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TICKS AND TICK-BORNE PATHOGENS FROM WILDLIFE IN THE FREE STATE PROVINCE, SOUTH AFRICA

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ABSTRACT: Eight ixodid tick species, associated with 59 free-ranging mammals belonging to 10 species, were collected at five different localities in the Free State Province, South Africa. Four of the study areas were nature reserves (Willem Pretorius, Sandveld, Tussen-die-Riviere, and Soetdoring), and one site was a private farm located in Senekal district. The collection was performed from March 2006 until June 2006. Ticks ($n=569$) and tissues from animals ($n=52$) were analyzed by polymerase chain reaction, reverse line blot, and sequencing for various tick-borne pathogens belonging to the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia*. *Rhipicephalus* (*Boophilus*) *microplus*, the known vector of *Babesia bovis* responsible for Asiatic redwater in South Africa, was found for the first time in the Free State Province. *Rhipicephalus appendiculatus* also was collected in areas in the Free State where it has not been previously described. *Anaplasma marginale* was detected for the first time in a gemsbok (*Oryx gazella gazella*). Gene sequences recovered in this study were 98–100% homologous with GenBank sequences for *Anaplasma bovis*, *Theileria separata*, and *Theileria* sp. Malelane sable antelope.

Key words: *Anaplasma*, *Babesia*, *Ehrlichia*, *Rhipicephalus* (*Boophilus*) *microplus*, *Theileria*.

INTRODUCTION

Wild ruminants have long been suspected to be involved in the epidemiology of tick-borne pathogens affecting cattle and small domestic ruminants in Africa (Löhr and Meyer, 1973; Bigalke, 1994; Peter et al., 1998). In the 1930s, the susceptibility of game species for anaplasmosis was reported (Neitz and Du Toit, 1932; Neitz, 1935). Later, it was demonstrated that tick-borne protozoan and bacterial pathogens were widespread in African wildlife species (Löhr et al., 1974; Carmichael and Hobday, 1975) and for many of these important veterinary pathogens, transmission from wildlife to livestock was demonstrated (Löhr and Meyer, 1973; Young, 1977; De Vos and Potgieter, 1994; Peter et al., 1998). Several wild ruminant species have been identified as reservoirs for tick-borne pathogens; African buffalo (*Syncerus caffer*) are a reservoir for East Coast fever, corridor diseases, and heartwater (Young, 1977; Lawrence et al., 1994a; Allsopp et al., 1999). Large populations of game animals on reserves and game farms can support large populations of tick vectors associated with these pathogens throughout the year (Purnell, 1980). In southern Africa, the majority of the livestock animals are in contact with wildlife species (including small mammals; Purnell, 1980; Peter et al., 1998), suggesting a complex circulation dynamic of tick-borne pathogens between wild and domestic animals.

Currently, the roles of many wildlife species in the epidemiology of intracellular tick-borne pathogens of the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* in endemic areas are poorly defined. Such information has application to both domestic and wild animal health, as wild animals are not resistant to these arthropod-borne diseases (Carmichael and Hobday, 1975) and mortality of wild animals due to tick-borne diseases has been reported (Nijhof et al., 2005). These fatal cases mostly occur when wild animals are translocated to endemic areas, and this practice is increasing through international conservation efforts and game farming. Because many aspects of the ecology and epidemiology of these pathogens, such as host range, vector range, and their geo-

