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Ticks and tick-borne pathogens at the interplay of game and livestock animals in South Africa

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1 Abstract

To evaluate the exposure of wild and domestic ungulates to tick borne-pathogens, and hence to study the pathogen exchange at the wildlife-livestock interface, blood from 181 individual hosts assigned to 18 species and 7364 ticks belonging to 13 species were collected. Samples originated from nine localities in four South African Provinces (Free State, Mpumalanga, Gauteng and Limpopo). Polymerase chain reaction followed by reverse line blotting and sequencing was used to screen host blood and tick salivary glands for protozoan pathogens of the genera *Babesia* and *Theileria* as well as for bacterial pathogens of the genera *Anaplasma* and *Ehrlichia*. From each individual infested host, a maximum of ten males and ten females of each tick species were dissected to isolate the salivary glands, this led to 2117 analysed ticks. Three hundred twenty nine ticks (15.5%), belonging to eight species, were infected and harboured 397 infections among which 57.7% were identified to species level and were assigned to 23 pathogen species. While, 110 / 181 individual hosts were infected and harboured 210 infections and 163 were identified to species level and belonged to 16 pathogen species. Screening such a large variety of host and tick species allowed describing 30 new host-pathogen combinations, involving ten pathogen species, and 23 new vector-pathogen combination which involved 14 pathogen species. Principal component analysis (PCA) assigned the 163 infections, identified to species level in host blood, to four groups. Three groups were associated to sheep, cattle and horses and their respective wild counterparts. Each group was characterized by high homogeneity in pathogen assemblage and host phylogenetic status. These groups characterized the most privileged transmission routes between and among wild and domestic ungulates. Within six 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. Interestingly, salivary glands from ticks infesting wild ruminants displayed significantly higher infection prevalence and pathogen mean density than salivary glands from ticks infesting livestock animals. This suggests that wild ungulates are more refractory to tick-borne pathogen infections than domestic ones, given that the infection prevalence and intensity of infection displayed similar values in host blood of wild and domestic ungulates. However, both animal types could act as equally 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.

Keywords: tick-borne pathogens; African wildlife; Livestock; Co-infections; *Theileria, Babesia, Ehrlichia; Anaplasma.*

Résumé

Nous avons étudié les pathogènes transmis par les tiques en Afrique du Sud (Free State, Mpumalanga, Gauteng et Limpopo), plus précisément, l'échange de pathogènes se produisant entre ongulés sauvages et domestiques. Un total de 7364 tiques appartenant à 13 espèces a été récolté sur 181 hôtes. Les protozoaires *Babesia* et *Theileria* ainsi que les bactéries *Anaplasma* et *Ehrlichia* ont été recherchés dans le sang des hôtes et dans les glandes salivaires des tiques par PCR, « Reverse Line Blot » et séquençage. Un maximum de dix tiques mâles et de dix tiques femelles de chaque espèce a été analysé pour chacun des individus, soit 2117 tiques. Celles-ci présentaient un taux d'infection de 15% comprenant 397 infections appartenant à 23 espèces de pathogène. Quant aux hôtes vertébrés, près de 61% d'entre eux étaient infectés par 16 espèces de pathogène. Ce travail a permis d'observer des infections non encore décrites : 30 impliquant dix espèces de pathogènes chez les hôtes et 23 impliquant 14 espèces de pathogènes chez les tiques. L'analyse en composante principale (ACP) a permis d'attribuer les 163 infections dans le sang des hôtes à quatre groupes dont trois sont constitués des ovins, bovins et équidés domestiques et de leurs équivalents sauvages. Ces groupes représentent les voies privilégiées de transmission des pathogènes parmi et entre la faune sauvage et les animaux domestiques. Les glandes salivaires des tiques des hôtes sauvages (n=64) présentent des taux et des densités d'infection significativement plus élevés que celles des tiques des animaux domestiques (n=64) vivant à proximité. Les animaux sauvages sont donc davantage exposés aux pathogènes se transmettant par les tiques que les animaux domestiques. Pourtant, l'infection du sang (prévalence et densité d'infection) n'est pas différente chez les animaux sauvages et domestiques exposés à ces mêmes tiques. La faune sauvage semble donc plus réfractaire aux pathogènes véhiculés par les tiques que les animaux de rentes. Néanmoins, les animaux sauvages et domestiques sont des sources d'infections tant pour eux-mêmes qu'entre eux. Cette étude apporte un éclairage nouveau sur les pathogènes transmis par les tiques à l'interface entre la faune sauvage et les animaux domestiques dans certaines régions d'Afrique du Sud.

Mots clés: Pathogènes ; tiques ; faune sauvage africaine ; animaux de rentes ; coinfections; *Theileria, Babesia, Ehrlichia, Anaplasma.*

2 Introduction

2.1 Tick biology

Ticks represent the most important group of arthropod vectors for wild and domestic ungulates, they transmit a wide spectrum of pathogenic microorganisms such as viruses, bacteria, and protozoa (Uilenberg, 1995; Jongejan and Uilenberg, 2004). They are a highly specialized group of obligate ectoparasites infesting mammals, birds and reptiles consisting in about 900 species from which approximately 10% transmit pathogens (Barker and Murrell, 2004, Jongejan and Uilenberg, 2004).

Ticks belong to the subphylum Chelicerata, class Arachnida, subclass Acari, and suborder Ixodida. Three families are recognized: Ixodidae (hard ticks) with 692 species, Argasidae (soft ticks) with 186 species and Nuttalliellidae with only one species (Barker and Murrell, 2004; Nava et al., 2009) (Figure 1).

Ixodidae (692 species)

Amblyomma
Anomalohimalaya
Bothriocroton
Cosmiomma
Cornupalpatum
Dermacentor
Haemaphysalis
Hyalomma
Ixodes
Margaropus
Nosomma
Rhipicentor
Rhipicephalus

Argasidae (186 species)

Argas
Carios
Ornothodoros
Otobius

Nuttalliellidae (one species)

Nuttalliella

Figure 1: The current list of valid tick **families** and genera according to Barker and Murrell (2004) and Nava et al. (2009).

In the past decade several changes in the nomenclature have been made through molecular phylogeny (Barker and Murrell, 2004; Nava et al., 2009). The genus *Boophilus* appears paraphyletic to the genus *Rhipicephalus* and was placed within *Rhipicephalus* as subgenus (Murrell and Barker, 2003). Furthermore, the genus *Aponomma* is not considered as valid any longer, some species were included in the genus *Amblyomma* while a new subfamily Bothriocrotoninae containing a single genus *Bothriocroton* was created for the other species (Barker and Murrell, 2004). Finally, the subfamily Hyalomminae is now part of the subfamily Rhipicephalinae.

Hard tick species have three development stages (larvae, nymphs and adults), all of them are parasitic and feed on hosts (Walker et al., 2003). The majority of Ixodidae species require three different hosts to complete their life cycle (three-host life cycle), being most probably the plesiomorphic life cycle of hard ticks (Barker and Murrell, 2004). Briefly, questing larvae climb a host and feed (approximately 3-5 days), drop to the ground where they hide in the soil or vegetation to moult to nymphs (Figure 2). The latter attach to a second host (belonging to the same or different species) and take a blood meal (approximately 4-8 days), detach from the host and moult to adults on the ground. Females and males infest a third host (belonging to the same or different species), females feed (approximately 5-20 days), mate and lay eggs on the ground (usually several thousands) and die. The males usually take several small meals, mate several times and die. This primitive live cycle is slow, it lasts from six months to several years. Three-host life cycles are displayed, for example, by *Rhipicephalus appendiculatus, R. zambeziensis, R. gertrudae, R. warburtoni, Amblyomma hebraeum, Haemaphysalis silacea* and *Ixodes rubicundus*.

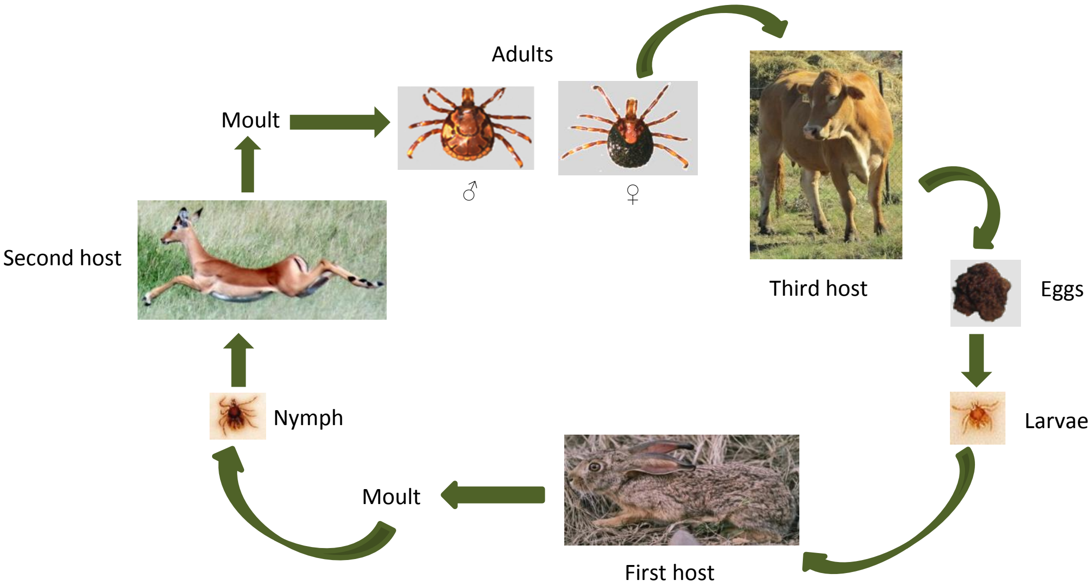

Figure 2: Three-host life cycle (example: *A. hebraeum*).

Another type of life cycle implicates only two individual hosts (two-host life cycle), larvae and nymphs feed on the same individual host, while adults infest a second individual host (Walker et al., 2003). This life cycle occurs in the same manner as the three-host life cycle with the difference that moulting from larvae to nymphs occurs on the host. *Rhipicephalus evertsi evertsi* and *Hyalomma marginatum rufipes* are examples of ticks with a two-host life cycle.

In the third type of life cycle (one-host life cycle) all three life stages feed and moult on the same individual host (Figure 2). After mating, females detach from the host and eggs are laid on the ground. This is the less represented and fastest life cycle, it can be completed in about two months. One-host life cycles are observed, for example, in *Rhipicephalus (Boophilus) decoloratus, R. (B.) microplus* and *Margaropus winthemi*.

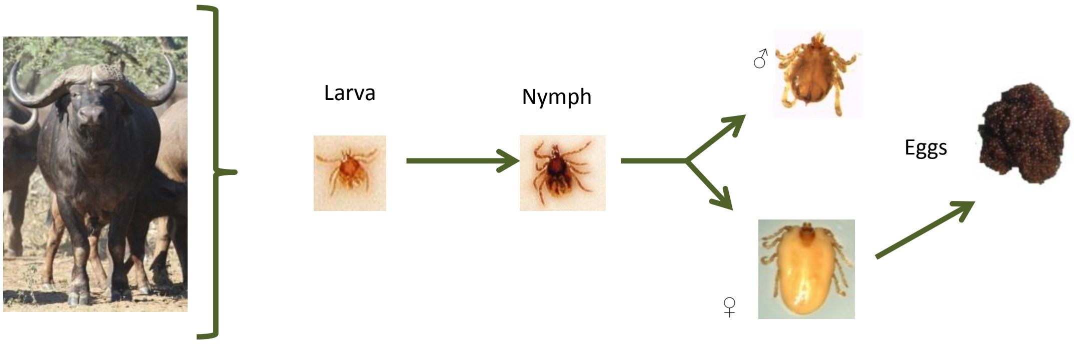

Figure 3: One-host life cycle (example: *R. (B.) decoloratus*).

The development of soft ticks differs in several ways in that the majority of the species requires multiple hosts to complete their life cycle, except *Otobius megnini* that develops on a single host (Walker et al., 2003). The behaviour of larvae differs between species, in some species they feed quickly, while in others they feed during several days using different host species. In contrast to hard ticks all Argasidae have multiple nymphal stages (their number varies between species) and feeding generally occurs on several different individual hosts. Females lay several small batches of eggs after each of their multiple blood meals. While hard ticks (except some *Ixodes* species) mate on their hosts, soft ticks mate off hosts.

In South Africa, the main tick species transmitting pathogens of veterinary importance affecting domestic and wild Ungulates are principally found in the genera *Rhipicephalus,* *Amblyomma* and *Hyalomma*. In addition to pathogen transmission, ticks can also cause harm to their host through injection of toxins inducing paralysis, loss of condition due to heavy infestations and secondary bacterial infections for some species of the genera *Ixodes, Margaropus* and *Otobius*. The most important species of these genera threatening domestic and wild ungulates being of interest for the present study are described below.

2.1.1 *Rhipicephalus* species

In the South African context the two-host tick *R. evertsi evertsi* belongs to the most widely distributed species (Appendix A1) (Walker et al., 2000; Walker et al., 2003; de Matos, 2008). This is probably due to its very broad host range including mainly Ungulates but also Carnivores, Lagomorphs, Primates and several bird taxa. The immature stages infest all host species whereas adults are principally recorded from larger animals (Walker et al., 2000; Walker et al., 2003). Adults preferably attach on the peri-anal area, but are also found on the groin region while the immature stages principally attach on the external ear canals. *R. e. evertsi* is able to accomplish more than one generation per year and all live stages are found on hosts throughout the year (Horak et al., 1991). It transmits the rickettsial pathogens *Anaplasma marginale* (Potgieter, 1981), *A. ovis* (Kaufman, 1996) and *Ehrlichia ovina* (Neitz, 1956) as well as the protozoan pathogens *T. equi* (De Waal and Potgieter, 1987; De Waal and Heerden, 1994), *T. separata*, *T. ovis* (Jansen and Neitz, 1956), *Theileria* sp. (sable) (Steyl et al., 2012), *T. taurotragi* (Theiler, 1907), *T. parva parva* (Lounsbury, 1906) and *B. bigemina* (Büscher, 1988). *R. e. evertsi* is not an important vector for *T. p. parva* (Walker et al., 2000) and its exact role in the epidemiology of redwater due to *B. bigemina* remains unclear (De Vos and Potgieter, 1994). It seems that only nymphs transmit *B. bigemina* and that transovarial transmission does not occur (Büscher, 1988).

The three-host ticks *R. appendiculatus* and *R. zambeziensis* are closely related, consequently they show similarities in their morphology, host range, seasonal occurrence and attachment sites (Norval, 1994). Among ungulates, cattle and wild members of the Tragelaphinae (e.g. greater kudu, bushbuck, nyala) belong to the preferred hosts of both species for all life stages, several wild carnivore species are also infested and hares represent important hosts for the immature stages (Walker et al., 2000). Impala are infested by all stages of both species but seem especially important for the maintenance of *R. zambeziensis* populations

(Walker et al., 2000, 2003). Preferred attachment sites of *R. appendiculatus* adults are the ear pinnae and the head, but when infestation increases they are also found elsewhere. In addition to those sites the immature stages also attach on legs and feet even in light infestations. On the majority of their hosts, *R. zambeziensis* displays a similar behaviour, nevertheless on impala they principally attach on the muzzle. In Southern Africa both species have a seasonal development cycle: adults mainly occur at the end of the summer during the rainy period, larvae in autumn and early winter and nymphs during winter and early spring (Walker et al., 2003). Both species transmit *T. parva parva, T. p. lawrencei* (De Vos, 1981; Lawrence et al., 1983), *T. p. bovis* (Fivaz et al., 1989; Lawrence et al., 1983), *T. taurotragi* (Lawrence and MacKenzie, 1980; Lawrence et al., 1983) and *A. bovis* (Norval, 1979; Scott, 1994). Despite the fact that both species are able to transmit these pathogens, *R. appendiculatus* is associated to most outbreaks due to the *T. parva* subspecies complex in the field (Norval, 1994). Transmission of *T. p. bovis* and *T. p. parva* by *R. zambeziensis* was only observed in the laboratory (Walker et al., 2003). Thus, *R. appendiculatus* must be considered as a more important vector. Furthermore, *R. appendiculatus* has a much broader distribution in Southern Africa than *R. zambeziensis* (Appendix A2, A3). *R. zambeziensis* mainly occurs in dry and hot river valleys such as the Limpopo, Sabi, Zambezi and Luangwa valleys which are not suitable for *R. appendiculatus*.

Two one-host ticks of the subgenus *Boophilus* occur in South Africa: *R. (B.) decoloratus* and *R. (B.) microplus* (Appendix A4, A5). *R. (B.) decoloratus* is indigenous to the African continent, while *R. (B.) microplus* is an Asian tick which was introduced into South Africa from Madagascar and East Africa with cattle imported at the end of the 19th century (Theiler, 1962). Both species infest cattle heavily, which are probably the only effective hosts for *R. (B.) microplus* (Walker et al., 2003). On the other hand, *R. (B.) decoloratus* was reported from numerous wild ungulate species (Latif and Walker, 2004). Both species are found all over the host body: back, bally, neck, shoulders, dewlap, thigh and legs. Due to their one-host life cycle several generations can be produced per year (Walker et al., 2003). Under optimal conditions the entire development of *R. (B.) decoloratus* only lasts about two months with approximately three weeks on the host. *R. (B.) microplus* has even a shorter cycle and lays more eggs than its endemic relative. Both species require similar climatic conditions (Estrada-Peňa et al., 2006). But the shorter life cycle gave *R. (B.) microplus* an advantage in areas with higher rainfall resulting in the replacement of *R. (B.) decoloratus* by *R. (B.) microplus* in these areas of Southern Africa (Sutherst, 1987). Both species transmit *B. bigemina* responsible for African redwater and *A. marginale*, in addition to *R. (B.) decoloratus, R. (B.) microplus* also transmits *B. bovis* the agent of the devastating Asiatic redwater (Walker et al., 2003).

R. gertrudae and *R. warburtoni*, both having three-host life cycles, occur in the Highveld grasslands of South Africa (Walker et al., 2000). Their geographic distribution is relatively restricted compared to the previous described species (Appendix A6, A7). Cattle and small domestic ruminants are the principal domestic hosts of the adults of both species. Among wild hosts, records from several large ruminant species attest to their importance for the adults of *R. gertrudae*, in addition they demonstrate affinities for primates and carnivores, while scrub hares (*Lepus saxatilis*) appear to be important wild hosts of all *R. warburtoni* stages. The immature stages of *R. gertrudae* (feeding on different host species than adults) mainly infest small rodents (Fourie et al., 1992). According to observations on goats, *R. warburtoni* mainly attach on head and ears on young animals, in addition to these sites neck and breast are target sites on older animals (Fourie et al., 1991). On large ruminants *R. gertrudae* is principally found on the neck, groin and tail regions (Hlatshwayo et al., 2000). Both species display differences in abundance throughout the year. In the Free State, adults of both species are most abundant from early spring to the end of the summer (Fourie et al., 1996; Fourie and Horak, 1991). To date none of these two Rhipicephalids have been associated with pathogens, but paralysis has been reported in young goats heavily infested by *R. warburtoni* (Fourie et al., 1988) and *R. gertrudae* was associated to death in Chacma baboons (*Papio cynocephalus ursinus*) caused by acute inflammation (Brain and Bohrmann, 1992).

