo | 1/2 (50%) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Common eland | 3/4 (75%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1[a] | 0 | 4 |
| Greater kudu | 1/1 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Plain zebra | 6/6 (100%) | 0 | 5 | 0 | 0 | 0 | 0 | 1[c] | 0 | 0 | 0 | 6 |
| Domestic horse | 2/2 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 2[e] | 0 | 0 | 0 | 2 |
| Warthog | 1/3 (33.3%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| **Total** | **27/53 (50.9%)** | **1 (1.9%)** | **5 (9.4%)** | **6 (11.3%)** | **7 (13.2%)** | **5 (9.4%)** | **11 (20.8%)** | **3 (5.7%)** | **8 (15.1%)** | **1 (1.9%)** | **2 (3.8%)** | **49** |

*B/T*: Includes infections reacting with this probe only; considered as belonging to the genus *Babesia*.
Locations of the infections identified at species level:

[a] Tüssen-Die-Riviere.
[b] Sterkfontein.
[c] Seekoeivlei.
[d] Sandveld.
[e] Pretoria.

**Table 3**
Coinfections, constrained to locations, of pathogen species belonging to the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* involved in coinfections observed among 181 wild and domestic ruminants (datasets 1 and 2).

| Pathogens | *T.* sp. (sable) | *T. separata* | *T. ovis* | *T. bicornis* | *A. ovis* | *A. centrale* | *A. platys* | *T. mutans* | *T. velifera* | *T. taurotragi* | *T. buffeli* | *A. marginale* | *B. bigemina* | *E.* sp. (Omatjenne) | No. of infections/species[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T.* sp. (sable) | / | **19*** | **8*** | **14*** | 3 | 4 | 1 | 5 | 5 | 1 | **15*** | 0 | 1 | 2 | 39 |
| *T. separata* | **19*** | / | **6*** | **7*** | 3 | 1 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 25 |
| *T. ovis* | 8 | **6*** | / | **4*** | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| *T. bicornis* | **14*** | **7*** | **4*** | / | 2 | 1 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 2 | 16 |
| *A. ovis* | 3 | 3 | 2 | 2 | / | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| *A. centrale* | 4 | 1 | 3 | 1 | 0 | / | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 6 |
| *A. platys* | 1 | 1 | 0 | 0 | 0 | 0 | / | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 1 |
| *T. mutans* | 5 | 0 | 0 | 0 | 0 | 0 | 0 | / | 6 | 1 | 2 | 0 | 1 | 0 | 10 |
| *T. velifera* | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | / | 1 | 2 | 0 | 1 | 0 | 6 |
| *T. taurotragi* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | / | 0 | 0 | 0 | 0 | 2 |
| *T. buffeli* | **15*** | 4 | 0 | 5 | 0 | 0 | 0 | 2 | 2 | 0 | / | 1 | 2 | 2 | 17 |
| *A. marginale* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | / | 2 | 0 | 13 |
| *B. bigemina* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 2 | / | 0 | 4 |
| *E.* sp. (Omatjenne) | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 6 |
| No. of infections/species[a] | 39 | 25 | 11 | 16 | 3 | 6 | 1 | 10 | 6 | 2 | 17 | 13 | 4 | 6 | 159 |

[a] Observed among the 181 ruminants.
* Significant associations (Bonferroni corrected *P* values < 0.0004) given by the permutation test (Raup and Crick, 1979; modified by Clua et al., 2010).




**Fig. 3.** Principal component analysis (PCA) on Hellinger-transformed data: Infections by 16 pathogen species (163 infections) of 181 hosts belonging to 18 species. 3a Groups of host species deriving from pathogen assemblage. Group 1 (in black): sk, springbok ($n=6$); bw, blue wildebeest ($n=5$); bkw, black wildebeest ($n=18$); rh, red hartebeest ($n=15$); bk, blesbok ($n=3$); sa, sable antelope ($n=2$); ro, roan antelope ($n=5$); ge, gemsbok ($n=1$); sh, sheep ($n=14$). Group 2 (in red): b, buffalo ($n=17$); e, common eland ($n=13$); gk, greater kudu ($n=9$); c, cattle ($n=50$). Group 3 (in blue): Z, zebra ($n=6$); hor, horse ($n=2$). Group 4 (in green): I, Impala ($n=7$); g, giraffe ($n=5$). 3b Contribution of the pathogen species to the definition of host groups via pathogen assemblage. *T. buffeli* ($n=17$), *Theileria* sp. (sable) ($n=39$), *T. bicornis* ($n=16$), *T. separata* ($n=25$), *T. ovis* ($n=11$), *A. ovis* ($n=3$), *A. platys* ($n=1$), *T. velifera* ($n=6$), *T. mutans* ($n=10$), *T. taurotragi* ($n=2$), *B. bigemina* ($n=4$), *A. marginale* ($n=13$), *B. bovis* ($n=1$), *Ehrlichia* sp. (Omatjenne) ($n=6$), *A. centrale* ($n=6$), *T. equi* ($n=3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.ttbdis.2013.10.007.