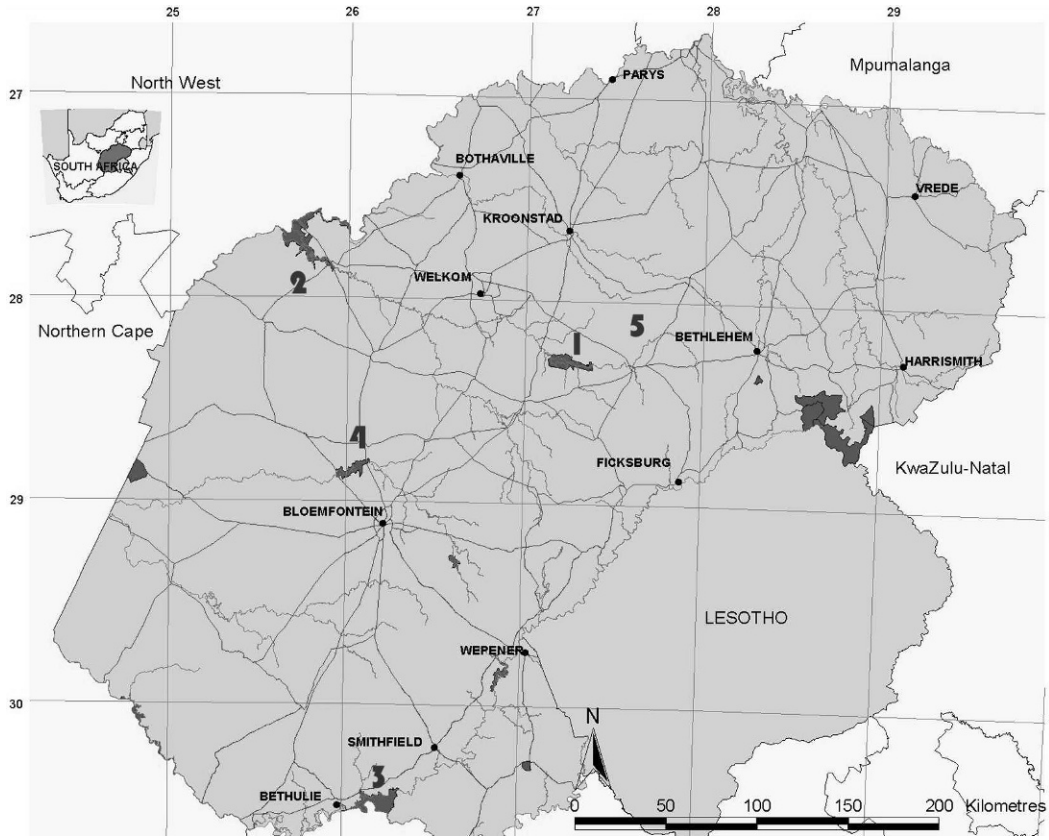

FIGURE 1. Study areas in the Free State Province of South Africa. 1, Willem Pretorius; 2, Sandveld; 3, Tussen-die-Riviere; 4, Soetdoring; 5, Senekal (modified from http://www.environment.gov.za).

graphic distribution, remain obscure, investigations of the relations between ticks, pathogens, and wildlife are of interest to those involved in both nature conservation and cattle farming, especially in areas such as the Free State that have both high cattle production and game farming (Anonymous, 2006).

The objective of this study was to obtain more information on host and vector ranges, as well as the geographic distribution of pathogen species belonging to the genera *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* infecting wild animals.

MATERIALS AND METHODS

Study area

Sampling of ticks and hosts was undertaken from March 2006 until June 2006 in the Free State Province of South Africa at five different locations, including four nature reserves and one private farm. The game reserves were Willem Pretorius (28°15′S, 27°13′E), Sandveld (27°39′S, 25°42′E), Tussen-die-Riviere (30°28′S, 26°07′E), and Soetdoring (28°50′S, 26°02′E; Fig. 1). These study areas are located at altitudes of 1,382, 1,254, 1,311, and 1,263 m above sea level, respectively. The private farm is located in Senekal district (28°09′S, 27°35′E) and lies at an altitude of 1,547 m above sea level.

Animal captures and tissue sampling

Ten wildlife species were examined in this study; Gemsbok (*Oryx gazella gazella*), common eland (*Taurotragus oryx*), springbok (*Antidorcas marsupialis*), black wildebeest (*Connochaetes gnou*), red hartebeest (*Alcelaphus buselaphus caama*), blesbok (*Damaliscus dorcas phillipsi*), greater kudu (*Tragelaphus strepsiceros*), roan antelope (*Hippotragus equinus*), plain zebra (*Equus burchellii*), and lion (*Panthera leo*). These animals were either