2.1.2 *Amblyomma* species

The three-host tick *A. hebraeum* is one of the most important South African vectors of veterinary importance. The main hosts of the adults are large wild and domestic ungulates, the immature stages infest the same host species as the adults and in addition they feed on various smaller ruminants, lagomorphs, birds and reptiles (Norval, 1994; Walker et al., 2003). Adults principally attach to the hairless body parts as the perianal and groin regions, around the genitalia and the axillae. Larvae are principally found on the legs, feet and muzzles. These sites are shared with the nymphs, which also attach to the breast, groin and neck. The development cycle can be completed in one year under optimal conditions. Seasonal occurrence of the different life stages varies throughout the species distribution range (Appendix A8) (Norval, 1994). Usually all stages are found on their hosts at all seasons, but the immature stages are generally more abundant in the drier months and adults in the rainy season. In Southern Africa, *A. hebraeum* is the most important vector for the virulent *E. ruminantium* (Bezuidenhout et al., 1994) and also transmits the benign cattle parasites *T. mutans* (Lawrence et al., 1994d) and *T. velifera* (Uilenberg, 1983).

2.1.3 *Hyalomma* species

Among the several *Hyalomma* species occurring in Southern Africa, the two-host species *H. marginatum rufipes* is probably one of the most widely distributed species in the area (Appendix A9) and belongs to the most important species of this genus in terms of pathogen transmission in the region (Norval, 1994). Adults principally feed on cattle, sheep, goats and horses among domestic animals and various species of large ungulates are infested among wild animals (Walker et al., 2003; Latif and Walker, 2004). These hosts are not infested by the immature stages which use hares and ground-frequenting bird species as hosts. The adults principally attach to the same sites as *A. hebraeum* (Norval, 1994; Walker et al., 2003). All life stages are found throughout the year, adults reach their population peak in the wet season and the immature stages in the dry season (Walker et al., 2003). *H. m. rufipes* transmits the rickettsial pathogen *A. marginale* (Norval, 1994) and the protozoan *B. occultans* (Thomas and Mason, 1981) affecting cattle. In addition, it is the principal vector of the virus causing the fatal Crimean-Congo haemorrhagic fever in humans in South Africa (Walker et al., 2003).

2.1.4 *Haemaphysalis* species

Most species of the genus *Haemaphysalis* occurring in South Africa are specialized on carnivores (Apanaskevich and Horak, 2008; Horak et al., 2000, Horak et al., 2010). Nevertheless a few species as the three-host tick *H. silacea* mainly feed on ruminants (Norval, 1975; Horak et al., 2007). This species seems to have a limited geographic distribution (Appendix A10), since it was exclusively reported from the Eastern Cape Province (river valleys of the Fish River and coastal bush complex) and to a lesser extent from the north-eastern KwaZulu-Natal Province (Norval, 1975; Walker, 1991; Horak et al., 1991; Horak et al., 2007). *H. silacea* is associated with a vegetation type called "Valley Bushveld" (Horak et al., 2007) and with hosts belonging to the Tragelaphinae (e.g. greater kudu, bushbuck, nyala) representing the preferred hosts of the adults (Norval, 1975; Walker, 1991). In addition, sheep, goats and cattle as well as medium to large wild ruminants including the African buffalo (*Syncerus caffer*), are infested (Walker et al., 1991; Horak et al., 2007). The immature stages are mainly found on smaller carnivores, on several larger ground frequenting birds and occasionally, on hares and rodents (Norval, 1975; Walker et al., 1991). Adults principally attach to the limbs, belly, groin and perianal regions while larvae and nymphs are mainly found on the lower parts of the host limbs (Norval, 1975). According to Norval (1975) larvae are active during autumn and the beginning of the winter, nymphs in the late winter and spring, and adults during summer. It is probable that *H. silacea* completes more than one live cycle per year (Horak et al., 1991). To date, no pathogen transmission has been attributed to *H. silacea* (Norval, 1971).

2.1.5 *Ixodes* species

The three-host ticks *I. rubicundus* (Appendix A11) and *I. pilosus* are the most represented species of the genus *Ixodes* in South Africa. In terms of veterinary importance *I. rubicundus* is definitely more important than *I. pilosus* due to the fact that females of this species induce severe paralysis mainly in small domestic ruminants (Walker et al., 2003). Among livestock animals, cattle are infested by adult *I. rubicundus* in addition to small domestic ruminants, however, their main natural hosts are caracal (*Caracal caracal*) and various medium size wild ungulates also serve as hosts (Walker et al., 2003; Latif and Walker, 2004). The immature stages feed preferably on elephant shrews (*Elephantulus* spp.) as well as on red rock rabbits (*Pronolagus* spp.) (Norval, 1994). According to observations on sheep, females mainly attach on the limbs and belly (Walker et al., 2003). The development cycle of *I. rubicundus* takes two years to be completed and is tied to the seasons, adults appear from autumn and their eggs will only hatch the following autumn (Walker et al., 2003). *I. rubicundus* is not associated to pathogens, notwithstanding that it can cause losses reaching 15% in sheep flocks if no adequate preventive measures are applied (Howell et al., 1978). Paralysis is induced by a toxin injected into the host with the saliva of female ticks (Walker et al., 2003). It begins in the legs and can extend to the respiratory system, effects are reversible if ticks are removed.

2.1.6 *Margaropus* species

The one-host tick *M. winthemi* is the only representative of this genus in South Africa, where it mainly occurs in temperate area of the highlands (Appendix A12) (Walker et al., 2003). Its preferred hosts are wild and domestic equids, cattle and wild ruminants such as common eland (*Tragelaphus oryx*) are also good hosts. In heavy infestations *M. winthemi* is usually found all over the host body, but neck, flanks and face are the preferred attachment sites. *M. winthemi* is adapted to low temperatures and appears on hosts exclusively in winter where this species reaches high population densities. It is not known to be associated to pathogen transmission but it causes such heavy infestations, principally on horses, that loss of condition occurs.

2.1.7 Argasidae species

Among Argasidae, tick species of veterinary importance are found in the genera *Argas, Ornithodoros* and *Otobius* (Jongejan and Uilenberg, 2004), while some species represent constraints in poultry production others like *Otobius megnini* are pests of cattle and horses (Walker et al., 2003; Keirans and Pound, 2003). *O. megnini* has its origins in America, it was imported into South Africa with horses or cattle, probably at the end of the 19th century. It has mainly colonialized arid areas such as the Kalahari, Karoo and parts of the Free State (Appendix A13) (Walker et al., 2003). In addition to cattle and horses, *O. megnini* also feeds on sheep, goats and on carnivores. The parasitic stages (larva and two nymphal stages) feed deep in the ear canal of their single host on which they can be found throughout the year. Adults are not in contact with hosts. Pathogen transmission was never observed in this species which affects its hosts by their feeding habits (Walker et al., 2003). The spines on the tick bodies induce painful irritations of the ear resulting in appetite loss, in some cases irritation is followed by inflammation, necrosis and secondary bacterial infections.

2.2 Protozoan pathogens

The protozoa of the genera *Babesia* and *Theileria* are intra-cellular, tick-transmitted parasites infecting a huge range of wild and domestic vertebrates worldwide. Some species are non-pathogenic, while others are highly virulent causing great economic losses to the livestock and game ranching industries, especially in the southern hemisphere (Allsopp et al., 1994; Bishop et al., 2004; Bock et al., 2004). The resulting diseases are given specific names according to the affected hosts and the geographic area in which they occur (De Vos et al., 1994).

Babesia spp. and *Theileria* spp. were classically placed within the phylum Apicomplexa, class Piroplasmasida, order Piroplasmorida and families Babesiidae and Theileriidae, respectively (Levine, 1971). According to Bishop et al. (2004) the taxonomic validity of the Apicomplexa, based on the common possession of an apical complex involved in the invasion of host cells, remains unclear since the evolutionary and functional origins between different taxa of the apical complex diverge. The systematic position of many species remained doubtful for long; for instance, *T. equi* was considered as *Babesia* sp. before and it was named *B. equi*, it was reclassified as *Theileria* sp. by Mehlhorn and Schein (1998). Furthermore, species were reclassified in different genera, *Cytauxzoon taurotragi* became *T. taurotragi*, *Gonderia mutans* became *T. mutans* and *G. ovis* became *T. ovis* (Levine, 1971). In the recent study of Schnittger et al. (2012) six monophyletic piroplasmid clades are recognised. Clade 1 "*B. microti*-group" contains *Babesia* spp. with ancient characteristics as lower host specificity, many rodent and feline *Babesia* spp. belong to this group. Clade 2 contains canine and human *Babesia* spp., clade 3 *Cytauxzoon* spp. as well as *Theileria* spp. and *Babesia* spp. of uncertain classification. Clade 4 consists of *Theileria* sensu lato (s.l.), which can neither be considered as *Theileria* sensu stricto (s.s.) nor as *Babesia* s.s., namely *B. bicornis* and *T. equi*. Clade 5 and clade 6 are composed of all known *Theileria* s.s. and *Babesia* s.s., respectively. Phylogenetic relationships of *Babesia* and *Theileria* species are shown in Appendix B.

2.2.1 Life-cycle of *Babesia* species

The life-cycle of *Babesia* spp. takes place partly in hosts and partly in ticks (Figure 4). Numerous studies have aimed to understand the life-cycles of *Babesia* species, however our knowledge is still incomplete and variations in some species occur (Bock et al., 2004). Briefly, sporozoites (Sz) are injected with the saliva of an infected tick into the bloodstream of a vertebrate host where erythrocytes are the only cells which are invaded (Figure 4). Once inside the erythrocytes, the sporozoites differentiate into trophozoites (T), which become merozoites (M) through asexual division. The majority of the merozoites carry on with the replication cycle in the host by invading new erythrocytes, while a small proportion of the merozoites stop dividing to become gametocytes (G) which produce gamonts. The second half of the life cycle can only occur if gamonts are taken by ticks during their blood meal. In the midgut of the tick the sexual division takes place with the gamonts differentiating into gametes, called ray bodies (Sk) transforming into diploid zygotes (Z). Haploid mobile kinetes, produced through meiosis from zygotes, multiply and invade several organs of the tick such as salivary glands, where the replication is carried on. Most *Babesia* s.s. invade tick ovaries and eggs, perpetuating the infection to the larvae of the next generation (transovarial transmission, To). Finally, kinetes differentiate into sporozoites; this happens after the tick moults into the next development stage (nymph or adult), which will inject sporozoites into a host (transstadial transmission, Ts).

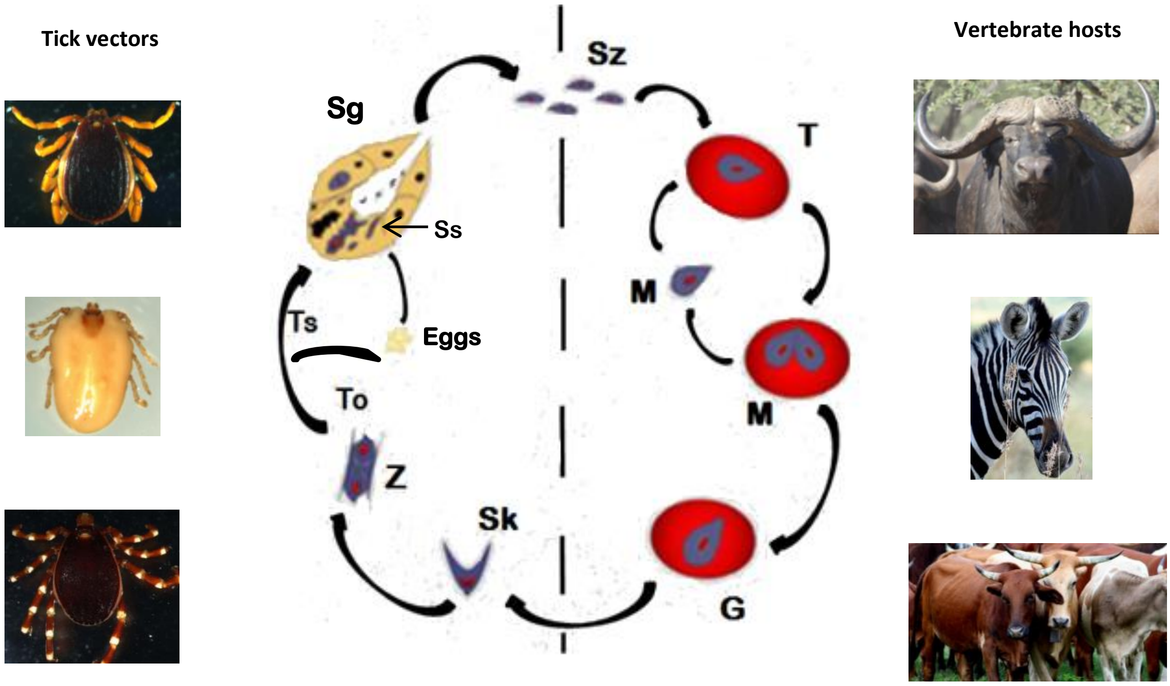

Figure 4: Life-cycle of *Babesia* spp., **Sz:** sporozoites; **T:** trophozoites; **M:** merozoites; **G:** gametocytes ; **SK:** ray bodies (Strahlenkörper); **Z:** zygote ; **To:** transovarial transmission; **Ts:** transstadial transmission; **Ss:** only in *Babesia* sensu stricto **SG:** Salivary glands (Schnittger et al. (2012) modified).

2.2.2 Life-cycle of *Theileria* species

The life-cycle of *Theileria* spp. has similarities with that of *Babesia* spp., nevertheless important differences must be noticed concerning the target cells as well as the transmission pathways between the vectors. Theilerial parasites also undergo multiple transformations in hosts and vector ticks (Figure 5). Sporozoites injected with tick saliva infect lymphocytes, in which they develop into schizonts. As the lymphocytes divide, the schizonts also divide asexually resulting in a clonal expansion of parasitized cells within the lymphoid system of the host. While most schizonts continue replication, a few develop into merozoites which are released into the bloodstream where they invade erythrocytes in which they transform into pear-shaped structures called piroplasms. Similar to *Babesia* spp., sexual reproduction only takes place if infected erythrocytes are ingested by a vector tick. Piroplasms differentiate to male and female gamonts in the tick midgut and become zygotes which differentiate into mobile kinetes. Kinetes move to the salivary glands where sporoblasts are formed producing thousands of sporozoites which will be injected into a host by the next tick stage (nymph or adults) resulting in transstadial transmission. Contrary to *Babesia* spp., ovaries and eggs are not invaded by kinetes, thus transovarial transmission does not occur.

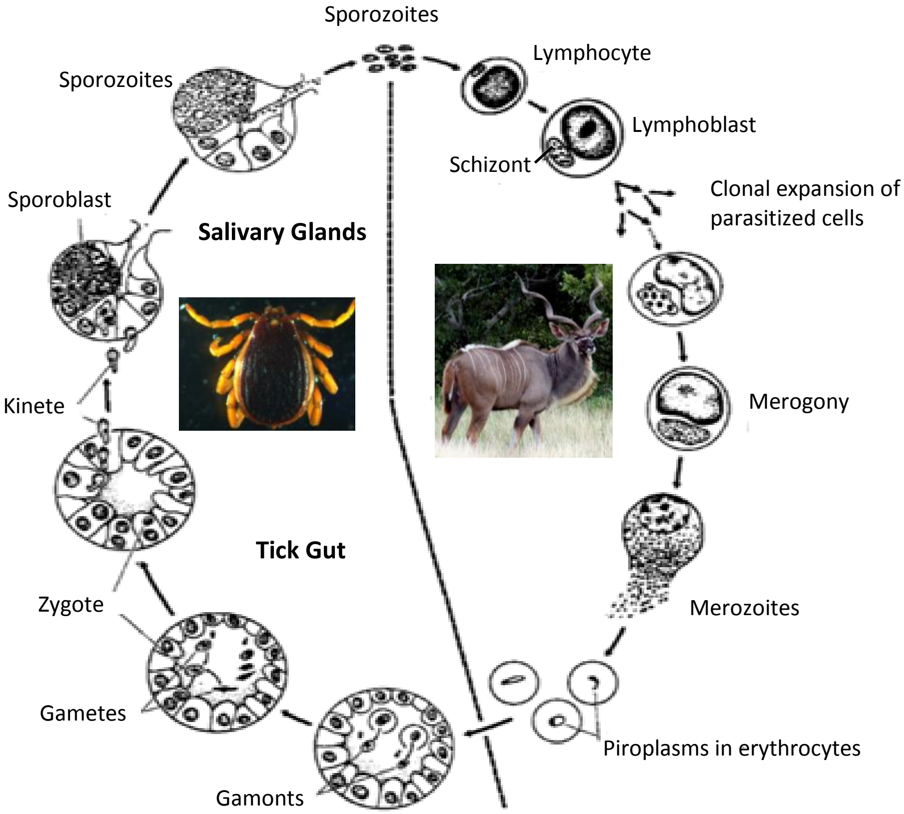

Figure 5: Life-cycle of *Theileria* spp. (Bishop et al., (2004) modified).

2.2.3 Clinical manifestations of *Babesia* and *Theileria* species

Clinical manifestations induced by *Babesia* spp. and *Theileria* spp. are a result of massive erythrocyte and lymphocyte destruction, respectively, resulting from multiplication in vertebrate hosts. Therefore, babesiosis is characterised in varying degrees by intravascular haemolysis (due to haemoglobin in the plasma), haemoglobinaemia (due to free haemoglobin proteins in the plasma), haemoglobinuria (due to haemoglobin in the urine) and icterus (due to bilirubin, a yellow breakdown product of the heme catabolism, in the blood) (De Vos et al., 1994). While, theileriosis is characterised in variable degrees by symptoms such as lymph node enlargement (resulting through hyperplasia and necrosis of lymphocytic cells), opacity of the cornea (due to infiltrations of lymphocytic cells), hyperplasia of lymphoid tissues and lymphoid infiltration into organs such as kidneys as well as diarrhea with presence of blood in faeces and mucus are also observed (Lawrence et al., 1994a). Death due to virulent *Babesia* spp. and *Theileria* spp. is frequent in naïve animals. Within both genera, the general clinical manifestations are similar in all vertebrate species (De Vos et al., 1994).

2.2.4 Piroplasm species

Both genera possess a high species richness, the genus *Babesia* alone include more than a hundred described species (Schnittger et al. 2012) and through the progress in molecular diagnostic tools the number of known species is increasing. Different *Babesia* spp. and *Theileria spp.* occurring in Southern Africa in wild and domestic ruminants of interest for the present study are discussed below.

***Babesia* species recorded from game animals**

***Babesia* sp. (sable)**

This parasitic tick-borne protozoan might have caused the first fatal case, due to piroplasmosis, described in an antelope species (Oosthuizen et al., 2008). In 1930, Martinaglia observed *Babesia*-like parasites in blood smears from a sable antelope (*Hippotragus niger*), which succumbed to a disease with similar post-mortem findings as for bovine babesiosis, six weeks after its arrival in the Johannesburg Zoological Garden. The parasite was named *B. irvinesmithi*. Subsequently, other cases of babesiosis in sable antelope, most probably due to the same parasite, were reported by mean of microscopic observations by Wilson et al. (1974), Thomas et al. (1982) and McInnes et al. (1991). The transmission of the parasite to splenectomized cattle was unsuccessful, reciprocally the virulent bovine parasites *B. bovis* and *B. bigemina* were not transmissible to sable antelope (Thomas et al., 1982). Recently, Oosthuizen et al. (2008) reported a case of a sable antelope from a game farm in the Limpopo Province which died during immobilization. The veterinarians observed small piroplasms in erythrocytes. Molecular analysis revealed a new species or a variant of a species, which belongs to the *Babesia* s.s. clade (Oosthuizen et al., 2008). In this clade, the now called *Babesia* sp. (sable), forms a monophyletic group with the *B. orientalis* group, *B. occultans*, the unnamed *Babesia* sp. (accession number: U09834) and *Babesia* spp. from China *B.* sp. Kashi 1 and 2. Unfortunately, until now, no vector ticks of the pathogen could be identified. *Hyalomma marginatum rufipes* is the proven vector of the closely related *B. occultans* (Thomas and Mason, 1981). This suggests that *H. m. rufipes* could also transmit the new parasite, *Babesia* sp. (sable) (Oosthuizen et al., 2008). No molecular evidence could be brought, due to lack of exploitable biological material, that *Babesia* sp. (sable) is *B. irvinesmithi*, but it seems probable.