## Discussion

In this study, tick-borne pathogens were detected by RLB hybridisation using probes allowing identification at the genus and at the species level. Most infections were identified at the species level, nevertheless some of them ($n=47$) were identified at the genus level only even after sequencing, due probably to the presence of multiple sequences of different species and/or variants (Microsynth, pers. communication) belonging mainly to *Anaplasma* spp. and *Ehrlichia* spp. for which no probes were

available. Nevertheless, sequencing of 7 samples allowed species identification: one *A. platys*, 3 *A. ovis*, and 3 *A. marginale*. Absence of hybridisation of the last 6 samples with the specific oligonucleotide probes included in the assay was due to the forward position of the probes on the amplified sequence.

The great majority of infections identified at the species level (77.9%) involved more than one pathogen species, with the most frequent combination involving 3 species (37%). This corroborates previous observations in cattle in South Africa where most individuals carried coinfections with mainly 3 species of piroplasms (Yusufmia et al., 2010). Similarly, the majority of the infections observed in nyala by Pfitzer et al. (2011) were coinfections, with combination of 4 haemoparasites being the most frequent. In the present study, coinfections with 6 *Theileria* species, mainly associated with sheep-related host species and with impala, were significant. However coinfections involving pathogens of different genera were also detected (e.g. with *A. marginale* and *B. bigemina*). Yusufmia et al. (2010) reported coinfection involving *T. velifera*, *Theileria* sp. (sable), and *T. mutans* (but no further details of species associations are available). Pfitzer et al. (2011) observed combinations with *Theileria* sp. (kudu), *T. buffeli*, *T. bicornis*, *Theileria* sp. (sable), *Ehrlichia* sp. (Omatjenne), and *A. marginale*. Stoltsz (1989) reported that *T. velifera* is rarely found in single infection, but more frequently associated with other piroplasm species. Binta et al. (1998) observed high cattle morbidity resulting from dual infections of *T. mutans* with *T. taurotragi*, while single infections by either one of these species generally cause mild symptoms. These authors suggested that the synergistic effects of the 2 infections increased pathology and might induce immunodepressive effects, enhancing infection by additional pathogen species. This might explain coinfection with up to 4 pathogen species, as observed here.

Among the 16 identified pathogen species, 9 were *Theileria* species and 4 (*T. taurotragi*, *T. mutans*, *T. velifera*, and *T. equi*) displayed a host range congruent with the literature. In the present study, *T. taurotragi*, *T. mutans*, and *T. velifera*, which differ in their virulence to cattle, were only detected in cattle. *Theileria taurotragi* is generally benign, but can occasionally cause cerebral theileriosis (De Vos et al., 1981), southern African *T. mutans* strains induce a mild form of the diseases in contrast to some of the eastern African strains (Lawrence et al., 1994c), and no clinical signs are generally recorded for *T. velifera* (Uilenberg and Schreuder, 1976). Other studies detected these 3 pathogen species in wild Bovinae: *T. taurotragi* in the common eland (Martin and Brocklesby, 1960), *T. mutans* (Uilenberg, 1981) and *T. velifera* (Uilenberg, 1970) in the African buffalo. Finally, *T. equi* the agent of equine piroplasmosis (De Waal and van Heerden, 1994) is known to infect horses, donkeys, mules, and zebras (Alsaad et al., 2012) and was detected in horses and zebras in the present study.