live caught and tranquilized with perphenazine, or hunted by the Free State game capture team, or anesthetized by veterinarians. The drug mixtures were administrated intramuscularly. Gemsbok were anesthetized with the use of 0.03 mg/kg of etorphine hydrochloride (M99®, 10 mg/ml, Krüger-Med Pharmaceuticals Pty. Ltd., Johannesburg, South Africa), 0.025 mg/kg of detomidine (Domosedan®, 10 mg/ml, Pfizer Laboratories [Pty.] Ltd., Sandton, South Africa), and 0.15 mg/kg of xylazine (Rompun®, Kyron Laboratories, Benrose, South Africa). The antidote used was diprenorphine hydrochloride (M5050®, 12 mg/ml, Krüger-Med). Lions were anesthetized with two different anesthetics. The first one was 0.03 mg/kg of medetomidine hydrochloride (20 mg/ml, Wildlife Pharmaceuticals, Karino, South Africa) and 0.4 mg/kg of an equivalent mixture of tiletamine and zolazepam (Zoletil 100®, 100 mg/ml, VIRBAC, Pretoria, South Africa). The antidote used was atipamezole hydrochloride (Antisedan®, 5 mg/ml, Wildlife Pharmaceuticals) with two to four times the medetomidine dose used. The second anesthetic was 3 mg/kg of a 1:1 mixture of tiletamine and zolazepam. No antidote was needed. Samples from heart, liver, and spleen (approximately 1–10 g) were obtained from the hunted animals and stored in tubes containing 100% alcohol.

Tick sampling

Animals were visually examined for ticks, with special attention to the abdomen, back, anal area, and hind legs. In addition, palpation was used to locate immature ticks on the front and hind legs. Ticks were removed with forceps and pooled per animal and per stages in tubes containing 100% alcohol. Ticks were identified to species, life stage, and sex according to Matthysse and Colbo (1987) and Walker et al. (2004).

DNA extraction

DNA was extracted from the ticks with the use of ammonium hydroxide, as described previously (Moran Cadenas et al., 2007). Organ samples were washed twice in phosphate-buffered saline, pH 7.2 (50 mM potassium phosphate; 150 mM NaCl) prior to extraction with the use of a commercially available kit (DNeasy Blood and Tissue Kit, QIAgen, Hombrechtikon, Switzerland); two elution steps provided in manufacturer's protocol were modified; the first elution step was performed with 150 µl of elution buffer instead of 200 µl and the second with 100 µl instead of 200 µl of elution buffer, in order to increase DNA concentration in the eluate.

Amplification of tick-borne pathogen DNA

Polymerase chain reaction (PCR) was performed in a reaction volume of 50 µl containing 10 µl of the DNA sample with the use of two sets of primers. Primers 16S8FE and B-GA1B-new were used to amplify a fragment of approximately 500 base pairs (bp) of the 16S rRNA gene spanning the V1 region of *Anaplasma* spp. and *Ehrlichia* spp., as described by Schouls et al. (1999) and modified by Bekker et al. (2002). Primers RLB-F2 and RLB-R2 were used to amplify a fragment of approximately 400 bp of the 18S SSU rRNA gene spanning the V4 region of *Babesia* spp. and *Theileria* spp. (Georges et al., 2001). All tested samples were amplified twice, once with the *Anaplasma* spp. and *Ehrlichia* spp. primer set and once with the *Babesia* spp. and *Theileria* spp. primer set. All the primers were obtained from Microsynth AG (Balgach, Switzerland).

Polymerase chain reactions were performed in a Whatman Biometra® Tgradient basic thermocycler 96 (Göttingen, Germany) by using a touchdown PCR program (Bekker et al., 2002) with the following modifications: Uracil DNA glycosylase was not used and the annealing temperature was lowered by 1 C instead of 2 C.