***Babesia* species principally recorded from cattle**

B. bovis* and *B. bigemina

B. bovis causing Asiatic redwater and *B. bigemina* causing African redwater, belong to the economically most important tick-borne pathogens affecting cattle worldwide (Uilenberg, 1995). Both species occur in South Africa. *B. bovis* was first reported in this country in 1941 (Neitz, 1941), where it was introduced with the Asiatic blue tick *R. (B.) microplus*, at the end of the 19th century (Theiler, 1962). *R. (B.) microplus* is the only vector of *B. bovis* in southern Africa (Tonnesen, 2004). *B. bigemina* is endemic to Africa and probably evolved among wild ruminants (Bigalke, 1994). It is likely that it reached the south of the continent, along with its main vector *R. (B.) decoloratus*, with migrations of ancient tribes and their cattle, long before the arrival of Europeans (Henning, 1956). In contrast to *B. bovis*, *B. bigemina* has more than one vector in southern Africa. In addition to *R. (B.) decoloratus*, the invasive *R. (B.) microplus* (Tonnesen, 2004) and the common African tick *R. e. evertsi* are known to transmit *B.*

bigemina (Büscher, 1988). The exact role of *R. e. evertsi* in the epidemiology of African redwater is still unclear (De Vos & Potgieter, 1994). It seems that only *R. e. evertsi* nymphs transmit *B. bigemina* and that transovarial transmission does not occur (Büscher, 1988). Nymphs and adult stages of *R. (B.) decoloratus* transmit the infection which persists for at least two generations. *B. bovis* is recognised as being more virulent than *B. bigemina*. However, in South Africa, *B. bigemina* might be more important due to its wider distribution (De Vos & Potgieter, 1994). In fact, pathogen species follow the distribution of their vectors and *R. (B.) decoloratus* has a much wider distribution in South Africa than *R. (B.) microplus* (Walker et al., 2003). Furthermore, *B. bigemina* is transmitted by three tick species in Southern Africa while *B. bovis* only by one. The role of wildlife in the epidemiology of redwater is still unclear (Geleta, 2005). *B. bigemina* antibodies were detected in African game species (Löhr et al., 1974), but no wildlife reservoir of importance was found (Friedhoff and Smith, 1981). Severe clinical signs of both pathogen species seem to be restricted to cattle and to a certain extent to small domestic ruminants (Friedhoff and Smith, 1981; De Vos & Potgieter, 1994). Latent *B. bigemina* infections in African buffalo, lasting up to 4 month, were also demonstrated (Karbe et al., 1979).

B. occultans

B. occultans was first described in *H. m. rufipes* ticks originating from a South African farm near Lephalale in 1981 by Thomas and Mason. These authors demonstrated that the parasite is transovarially transmitted by *H. m. rufipes* and that it is transmitted to cattle by this tick species. The infection results in a mild febrile reaction in cattle, while horses seem refractory to the infection. According to Gray and De Vos (1981) the parasite is highly invective to *H. m. rufipes* ticks since over 50% of engorged female ticks become infected on cattle with very low parasitaemias. *B. occultans* reaches high herd and within-herd prevalence in cattle and is widespread in South Africa (Gray and De Vos, 1981). It was recently isolated from *H. marginatum* in Tunisia by Ros-Garcia et al. (2011). According to these authors the parasite might have a much wider distribution than previously thought since unnamed *Babesia* spp., with highly similar DNA sequences to *B. occultans*, were reported from cattle in Turkey (Ica et al., 2007) and in China (Luo et al., 2005). Therefore, the parasite is probably transmitted by several tick species of the genera *Hyalomma* such as *H.*

anatolicum (Luo et al., 2005; Ica et al., 2007) and *H. truncatum* (Gray and De Vos, 1981). *B. occultans* is genetically very close to *Babesia* sp. (sable). To establish whether it could be the same organism, *B. occultans* was sequenced by Oosthuizen et al. (2008) in addition to *Babesia* sp. (sable). These authors showed that *B. occultans* and *Babesia* sp. (sable) belong to distinct species. Currently, only records from cattle and from *Hyalomma* spp. ticks are known. No infection in wildlife animals has been described. Nevertheless, its high infectivity to *H. m. rufipes* and its low pathogenicity shows a well-developed relationship to vectors and hosts. Grey and De Vos (1981) speculated that *B. occultans* might originate from an African antelope.

***Babesia* species recorded from Equids**

B. caballi

B. caballi is one of the ethologic agents of equine piroplasmosis, also called “biliary fever”, it is considered as less pathogenic than the other agent of the disease *T. equi* (Knowles, 1996; Katz et al., 2000). Horses, donkeys, mules and zebra are affected by the diseases and economic losses are induced (Alsaad and Al-Obaidi, 2012). Piroplasmosis represents the most frequent infectious diseases in equids in Southern Africa (Gummow et al., 1996). The disease is probably known in South Africa for more than one century, since the first report thought to refer to equine piroplasmosis is from Wiltshire in 1883 (De Waal and Heerden, 1994). Equine piroplasmosis is widespread throughout the world, it occurs in parts of Africa, Asia, Middle East, South and central America, the Caribbean and in temperate areas where it is less prevalent (Knowls, 1996; Mark, 2010). *B. caballi* is probably less prevalent than *T. equi* in endemic areas (Knowles, 1996; Katz et al., 2000). In South Africa, *B. caballi* is transmitted by *R. e. evertsi* (De Waal et al., 1988). Worldwide, 14 tick species belonging to the genera *Dermacentor, Hyalomma* and *Rhipicephalus* were identified as vectors for either *B. caballi* or *T. equi* (De Waal and Van Heerden, 1994). Horses infected with *B. caballi* remain infective for ticks for up to four years (De Waal and Van Heerden, 1994).

***Theileria* species principally recorded from game animals**

***Theileria* sp. (sable)**

The story of the discovery of this parasite probably begins in 1912 in Gambia where Todd and Wolbach observed a *Theileria* species in a blood smear from one sable antelope, they called it *T. hippotragi* (Todd and Wolbach, 1912). Half a century later, a similar organism was observed in blood smears of sable and roan antelope (Neitz, 1957; Wilson et al., 1974; Thomas et al., 1982). More recently, Stoltsz and Dunsterville (1992) cultivated a *Theileria* sp. originating from a sable antelope which died from theileriosis, and named it *Theileria* sp. (sable). A few years ago, Nijhof et al. (2005) described a *Theileria* species associated with the death of the now endangered roan and sable antelope. To their surprise, the organism was *Theileria* sp. (sable) described by Stoltsz and Dunsterville (1992) (GenBank accession number L19081). It could not be proven that the piroplasms described in previous reports were *Theileria* sp. (sable) but it is most likely (Nijhof et al., 2005). It has now been experimentally proven that *Theileria* sp. (sable) is responsible for severe illness in roan antelope which can lead to death, especially in calves (Steyl et al., 2012). In addition, these authors could demonstrate experimentally that *R. e. evertsi* and *R. appendiculatus* transmit *Theileria* sp. (sable). Recent field studies showed that this parasite has a broad host range and reaches high infection prevalence. Spitalska et al. (2005) described it in 22/23 red hartebeests in Namibia, Yusufmia et al. (2010) observed that 46.8% of 60 examined cattle carried *Theileria* sp. (sable) in South Africa. In the same country Pfitzer et al. (2011) detected it in 58.8% of 97 tested nyala. Phylogenetic studies demonstrated that *Theileria* sp. (sable) is closely related to *T. separata*, a benign parasite of sheep (Yin et al., 2007), and that the species is composed of several genotypes among which one genotype is adapted to cattle (Mans et al., 2011).

***Theileria* sp. (kudu)**

The first description of *Theileria* sp. (kudu) and its associated illness was most probably from Neitz in 1957. A young kudu bull in weak condition arrived from a farm in Vischgat near Vaalwater (Limpopo Province) to the Onderstepoort research station and died nine days after arrival from its illness (Neitz, 1957). The haemoparasites observed in the kudu looked like *Cytauxzoon sylvicaprae* previously described in a grey duiker. Neitz named the kudu parasite *C. strepsicerosi*. In previous observations Neitz (1933) saw undistinguishable pathogens in kudu erythrocytes from Zululand and Transvaal which let him conclude that the infection might be more widespread than first admitted. Calves and sheep could not be infected by intravenous injection of infected kudu blood (Neitz, 1957). Recently, Nijhof et al. (2005) analysed the blood of one out of several kudus which had died after developing clinical signs similar to those described by Neitz (1957). The sequence obtained by Nijhof et al. (2005) showed the highest homologies with a *Theileria* sp. from Thailand (GenBank accession number AB000270). Nijhof et al. (2005) called the kudu parasite *Theileria* sp. (kudu) and suggested that it might be the same species previously described by Neitz (1957). Unfortunately, the original samples from Neitz (1957) were not available and therefore it could not been proven that the observed pathogens belonged to the same species, nevertheless it seems likely. Pfitzer et al. (2011) detected *Theileria* sp. (kudu) in nyala, a species closely related to the greater kudu. Currently, no vector ticks were identified for this parasite, but future investigations will need to understand the transmission of *Theileria* sp. (kudu).

***Theileria* sp. (giraffe)**

During the last century, reports describing piroplasms in giraffe in southern and eastern Africa appeared in the literature. The first report seems to be from Brocklesby and Vidler (1965) who observed *Theileria* spp. in Masai giraffe in Kenya. A few years later, McCully et al. (1970) reported a case from one giraffe originating from Namibia imported into South Africa which died a few months after importation and the authors diagnosed it as cytauxzoonosis. Krecek et al. (1990) found haemoparasites in giraffe living in the Etosha National Park in Namibia and suspected that the parasites belonged to the genus *Cytauxzoon*. Recently, Oosthuizen et al. (2009) described a *Theileria* sp. with the use of reverse line blotting in 3 giraffe, 2 were sampled during translocation in South Africa and one died in South Africa after being imported from Namibia. Phylogenetic analysis showed that the parasite belongs to the *Theileria* s.s. clade. In accordance with previous reports, Oosthuizen et al. (2009) observed the susceptibility of giraffe to piroplasm infections, but whether several species are involved remains to be investigated. Future studies on the epidemiology of *Theileria* sp. (giraffe) especially on its transmission are needed since no vector ticks have been identified.

T. bicornis

One of the first reports of this parasite was probably from Brocklesby (1967) who observed small piroplasms in blood smears from black rhinoceros (*Diceros bicornis*) in Kenya. Later Bigalke (1970) reported similar structures in blood smears from white rhinoceros (*Ceratotherium simum*) in South Africa. Recently, through the use of molecular tools, Nijhof et al. (2003) identified a *Theileria* sp. from healthy black rhinoceros in South Africa and called it *T. bicornis*. Nijhof and colleagues findings were confirmed by Penzhorn et al. (2008) who reported the same parasite from black rhinoceroses in Namibia, indicating that the species could be widely distributed. In fact, two years later it was detected in Uganda (Muhanguzi et al., 2010). *T. bicornis* seems not to be pathogenic (Nijhof et al., 2003; Penzhorn et al., 2008; Govender et al., 2011). In contrast to what could be expected, it is not specific to black and white rhinoceroses since it was detected from cattle by Muhanguzi et al. (2010) and from nyala by Pfitzer et al. (2011). No vector tick species could currently be identified although *A. rhinocerotis* and *D. rhinocerotis* specifically feed on rhinoceroses (Nijhof et al., 2003; Zimmermann, 2009). Future studies will need to identify vector ticks for this pathogen. Genetically, *T. bicornis* is close to *T. youngi* and *T. equi* (Nijhof et al., 2003).

T. taurotragi

In 1960 Martin and Brocklesby described a piroplasm species in common eland (*Tragelaphus taurotragi*). Martin and Brocklesby (1960) concluded that the parasite belongs to the genus *Theileria* and should therefore be called *T. taurotragi*. In an unpublished work, Young and Martens could prove that *T. taurotragi* was transmitted, under natural conditions, to cattle (Grootenhuis and Young, 1980). It is now known that cattle are suitable hosts for this parasite (Lawrence et al., 1994c) in which it usually causes mild symptoms (Grootenhuis et al., 1980). However, it can occasionally be responsible for cerebral theileriosis in cattle (De Vos and Roos, 1981a). In common eland the effects of the parasite are more virulent, since it causes severe diseases and even death. This host species is considered as the main wild reservoir (Grootenhuis et al., 1980). *T. taurotragi* has probably a wide geographic distribution (Lawrence et al., 1994c). Indeed, it occurs in South Africa (Martin and Brocklesby, 1960), in Kenya (Grootenhuis et al., 1980), in Botswana (Binta et al., 1998) and in Uganda (Muhanguzi et al., 2010). The proven tick vectors of *T. taurotragi* are *R. e. evertsi*, *R. appendiculatus*, *R. pulchelus* and *R. zambeziensis* (Lawrence et al., 1994c). Binta et al. (1998)

observed several tick species on cattle infected with *T. taurotragi* and *T. mutans*, including other species than the established vectors like *A. hebraeum, H. truncatum* and *H. m. rufipes*. These authors suggested that these species should be investigated for their ability to transmit *T. mutans* and *T. taurotragi*. Interestingly, Binta et al. (1998) observed high morbidity in cattle, probably due to the dual infections of *T. taurotragi* and *T. mutans*. It seems that the species (*T. taurotragi*) includes different strains adapted to different hosts (cattle and common eland) (Uilenberg et al., 1977; Grootenhuis et al., 1980; Mans et al., 2011).

Theileria **species principally recorded from cattle**

T. annulata

T. annulata the agent of tropical theileriosis (Robinson, 1982), is highly pathogenic (Spitalska et al., 2005) and it is widely distributed throughout a belt of tropical and subtropical areas (Dolan, 1989). It occurs in Europe where it is found in Portugal, Spain as well as in south-east Europe from where it sprawls into the Near East and Middle East, throughout southern Russia and across India and China and the Far East (Dolan, 1989). It is as well established in North Africa and more southwards in Sudan and Eritrea. *T. annulata* was never reported from southern Africa (Pipano, 1994). Due to its wide distribution, 250 million cattle are estimated to be at risk (Bishop et al., 2004). According to these authors the original hosts of this parasite are Asiatic buffalos, which together with cattle maintain the pathogen in nature (Pipano, 1994). This pathogen and its associated disease are known for more than a century since it was first described in 1904 by Dschunkowsky and Luhs in the Caucasus in cattle (Pipano, 1994). *T. annulata* is mainly transmitted by ticks of the genus *Hyalomma*, among which 15 species were incriminated as vectors, among those the most important species are *H. anatolicum, H. detritum, H. dromedarii, H. excavatum* and *H. scupense* (Robinson, 1982). More recently, it was shown by Kok et al. (1993) that *H. m. marginatum* is able to transmit the parasite and that *R. bursa* harbours it in Spain (Fernandez et al., 2006), but according to theses authors *H. lusitanicum* is the main vector in this country. Furthermore, *Dermacentor marginatus* was suspected to transmit the parasite by Habela et al. (1993) and Jacquiet et al. (1994) suspected *R. e. evertsi* to be involved in its epidemiology in Mauritania. The latter authors observed a correlation between high *T. annulata* seroprevalence and predominance of *R. e. evertsi*. This hypothesis was tested by d'Oliveira et al. (1997) but transmission of *T. annulata* by *R. e. evertsi* could not been proven, though it was demonstrated for *H. m. rufipes*.

T. buffeli

T. buffeli is part of the *T. orientalis/sergenti/buffeli* group consisting mainly in benign bovine pathogens (Kamau et al., 2011). However, these authors observed severe symptoms in Australian cattle due to this parasite and hypothesized that a mutation might have enhanced the pathogenicity of the pathogen. The geographic distribution of *T. buffeli* includes Europe, Asia (Georges et al., 2001), Australia (Kamau et al., 2011), North America (Cossio-Bayugar et al., 2002) and parts of Africa (Allsopp et al., 1999). This vast distribution suggests a broad vector range. Indeed, ticks of the genera *Haemaphysalis, Dermacentor* and *Amblyomma* transmit this parasite and additional vectors should exist (Gubbels et al., 2000). According to Gubbels et al. (2000), *T. buffeli* is an Asiatic water buffalo (*Bubalus bubalis*) derived parasite. Thus it must have been introduced into South Africa. One could imagine that this happened through cattle transport. To our knowledge the South African vectors remain unknown to date. In South Africa, *T. buffeli* not only infects cattle, it has found its way into wildlife since it was detected also in African buffalo (Allsopp et al., 1999) and in nyala (*Tragelaphus angasii)* (Pfitzer et al., 2011).

T. mutans

Originally this parasite was described by Theiler (1906) in South Africa, but it might have been confused with *T. taurotragi*, the other benign parasite infecting cattle in South Africa (Stoltsz, 1989). *T. mutans* was defined by Uilenberg (1981) as an African buffalo pathogen infective for cattle with generally low pathogenicity. In an unpublished work from Stoltsz in 1985, *T. mutans* could successfully be transmitted from African buffalo to cattle by inoculation of infected buffalo blood (Stoltsz, 1989). *T. mutans* is widely distributed on the African continent, occurring in eastern, western and southern Africa (Lawrence et al., 1994d) including countries like Botswana (Binta 1998), Zambia (Simuunza et al., 2011) and Uganda (Muhanguzi et al., 2010). However, it seems to occur further North since Gueye et al. (1994) observed it in Senegal. It was also introduced into the Caribbean islands with cattle imports from Africa (Lawrence et al., 1994d). It seems to be mainly transmitted by ticks of the genus

Amblyomma like *A. variegatum, A. cohaerens, A. gemma, A. hebraeum* and *A. astrion* (Lawrence et al., 1994d). Theiler (1909) reported that *R. e. evertsi* and *R. appendiculatus* can transmit this protozoan, since *T. mutans* might have been confused with *T. taurotragi*, further studies aimed to verify Theiler's observations. *R. appendiculatus* and *R. e. evertsi* were investigated as vectors for *T. mutans* by Purnell et al. (1970) and by De Vos and Ross (1981b), but attempts to transmit this parasite through these ticks to cattle failed. Ross and De Vos concluded that no conclusion can be drawn concerning their single transmission attempt. Young et al. (1978) observed that East African strains of *T. mutans* seem more virulent than the South African strains, therefore it seems possible that the species is composed of different genotypes with different levels of pathogenicity. Recently, Mans et al. (2011) confirmed that isolates from Kenya represent a different genotype than isolates from South Africa. Furthermore, these authors showed than some sequences of *T. mutans* are unique to African buffalo.

T. velifera

The first report of *T. velifera* came from Uilenberg (1964) who described it in Madagascar as a benign cattle parasite characterized by veil-like structures which allow an easy identification of this protozoan. Berger (1979) was the first to observe *T. velifera* in South Africa. The parasite does not induce clinical signs in healthy animals while anaemia was observed in splenectomized calves (Uilenberg and Schreuder, 1976). *T. velifera* seems to have a broad geographic distribution including eastern and southern Africa as well as the Caribbean (Lawrence et al., 1994e). Furthermore, it was reported from Uganda (Muhanguzi et al., 2010), Senegal (Gueye et al., 1987a), Nigeria (Folkers and Kuil, 1967), Mali (Uilenberg, 1970) and Ivory Coast (Uilenberg and Schreuder, 1976). According to Stoltsz (1989), *T. velifera* is often seen in mixed theilerial infections. In addition to cattle, the parasite seems to be common in African buffalo since it was observed in blood smears of these hosts in several South African localities (Stoltsz, 1989). Several ticks from the genus *Amblyomma* transmit *T. velifera* including *A. variegatum, A. hebraeum, A. lepidum* and *A. astrion* (Lawrence et al., 1994e). Mans et al. (2011) reported that some genotypes are limited to African buffalo, suggesting that this parasite evolved among wild African buffalo and adapted to cattle.