Screening a large variety of wild animals, we detected a broader host range than reported in the literature. This concerned 5 *Theileria* species. *Theileria* sp. (sable) was the most frequently detected pathogen. It was observed in 5 new host species (sheep, black wildebeest, springbok, gemsbok, and impala). It was also detected in 6 known host species such as cattle (Yusufmia et al., 2010), blue wildebeest, blesbok (Steyl et al., 2012), sable antelope, roan antelope (Nijhof et al., 2005), and red hartebeest (Spitalska et al., 2005). *Theileria buffeli*, a worldwide pathogen in cattle (Gubbels et al., 2000) usually considered as benign, although pathogenic variants may exist (Kamau et al., 2011), was also described in African buffalos (Allsopp et al., 1999) and nyala (Pfitzer et al., 2011). In the present study, we detected *T. buffeli* in cattle as well as in 6 new host species (greater kudu, impala, blue wildebeest, black wildebeest, sable antelope, and roan antelope). According to the literature, *T. bicornis* mainly infects white and black rhinoceroses (Nijhof et al., 2003; Govender et al., 2011), but also cattle (Muhanguzi et al., 2010) and nyala (Pfitzer et al., 2011). In the present study, it was detected in 5 additional animal species (sheep, sable antelope, roan antelope, black wildebeest, and impala). The main hosts for *T. separata*, the agent of benign ovine and caprine theileriosis, are small domestic ruminants (Lawrence et al., 1994a). However, *T. separata* was also reported in the common grey duiker (Nijhof et al., 2005) and in tsessebe (Brothers et al., 2011). Tonetti et al. (2009) suggested that wildlife could be a source of *T. separata* infection for sheep. In the present study, we detected *T. separata* not only in sheep, but also in 5 new wild animal species (blue wildebeest, black wildebeest, springbok, sable antelope, and red hartebeest). This corroborates the previous observations of this parasite in wildlife. Overall, *T. separata* appears thus as a common infection of wild ruminant species closely related to Caprinae. Finally, *T. ovis*, the other agent of benign ovine and caprine theileriosis, was identified in its known host (sheep) as well as in 3 new host species (blesbok, blue wildebeest, and sable antelope).

Four *Anaplasma* species were identified, namely *A. ovis*, *A. centrale*, *A. marginale*, and *A. platys*, and 3 were involved in new host–pathogen combinations. *Anaplasma ovis*, the agent of ovine and caprine anaplasmosis, causes subclinical to severe illness even leading to death, although rarely in southern Africa (Stoltsz, 1994). We detected *A. ovis* only in sheep, but it was previously reported in sable antelopes (Thomas et al., 1982). *Anaplasma centrale* that usually produces a mild form of gallsickness was identified in its known hosts, sheep (Potgieter and Van Rensburg, 1987) and buffalo (Brocklesby and Vidler, 1966) as well as in a newly reported wild animal species, the impala. *Anaplasma* spp. antigens were previously reported in the impala (Löhr et al., 1974), but it is not known whether *A. centrale* was involved. In contrast to the other *Anaplasma* species detected here, *A. marginale*, the agent of gallsickness, is known to infect various wild animal species (Neitz and Du Toit, 1932; Neitz, 1935; Löhr et al., 1974; Tonetti et al., 2009) including African buffalo and common eland (Potgieter, 1979; Ngeranwa et al., 1998). We detected *A. marginale* in cattle, African buffalo, common eland, and greater kudu. No previous report for greater kudu was found in the literature, but it was described in the closely related nyala (*T. angassii*) (Pfitzer et al., 2011). The infection is usually subclinical in wild animals, except in giraffe, which is thus far the only wild ruminant seriously affected by this pathogen (Augustyn and Bigalke, 1972). Finally, *A. platys*, previously *E. platys* (Dumler et al., 2001), responsible for canine infectious thrombocytopenia (Huang et al., 2005) was surprisingly identified in one sheep. The sequence (GenBank accession number KF414713) showed 99% similarity to *A. platys* (GenBank accession number AY040853.1) detected in one Italian dog by Sparagano et al. (2003). However, Chochlakis et al. (2009) also detected *A. platys* in Caprinae (goats). Inokuma et al. (2005) showed that *A. platys* is closely related to *Anaplasma* sp. (Omatjenne) (GenBank accession number U54806) from sheep in South Africa (Allsopp et al., 1997) and to *Anaplasma* sp. (BomPastor) (GenBank accession number AF318023) from goat in Mozambique (Bekker et al., 2001). Thus, our finding of *A. platys* in sheep is not so surprising, but needs further studying to clarify the situation.