Reverse line blot hybridization

Reverse line blot (RLB) hybridization was used on PCR products to detect *Anaplasma*, *Ehrlichia*, *Babesia*, and *Theileria* species according to Gubbels et al. (1999) with the following modifications: Genus-specific oligonucleotides (catch-all) and species-specific oligonucleotides were diluted in 500 mM $NaHCO_3$ (pH 8.4) at concentrations of 50 pmol/150 µl for the *Babesia/Theileria* (B/T) catch-all probe, 100 pmol/150 µl for the *Anaplasma/Ehrlichia* (A/E) catch-all probe and the *Babesia bigemina* and *Theileria parva* species–specific probes, and at 500 pmol/150 µl for all the other species-specific probes. A volume of 10 µl of PCR product was diluted to a final volume of 150 µl in 2×SSPE/0.1% SDS for RLB hybridization. The posthybridization temperature was set to 52 C. Catch-all probes A/E (Bekker et al., 2002) and B/T (Gubbels et al., 1999) were used in this study. Thirteen different specific probes were used to detect common tick-borne pathogens of veterinary importance in southern Africa. *Anaplasma*-spp.– and *Ehrlichia*-spp.–specific probes for *Ehrlichia ruminantium*, *Anaplasma*

centrale, *Anaplasma phagocytophilum*, *Anaplasma marginale*, *Anaplasma bovis*, and *Anaplasma ovis* were designed by Bekker et al. (2002). *Babesia*-spp.– and *Theileria*-spp.–specific probes for *Theileria velifera*, *Theileria parva parva*, *Theileria taurotragi*, *Theileria mutans*, *Babesia bovis*, *Babesia bigemina*, and *Babesia divergens* were designed by Gubbels et al. (1999). All probes were obtained from Microsynth AG.

Gene sequencing

Polymerase chain reaction products that reacted only with the catch-all A/E and B/T probes were purified with the use of a commercially available kit (QIAquick PCR Purification Kit, QIAgen) and sequenced. Sequencing was done by Microsynth AG. Each obtained sequence was compared with available sequences from the international data bank (NCBI BLAST) with the use of a software package (Bioedit, Tom Hall Ibis Biosciences, Carlsbad, California).

RESULTS

Tick infestation

Eight ixodid tick species were collected from 59 individuals belonging to 10 species at the five study areas (Table 1). *Rhipicephalus evertsi evertsi* and *Hyalomma marginatum rufipes* were found at all five of the collection areas; *Rhipicephalus* (*Boophilus*) *decoloratus* was detected on all study areas except the Tussen-die-Riviere nature reserve (Fig. 1). *Rhipicephalus appendiculatus* was collected at the Tussen-die-Riviere, Soetdoring, and Willem Pretorius nature reserves and *Margaropus winthemi* at the Soetdoring, Sandveld, and Willem Pretorius nature reserves. *Ixodes rubicundus* was found in the Tussen-die-Riviere and the Soetdoring nature reserves. Finally, *Rhipicephalus* (*B.*) *microplus* and *Haemaphysalis leachi* were collected at the Sandveld and Soetdoring nature reserves, respectively.

The prevalence of infestation was 100% for all species except the springboks (40%) and red hartebeests (80%; Table 1). Common eland and gemsbok had the highest mean density of ticks per host and the largest number of tick species. Low mean densities of ticks per host were observed on springbok, black wildebeest, red hartebeest, blesbok, and lion. Mean densities of ticks per host were intermediate to high for greater kudu, roan antelope, and plain zebra; however, only one roan antelope and plain zebra and two greater kudus were examined. Interestingly, *H. m. rufipes* was collected on black wildebeest, *I. rubicundus* on blesbok, greater kudu and red hartebeest, and *R.* (*B.*) *microplus* on gemsbok.

Hosts and tick infection

A total of 114 tissue biopsies from different organs from eight of 10 host species were analyzed by RLB; 11 (9.7%) tested positive with at least one probe (Table 2). Ten samples reacted with the catch-all probes only. One liver sample from a common eland tested positive with the A/E catch-all probe and heart samples from eight gemsboks and a liver sample from a roan antelope tested positive with the use of the B/T catch-all probe. Finally, the heart sample of one gemsbok hybridized with the B/T catch-all probe and with the specific probe for *A. marginale*.