***Theileria* species principally recorded from small domestic ruminants**

T. separata

T. separata is a benign parasite of small domestic ruminants, it is commonly found in the eastern half of southern Africa within the range of its vector ticks *R. e. evertsi* and *R. e. mimeticus* (Lawrence et al., 1994f). Jansen and Neitz (1956) might have been the first to describe the relation of this pathogen with *R. e. evertsi*. In their original paper the sheep parasite was called *T.* ovis, but according to Uilenberg (1976) *T. separata* is the only benign theilerial species infecting sheep in southern Africa. Therefore, considering Uilenberg's interpretation, Jansen and Neitz (1956) might have confused *T. ovis* with *T. separata*. Conversely, Stoltsz (1989) supported the hypothesis that there might be more than one theilerial parasites infecting sheep in South Africa. Future investigations will have to clarify the situation with the use of molecular tools. Recently, observations showed that *T. separata* also infects wild ruminants, as it was isolated from grey duiker (*Sylvicapra grimmia*) (Nijhof et al., 2005) and from Tsessebe (*Damaliscus lunatus*) (Brothers et al., 2011). Tonetti et al. (2009) obtained evidence that wildlife might be a source of *T. separata* infections for *R. e. evertsi*, the main vector of *T. separata*. Furthermore, Yin et al. (2007) showed that *T. separata* is closely related to the sable antelope parasite *Theileria* sp. (sable). These authors hypothesized that if these two parasites would be transmitted by the same vector tick, it would support the hypothesis that the parasite originated from sable antelope and adapted to domestic sheep. Currently it is established that *R. e. evertsi* transmits *Theileria* sp. (sable) (Steyl et al., 2012).

T. ovis

T. ovis causes ovine theileriosis in small domestic ruminants and the infection is usually subclinical (Altay et al., 2005). As discussed above, the taxonomy of benign theileria in domestic ovine is controversial, therefore it is difficult to account for the early reports of this parasite and to understand its geographic distribution. Except for the controversial report of Jansen and Neitz (1956), no mention is made concerning the occurrence of *T. ovis* in southern Africa. According to more recent reports, *T. ovis* occurs in the Middle East where it was reported in Iran (Heidarpour Bami et al., 2010) and in Turkey (Altay et al., 2005). Other studies report it from central European countries like Spain (Nagore et al., 2004) and Asiatic countries like Southern Korea (Han et al., 2009). It also occurs in northern Africa where it was observed in Ghana (Bell-Sakyi et al., 2004) and in Sudan (Latif, 1977). According to Li et al. (2010) *H. a. anatolicum* transmits *T. ovis*, in addition to *Hyalomma* ticks the non-pathogenic *T. ovis* is transmitted by ticks of the genus *Rhipicephalus* like *R. bursa* (Uilenberg, 1997). To our knowledge *T. ovis* was only reported in small domestic ruminant and no mention is made in the literature that it infects wild ruminants.

***Theileria* species principally recorded from Equids**

T. equi

T. equi, the more virulent agent of equine piroplasmosis, is mainly responsible for the clinical cases of the disease in Southern Africa (De Waal and Heerden, 1994; Knowles, 1996; Katz et al., 2000). It was recently reclassified by Mehlhorn and Schein (1998). Previously it belonged to the genus *Babesia* and was called *B. equi*. Like *B. caballi* it infects horses, donkeys, mules and zebras (Alsaad et al., 2012). In addition it was recently detected in white rhinoceros from the Kruger National Park where it was found in mixed infections with *T. bicornis* (Govender et al., 2011). In contrast to *B. caballi* infections, horses infected with *T. equi* may remain carrier for a life-time (De Waal and Van Heerden, 1994). In Southern Africa, *R. e. evertsi* is the only confirmed vector of *T. equi* and transplacental transmission does occur for this parasite (De Waal and Van Heerden, 1994). The geographic distribution of the disease is described above (see *B. caballi*). The spread of the disease from its endemic areas (tropical and sub-tropical zones) to more temperate areas occurred through global horses transport (Sluyter, 2001). Interestingly, Bhoora et al. (2009) detected different *T. equi*-genotypes from horses and zebra at different South African localities. One genotype is unique to zebra, but to confirm this hypothesis more samples would be needed as mentioned by the authors (Bhoora et al., 2009). If this hypothesis is confirmed it would suggest that zebras might be the original host of this theilerial pathogen.

2.3 Bacterial pathogens

Tick-borne pathogens belonging to the genera *Anaplasma* and *Ehrlichia* are obligate intracellular bacteria infecting vertebrate hosts from various taxa and their life cycles, their morphology, their epidemiology as well as their genome organisation show similarities

(Rymaszewska and Grenda, 2008; Rar and Golovljova, 2011). Both genera consist of gram-negative bacteria living in the cytoplasm of various blood cells (macrophages, neutrophils, monocytes and erythrocytes) or endothelial cells of blood vessels, depending on the species (Rar and Golovljova, 2011). Inside the cell the bacterium is situated in vacuoles which bind to the cell-membrane and both genera appear in two distinct morphological forms: Reticulate cells (vegetative form) and dense-cored cells (infective form) (Ismail et al., 2010). Species of both genera possess comparatively small genomes (1.2-1.5 x 10^6 bp) consisting in one circular chromosome. This small size can be explained by the development of dependence on host cell metabolism for elementary functions (Ismail et al., 2010; Rar and Golovljova, 2011). *Anaplasma* and *Ehrlichia* species are found worldwide in wild and domestic animals causing economically important diseases (e.g. heartwater and gallsickness) inducing considerable losses to the livestock industry, especially in the southern hemisphere (Kawahara et al., 2006; Rymaszewska and Grenda, 2008).

Anaplasma spp. and *Ehrlichia* spp. are classified within the Phylum Proteobacteria, Class Alphaproteobacteria, Order Rickettsiales, both genera are part of the Family Anaplasmatacea and share a common ancestor with the species belonging to the genera *Rickettsia, Neorickettsia, Wolbachia* and *Orientia* (Dumler et al., 2001; Ismail et al., 2010; Rikihisa, 2010). Important reorganisation occurred in both genera thanks to recent improvements in molecular and genetic analysis techniques (Dumler et al., 2001). Some of these reorganisations are of concern for species affecting wild and domestic animals in southern Africa. Dumler et al. (2001) obtained solid evidence that some *Ehrlichia* species (*E. bovis* and *E. platys*) should be placed in the genus *Anaplasma* and that the genus *Ehrlichia* should include *Cowdria ruminantium*. Phylogenetic relationships of *Anaplasma* and *Ehrlichia* species are shown in Appendix C.

2.3.1 Life cycle of *Anaplasma* species

Despite numerous studies aiming to understand the life cycles of *Anaplasma* spp. uncertainties remain (Marcelino et al., 2012) and variations from one species to the other occur like the target host cell types for example. Due to its economic importance *A. marginale* is well studied and therefore represents a good example to illustrate the life cycle of an *Anaplasma* species. Briefly, vector ticks (e.g. *R. e. evertsi*, *R. (B.) decoloratus, R. (B.)*

microplus) ingest infected erythrocytes from a host. Within the tick *A. marginale* develops in the tick midgut where the vegetative form (reticulate cells) undergo their first replication cycle through binary fission, colonies are formed (Figure 6) (Rar and Golovljova, 2011; Marcelino et al., 2012). The reticulate cells are able to change into the dense infective form (dense-cored cells), and can survive outside host cells and invade other tick organs like the salivary glands where the epithelial cells are colonialized. The second replication cycle takes place in the epithelial cells of the salivary glands and from there the infective form enters the salivary gland secretion when the tick infests the next host (Rar and Golovljova, 2011). *A. marginale* is transmitted transstadially (from life stage to life stage), as occurs in all *Anaplasma* spp., while transovarial transmission (from female to eggs) was never observed in any of the studied *Anaplasma* species (Dumler et al., 2001; Rar and Golovljova, 2011; Marcelino et al., 2012). *Anaplasma* spp. can be transmitted by iatrogenic means (i.e. vaccination, castration, collection of blood samples and dehorning) (Marcelino et al., 2012).

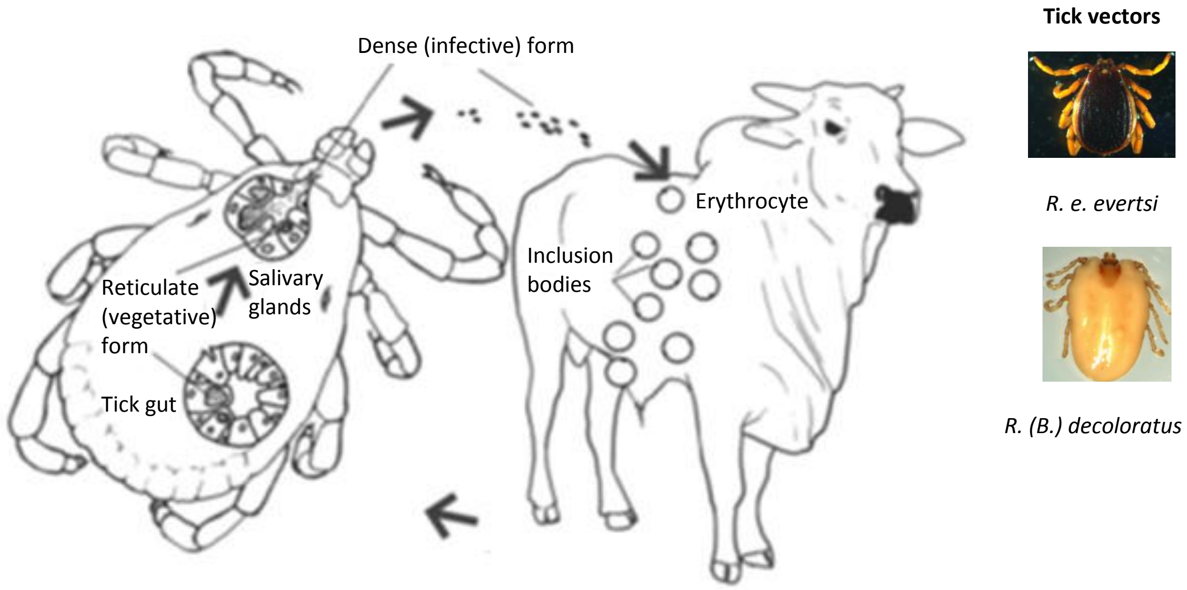

Figure 6: Life cycle of *A. marginale* (Marcelino et al., 2012 modified).

2.3.2 Life cycle of *Ehrlichia* species

The life cycles of *Ehrlichia* spp. display similarities with those of *Anaplasma* spp., nevertheless host cell invasion mechanisms and target host cell types differ. The life cycle of *Ehrlichia* spp. will be illustrated by the well-studied ruminant pathogen, *E. ruminantium*. The part of the life cycle occurring in the endothelial cells of the host is well understood while development in vector ticks (*Amblyomma* spp.) remains unclear (Marcelino et al., 2012). Similar to *Anaplasma* spp., *E. ruminantium* initially undergoes its development in epithelial cells of the tick midgut and invades other tick organs in which it will develop, among which are salivary glands (Figure 7). Hosts become infected through tick saliva injected during the blood meal. In the host the pathogen multiplies in macrophages, neutrophils and vascular endothelial cells by a biphasic development cycle involving two morphological forms here called elementary bodies (EBs) and reticulate bodies (RBs) (Marcelino et al., 2012). Differently to *Anaplasma* spp., the cells are entered by a process resembling to phagocytosis, within the host cells pathogenic cells multiply by binary fission forming a morulae (colonies of RBs), RBs are released by lysis of the host cell. *Amblyomma* ticks become infected with *E. ruminantium* as larvae and nymphs. The infection is acquired transstadially by the next life stages (nymphs and adults) which transmit the infection to the host. Transovarial transmission does most probably not occur in nature, even if it was observed under laboratory conditions (Marcelino et al., 2012).

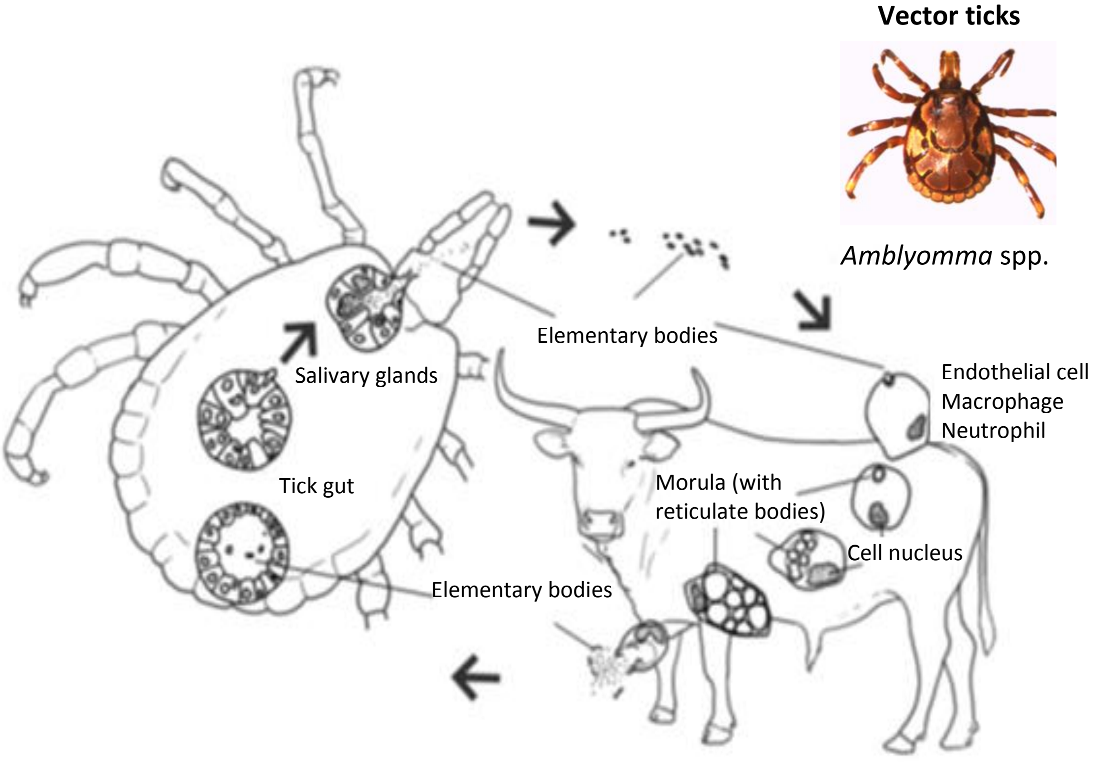

Figure 7: Live cycle of *E. ruminantium* (Marcelino et al., 2012 modified).

2.3.3 Clinical manifestations of *Anaplasma* and *Ehrlichia* species

Clinical signs induced by *Anaplasma* spp. and *Ehrlichia* spp. are a result of target host cell destruction due to pathogen proliferation. The effect of the infection in mammalian hosts varies from inapparent infection to severe illness, morbidity and mortality (Potgieter and Stoltsz, 1994; Scott, 1994). Generally, anaplasmosis symptoms include pyrexia, anaemia and jaundice (Potgieter and Stoltsz, 1994). Animals which recovered from the disease remain, usually, livelong carriers of the bacteria. Clinical signs due to ehrlichiosis are generally characterized by hydropericardium, hydrothorax, hyperaemia, splenomegaly, nephrosis and lung oedema (Scott, 1994).

2.3.4 Rickettsiales species

The genera *Anaplasma* and *Ehrlichia* display much lower species richness than the discussed protozoan pathogens. In southern Africa the main *Anaplasma* and *Ehrlichia* species of concern for domestic and wild ruminants include *A. marginale, A. centrale, A. bovis, A. ovis, E. ruminantium* and *E. ovina* (Potgieter and Stoltsz, 1994; Stoltsz, 1994; Bezuidenhout et al., 1994; Scott, 1994). These species are briefly reviewed as well as the recently described *Ehrlichia* sp. (Omatjenne) (Du Plessis, 1990) and *A. platys* (Chochlakis et al., 2009) detected in ruminants.

***Anaplasma* species principally recorded from cattle**

A. marginale

A. marginale, the etiologic agent of bovine anaplasmosis, also called “gallsickness”, is South Africa’s most widespread, economically important tick-borne infection (Potgieter and Stoltsz, 1994). This bacterium was first observed at the beginning of the last century by Theiler who described “marginal points” in cattle erythrocytes (Theiler, 1910). Usually, calves (up to 9 months) are protected by colostral and non-specific immunity, the severity of the infection increases with age (Potgieter and Stoltsz, 1994). This bacterium can be quite easily transmitted mechanically (e.g. through the use of needles), even hematophagous insects were suspected to be involved in its mechanical transmission (Potgieter and Stoltsz, 1994). Nevertheless, Ixodid ticks are the main biological vectors of *A. marginale*. In South Africa, *R.*

(B.) decoloratus is its principal vector, but *R. (B.) microplus, R. e. evertsi, R. simus* and *H. m. rufipes* are also involved in its epidemiology (De Wall, 2000). Game animals have long been suspected to serve as a reservoir for bovine anaplasmosis and several species were shown to be susceptible to *A. marginale* infections including black wildebeest (*Connochaetes gnou*) (Neitz, 1935), blue wildebeest (*C. taurinus*) (Smith et al., 1974), blesbok (*Damaliscus pygarus pygarus*), common duiker (Neitz and Du Toit, 1932), African buffalo (Potgieter, 1979) and nyala (Pfitzer et al., 2011). Furthermore, unidentified *Anaplasma* spp. were detected serologically or observed on blood smears in a wide range of game species (Brocklesby and Vidler, 1965; Löhr et al., 1974; Carmichael and Hobday, 1975). These observations suggest that *A. marginale* is originally a pathogen of wild ruminants (Potgieter and Stoltsz, 1994) which adapted to domestic ruminants.

A. centrale

A. centrale, the other agent of gallsickness induces a much milder form of the diseases (Potgieter and Stoltsz, 1994). It was first observed by Theiler, one year after his description of *A. marginale* in 1910, he found another slightly smaller bacterium located at the periphery of cattle erythrocytes (Theiler, 1911). Theiler observed the lower virulence of *A. centrale* and due to its incomplete cross-immunity to *A. marginale*, he understood that it can be used as a live vaccine against the virulent *A. marginale* (Potgieter and Stoltsz, 1994). Currently, Theiler's isolate is still in use as a live-blood vaccine in South Africa as well as in other countries. It seems that the epidemiology of *A. centrale* is less intensively studied than the epidemiology of *A. marginale*, due to its lower pathogenicity. It is generally admitted that *R. (B.) decoloratus* is the principal vector of *A. centrale* based on the fact that its distribution overlaps with the distribution of this tick species (Bigalke et al., 1976). In addition, the other tick species transmitting *A. marginale* are incriminated to serve as vectors for the closely related *A. centrale* (Rymaszewska and Grenda, 2008; Potgieter and Stoltsz, 1994). *A. centrale* was detected in blesbok (Neitz and Du Toit, 1932) as well as in African buffalo (Brocklesby and Vidler, 1966) and it is possible that some *Anaplasma* spp. detected by Brocklesby and Vidler (1966), Löhr et al. (1974) and Carmichael and Hobday (1975) belonged to this pathogen species. In addition to cattle, *A. centrale* also infects sheep and goats (Potgieter and Van Rensburg, 1987).