Two *Babesia* species, *B. bovis*, the aetiological agents of Asiatic redwater, and *B. bigemina* responsible for African redwater were detected in host blood. *Babesia bovis* was identified only in one cow that had succumbed to the disease. The extremely low prevalence of *B. bovis* can probably be explained by the fact that blood was taken from the jugular or the coccygeal vein and not from capillary blood for all sampled cattle except the cow that was infected with *B. bovis*. In this specific case, blood was taken from the throat of the dead animal probably contaminated with capillary blood. The main vector of *B. bovis* in South Africa, *Rhipicephalus (Boophilus) microplus* (Tonnesen et al., 2004), was observed on this animal (Berggoetz et al., 2013). *Babesia bigemina* was detected in its usual hosts (i.e. cattle and impala), which confirms previous finding (Löhr et al.,

1974). However, we also detected *B. bigemina* in one greater kudu. To our knowledge, this is the first report for this host species. Usually, clinical signs are restricted to cattle, and the role of wildlife in the epidemiology of bovine babesiosis needs to be determined (Geleta, 2005).

The recently described and still fairly unknown species *Ehrlichia* sp. (Omatjenne) was previously observed in cattle (Muhanguzi et al., 2010), sheep (Du Plessis, 1990), goat (Bekker et al., 2001), and nyala (Pfitzer et al., 2011). We identified this microorganism in cattle and for the first time in roan antelope and Southern giraffe. *Ehrlichia* sp. (Omatjenne) is considered as non-pathogenic, but Du Plessis (1990) observed that after several passages through *Amblyomma* ticks, this pathogen species might induce severe clinical signs in sheep similar to those of heartwater.

To sum up, among the 16 pathogen species identified in this study, 10 had a broader host range than previously known and were involved in 30 new host–pathogen combinations. This gives a new perspective of the circulation of some of these pathogens in nature. For example, *Theileria* sp. (sable), *T. bicornis*, and *T. buffeli* appear as more generalist species in the present study than usually recognised in the literature.

PCA showed that the 16 detected pathogen species constitute 4 different pathogen assemblages corresponding to 4 groups of host species. *Theileria separata*, *T. ovis*, *A. ovis*, *T. bicornis*, and *Theileria* sp. (sable) were significantly associated with Group 1, which contains 9 host species, namely: blue wildebeest, black wildebeest, red hartebeest, blesbok (subfamily Alcelaphinae), sable antelope, roan antelope, gemsbok (subfamily Hippotraginae), sheep (subfamily Caprinae), and springbok (subfamily Antilopinae). Interestingly, these host species share evolutionary relationships. Indeed, the subfamilies Alcelaphinae and Hippotraginae are closely related to one another and constitute the sister clade of the Caprinae (Matthee and Davis, 2001; Fernandez and Vrba, 2005). Some authors placed the springbok in the tribe Antilopini within the subfamily Antilopinae together with the Alcelaphini, Hippotragini, and Caprini (Hassanin and Douzery, 1999; Ropiquet and Hassanin, 2005), thus supporting the inclusion of springbok in Group 1. The affiliation of *T. separata*, *T. ovis*, and *A. ovis* to Group 1 is not surprising as these species are known as pathogens of domestic Caprinae. Despite their preference for Group 1 animals, *Theileria* sp. (sable) (Yusufmia et al., 2010; Pfitzer et al., 2011) and *T. bicornis* (Muhanguzi et al., 2010; Pfitzer et al., 2011) were reported in Bovinae. We suggest that Bovinae are infected by other genotypes of these pathogens, as reported by Mans et al. (2011) who observed one *Theileria* sp. (sable) genotype adapted to cattle. Whether a similar situation occurs for *A. centrale* and *A. platys* remains to be evaluated. It is surprising to see *T. buffeli*, a classic parasite of the Bovinae, affiliated to the Group 1 of pathogens. Further genetic studies are required to clarify the situation all the more so since Mans et al. (2011) reported different genotypes in this *Theileria* species. This probably highlights an ongoing evolutionary process of this species.