With the exception of ticks from gemsboks, only ticks from infected animals were analyzed (Table 3). A total of 39 samples (representing 37 ticks) belonging to three tick species, *R.* (*B.*) *decoloratus*, *H. m. rufipes*, and *R. e. evertsi*, reacted with at least one probe. Two *R. e. evertsi* ticks, both from gemsboks, were infected with two different pathogens identified at the genus level with the catch-all probes A/E and B/T. Protozoan pathogens were mainly detected in *R. e. evertsi* ticks, and bacterial pathogens were mainly detected in *H. m. rufipes* (Table 3). These observations coincide with host infections. Gemsbok and roan antelope were predominantly infected by protozoan pathogens and infested by *R. e. evertsi*, and common eland were primarily infected by bacterial pathogens and infested with *H. m. rufipes*.

TABLE 1. Host infestation by various ixodid tick species in the Free State.

Tick and host species[a]	No. infested/ examined (%)	Number of ticks				
		Larvae	Nymphs	Males	Females	Total
Rhipicephalus (*Boophilus*) *decoloratus*						
Gemsbok	12/13 (90)	86	70	80	101	337
Common eland	4/13 (31)	4	71	17	23	115
Springbok	1/10 (10)	18	7	0	0	25
Black wildebeest	1/8 (12,5)	0	3	0	0	3
Red hartebeest	2/5 (40)	1	5	2	6	14
Blesbok	1/3 (33,5)	0	4	0	16	20
Greater kudu	2/2 (100)	0	9	9	6	24
Roan	1/1 (100)	0	1	1	3	5
Plain zebra	1/1 (100)	0	17	1	1	19
Rhipicephalus evertsi evertsi						
Gemsbok	13/13 (100)	0	0	106	34	140
Common eland	11/13 (84,5)	0	0	233	37	270
Springbok	3/10 (30)	0	0	2	5	7
Black wildebeest	8/8 (100)	0	0	28	15	43
Red hartebeest	1/5 (20)	0	0	1	2	3
Blesbok	2/3 (66,5)	0	0	1	2	3
Greater kudu	2/2 (100)	0	0	5	0	5
Roan	1/1 (100)	0	0	26	10	36
Plain zebra	1/1 (100)	0	0	22	4	26
Hyalomma marginatum rufipes						
Gemsbok	7/13 (54)	0	0	18	2	20
Common eland	13/13 (100)	0	0	448	49	497
Black wildebeest	2/8 (25)	0	0	3	1	4
Roan	1/1 (100)	0	0	1	0	1
Plain zebra	1/1 (100)	0	0	7	0	7
Ixodes rubicundus						
Common eland	6/13 (46)	0	0	11	31	42
Springbok	2/10 (20)	0	0	2	3	5
Red hartebeest	2/5 (40)	0	0	4	5	9
Blesbok	1/3 (33,5)	0	0	1	1	2
Greater kudu	1/2 (50)	0	0	0	1	1
Rhipicephalus appendiculatus						
Common eland	11/13 (84,5)	0	4	123	10	137
Margaropus winthemi						
Gemsbok	5/13 (38,5)	14	4	17	4	39
Common eland	1/13 (7,5)	0	0	1	0	1
Rhipicephalus (*Boophilus*) *microplus*						
Gemsbok	3/13 (23)	0	0	0	8	8
Haemaphysalis leachi						
Lion	3/3 (100)	0	0	2	3	5

[a] Gemsbok (*Oryx gazella gazella*), common eland (*Taurotragus oryx*), springbok (*Antidorcas marsupialis*), black wildebeest (*Connochaetes gnou*), red hartebeest (*Alcelaphus buselaphus caama*), blesbok (*Damaliscus dorcas phillipsi*), greater kudu (*Tragelaphus strepsiceros*), roan antelope (*Hippotragus equinus*), plain zebra (*Equus burchellii*), and lion (*Panthera leo*).

TABLE 2. Host infection rates according to pathogen groups.