A. bovis

The bovine pathogen, *A. bovis* belongs to the Rickettsiales species which had been reclassified by Dumler (2001), before it belonged to the genus *Ehrlichia*. It is generally admitted that *A. bovis* has low pathogenicity, nevertheless severe symptoms and even death occurred in stressed or exotic cattle (Scott, 1994). *A. bovis* was first described by Donatien and Lestoquard (1936) in *Hyalomma* ticks in Iran and one year later it was identified in South Africa by De Kock et al. (1937). This pathogen has a wide distribution in Africa including southern, central and western parts of the continent, it was also reported from the Middle East, India and South America (Scott, 1994). Recently, *A. bovis* was reported in North America (Goethert and Telford, 2003) and Japan (Kawahara et al., 2006; Sashika et al., 2011). In southern Africa, *A. bovis* is transmitted by *R. appendiculatus* and in West Africa by *A. variegatum* (Scott, 1994). Recently, Tonetti et al. (2009) identified *A. bovis* in *R. e. evertsi* infesting a gemsbok (*Oryx gazella gazella*) in South Africa. This finding suggests that *R. e. evertsi* could be an additional vector in southern Africa. Unfortunately, their finding did not allow distinguishing whether the pathogen was associated to the tick itself or to an infected blood meal from the gemsbok. Tonetti et al. (2009) findings also indicated that *A. bovis* could occur in African wild ruminants, indeed, this was confirmed a few years later by the isolation of *A. bovis* from nyala in South Africa (Pfitzer et al., 2011).

Anaplasma species principally recorded from small domestic ruminants

A. ovis

Anaplasmosis in sheep and goats is caused by *A. ovis* and generally it induces mild symptoms but severe illness and even occasional death were observed although rarely in South Africa, where it is considered to have little economic importance (Stoltsz, 1994). It seems that it was first reported in Zimbabwe by Bevan (1912) although the first authentic description of anaplasmosis in another host than cattle is generally attributed to Di Domizio (1919). The name *A. ovis* was given by Lestoquard (1924). *A. ovis* was reported from all South African countries, except Namibia (Stoltsz, 1994). Elsewhere in the world the disease is widely distributed since it occurs in Europe, Asia, North and South America as well as in other parts of Africa. According to Stoltsz (1994) no vector tick has been identified in southern Africa, however *A. ovis* may have common vectors with *A. marginale* (Ryff et al. 1964; Stiller et al.

1989). Elsewhere, experimental transmission of *A. ovis* was successful with *Ornithodoros lahorensis* (Rastegaieff, 1935), *R. bursa* (Rastegaieff, 1933), *R. turanicus* (Splitter et al., 1956) and *D. silvarum* (Rastegaieff, 1937). In addition, the pathogen was detected in *D. andersoni* (Ryff et al., 1964) and *H. plumbeum, H. otophila* (Pipano, 1966), *D. albipictus, D. occidentalis* and *D. variabilis* (Stiller et al., 1989) were suspected to be implicated in its epidemiology. Stoltsz (1994) reported that experimental transmission with *R. e. evertsi* failed. However, Kaufmann (1996) reported that *A. ovis* is transmitted by *R. e. evertsi* in tropical Africa. Wild African ruminants like common eland and blesbok are susceptible to *A. ovis* infections (Enigk, 1942) and Thomas et al. (1982) reported it from sable antelope.

***Anaplasma* species usually recorded from canids**

A. platys

A. platys is essentially known to infect canids in which it is responsible for canine infectious thrombocytopenia (Huang et al., 2005). It is distributed worldwide following the distribution of its vector *R. sanguineus* (Hoskins et al., 1991). Recently, Chochlakis et al. (2009) isolated an *Anaplasma* sp. (GenBank accession number EU090182) from one goat in Cyprus which revealed 100% homology with *A. platys* (GenBank accession number EF139459). This is the first report of this bacterium in a non-canine host and it seems surprising to isolate it from a ruminant. Nevertheless, Inokuma et al. (2005) demonstrated that it is closely related to *A.* sp. (Omatjenne) (GenBank accession number U54806) isolated from sheep in South Africa (Allsopp et al., 1997) and to *A.* sp. BomPastor (GenBank accession number AF318023) isolated from one goat in Mozambique (Bekker et al., 2001). Furthermore, Allsopp and Allsopp (2001) isolated a novel *Ehrlichia* genotype in dogs from South Africa closely related to *E. ruminantium* infecting cattle, sheep and goats.

***Ehrlichia* species principally recorded from cattle**

E. ruminantium

Heartwater is one of the principal causes of stock loss in southern Africa, in terms of economic importance it is only surpassed by East coast fever and trypanosomiasis. It is caused by a single organism: *E. ruminantium* (Bezuidenhout et al., 1994). From an economic point of view heartwater mainly affects cattle but sheep, goats as well as some wild ruminants also suffer from the disease which includes a wide spectrum of symptoms and ends almost invariably with death if not treated adequately. Reports from stock loss, most probably due to heartwater, appeared in the first half of the eighteenth century (Bezuidenhout et al., 1994). Nevertheless, the causative agent was only demonstrated in host tissues and a vector tick in 1925 by Cowdry. *E. ruminantium* is widely distributed on the African continent, it occurs in almost all countries situated southwards of the Sahara desert and on numerous islands: Réunion, Zanzibar, Mauritius, Sao Tomé, Madagascar and Caribbean (Provost and Bezuidenhout, 1987; Flach et al., 1990). *E. ruminantium* is transmitted by ticks belonging to the genus *Amblyomma*, among which *A. variegatum* is the principal vector in West, Central and East Africa while *A. hebraeum* plays the major role in its transmission in southern Africa (Bezuidenhout et al., 1994). Vectors of secondary importance include *A. pomposum, A. lepidum, A. gemma* and *A. cohaerens*. Among wildlife species, African buffalo is a confirmed reservoir of *E. ruminantium* (Allsopp et al., 1999). Game species susceptible to develop clinical signs include springbok (*Antidorcas marsupialis*), black wildebeest, blesbok and eland (Oberem and Bezuidenhout, 1987).

***Ehrlichia* sp. (Omatjenne)**

This fairly recently discovered bacterium was first described by Du Plessis (1990). This author obtained *H. truncatum* ticks originating from a Namibian farm on which cattle were found seropositive for heartwater. Surprisingly, the animals did not manifest any symptoms and no heartwater vectors occurred in the area. Du Plessis (1990) observed that this apparently apathogenic tick-transmitted bacterium is able to cause severe clinical signs (similar to heartwater) in sheep. *Ehrlichia* sp. (Omatjenne) was isolated from one goat in Mozambique (Bekker et al., 2001) as well as from cattle in Uganda (Muhanguzi et al., 2010) and Turkey (Aktas et al., 2011) indicating that this pathogen displays a wide geographic distribution. Aktas et al. (2012) isolated *Ehrlichia* sp. (Omatjenne) from *R. turanicus* ticks infesting cattle in Turkey adding a potential vector. Pfitzer et al. (2011) obtained evidence that *Ehrlichia* sp. (Omatjenne) occurs in African wildlife species since these authors isolated it from nyala in South Africa. Future investigations will have to show whether *Ehrlichia* sp. (Omatjenne) infects a wide range of game animals and if clinical manifestations occur.

Ehrlichia recorded from small domestic ruminants

E. ovina

E. ovina, the agent of ovine ehrlichiosis, is poorly understood (Scott, 1994) and there seems to be a lack of recent data. Therefore, its economic impact in South Africa remains unclear, however, it is known to occur in South African sheep flocks (Scott, 1994). *E. ovina* was incriminated to have caused epidemic outbreaks in sheep more than half a century ago. Schulz (1940) documented an outbreak from a farm in north-eastern Namibia, the farmer claimed important sheep losses lasting for approximately one year while cattle and goats were not affected. Schulz received blood and lung smears, he observed *E. ovina* in the material (1940). Important numbers of *R. (B.) decoloratus* and *H. impressum* were reported from the animals. The sheep were in poor conditions (due to parasitic worms and malnutrition) which certainly contributed to the high losses (Schulz, 1940). Two other outbreaks due to *E. ovina* were previously reported in Algeria and Turkey (Donatien and Lestoquard, 1935, 1936). In addition, *E. ovina* was reported from Iran (Rafyi and Maghami, 1966), Sudan (Neitz, 1968), Sri Lanka (Seneviratna and Jainudeen, 1967), Nigeria (Leeflang and Ilemobade, 1977) and Senegal (Gueye et al., 1987b). Neitz (1956) demonstrated that *R. e. evertsi* transmits *E. ovina* transstadially. *R. bursa* is an additional vector of this bacterium (Stoenner, 1980).

2.4 Tick-borne pathogen circulation between livestock and game animals

Given the great species richness of vertebrate hosts in South Africa, ticks are widespread in this country, where 80 hard tick species, 25 soft tick species and one Nuttalliellidae species have been listed (Horak, 2009). Their numerous associated diseases constitute a considerable challenge to the livestock industry which contributes up to 49% of the South African agricultural output (http://www.info.gov.za/aboutsa/agriculture.htm). Tick-borne diseases also affect game farming which has become an important economic sector in South Africa (http://www.southafrica.info/business/economy/sectors/542547.htm). Furthermore, tick-borne diseases could represent one of the constraints in game management and species conservation.

In South Africa, Babesiosis, Theileriosis, Anaplasmosis and Ehrlichiosis are some of the main tick-borne diseases that threaten animal health. In this country, the great majority of the livestock animals is in contact with wild animals (Purnell, 1980; Young et al., 1988; Peter et al., 1998). The large wildlife populations occurring in nature reserves and game farms as well as the estimated 13.5-millon cattle and the 35.9-million sheep and goats (FAO, 2005) support large tick populations at any time of the year, as well as their pathogens. This leads to a complex eco-epidemiological situation involving pathogens of the genera *Babesia, Theileria, Anaplasma* and *Ehrlichia*, their vectors and numerous wild and domestic animal hosts.

At present, knowledge on the circulation of intracellular tick-borne pathogens of the above mentioned genera between wild and domestic ungulates remains lacunar. To bring more light on these relations it is necessary to look into the evolutionary history uniting these organisms. Wild ungulates and the pathogenic organisms present in their environment evolved together over many millions of years resulting in an evolutionary arms race between them, a phenomenon known as Red Queen Effect (Van Valen, 1973). The acquired reciprocal adaptations between mammalian hosts and pathogenic microorganisms generally resulted in a state of equilibrium between the parasite and host populations (Jongejan and Uilenberg, 2004). This implicates that wild animals act as reservoirs for certain pathogen species (Bigalke, 1994). On the other hand, livestock animals were first established in Africa throughout the Sahara. From there, herded by ancient tribes, they started their way southwards around 4’500 years ago when the Sahara became drier (Du Toit, 2008). It is believed that these herds moved through tsetse-free areas into East and West Africa. The current South African territory was only reached, by fat-tailed sheep herded by the Khoikhoi, about 2000 years ago (Du Toit, 2008). Sanga cattle arrived to the area about 500 years later, herded by Bantu-speaking farmers. These ancestral livestock animals were, to a certain extent, susceptible to indigenous wildlife-associated diseases (Bigalke, 1994). Nevertheless, the exposure of these animals to indigenous African diseases over several thousand years has conferred them certain degrees of resistance to some of these diseases (Kroger, 1981). The early European settlers bartered their first livestock from the Khoikhoi (http://countrystudies.us/south-africa/6.htm) before European *Bos taurus* breeds were imported from Europe from 1706 onwards (Verbeeck, 1971). In contrast to the indigenous livestock animals, the introduced productive European breeds had not experienced exposure to indigenous wildlife-associated tick-borne diseases (among others diseases) and appeared to be highly susceptible (Bigalke, 1994). Therefore, the establishment and the development of the South African livestock industry were heavily constrained by indigenous tick-borne wildlife-associated diseases.

The majority of the indigenous tick-borne pathogens were first discovered through losses induced in livestock animals, and wildlife animals have been thought to be the cause of these outbreaks (Nestel, 1980; Bengis and Erasmus, 1988; Bigalke, 1994). This was the case for the economically most important tick-borne pathogens affecting cattle such as *T. parva parva, T. p. lawrencei, E. ruminantium, B. bigemina* and *A. marginale* causing East coast fever, corridor disease, heartwater, African redwater and gallsickness, respectively (Bigalke, 1994; Uilenberg, 1995). It is now generally accepted that *T. p. parva* has its origin in eastern African buffalo populations (Lawrence et al., 1994b). Likewise, *T. p. lawrencei* causing corridor diseases (also called buffalo diseases) is clearly a buffalo-derived pathogen, it occurs throughout Eastern and Southern Africa in areas where cattle and wild buffalos are in contact (Lawrence et al., 1994b). *E. ruminantium, B. bigemina* and *A. marginale* are recognized as typically African pathogens and obviously had evolved from wild African ruminants (Bigalke, 1994). Similarly, less pathogenic protozoan such as *T. mutans* and *T. velifera* infecting wild and domestic members of the Bovinae most probably evolved among African buffalo (Mans et al., 2011), but were first observed in cattle (Theiler, 1906; Uilenberg, 1964). Similar phenomena seem to occur for ovine and equine pathogens such as *T. separata* and *T. equi*. According to Yin et al. (2007), *T. separata* might have passed from sable antelope to sheep since it is closely related to the sable antelope parasite *Theileria* sp. (sable) and a unique *T. equi*-genotype seems to occur in zebra (Bhoora et al., 2009). Conversely, other pathogens such as *B. bovis*, the causative agent of Asiatic redwater, and certainly the Asiatic water buffalo (*Bubalus bubalis*) derived parasite *T. buffeli* were introduced into South Africa from Asia (Tonnesen et al., 2004; Gubbels et al., 2000).

Wildlife-associated diseases have contributed to a human-wildlife conflict in the areas where livestock and wildlife are in contact (Ferguson and Hanks, 2012). Some of the first attempts to reduce transmission of diseases originating from wildlife, such as killing of game animals, represented an important threat to the wildlife populations and resulted in confrontation with nature conservationists (Nestel, 1980; Bigalke, 1994). Less controversial protection measures arose out of veterinary research. The East coast fever eradication campaign at the beginning of the 20th century is an example (Lawrence et al., 1994a). Other protection measures, like the erection of game-proof veterinary fences took place since the 1950s (Ferguson and Hanks, 2012). Those have the advantage to physically reduce contact between game and livestock animals, but the disadvantage is to fragment the territory and cut-off migratory routes of wildlife populations.

Currently, it is well known that game animals can suffer from tick-borne diseases. In fact, many cases of death of wild animals due to tick-borne diseases are known and well documented (McCully et al., 1970; Basson et al., 1970; Carmichael and Hobday, 1975; Nijhof et al., 2003, 2005). However, most of the fatal cases in game animals are associated with translocations, when naïve animals or pathogens are introduced into new regions (Oosthuizen et al., 2009). Such practices became necessary in game management and nature conservation since the majority of the conservation areas are not connected to each other (Ferguson and Hanks, 2012). Fatal cases linked to translocations were, for example, observed in the highly endangered black rhinoceroses (*Diceros bicornis*) due to babesiosis (*B. bicornis*) (Nijhof et al., 2003). Similarly, fatal cases linked to translocation occurred in roan antelope (*H. equinus*) due to *Theileria* sp. (sable) (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). Some of the pathogens threatening game animals as *T. bicornis, Theileria* sp. (sable) and the common eland parasite *T. taurotragi* also occur, generally asymptotically, in cattle (Muhanguzi et al., 2010, Yusufmia et al., 2010; Lawrence et al., 1994c). This implies that these pathogenic microorganisms can be spread throughout the country through cattle transport and shows that both animal groups represent an infection source for each other.

The tourism industry, in which eco-tourism plays a crucial role has become a key sector of the South African economy and it has a great potential to grow (http://www.southafrica.info/business/economy/sectors/tourism-overview.htm). This implicates that conservation has become increasingly important. South Africa is one of the leading countries in the development of Transfrontier Parks (TP) and Transfrontier Conservation Areas (TFCAs) which aim to connect various former reserves. The Great Limpopo Transfrontier Park (Mozambique, South Africa, and Zimbabwe) and the Kgalagadi Transfrontier Park (Botswana and South Africa) are good examples of such initiatives. TFCAs and TPs are growing in acceptance, 14 are already established throughout Southern Africa which implies the removal of some of the former game-proof fences, leading to a mixed economy which associates wildlife and agriculture (Ferguson and Hanks, 2012). As such, the understanding of tick-borne pathogens (among other diseases) at the interplay of wild and domestic ungulates is crucial and represents a prerequisite for the peaceful co-existence of Africa's unique wildlife populations and livestock animals.

2.5 Objectives

The general goals of this study were (I) to evaluate the exposure of game and livestock animals to tick-borne pathogens in their respective environments and overall, (II) to shed more light on the nature of the interplay existing between the pathogens and their wild and domestic hosts living in close vicinity.

Specific objectives

- Clarify the host range of recently described pathogen species.
- Evaluate the role of common tick species in the transmission of recently described pathogen species.
- Evaluate and compare host infections in an equal number of wild and domestic ungulates living in close vicinity.
- Evaluate and compare tick salivary glands infections of various tick species collected from an equal number of wild and domestic ungulates.
- Describe associations between hosts and pathogen species.
- Examine co-infections of pathogen species in hosts and ticks.

3 Materials and methods

3.1 Study areas

Sampling of ticks and / or host blood was undertaken during three field work sessions: May-July 2009; January-May 2010 and April-June 2011. It was performed at nine different locations in South Africa (Figure 8). Five provincial nature reserves namely Tüssen-Die-Riviere, Willem Pretorius, Sterkfontein, Seekoeivlei and Sandveld were investigated in the Free State Province (1, 2, 3, 4, 5 Figure 8). Private farms located at the surroundings of the Tussen-Die-Riviere, Willem Pretorius and Sandveld nature reserves were also investigated. A private hunting farm and neighbouring cattle farms were investigated near Bethal in the North-west of the Mpumalanga Province (6, Figure 8). Several private game farms and cattle farms were investigated in the Thabazimbi and Lephalale areas in the North-west of the Limpopo Province (7, 8, Figure 8). Finally, two livestock farms were investigate near Pretoria.

Figure 8 : Study areas: 1, Tüssen-Die-Riviere; 2, Willem Pretorius; 3, Sterkfontein; 4, Seekoeivlei; 5, Sandveld; 6, Bethal; 7, Thabazimbi; 8, Lephalale; 9, Pretoria (modified from http://www.phonebookoftheworld.com).

3.1.1 Free State provincial nature reserves and surrounding farms

Tussen-Die-Riviere Nature Reserve: (30°25’-30°35’ South to 26°03’-26°20’ East)

Figure 9: Riverside of the Orange River (VW).

This area was proclaimed as nature reserve in 1972 (Vrahimis, 1991). It is situated in the Southern Free State between the Caledon and the Orange Rivers (Figure 9), 15 km away from the small town Bethulie. The reserve covers about 23 000 ha, its altitude varies between 1150-1480 m above sea level (Watson, 2006). The climate is transitional between the Karoo and the Highveld, cold winters and hot summers with thunderstorms are characteristic for the region. The reserve experiences maximum and minimum temperatures of 38.3°C and -9.3°C respectively, and mean annual precipitations of 441 mm (Vrahimis 1991). Tüssen-Die-Riviere falls under the Nama Karoo Biome (Low and Robelo, 1998). The reserve is characterized by dolomite koppies (Figure 10) mainly occupied by trees of the genera *Olea, Rhus, Celtis* and *Maytenus* (Watson, 2006). Grass plains are found in between the koppies (Figure 10), consisting of species part of the genera *Themeda, Aristida, Cymbopogon, Eragrostis, Chrysocoma, Lesertia, Osteospermum, Salsola* as well as *Nestlera*. The riversides (Figure 9) of the Orange and Caledon rivers, two of South Africa’s main watercourses, represent a good habitat for *Diospyros, Phragmites, Salix, Acacia, Lycium* and *Celtis* species (Watson, 2006). Originally, the reserve was used exclusively for hunting proposes, more recently it turned into a conservation area of great importance for the Southern Free State. It’s management has changed according to its new functions. Nevertheless, hunting is still used as a game management action in the reverse. Tüssen-Die-Riviere harbours 65% of the 101 indigenous mammals of the Free State (Watson, 2006). Indeed, 66 species are recorded in the reserve, among them one species, the impala *(A.*

Figure 10: Grass plains in front of dolomite koppies (VW).

melampus), is not indigenous to the area. Large and medium game species such as greater kudu *(T. strepsiceros)*, common eland *(T. oryx)*, African buffalo *(S. caffer)*, black wildebeest *(C. gnou)*, red hartebeest *(A. buselaphus)*, and springbok *(A. marsupialis)* represent the main host species for adult Ixodida tick species. The reserve harbours between 5500 and 7000 heads of game species at any time of the year.