*Theileria taurotragi*, *T. velifera*, *T. mutans*, and *A. marginale* are the pathogen species defining the Group 2, each with significant *P* values. This group is also supported by *B. bovis* and *B. bigemina*, although not significantly. Regarding the host species, Group 2 included African buffalo, common eland, greater kudu, and cattle, i.e. members of the subfamily Bovinae (Matthee and Davis, 2001; Fernandez and Vrba, 2005; Ropiquet and Hassanin, 2005). *Theileria taurotragi*, *T. velifera*, and *T. mutans* are known to occur in wild Bovinae, eland and buffalo, respectively, with the genotypes infecting such wild hosts considered as representing the origin of the genotypes infecting cattle (Mans et al., 2011). It is possible that other genotypes of *A. marginale* are involved in the infections of non-bovine host species such as blesbok, common duiker, and black wildebeest (Neitz and Du Toit, 1932; Neitz, 1935).

Horses and zebras, members of the Equidae family, formed the Group 3 characterised by the presence of *T. equi*. By contrast with the 3 former groups, the Group 4 is not well characterised. This group is neither characterised by a cohort of pathogen species nor by any closer phylogenetic relationships among its host species. Group 4 exhibits very heterogeneous infection profiles encountered in impala and giraffe. Giraffe is part of a distinct family referred to as Giraffidae (Fernandez and Vrba, 2005). Impala was successively affiliated to the Alcelaphinae (Vrba, 1984), Antilopinae (Kingdon, 1989), and to the Reduncinae (Murray, 1984). It is currently recognised as a distinct subfamily, Aepycerotinae (Matthee and Davis, 2001; Fernandez and Vrba, 2005; Ropiquet and Hassanin, 2005). In the present study, impala shared pathogens affiliated to hosts of Groups 1 and 2. This suggests a frequent spillover of the pathogens usually associated with Caprinae and Bovinae into the impala species.

Overall, the host–pathogen combinations detected in the present study suggest that transmission of tick-borne pathogen species remains mainly restricted to genetically related host species, except for impalas which may represent a bridge species between several transmission routes. Such importance of the phylogenetic status of the host has previously been observed in experimental infection of domestic animals with pathogens isolated from wild animals (Penzhorn, 2006). However, the present study is the first to our knowledge to corroborate the host–pathogen compatibility dependence on host phylogeny from a wide-scale sampling design involving 18 host species and 16 pathogen species. This highlights one aspect of the nature of the interplay existing between domestic and wild animals. The observation that closely related host species, domestic or wild, were mainly infected by the same pathogen species is a crucial point to bear in mind in game and livestock management.

The common belief is that game animals harbour more pathogens than domestic animals because they are seen as the main infection sources for domestic animals (Bigalke, 1994). Inversely, pathogens could be more frequent in domestic animals since they are known to be more susceptible to infections (Jongejan and Uilenberg, 2004). When we evaluated the infection load in wild ($n = 64$) and domestic ($n = 64$) animals living in close vicinity, within each of 6 localities, we observed similar ranges in infection prevalence and in intensity of infection in both host types using the same detection method. Knowing that game usually has subclinical infections (Jongejan and Uilenberg, 2004), while remaining carrier, wild animals with a very low parasitaemia could have been overlooked using RLB. From an epidemiological point of view, animals with such a low parasitaemia are not very relevant. Indeed, those animals are, most probably, not very infective for vector ticks. Taken into account that livestock is protected by acaricide treatments, vaccination, and field management measures and still display similar infection profiles as game, wild ungulates appear less susceptible to tick-borne infections than livestock animals. Our results strongly suggest that the interplay between wild and domestic ungulates living in close vicinity is very close, with both animal types consisting in efficient infection sources for ticks infecting themselves and each other. Nevertheless, livestock animals should not be underestimated as infection source for game animals. Löhr et al. (1974) reported that *Theileria*, *Babesia*, and *Anaplasma* antibodies were much more frequent in antelopes grazing in the vicinity of non-dipped cattle than in antelopes grazing in areas where cattle were regularly dipped or were absent.

The present study revealed infection patterns among host groups, which defined transmission pathways between given host species, among and between domestic and wild ungulates. Furthermore, we showed that pathogen species like *Theileria* sp. (sable), *T. buffeli*, *T. bicornis*, *T. separata*, *T. ovis*, *A. centrale*, *A. marginale*, *A. platys*, *Ehrlichia* sp. (Omatjenne), and *B. bigemina* have a broader

host range, mainly among wild animals, than previously known. We described previously unknown coinfection patterns, an information which is important since immunodepressive effects could enhance infection by additional pathogen species. Finally, we suggest that livestock animals should not be underestimated as infection sources for their congenerics as well as for game animals.

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