Hosts[a]	Infection rates	
	Babesia/ Theileria	*Anaplasma/ Ehrlichia*
Gemsbok	8/9	1/9[b]
Eland	0/13	1/13
Roan	1/1	0/1
Springbok	0/7	0/7
Red hartebeest	0/2	0/2
Blesbok	0/3	0/3
Kudu	0/2	0/2
Black wildebeest	0/8	0/8

[a] Gemsbok (*Oryx gazella gazella*), common eland (*Taurotragus oryx*), roan antelope (*Hippotragus equinus*), springbok (*Antidorcas marsupialis*), red hartebeest (*Alcelaphus buselaphus caama*), blesbok (*Damaliscus dorcas phillipsi*), greater kudu (*Tragelaphus strepsiceros*), and black wildebeest (*Connochaetes gnou*)

[b] *Anaplasma marginale* infection. The animal was infected with the two pathogen groups. The amplified DNA reacted with the *Babesia/Theileria* catch-all probe and with the *A. marginale*–specific probe.

Species identification by sequencing

Among PCR products reacting with A/E and B/T catch-all probes only, four PCR products were sequenced to identify the pathogenic organisms up to species level. Three sequences of the genus *Theileria*, detected in three *R. e. evertsi* ticks, were identified. The first one (GenBank accession number FJ155995) is closely related to *Theileria separata* (GenBank accession number AY260175.1) and the remaining two sequences (GenBank accession numbers FJ155996 and FJ688065) are closely related to *Theileria* sp. Malelane sable antelope (GenBank accession number AY748462.1). The first sequence (homology of 99% with *T. separata*) was isolated from a tick infesting a gemsbok in the Willem Pretorius nature reserve. The other two sequences (homology of 98% with *Theileria* sp. Malelane sable antelope) were detected in ticks infesting gemsbok and showed 100% homology between them, which were found in the Senekal district farm and the Soetdoring nature reserve, respectively. Finally, one gene sequence detected in a *R. e. evertsi* collected on a gemsbok from the Sandveld nature reserve (GenBank accession number FJ155997), showed homology to *A. bovis* (99% similarity; GenBank accession number U03775.1). The heart sample from the gemsbok, which hybridized with

TABLE 3. Tick species and infection rates for ticks recovered from gemsbok (*Oryx gazella gazella*), common eland (*Taurotragus oryx*), and roan antelope (*Hippotragus equinus*).

Hosts/Ticks	*Babesia/Theileria*			*Ehrlichia/Anaplasma*			Total/tick species
	Gemsbok ($n=13$)	Eland ($n=2$)	Roan ($n=1$)	Gemsbok ($n=13$)	Eland ($n=2$)	Roan ($n=1$)	
Rhipicephalus (Boophilus) decoloratus	1/250 (0.4)[a]	–	0/5	2/250 (0.8)[a]	–	0/5	3/255 (1.2)[a]
Rhipicephalus evertsi evertsi	17/136 (12.5)[a]	–	4/33 (12.1)[a]	5/136 (3.7)[a]	–	0/33	26/169 (15.4)[a]
Hyalomma marginatum rufipes	0/20	1/80 (1.3)[a]	0/1	0/20	9/80 (11.3)[a]	0/1	10/101 (9.9)[a]
Ixodes rubicundus	–	0/1	–	–	0/1	–	0/1
Margaropus winthemi	0/31	–	–	0/31	–	–	0/31
Rhipicephalus appendiculatus	–	0/5	–	–	0/5	–	0/5
Rhipicephalus (Boophilus) microplus	0/7	–	–	0/7	–	–	0/7
Total/host species	18/444 (4.1)[a]	1/86 (1.2)[a]	4/39 (10.3)[a]	7/444 (1.6)[a]	9/86 (10.5)[a]	0/39	39/569 (6.9)[a b]

[a] Number infected/number tested (% positive).

[b] Including two ticks with mixed infection.

the species-specific probe for *A. marginale*, was also sequenced for identity confirmation (FJ155998) and showed 99% homology with *A. marginale* (GenBank accession number AF414871).