Private farms located at the surrounding of the Tüssen-Die-Riviere Nature Reserve:

Figure 11: Entry gate of the Fairydale farm (VW).

Four private farms, neighbouring the nature reserve, were investigated namely Brandewynsgat, Carmel, Katfontein and Fairydale (Figure 11). Two farms, Brandewynsgrat and Katfontein, have a common border with the Tüssen-Die-Riviere Nature Reserve. The headquarter of the Brandewynsgrat farm is located a few kilometres away from the Tüssen-Die-Riviere emergency gate on the gravel road towards Aliwal North. The main buildings of the Katfontein farm are situated on the tar road between Smithfield and Bethulie running parallel to the border of the reserve. The Carmel farm is located more or less 20 km away from the Tüssen-Die-Riviere emergency gate on the tar road towards Smithfield. Finally, the land of the Fairydale farm is situated on the north side of the tar road between Smithfield and Bethulie, close to the Katfontein farm, about one km away from the border of the nature reserve. On all four farms cattle and sheep are bred extensively, the main income from the cattle breeding is generated by the sale of the weaners to feeding lots (Figure 12). In addition, a few cattle were sampled directly in the nature reserve, near the emergency gate where the watchman of the gate keeps a small cattle herd.

Figure 12: Bonsmara Weaners ready for sale (VW).

Willem Pretorius Nature Reserve: (28°16’-28°21’ South to 27°07’-27°23’ East)

As many of the Free State Provincial nature reserves, Willem Pretorius does not owe its existence for nature protection proposes, but for the building of a dam (Personnel communication: Frans Marais). In 1960 the building of the Allemanskraal Dam (Figure 10) was accomplished, the surrounding farmland was bought by the State, the acquired land was then used by the Provincial Administration to establish a nature reserve (Winterbach, 1999). The area was finally declared as nature reserve the 18 December 1970 (Jordaan, 2010). Willem Pretorius is 12091 ha in size, about 2648 ha are occupied by the Allemanskraal Dam when its full capacity is reached, the reserve is located in the heart of the Free State between Winburg and Ventersburg. Being part of the Highveld, the altitude of the reserve varies between 1375 and 1510 m above sea level (Winterbach, 1999). The area sustains important temperatures variation from below 0°C in the winter months (May-July) to over 30°C in summer (November-February). Since 1976 rainfall is recorded, resulting in an average of 578 mm per year and most of the precipitations are recorded in summer (Jordaan, 2010). According to Low and Rebelo (1998) the region falls within the Grassland Biome, the reserve offers a wide variety of habitats, Müller (1986) described five main vegetation types. The thornveld and riverbank communities (Figure 13), representing 11.5% of the reserve surface, are mainly situated in the Northern and Eastern parts of the reserve, on the shore of the Allemanskraal Dam, on the Doringberg and surroundings as well as on the riverbanks of the Sand River. Müller (1986) described *Acacia Karroo* as the only characteristic woody plants of these communities, species as *Protasparagus laricinus, Cynodon hirsutus, Panicum maximum, Atriplex semibaccata* and *Urochlora panicoides* are typically found in these communities without being exclusive. The tree and shrub communities of the ridges cover about 10% of the reserve land and these communities are neighbouring the previous

Figure 13: View on the thornveld and riverbank communities between the Doringberg and the Allemanskraal Dam on the Northen part of Willem Pretorius, the grassland communities can be perceived behind the Dam in the South of the reserve (MB).

described communities in the North and Eastern parts of Willem Pretorius. *Rhus burchellii* is the dominant species, the ground layer is characterized by *Cheilanthes hirta, Cheilanthes eckloniana, Clutia pulchella* and *Crassula schimperi* (Müller, 1986). With 70.6% of the terrestrial surface of the reserve, the grassland communities (Figure 13 and 14) are the main vegetation type of Willem Pretorius, mainly occurring in the South. Characteristic species includes *Themeda triandra, Eragrostis curvula, E. chloromelas, E. obtuse, Heteropogon contortus, Cymbopogon plurinodis* and *Hermannia coccocarpa* (Müller, 1986). The hygrophilous communities (Figure 14) only represent 7.5% of the reserve surface, they are found in the vicinity of the Sand River and the Dam in the "vlei" (swamps) and natural drainage areas. *Setaria incrassata* and *Pennisetum sphacelatum* are the most characteristic plants with 96% and 52% of frequency occurrence, respectively (Müller, 1986). With only 0.4%, the rocky outcrops communities are the less represented communities of Willem Pretorius and this vegetation type is found at the exposed rocky outcrops in the fissures of the dolerite rock. Among the characteristic species *Oropetium capense, Euryops empetrifolius, cyperus usitatus, Digitaria eriantha* and *Cheilanthes eckloniana* are found (Müller, 1986). According to Kietzman (1998) 56 mammal species have been identified on the reserve land among those 19 game species which had been reintroduced from other reserves over the years. Primary, known to harbour the biggest black wildebeest *(C. gnou)* herd of the world, big game species such as white rhinoceros *(C. simum),* plain zebra *(E. quagga),* sable antelope *(H. niger)* and African buffalo *(S. caffer)* can be encountered at Willem Pretorius.

Figure 14: Sand River fringed by the hygrophilous communities flowing through the grassland (MB).

Private farm located at the surrounding of Willem Pretorius Nature Reserve:

One farm sharing a common border with the Willem Pretorius game reserve was investigated in the area. It is located about 30 km east from the nature reserve offices. Among other agricultural activities, beef cattle are bred extensively on the farmland, situated mainly on grassland.

Sterkfontein Dam Nature Reserve: (28°24’ South to 29°02’ East)

Figure 15: Cabbage tree in front of the Sterkfontein Dam (MB).

This conservation area lies around South Africa’s third largest dam, this vast expanse of water is called Sterkfontein Dam (Figure 15). The reserve is located in the North-Eastern Free State, close to Harrismith, a few kilometres away from the Drakensberg Escarpment, not far from the famous Golden Gate Highlands National Park. The reserve covers 17770 ha of which 6940 ha are occupied by the Dam (Figure 15). The altitude of the Sterkfontein Dam Nature Reserve varies between 1700 and 2350 m above sea level, its average annual temperature reaches 17°C, occasionally snow is seen in winter (Taylor et al., 2005). The rainfall gradient varies from 720 mm per year to 1400 mm at the Lesotho side of the reserve (http://www.southafrica.net). Sterkfontein is characterised by rugged mountains and endless plains (Figure 16), it falls mainly under the Moist Cool Highveld Grassland (Bredenkamp and van Rooyen, 1998a). On the grass plains (Figure 16, redgrass *(Themeda triandra)* is the dominant species, in pristine condition it can dominate entirely, on the other hand, when overgrazing occurs weeping lovegrass *(Eragrostis curvula)* takes over. Other grasses as broom needlegrass *(Triraphis andropogonoides)*, sawtooth lovegrass *(Eragrostis superba)* and velvet signalgrass *(Brachiaria serrata)* are often encountered. On the mountain slopes

Figure 16: Grass plains at Sterkfontein (MB).

species like the silver sugarbush *(Protea roupelliae roupelliae)*, the highveld protea *(Protea caffra caffra)* and the oldwood *(Leucosidea sericea)* are found (http://www.sa-venues.com). In the mountains, the precipitous ravines are populated with trees like the wild peach *(Kiggelaria africana)*, silky bark *(Maytenus acuminata)*, bush guarri *(Euclea racemosa)* and cabbage tree *(Cussonia* sp.*)* and yellow wood (*Podocarpus* spp.) (Figure 15). Game species such as black wildebeest *(C. gnou)*, red hartebeest *(A. buselaphus),* springbok *(A. marsupialis),* blesbok (*D. p. phillipsi*) and plain zebra *(E. quagga)* are encountered on the plains. In the mountains grey rhebok *(Pelea capreolus)* and mountain reedbuck *(Redunca fluvorufula)* are commonly seen.

Seekoeivlei Nature Reserve: (27°27’-27°41’ South to 29°34’-29°37’ East)

Seekoeivlei Nature Reserve has an outstanding ecological role. It secures a large portion of one of the largest wetlands of the southern African Highveld. The area falls under the Ramsar convention, it was proclaimed as nature reserve in 1978 (Du Preez and Marneweck, 1996). Subsequently, farm land containing large sections of the wetland was purchased in order to expand the reserve in 1993. Presently, the reserve covers 4415 ha of this particular important wetland. This protected area lies in the north-eastern corner of the Free State close to the Mpumalanga border, in the Memel District. Memel is the closest town (500 m SSW). The altitude of the Seekoeivlei Nature Reserve ranges from 1680 to 1700 m above sea level (Du Preez and Marneweck, 1996). The maximum and minimum mean monthly temperatures registered at Memel are 23.5°C and 9.8°C respectively (http://www.worldweatheronline.com). Seekoeivlei lies in the summer rainfall zone, precipitation occurs mainly under the form of thunderstorms between November and March, the average rainfall recorded at Memel is of 732 mm per year. Seekoivlei Nature Reserve consists of a floodplain ecosystem (Figure 17) irrigated by the Klip River, about 220 small oxbow lakes are seasonally flooded (Du Preez and Marneweck, 1996). According to Low and Rebelo (1998) the region falls within the Grassland Biome. The reserve land

Figure 17: Klip River surrounded by floodplains and oxbow lakes. Grassland can be perceived beyond the river (MB).

is occupied by three vegetation communities: Grassland, woodland and thicket as well as hygrophilous communities (Du Preez and Marneweck, 1996). The grassland in the floodplains and the drier bottomland is dominated by the two grass species *Themeda triandra* and *Eragrostis curvula*. The woodland and thicket communities uncounted on the reserve are mainly found along the streams, this vegetation type is characterised by trees of the species *Rhus pyroides* and *Leucosidea sericea*. Finally, the hygrophilous communities are confined to the streambanks and streambeds of the wetland. In general, this vegetation can be seen as a *Eragrostis plana-Agrostis lachnantha* wetland community. Seekoeivlei Nature Reserve mostly owes its important conservation status to the fact that it represents a key habitat for more than a quarter of the Free State's endangered bird species (http://www.places.co.za). Nevertheless, it also plays an important role for the conservation of mammal species. As on most of the Free State provincial reserves the endemic mammal species experienced a dramatic history, most of the game species were driven away by farming practices or hunted to local extinction. Over the years most of the original occurring game species were reintroduced. Actually, species such as the hippopotamus *(Hippopotamus amphibious),* the black wildebeest *(C. gnou),* the red hartebeest *(A. buselaphus),* the African buffalo *(S. caffer),* and the plain zebra *(E. quagga)* can be observed on the reserve.

Sandveld Nature Reserve: (27°37' South to 25°46' East)

Figure 18: Sandveld Nature Reserve harbors a remaining patch of the eastern Kalahari Thornveld (VW).

Named after its mostly sandy soils, the Sandveld Nature Reserve (Figure 18) is situated in the north-western Free State on the border with the North-West Province, between Hoopstad and Bloemhof. It surrounds the Bloemhof Dam which lies at the confluence of the Vaal and Vet Rivers. The area was proclaimed as nature reserve in 1980, it covers 37823 ha of which 22300 ha are covered by the Bloemhof Dam, 15523 ha land surface are available for conservation. Referring to its total size, Sandveld

Nature Reserve is the largest provincial nature reserve of the Free State. The reserve altitude varies from 1231 to 1271 m above sea level (Google Earth). The maximum and minimum average monthly temperatures recorded at Bloemhof are 21.5°C and 7.2°C respectively (http://www.worldweatheronline.com). The area receives an annual average rainfall of 417 mm. Sandveld nature reserve plays a major role in the conservation of a remaining patch of the eastern Kalahari Thornveld (Figure 18), also called Kimberley Thorn Bushveld a vegetation type of the Savannah Biome which projects into the Free State Grassland on the western border of the province (van Rooyen and Bredenkamp, 1998a). This vegetation type is characterized by open savannah, with umbrella thorn *(Acacia tortilis)* and camel thorn *(Acacia erioloba)* as dominant tree species. Untidily individuals of Shepherd's tree *(Boscia albitrunca)* and sweet torn *(Acacia karroo)* are also uncounted in this savannah type. The grass layer is usually well developed, species as redgrass *(Themeda triandra),* common nine-awn grass *(Enneapogon cenchroides)* and Lehmann's lovegrass *(Eragrostis lehmanniana)* are common in this habitat (van Rooyen and Bredenkamp, 1998a). In the central parts of the reserve these vegetation communities are well preserved (http://www.birdlife.org). The remaining parts of the reserve are dominated by open short grassveld with thickets of trees of the genera *Ziziphus, Rhus* and *Acacia*. Areas which were used for cultivation or grazing before the proclamation of the nature reserve are also found. These areas remain open as grassland; some tend to turn into woodland again. Otherwise, open water, shoreline, marsh areas (Figure 19) and a few rocky areas are encountered on the reserve. Game species like the endangered roan antelope *(H. equinus),* sable antelope *(H. niger),* gemsbok *(O. g. gazella),* tsessebe *(Damaliscus lunatus),* blue wildebeest *(C. taurinus),* greater kudu *(T. strepsiceros),* bushbuck *(T. scriptus),* common eland *(T. oryx),* giraffe *(G. camelopardalis),* African buffalo *(S. caffer),* white rhinoceros *(C. simum)* and springbok *(A. marsupialis)* populate the reserve.

Figure 19: Marsh area of the Sandveld Nature Reserve (VW).

Private farms located at the surrounding of the Sandveld Nature Reserve:

Three farms located in the close vicinity of the Sandveld Nature Reserve were investigated to sample cattle and sheep. The majority of the samples were taken at the Groenvlei farm, which shares a common border with the nature reserve. The farm is located on the southern side of the reserve a few kilometres away from Hoopstad. The Rietvlei farm also shares a border with the conservation area. The farm buildings are situated close to the tar road which runs parallel to the reserve fence between Bloemhof and Hoopstad, a few kilometres away from the Sandveld resort. Finally, the Gideon farm lies a few kilometres away from the southern part of the nature reserve in the Hoopstad district. As described previously, livestock animals are bred extensively on these farms, cattle and sheep are pastored on large camps throughout the year.

3.1.2 Farms in the Bethal area, Mpumalanga Province

Bethal (26°26’17.52’’ South 29°27’51.05’’ East) is situated in the north-western Mpumalanga Province, part of the Highveld, it lies at 1647 m above sea level (Google Earth). The area sustains annual average high temperatures of 21.5°C and annual average low temperatures of 8.2°C (http://www.worldweatheronline.com). The area receives in average 636 mm of rain per year, being part of the summer rainfall areas most of the rain falls between November and February. According to Bredenkamp and Van Rooyen (1998b) the region falls under the Moist Clay Highveld Grassland. The predominating soils are very clayey, the characteristic grass species of this vegetation type are three-awn rolling grass *(Artistida bipartita)*, blackseed fingergrass *(Digitaria ternata),* and largeseed setaria *(Setaria nigrirostris)* (Bredenkamp and Van Rooyen, 1998b). Among the prominent dicotyledonous forbs *Berkheya pinnatifida, Salvia repens, Abildgaardia ovata, Anthospermum pumilum* and *Chamaesyce inaequilatera* are typical for this community. These clay soils are rarely ploughed; they are mainly used for grazing by cattle and sheep. None of the Moist Clay Highveld Grassland occurs in official conservation area, well-managed private lands are the only examples of this vegetation type. Three private farms were investigated in this region, one hunting farm and two classic commercial farms where cattle are bred extensively. The private hunting farm, called Hunters Valley, lies approximately 20 km away from Bethal. Its owner keeps various game species on 1000 ha land for commercial hunting propose. The species composition varies over time since the owner also runs a private game capture business and some animals which have been caught are released on the farm. Species as blue wildebeest *(C. taurinus)*, black wildebeest *(C. gnou)* and blesbok *(D. p. phillipsi)* are usually found on the farm. The Bekkersrus farm is located approximately five kilometres away from Hunters Valley, and lies about 25 kilometres away from Bethal. Finally, the Goedgedaght farm lies about 15 km away from Hunters Valley and is situated approximately 30 Km outside of Bethal. The farming practices on these farms, regarding cattle breeding, are the same as described for the Free State farms. The main income generated by the breeding activities is made from the sale of the weaners to feeding lots.

3.1.3 Farms in the Thabazimbi and Lephalale areas, Limpopo Province

Thabazimbi: (24°35’23.90’’ South 27°24’35.25’’ East)
Thabazimbi is located in the south-western Limpopo Province; the altitude of the town is compromised between 950 and 1065 m above sea level (Google Earth). The area experiences mean maximum and mean minimum temperatures of 25°C and 9,5°C respectively, with an average of 540 mm of rainfall per annum (http://www.worldweatheronline.com). The region corresponds to the Savannah Biome, more precisely to the Waterberg Moist Mountain Bushveld, also called Sour Bushveld (Van Rooyen and Bredenkamp, 1998b). The area is surrounded by Mixed Bushveld, some parts of the Thabazimbi district falls within this vegetation type. As indicated by its name, the Sour Bushveld is characterised by an acidic sandy, loamy to gravelly soil. The tree layer of the Sour Bushveld is typically composed by species such as African beechwood *(Faurea saligna)*, common hookthorn *(Acacia caffra)* and red seringa *(Burkea Africana)* (Van Rooyen and Bredenkamp, 1998b). The moderately developed shrub layer is characterized by species as sandpaper raisin *(Grewia flavescens)*, peeling plane *(Ochna pulchra)* and blue guarri *(Euclea crispa)*. Wire grass *(Elionurus muticus)*, common russet grass *(Loudetia simplex)* and broadleaf *(Diheteropogon amplectens)* are usually found in the grass layer. This type of habitat is mainly used for game and cattle farming as well as ecotourism. These communities are fairly well preserved due to the Marekela National Park and the numerous private reserves and game farms. Two private farms were investigated in the Thabazimbi area, one game farm named “Enjanté” and one farm harbouring a small cattle herd called “Inniboss”.

These two farms are separated by approximately 20 kilometres. Game species such as impala *(A. melampus)*, blue wildebeest *(C. taurinus)*, greater kudu *(T. strepsiceros)* and bushbuck *(T. scriptus)* are found on the game farm. Inniboss is more a resort than a farm; nevertheless, its owner keeps a cattle herd on a few hectares.