DISCUSSION

The geographic distribution of all ticks collected in the present study corresponds to what was previously known in the Free State, except for *R.* (*B.*) *microplus* and *R. appendiculatus*. *Rhipicephalus* (*B.*) *microplus*, the vector of *B. bigemina*, the agent of African redwater, and the only known vector of the more virulent *B. bovis* responsible for Asiatic redwater in South Africa (De Vos and Potgieter, 1994; Tønnesen et al., 2004), was not previously detected in the Free State Province (Dreyer et al., 1998; Mbati et al., 2002; Mtshali et al., 2004) even though antibodies to *B. bovis* were reported in cattle (Dreyer et al., 1998). In the present study, eight *R.* (*B.*) *microplus* females were found on three gemsboks in the Sandveld nature reserve, situated at the border between the northwestern Free State Province and the North West Province. Based on such a small number of ticks, it is unclear if *R.* (*B.*) *microplus* is definitively established in this area, but this species has a well known capacity to colonize new areas. Although originally from India, *R.* (*B.*) *microplus* has been introduced and subsequently established in many areas in the world, often displacing indigenous *Rhipicephalus* species (Tønnesen et al., 2004; Estrada-Pena et al., 2006). In South Africa, *R.* (*B.*) *microplus* is now established along the southern and eastern coasts of the Western and Eastern Cape Provinces and of KwaZulu-Natal (Walker et al., 2003) and is also described in the Mpumalanga and Limpopo Provinces. Recently, Tønnesen et al. (2004) reported *R.* (*B.*) *microplus* in Soutpansberg (Limpopo Province), where it appeared to be widespread. Estrada-Pena et al. (2006) reported that there are large geographic areas at risk of colonization by *R.* (*B.*) *microplus* in Africa if the tick continues to spread from its present sites; the present study confirms the spread of this tick species in South Africa. Future studies to monitor the spread of *R.* (*B.*) *microplus* within South Africa are needed and our recovery of this species on gemsboks suggests that wildlife should be included in such surveillance.

In South Africa, *R. appendiculatus* was subject to intensive control treatments because of its capacity to transmit *T. parva parva*, causing East Coast Fever (Walker et al., 2003). This disease, if uncontrolled, can cause very high mortality in susceptible cattle, especially when introduced into a new area (Lawrence et al., 1994a). In South Africa, East Coast Fever was eradicated by 1955, and since then the historic distribution of *R. appendiculatus* (Walker et al., 2003) may not be accurate. In this context, new information on the distribution of *R. appendiculatus* is needed. In our study, *R. appendiculatus* specimens were only recovered from common eland collected at three study sites: Willem Pretorius nature reserve, Soetdoring Nature Reserve, and the Tussen-die-Riviere nature reserve. To our knowledge, this tick species is not considered as endemic in these areas of the Free State and it is unclear if *R. appendiculatus* had spread into these areas, or if it was there before without being reported. Several authors described an expansion of *R. appendiculatus* in southern Africa in the past decades (Chaka et al., 1999). In a study conducted in the Cape provinces and in the southwestern Free State, Horak et al. (1991) reported *R. appendiculatus* on hares and sheep in some of the Cape provinces; however, this species was not reported in the southwestern Free State.

Most of our observations on the host range for the tick species observed in the present study correspond to previously reported tick/host associations in the Free State (http://wwwold.icttd.nl/php/search_vtm.php; Horak et al., 1987; Cain et al., 2004). Exceptions include our recovery of *R.* (*B.*) *microplus* from gems-

bok; *H. m. rufipes* from black wildebeests; and *I. rubicundus*, the Karoo paralysis tick, from red hartebeest, kudu, and blesbok (Horak et al., 1987; http://wwwold.icttd.nl/php/search_vtm.php).

Among the seven tick species that were analyzed for pathogens, three species, *R.* (*B.*) *decoloratus*, *H. m. rufipes*, and *R. e. evertsi*, were infected with one or more of the tick-vectored pathogens that were included in our testing protocols (Table 3). Evidence of both protozoan and bacterial pathogens were detected in *R. e. evertsi*, *H. m. rufipes*, and *R.* (*B.*) *decoloratus*; however, *R. e. evertsi* were mainly infected with protozoan pathogens and *H. m. rufipes* with bacterial pathogens. Similarly, gemsboks and the roan antelope, which were the most common hosts for *R. e. evertsi*, were primarily infected by protozoan pathogens, whereas the common eland, the host of *H. m. rufipes*, was infected by bacterial pathogens. This may be partly because we had chosen to analyze ticks collected mainly from hosts that were infected, or partly because of the host preference of these tick species.

Among pathogens detected in ticks and host tissues, *A. marginale* was the only organism identified at the species level by RLB. In previous studies, *A. marginale*, or at least *Anaplasma* spp., was described in several wild ruminant species (Löhr et al., 1974; De Waal, 2000). In the present survey, we report this pathogen species, for the first time from a gemsbok in the Willem Pretorius nature reserve, belonging to the known geographic distribution of this pathogen (De Waal, 2000). *Rhipicephalus* (*B.*) *decoloratus*, *R.* (*B.*) *microplus*, *R. e. evertsi*, *H. m. rufipes*, and *Rhipicephalus simus* are vectors of *A. marginale* (De Waal, 2000). In our study, all of these tick species were found on gemsboks, except *R. simus*. Because the original distribution of the gemsbok covers Namibia, most of Botswana, southern Angola, and northwestern South Africa (Stuart and Stuart, 1997) and represents areas where *A. marginale* is not present, we suggest that the gemsboks kept in the Free State nature reserve, may be susceptible to *A. marginale* infections. It is well known that cattle are infected by *A. marginale* (De Waal, 2000). Therefore, with the high cattle stocking rates present in the Free State, gemsbok may be at risk.

In addition to *A. marginale*, *A. bovis* was identified in one *R. e. evertsi* tick collected from a gemsbok in the Sandveld nature reserve. This infection was not detected by the *A. bovis* probe used in our RLB technique (Bekker et al., 2002) probably due to a difference of one base pair difference in sequence. The known vector of *A. bovis* in South Africa is *R. appendiculatus* (Scott, 1994) and the gemsbok is not known to reservoir this pathogen. Our results do not allow us to distinguish if the detection of *A. bovis* DNA in *R. e. evertsi* is due to an infectious blood meal from the gemsbok or to the infection of the tick itself. Nevertheless our findings suggest new relationships of *A. bovis* either with the gemsbok as a reservoir host or with *R. e. evertsi* as a vector.

Concerning protozoan pathogens, *T. separata*, a benign pathogen of sheep, was detected in *R. e. evertsi* from a gemsbok at Willem Pretorius nature reserve. *Theileria separata* is common in southeastern Africa; small domestic ruminants are the only known reservoirs and it is transmitted by *R. e. evertsi* (Lawrence et al., 1994b). *Theileria separata* is maintained in tick populations only by transstadial transmission (Lawrence et al., 1994b) and infectious blood meals. Therefore, infected wildlife species, like gemsbok, could provide a source of infection for ticks at Willem Pretorius nature reserve, an enclosed environment, where small domestic animals were not present during the study period. Finally, DNA detected in two *R. e. evertsi* ticks infesting gemsboks showed 98% homology with a *Theileria* sp. Malelane sable antelope described by Nijhof et al. (2005). These authors described this specific pathogen in two roan antelopes originating from Togo

and Benin. These animals contracted theileriosis after being released on a private farm near Malelane, in the Mpumalanga Province of South Africa. This pathogen was also reported in a wide range of healthy wild ruminants (Nijhof et al., 2005), but apparently has not been detected before in ticks. Whether *R. e. evertsi* is the vector of *Theileria* sp. Malelane sable antelope remains unknown for the same reasons mentioned above for *A. bovis*.

In this study, we obtained evidence that several tick-borne pathogens that can infect both wildlife and domestic animals (*T. separata*, *A. marginale*, and *A. bovis*) are present in wild ruminants and/or in their associated ticks in the Free State Province of South Africa. Wild ruminants may play a reservoir role in the transmission cycles for some of these pathogens, which could increase difficulties in controlling tick-borne diseases of livestock. These pathogens also are relevant for wildlife health and conservation. Further studies on the potential health impact of these tick-borne pathogens of domestic animals on wild ruminants are encouraged. There also is a need to monitor the distribution of emerging tick species like *R. appendiculatus* and *R.* (*B.*) *microplus*. These tick species were collected from wild ruminants outside of their known distributions in South Africa and additional information of the distribution of these vectors of *T. p. parva* and *B. bovis* is needed to prevent their introduction into new areas.

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