Lephalale: (23°40’10.15’’ South 27°44’36.87’’ East)

Lephalale lies in the north-western part of the Limpopo Province not far from the Botswana border. The altitude of the town varies from 822 to 861 m above sea level (Google Earth). With an average annual temperature of 21.9°C the region benefits of a mild climate (Illgner, 2004). The area sustains very dry winters; almost the entire annual rainfall occurs in summer, the summer rainfall average ranges from 350 to 500 mm per year (Niemand, 2009). The region is part of the Savannah Biome, more particularly of an ecological type named Sweet Bushveld (Van Rooyen and Bredenkamp, 1998c). The vegetation structure of this ecological type is usually short and shrubby, as dominant species on sandy area trees such as silver clusterleaf *(Terminalia sericea)*, yellow pomegranate *(Rhigozum obovatum)* and umbrella thorn *(Acacia tortilis)* are found (Figure 20). Shallower, drier soils are populated by species such as common corkwood *(Commiphora pyracanthoides)*, wild raisin *(Grewia flava)* and Shepherd’s tree *(Boscia albitrunca),* very dense thickets of blue thorn *(Acacia erubescens)*, black thorn *(Acacia mellifera)* and Chinenes lantern tree *(Dichrostachys cinerea)* are characteristic for the Sweet Bushveld on drier soils (Van Rooyen and Bredenkamp, 1998c). The grass layer includes species like broom grass *(Eragrostis pallens)* and Kalahari sand quick *(Schmidtia pappophoroides)* on sandy areas and guinea grass *(Panicum maximum)* and blue buffalograss *(Cenchrus ciliaris)* on drier soils. In this area numerous game farms are encountered which have taken over cattle farms in the past decades. The samples which were taken in the area stem from six farms: Revierplass, Spreeuwal, Landmanrust, Unico (Figure 20), Grietasvlakte and Ekard’s Bordery, game animals were sampled on the first four farms and cattle on the two last farms. The distances between the farms range from 10 to 90 km. Revierplass, Spreeuwal, Unico, Grietasvlakte and Ekard’s Bordery are located relatively close together with distances ranging from 10 to 40 km, Landmanrust is farther away. Almost all South African game species are found on private game farms of the Limpopo province. Species such as giraffe *(G. camelopardalis)*, plain Zebra

(E. quagga) and white rhinoceros *(C. simum)* are very popular in the ecotourism business, species such as sable antelope *(H. niger)*, greater kudu *(T. strepsiceros)* and African buffalo *(S. caffer)* are sought-after by trophy hunters. The cattle kept extensively on the Grietasvlakte and Ekard's Bordery farms are in small herds, and represent a side income for the owners.

Figure 20: Tree layer in the Sweet Bushveld at Unico (VW).

3.2 Tick and blood sampling on wild and domestic ruminants

3.2.1 Game capture and culling in the Free State Provincial Nature Reserves

The Free State has 17 proclaimed Provincial Nature Reserves among those 14 are actively managed by the Department of Tourism, Environmental and Economic Affairs, Free State Province (DTEEA). The total land surface covered by these reserves represents 201025 ha of which 83921 ha are covered by water. Since no big predators are present on the reserves, game management is necessary. Game surveys are done on an annual basis by the Scientific Research Sub-Directorate of the DTEEA, on each reserve the carrying capacity is monitored by the reserve ecologist. When the carrying capacity for a certain species, on a certain reserve, is exceeded the animals are sold as live animals or as carcasses, at the annual game auction. These animals are removed by the game capture team or culling team of the DTEEA. Sampling on wild ruminants in the Free State was done by joining these teams. The live captures on which we participated were carried out by means of a dart gun and a helicopter.

Once the animal is anesthetised, it is carried by pick-up to a lorry (Figure 21, 22). Before the animal is loaded into the lorry (Figure 21), five to ten minutes are available to withdraw blood and to examine the animal for ticks. Usually only large game species are darted, smaller species are mass captured. The captured animals are kept in holding pens before being released on other reserves or game farms.

Figure 21: Buffalo *(Syncerus caffer)* on its way to the lorry (VW).

Figure 22: Plain zebra *(Equus quagga)* carried to the lorry (MB).

Major culling operations usually occur at night with spotlights, smaller operations are also done at day time. The target species are shot and carried by pick-up to the reserve butchery. Sampling of ticks and blood took place directly on the veld, on the pick-up or at the reserve butchery.

3.2.2 Game capture and hunting in the Mpumalanga and Limpopo Provinces:

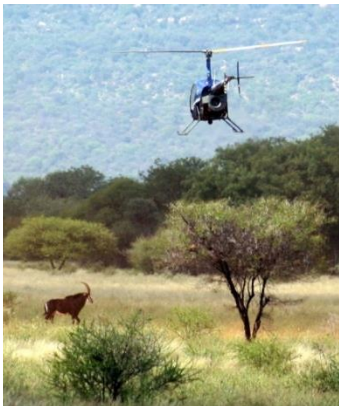

Figure 23: Private game capture team darting a Sable antelope, Lephalale area (VW).

On the game farms of the Bethal and Thabazimbi areas, sampling was done on wild ruminants which were shot in private hunts. The carrying capacity on private game farms are estimated by the owner or by a private ecologist. Game animals are sold to the hunters by the farm owner. Sampling usually took place at the farm butchery. In the Lephalale area all samples were taken with a private game capture team which we followed (Figure 23). Due to the high concentration of game farms in the North of the Limpopo Province many private game capture teams are active in the area. These companies also act as game seller, game animals are bought on farms where the carrying capacity is exceeded for a certain species, the animals are caught and sold to another game farmer. During our sampling campaign animals were darted from a helicopter (Figure 23). Sampling occurred directly after the animal falls down, due to the fact that mainly giraffes *(Giraffa camelopardalis)* were darted, this species was only lightly anesthetised to avoid harming its metabolism (Figure 24, 25).

Figure 24: Tick sampling on giraffe (VW).

Figure 25: Giraffe brought to the van after anaesthesia (VW).

3.2.3 Domestic ruminants

In six out of nine localities where wild ruminants were sampled (Tüssen-Die Riviere, Willem Pretorius, Sandveld, Bethal, Lephalale and Thabazimbi) domestic ruminants were sampled in equal numbers on farms located in the close vicinity of the reserves or game farms. Cattle were driven into kraals, with the agreement and / or help of the farmers, where holding facilities (Figure 26) were available to immobilise the animals. Sheep were brought into smaller camps and immobilised by hand.

3.2.4 Tick and blood collection

Figure 26: Cow in holding facility (DS).

Wild and domestic animals sampled in equal numbers were examined visually for ticks (Figure 26); palpation was used to locate smaller specimens and immature ticks. Ticks were removed with forceps and pooled per host, tick species and life stages in labelled tubes containing 100% alcohol. Ticks were identified to species, life stage and sex according to Matthysse and Colbo (1987), Walker et al. (2000) and Walker et al. (2003) under a stereomicroscope. In addition a microscope was used to count the hypostomal teeth of the *Rhipicephalus (Boophilus)* species. Blood was withdrawn from the jugular or the coccygeal vein for live animals. For hunted animals, blood was collected when the throat was cut. Blood was first placed in 5 ml tubes containing EDTA. Subsequently, 200 µl of blood was placed on FTA Classic Cards (Whatman®, Buckinghamshire, United Kingdom). Tick and blood samples were exported to Switzerland under the following permits: Export permit (354423606) and import permit (HP250.26).

3.3 Tick dissection and DNA extraction of salivary glands and blood samples

3.3.1 Tick dissection

Ticks were dried on filter papers prior to be dissected on microscope slides under a stereomicroscope to isolate the salivary glands. Ticks were maintained on the slides with forceps, the entire dorsal shields (conscutum or alloscutum and scutum) were removed with a regular scalpel with small blades. A drop of PBS (50 mM HK_2o_4P 150 mM NaCl) was placed on the open tick to avoid desiccation and to allow a better view on the organs. Salivary glands were carefully removed with forceps. Special attention was paid to avoid any contamination of the salivary glands by the midgut. The salivary glands were placed in 96 well plates (Milian®, Geneva, Switzerland) and washed in PBS (50 mM HK_2o_4P 150 mM NaCl). As described by Aktas et al. (2009) the instruments used for the dissection were sterilised by immersion in 5 M HCL followed by 5 M NaOH, for a few s each to avoid contamination between the ticks. For the same reasons microscope slides were change after each tick. DNA extraction of the salivary glands occurred directly after dissection to avoid deterioration of the tissues.

3.3.2 DNA extraction of the salivary glands

DNA was extracted from the salivary glands using a commercially available kit specially designed for isolation of genomic DNA from less than 10 mg tissue (QIAamp® DNA Micro Kit, Qiagen, Hombrechtikon, Switzerland). The provided protocol was followed with exception of the first two steps where the tissue lysis buffer and proteinase K solution were added into the 1.5 ml microcentrifuge tubes before the salivary glands. DNA was stored at -20°C in 1.5 ml tubes.

3.3.3 DNA purification of blood samples

Once dried, FTA Classic Cards were stored in dark boxes containing silicagel in order to avoid damages due to the growth of microorganism and sun light. As described by the manufacturers, 1.2 mm ø disc were cut into the cards containing the dried blood using the Harris Micro-Punch® (Whatman®, Buckinghamshire, United Kingdom). Between each sample, the tip of the Harris Micro-Punch® was rinsed in bleach and 70% alcohol and dried with a sterile wipe. Discs were washed three times in 200 µl FTA purification reagent (Whatman®, Buckinghamshire, United Kingdom) and twice in 200 µl homemade TE-1 buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 8.0) in 300 µl microcentrifuge tubes. Discs were dried at 56°C in a stove prior to polymerase chain reaction (PCR) which occurred directly after the DNA purification. The disc was included in the PCR reaction which took place in a reaction volume of 25 µl. The reaction volume was not changed due to the presents of the disc.

3.4 Amplification of tick-borne pathogen DNA

In order to amplify by PCR, a 492-498 base pair (bp) fragment of the 16S ribosomal RNA (rRNA) gene spanning the hypervariable V1 region of *Anaplasma* spp. and *Ehrlichia* spp. primers 16S8FE (Schouls et al., 1999) and B-GA1B-new described by Schouls et al. (1999) and modified by Bekker et al. (2002) were used (Table 1). Primers RLB-F2 and RLB-R2 were used for the amplification of an approximately 400 bp fragment of the 18S rRNA gene belonging to the V4 hypervariable region of *Babesia* spp. and *Theileria* spp. (Georges et al., 2001) (Table 1). Reverse primers of both primer sets were biotin-labelled in view of their utilisation in downstream manipulations. Primers were obtained from Microsynth AG (Balgach, Switzerland).

Table 1: Sequences of primers for the amplification of the genera *Anaplasma / Ehrlichia* and *Babesia / Theileria*.

Primers	**Locus**	**Sequences**
16S8FE	Part of the 16S rRNA gene	5’-GGAATTCAGAGTTGGATCMTGGYTCAG-3’
B-GA1B-new	Part of the 16S rRNA gene	biotin-5’-CGGGATCCCGAGTTTGCCGGGACTTYTTCT-3’
RLB-F2	Part of the 18S rRNA gene	5’-GACACAGGGAGGTAGTGACAAG-3’
RLB-R2	Part of the 18S rRNA gene	biotin-5’-CTAAGAATTTCACCTCTGACAGT-3’

Y = C or T, M = A or C

Amplification reactions were performed in a Master Mix reaction volume of 50 µl for DNA extracted from salivary glands and in 25 µl for reactions on the FTA 1.2 mm ø disc, designed by Bekker et al. (2002) and modified by Tonetti et al. (2009) where the Uracil DNA glycosylase was not used (Table 2).

Table 2: Master Mix composition for one sample to amplify DNA from salivary glands as well as from FTA cards.

Products	**Concentration**	**Volume: Salivary glands**	**Volume: FTA cards**
H_2O Nano pure 0,22 µm*[4]	/	29,75 µl	19,875 µl
PCR Buffer*[1]	10x	5 µl	2,5 µl
Mg Cl_2*[1]	25 mM	2 µl	1 µl
dNTPs*[2]	10 mM	1 µl	0,5 µl
Forward primer*[3]	10 µM	1 µl	0,5 µl
Reverse primer*[3]	10 µM	1 µl	0,5 µl
Taq DNA polymerase*[1]	5 units / µl	0,25 µl	0,125 µl
DNA	100-200 ng / µl	10 µl	1,2 mm ø disc
Total	**/**	**50 µl**	**25 µl**

*[1]Qiagen, Hombrechtikon, Switzerland. *[2]Promega, Madison, USA. *[3]Microsynth, Balgach, Switzerland. *[4]Millipak®, Darmstadt, Germany.

PCR reactions were made in a Whatman Biometra® **T**professional Basic Gradient (Göttingen, Germany) by using a touchdown PCR program described by Bekker et al. (2002), Tonetti et al. (2009) with the modification that the annealing temperature was lowered by 1°C instead of 2°C (Table 3).

Table 3: Touchdown PCR program.

Time	Temperatures	Phase	Number of cycles
3 min	94°C	initial denaturation	
20 s	94°C	denaturation	11 cycles whereby the hybridisation temperature was lowered from 67 to 57°C by steps of 1°C
30 s	67°C	hybridisation	
30 s	72°C	annealing	
20 s	94°C	denaturation	
30 s	57°C	hybridisation	40 cycles
30 s	72°C	annealing	
10 min	72°C	final extension	/

Positive and negative controls were included in each PCR run. The positive control used in the amplification reactions on DNA extracted from salivary glands consisted of DNA from *A. phagocytophilum* obtained from The Centro de Estudos de Vectores e Doenças Infecciosas, Instituto Nacional de Sande, Edificio Lemes, Lisboa, Portugal and from *B. divergens* obtained from The Institut Cantonal de Microbiologie, Bellinzone, Switzerland. Positive control used in amplification reactions on FTA cards were FTA cards containing infected cattle blood from the Ivory Coast with *A. centrale* or *A. marginale* and *B. bovis* (Rühle, 2008). The negative control consisted of Nano pure water for PCR reactions on DNA extracted from salivary glands and of clean 1.2 mm ø FTA discs for PCR reactions on FTA cards.

3.5 Reverse line blot (RLB) Hybridisation

The obtained PCR products derived from tick salivary glands and animal blood were analysed with the use of RLB hybridisation, a molecular technic with multiple applications like the detection of mutations, allele variations and species specific characterisation of prokaryote and eukaryote organisms. In the framework of the present study this last application of RLB hybridisation was used. The RLB technique was originally described by Kaufhold et al. (1994) to identify *Streptococci* serotypes and modified to detect *Ehrlichia* species (Schouls et al., 1999), *Babesia* and *Theileria* species (Gubbels et al., 1999) and finally also *Anaplasma* species (Georges et al., 2001). Subsequently, it was adapted and successfully used by many authors (Nijhof et al., 2005; Oosthuizen et al., 2009; Tonetti et al., 2009; Martins et al., 2010;

Pfitzer et al., 2011; Brothers et al., 2011) to describe tick-borne pathogens in various wild and domestic ruminants, and in some cases also in their associated ticks, in Southern Africa.

Reverse line blotting enables the non-radioactive hybridisation of 45 amplified PCR products with 45 different species-specific oligonucleotide probes. In a first working step the probes are covalently linked to a 6.6 nylon membrane (Biodyne C, Pall Gelman Laboratory) by a 5' terminal aminolinker in a miniblotter 45 apparatus (Immunetics) consisting of 45 parallel running slots. In a second working step, the membrane is rotated by 90° and the PCR products are deposited into the miniblotter, thereby the PCR products are crossing the probes perpendicularly (Figure 27). If the PCR products contains one of target species for which a species-specific oligonucleotide probe is linked to the membrane, hybridisation between the probe and the PCR product takes place (Figures 27, 28). This hybridisation is visualised using chemiluminescence (Figure 28). The biotin label attached to the 5' side of the primers which are hybridised to the probe is subsequently detected by a peroxidase, streptavidin-POD conjugate (Roche Diagnostics, Basel, Switzerland) (Figure 27). The blot is incubated rapidly with the peroxidase substrate, ECL (Amersham Biosciences, Europe, Switzerland), resulting in an oxidation-reduction generating light which can be detected on a film (Hyperfilm, GE Healthcare, UK) (Figure 28). Several washing steps are necessary to remove unbound PCR products.

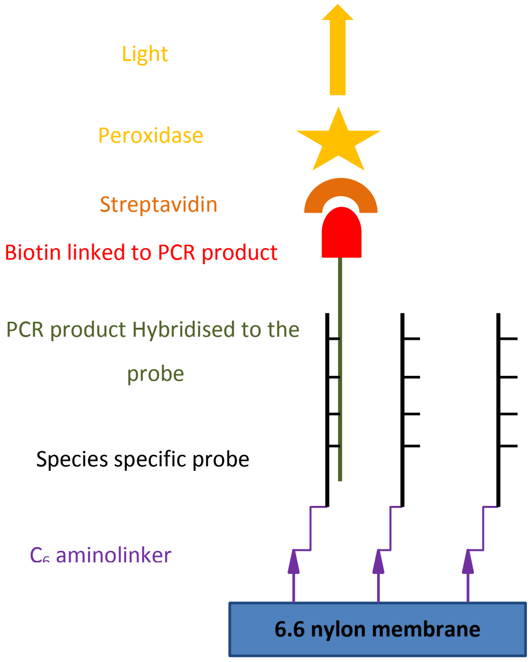

Figure 27: Hybridisation principle used in RLB (Taoufik et al. (2004) modified).

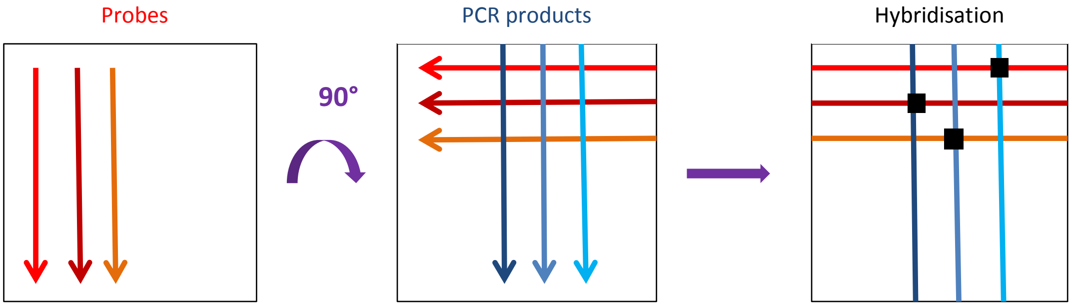

Figure 28: Representation of reverse line blotting: Species-specific probes are covalently linked to the membrane. Subsequently the membrane is rotated 90° and PCR products are deposited perpendicularly to the probes. Hybridisation occurs when one or several PCR products contain a target species for which a specific probe is available (Taoufik et al. (2004) modified).

Forty-one different oligonucleotide probes were used in this study (Table 4). Among those two were genera-specific, namely the Anaplasma / Ehrlichia catch all probe and the Babesia / Theileria catch all probe and another one was genus-specific namely the Theileria spp. probe. Those probes play an important role; they allow identification of non-target species with the use of further molecular analysis. Thirty-eight oligonucleotide probes were species-specific, among those 14 were specific for *Babesia* species, another 14 for *Theileria* species, eight for *Anaplasma* species and finally two for *Ehrlichia* species. Originally, the probes used in this study were the same as in Tonetti et al. (2009). Subsequently, other probes derived from various authors were added to the membrane and all previously obtained positive samples were re-examined with the newly added probes. Various concentrations (50 pmol, 100 pmol, 500 pmol and 800 pmol) were tested for the newly added probes and melting temperatures were empirically computed (http://www.operon.com).

Table 4: Oligonucleotide probes sequences used in this study.

Species	**Probe sequence**	**Reference**
Babesia/Theileria genus-specific	5’-amino-TAATGGTTAATAGGARCRGTTG-3’	Gubbels et al., (1999)
Babesia divergens	5’-amino-GTTAATATTGACTAATGTCGAG-3’	Gubbels et al., (1999)
Babesia bovis	5’-amino-CAGGTTTCGCCTGTATAATTGAG -3’	Gubbels et al., (1999)
Babesia bigemina	5’-amino-CGTTTTTTCCCTTTTGTTGG-3’	Gubbels et al., (1999)
Babesia major	5’-amino-TCCGACTTTGGTTGGTGT-3’	Georges et al., (2001)
Babesia ovis	5’-amino-TGCGCGCGGCCTTTGCGTT-3’	Schnittger et al., (2004)
Babesia bicornis	5’-amino-TTGGTAAATCGCCTTGGT-3’	Nijhof et al., (2003)

Babesia sp. (sable)	5'-amino-GCGTTGACTTTGTGTCTTTAGC-3'	Oosthuizen et al., (2008)
Babesia caballi	5'-amino-GTGTTTATCGCAGACTTTTGT-3'	Butler et al., (2008)
Babesia occultans	5'-amino-GTGTGCCTCTTTTGGCCCATC-3'	Ros-Garcia et al., (2011)
Babesia motasi	5'-amino-ATTGGAGTATTGCGCTTGCTTTTT-3'	Nagore et al., (2004)
Babesia crassa	5'-amino-TTATGGCCCGTTGGCTTAT-3'	Schnittger et al., (2004)
Babesia orientalis	5'-amino-CCTCTTTTGGCCGTCTCACT-3'	Hea et al., (2011)
Babesia gibsoni	5'-amino-CATCCCTCTGGTTAATTTG-3'	From Pfizer (2009)
Babesia rossi	5'-amino-CGGTTTGTTGCCTTTGTG-3'	Matjila et al., (2004)
Theileria genus-specific	5'-amino-GTTGAATTTCTGCTRCATYGC-3'	Nagore et al., (2004)
Theileria parva	5'-amino-TTCGGGGTCTCTGCATGT-3'	Gubbels et al., (1999)
Theileria taurotragi	5'-amino-TCTTGGCACGTGGCTTTT-3'	Gubbels et al., (1999)
Theileria mutans	5'-amino-CTTGCGTCTCCGAATGTT-3'	Gubbels et al., (1999)
Theileria velifera	5'-amino-CCTATTCTCCTTTACGAGT-3'	Gubbels et al., (1999)
Theileria sp. (greater kudu)	5'-amino-CTGCATTGTTTCTTTCCTTTG-3'	Nijhof et al., (2005)
Theileria buffeli	5'-amino-GGCTTATTTCGGWTTGATTTT-3'	Gubbels et al., (1999)
Theileria bicornis	5'-amino-GCGTTGTGGCTTTTTTCTG-3'	Nijhof et al., (2003)
Theileria sp. (sable)	5'-amino-GCTGCATTGCCTTTTCTCC-3'	Nijhof et al., (2005)
Theileria separata	5'-amino-GGTCGTGGTTTTCCTCGT-3'	Schnittger et al., (2004)
Theileria annulata	5'-amino-CCTCTGGGGTCTGTGCA-3'	Georges et al., (2001)
Theileria lestoquardi	5'-amino-ATTGCTTGTGTCCTC-3'	Schnittger et al., (2004)
Theileria ovis	5'-amino-TTGCTTTTGCTCCTTTACGAG-3'	Schnittger et al., (2004)
Theileria sp. (buffalo)	5'-amino-CAGACGGAGTTTACTTTGT-3'	Oura et al., (2004)
Theileria equi	5'-amino-TTCGTTGACTGCGYTTGG-3'	Butler et al., (2008)
Anaplasma/Ehrlichia genus-specific	5'-amino-GGGGGAAAGATTTATCGCTA-3'	Bekker et al., (2002)
Anaplasma ovis	5'-amino-ACCGTACGCGCAGCTTG-3'	Bekker et al., (2002)
Anaplasma centrale	5'-amino-TCGAACGGACCATACGC-3'	Bekker et al., (2002)
Anaplasma marginale	5'-amino-GACCGTATACGCAGCTTG-3'	Bekker et al., (2002)
Anaplasma bovis	5'-amino-GTAGCTTGCTATGAGAACA-3'	Bekker et al., (2002)
Anaplasma phagocytophila	5'-amino-TTGCTATAAAGAATAATTAGTGG-3'	From Pfizer (2009)
Anaplasma phagocytophila	5'-amino-TTGCTAT**G**AAGAATAATTAGTGG-3'	From Pfizer (2009)
Anaplasma phagocytophila	5'-amino-TTGCTATAAAGAATA**G**TTAGTGG-3'	From Pfizer (2009)
Anaplasma phagocytophila	5'-amino-TTGCTATA**G**AGAATAGTTAGTGG-3'	From Pfizer (2009)
Ehrlichia ruminantium	5'-amino-AGTATCTGTTAGTGGCAG-3'	Bekker et al., (2002)
Ehrlichia sp. (Omatjenne)	5'-amino-CGGATTTTTATCATAGCTTGC-3'	Bekker et al., (2002)

R = G or A, K = G or T, Y = T or C, W = A or T

3.6 Gene sequencing

Prior sequencing, PCR products which reacted only with genera-specific oligonucleotide probes (*Anaplasma / Ehrlichia* or *Babesia / Theileria*) and genus-specific probe (*Theileria* spp.) were purified using a commercial available kit (Wizard® SV Gel and PCR Clean-Up System, Promega, Madison, USA). The manufacturer's instructions were followed with the exception of the elution step which took place in 35 µl of Nuclease-Free Water instead of 70 µl in order to increase the DNA concentration. Sequencing was done by Microsynth AG (Balgach, Switzerland). The obtained sequences were corrected and compared between them with the use of a software packages: CLC Sequence Viewer 6 (CLC bio, Aarhus, Denmark) and Bioedit (Tom Hall Ibis Biosciences, Carlsbad). Sequences were compared with available sequences from the international data bank (NCBI BLAST).

3.7 Data analysis

Data was 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).

3.7.1 Pathogens in host blood

Two generalised linear models (GLM) were used on the pair-sample dataset involving wild and domestic animals that had been sampled within each of six localities. In each case, we assessed the significance of the factors LOCATION (six 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 an 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 adapted by Clua et al. (2010) was confined to locations for evaluating whether the adjusted associations between pathogen species in co-infections were significant. In this later case, *P*-value was considered significant when below 0.0004 (Bonferroni corrected).

3.7.2 Pathogens in tick salivary glands

A Generalised Linear Mixed Model (GLMM) with negative binominal errors was used to evaluate tick salivary gland infection (taking into account the four main infected tick species) collected from wild and domestic ruminants originating from six localities. At each locality, the significance of the factors LOCATION and HOST TYPE (i.e. ticks infesting wild *vs* domestic ruminants) as well as the LOCATION: HOST TYPE interaction was assessed, *P*-values were considered significant when below 0.05. In order to compute whether associations between pathogen species in co-infections were significant a permutation test proposed by Raup and Crick (1979), adapted to R by Clua et al. (2010) confined to locations was used. Here, *P*-values were considered significant when below 0.0001 (Bonferroni corrected).

4 Results

4.1 Paper 1

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

M. Berggoetz, M. Schmid, D. Ston, V. Wyss, C. Chevillon, A. M. Pretorius, L. Gern

Transmission of tick-borne pathogens at the wildlife-livestock interface was already a hindrance to cattle and sheep breeding in Africa in the 18th and 19th centuries (Nestel, 1980; Bigalke, 1994). Nevertheless, the first reports of wild ruminants harbouring tick-borne pathogens affecting cattle only appeared in the 1930th (Neitz and Du Toit, 1932; Neitz, 1935). In nineteen seventies the topic was addressed by several studies. Löhr and Meyer (1973) studied the possible interrelationship of intra-erythrocytic pathogens between cattle and antelopes and suggested that transmission of *Anaplasma* organisms from game to cattle might occur in nature. In addition, Löhr et al. (1974) screened the sera of more than 1500 game animals belonging to 19 species for antibodies to *A. marginale, B. bigemina* and *T. parva*. They noticed that wild animals are infected by either the same organisms as cattle or at least by organisms with similar antigenicity. Carmichael and Hobday (1975) reported the presence of piroplasms in eight game species. Young et al. (1977) showed experimentally that the African buffalo act as a reservoir for *T. p. lawrencei*. After these studies, a gap seems to reappear in the literature concerning this topic, until recently. Less than ten years ago, several studies, using molecular tools, reported pathogen species in game animals, which were either new species or were reported in a broader host range than previously known (Nijhof et al., 2003, 2005; Oosthuizen et al., 2008, 2009; Tonetti et al., 2009; Brothers et al., 2011; Pfitzer et al., 2011). Subsequently, some of these pathogens, which were thought to occur in game animals only, were detected in cattle as well (Muhanguzi et al., 2010; Yusufmia et al., 2010). However, none of these studies aimed at comparing the pathogen load in an equal number of wild and domestic animals living in close vicinity, which should allow understanding the nature of the interplay occurring between these animal types. Thus, our study aimed at filling this gap.

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

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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 four 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 30 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 four groups. Three groups were associated to sheep, cattle and horses and their respective wild counterparts. Each group was characterized by high homogeneity in pathogen assemblage and host phylogenetic status. These groups characterized the most privileged transmission routes between and among wild and domestic ungulates. The fourth group showed high heterogeneity in pathogen assemblage and host-phylogenetic status. This group seems to indicate frequent spill over events onto impala of pathogens that usually circulate among cattle- or sheep-related species. Within six 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 equally 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.

Keywords: tick-borne pathogens; African wildlife; Livestock; Co-infections; *Theileria, Babesia, Ehrlichia; Anaplasma.*

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 East coast fever, corridor disease, heartwater, African redwater and gallsickness which are caused by *Theileria parva parva, T. p. lawrencei, 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. p. parva, T. p. lawrencei, 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 spill-over from African sable antelope (*Hippotragus niger*) to sheep. Zebras looked also as the most likely ancestral hosts of *T. equi,* the agent 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 introduction of new pathogens species in South Africa. For instance, the causal agent of Asiatic redwater, *B. bovis* is of Asian origin and has been introduced into South Africa at the beginning 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 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 domestic 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) hybridization to screen blood samples from wild and domestic animals for tick-borne pathogens. In this study we aimed at a) evaluating the tick-borne pathogen load in wild and domestic ungulates living in close vicinity, b) studying associations between host and pathogen species, c) examining co-infection patterns of pathogen species, and d) 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 three fieldwork sessions (May-July 2009; January-May 2010 and April-June 2011) in four South African Provinces (Figure 1). A first dataset of 128 animals corresponds to a paired-sampling of wild and domestic hosts within each of six localities (Figure 1). For this dataset wild animal samples were collected at six localities: Tüssen-Die-Riviere, Willem Pretorius and Sandveld in the Free State Province (three provincial reserves), near Bethal in the Mpumalanga Province (one private game farm) and in the Lephalale and Thabazimbi areas in the Limpopo Province (five 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 six 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 at the same time as animals from the first sampling, and from three additional sites: Sterkfontein, Seekoeivlei in the Free State Province (two nature reserves) and Pretoria in the Gauteng Province (two 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 jugular vein 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 ø discs were punched into FTA card blood spot with the use of the Harris Micro-Punch® (Whatman®, Buckinghamshire, UK). Between each sample the tip of the Harris Micro-Punch® was rinsed in bleach and 70% alcohol and dried with sterile wipe. Discs were washed three 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® **T**professional Basic Gradient (Göttingen, Germany) by using a touchdown PCR program (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 three genus-specific probes Babesia/Theileria, Anaplasma/Ehrlichia and Theileria (Supplementary 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).

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 between them with the use of 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 was 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 six localities. In each case, we assessed the significance of the factors LOCATION (six 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 adapted by Clua et al. (2010) was confined to locations to evaluate whether the adjusted associations between pathogen species in co-infections were significant. In this later case, *P*-value was considered significant when below 0.0004 (Bonferroni corrected).

Results

Blood samples were collected from 181 animals belonging to 18 species (Figure 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 associations between host and pathogen species occurred.

Species identification was achieved by RLB except for seven *Anaplasma* 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, three identical sequences (GenBank accession number KF414721) were 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 infected and 161 infections were detected in these animals (Table 1). The intensity of infection (number of infections / per infected animal) reached 1.94 (161/83) pathogen. Thirty-three infections (20.5%) were identified at genus level only: 25 *Anaplasma* spp. and/or *Ehrlichia* spp., five *Theileria* spp. and three *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 (eight *Anaplasma* spp. and/or *Ehrlichia* spp., five *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). Figure 3a displays the groups of host-species deriving from pathogen assemblage. Figure 3b presents the relative contribution of the pathogen species to the definition of host-groups via pathogen assemblage. Group 1 was the largest host group. It included nine host species (springbok, blue wildebeest, black wildebeest, red hartebeest, blesbok, sable antelope, roan antelope, gemsbok and sheep) that showed relatively higher proportion of infections with *T. bicornis, T. separata* and *Theileria* sp. (sable), and to a lesser degree with *T. ovis* and *A. ovis*. These five pathogen species were strongly correlated with each other, with each of them being significantly correlated with the first axis (*P*-values < 0.001) (Figure 3b). *T. buffeli*, *A. platys* and *A. centrale* were also correlated with the pathogens characterizing Group 1 but were not significantly correlated with the first axis (Figure 3b). In this group, *T. buffeli* (*n* = 17) was detected 11 times, *A. platys* once and *A. centrale* four times out of a total of six 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 (Figure 3a). *T. taurotragi*, *T. velifera*, *T. mutans* and *A. marginale* were exclusively detected in these host species. *B. bigemina* and *B. bovis* were correlated with the other pathogens of Group 2 and with the first axis, although not significantly (*P* = 0.45) (Figure 3b). *B. bigemina* was detected in three out of the four host species constituting the Group 2 while *B. bovis* was detected only once in cattle (Table 1). Horses and zebras formed Group 3; this third group being characterized by the presence of *T. equi* (Figure 3a). Finally, impala and giraffe formed the Group 4 which is interestingly defined by a mix of the pathogen-assemblages defining the Groups 1 and 2 (Figure 3a). Impala was infected with four pathogen species that were abundant in Group 1 (*T. bicornis*, *Theileria* 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.

Co-infections

Among the 163 infections identified at the species level, 127 (77.9%) were co-infections involving more than one pathogen species (Supplementary Table S2). Co-infections were detected in 22.1% (40/181) individual-hosts (26.6%; 17/64 domestic animals and 20%; 23/117 wild animals). Among these co-infections, 22 (17.3%) involved two species, 47 (37%) were triple infections, 33 (26%) involved four pathogen species, and 25 (19.7%) involved five pathogen species. Associations among the 16 pathogen species are shown in Table 3. Significance of co-infections was computed using statistical analysis proposed by Raup and Crick (1979) and adapted by Clua et al. (2010) and confined to locations to decide whether the adjusted associations between pathogen species in co-infections were significant. Six pathogen associations, involving five *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 co-infections were observed in animals belonging to Group 1 as well as in Impala from Group 4.

Discussion

In this study, tick-borne pathogens were detected by RLB hybridisation using probes allowing identification at the genus and 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 personal communication) belonging mainly to *Anaplasma* spp. and *Ehrlichia* spp. for which no probes were available. Nevertheless, sequencing of seven samples allowed species identification: one *A.*

platys, three *A. ovis* and three *A. marginale.* Absence of hybridisation of the last six 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 three species (37%). This corroborates previous observations in cattle in South Africa where most individuals carried co-infections with mainly three species of piroplasms (Yusufmia et al. 2010). Similarly, the majority of the infections observed in nyala by Pfitzer et al. (2011) were co-infections, with a combination of four haemoparasites being the most frequent. In the present study, co-infections with six *Theileria* species, mainly associated to sheep-related host species and to impala, were significant. However co-infections involving pathogens of different genera were also detected (e.g., with *A. marginale* and *B. bigemina*). Yusufmia et al. (2010) reported co-infection involving *T. velifera, Theileria* sp. (sable) and *T. mutans* (but no further details of species associations are available). Pfitzer et al. (2011) observed combinations of *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 infections but more frequently associated to 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 two infections increased pathology and might induce immunodepressive effects, enhancing infection by additional pathogen species. This might explain co-infection with up to four pathogen species, as observed here.

Among the 16 identified pathogen species, nine were *Theileria* species and four (*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. *T. taurotragi* can cause cerebral theileriosis (De Vos et al., 1981), southern African *T. mutans* strains induce a mild form of the disease in contrast to the east African strains (Lawrence et al., 1994c) and no clinical signs are generally recorded for *T. velifera* (Uilenberg and Schreuder, 1976). Other studies detected these three pathogen species in wild Bovids: *T. taurotragi* in common eland (Martin and Brocklesby, 1960), *T. mutans* (Uilenberg, 1981) and *T. velifera* (Stoltsz, 1989) in African buffalo. Finally, *T. equi* the agent of equine piroplasmosis (De Waal and Heerden, 1994) is known to infect horses, donkeys, mules and zebras (Alsaad and Al-Obaidi, 2012) and was detected in horses and zebras in the present study.

Screening a large variety of wild animals, we detected broader host range than reported in the literature. This concerned five *Theileria* species. *Theileria* sp. (sable) was presently the most frequently detected pathogen. It was observed in five new host species (sheep, black wildebeest, springbok, gemsbok and impala). It was also detected in six 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). *T. 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 six 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 five 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 common grey duiker (Nijhof et al., 2005) and in tsessebe (Brothers, 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 five 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 three new host species (blesbok, blue wildebeest and sable antelope). These observations shed new light on the controversy regarding the presence of *T. ovis* in South Africa. Neitz (1957) used the name *T. ovis* Rodhain for the southern African pathogen, but, according to Uilenberg (1976), *T. separata* is the unique *Theileria* species infecting sheep in southern Africa, and the name *T. ovis* Rodhain should only be used for the parasite circulating in Central Africa and Madagascar. We identified *T. separata* and *T. ovis* in South Africa using RLB-probes designed by Schnittger et al. (2004) that do not cross-react. Future studies should confirm the presence of *T. ovis* in South Africa.

Four *Anaplasma* species were identified, namely *A. ovis, A. centrale, A. marginale* and *A. platys* and three were involved in new host-pathogen combinations*. A. 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). *A. 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 one new wild animal species, impala. *Anaplasma* spp*.* antigens were previously reported in 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, 1998). We detected *A. marginale* in cattle, African buffalo, common eland and greater kudu. No previous report in greater kudu was found in the literature, but it was described in the close 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 (Pending GenBank accession number) 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* was closely related to *A.* 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 studies to clarify the situation.

Two *Babesia* species, *B. bovis,* the etiologic agents of Asiatic redwater and *B. bigemina* responsible for African redwater were detected in host blood. *B. bovis* was identified only in one cow that had succumbed to the disease. Its vector, *Rhipicephalus (Boophilus) microplus* (Tonnesen et al., 2004), was observed on this animal (unpublished data). *B. bigemina* was detected in its usual hosts (i.e. cattle and impala), which confirms previous findings (Löhr et al., 1974). However, we also detected *B. bigemina* in one greater kudu. To our knowledge this is the first report in 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 this apparently apathogenic tick-transmitted bacterium is able to cause severe clinical signs (similar to heartwater) in sheep.

To sum-up, among the 16 pathogen species identified in this study, ten 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 recognized in the literature.

PCA showed that the 16 detected pathogen species constituted four different pathogen assemblages corresponding to four groups of host species. *T. separata, T. ovis, A. ovis, T. bicornis* and *Theileria* sp. (sable) were significantly correlated for their host use and defined Group 1. This group corresponds to nine 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 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); supporting thus 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 classical parasite of 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 on-going evolutionary process of this species.

T. 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). *T. taurotragi, T. velifera* and *T. mutans* are known to occur in the 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-Bovinae 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 that is characterized by the presence of *T. equi*. By contrast to the three former groups, the Group 4 is not well characterized. This group is neither characterized by a cohort of pathogen species nor by the phylogenetic relationships of the host species. Group 4 pools together 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 recognized 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 frequent spill over of the pathogens usually associated to 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 remain mainly restricted to genetically-related host-species, except for impala 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 close 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 ones 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 six localities we observed similar ranges in infection prevalence and in intensity of infection between both host types, despite the fact that livestock is protected by acaricide treatments and field management measures. Thus 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 tight, with both animal types consisting of efficient infection sources for ticks infecting themselves and each other. Therefore, 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 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 co-infection patterns, such information 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 congeneric as well as for game animals.

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References

Allsopp, M.T.E.P., Visser, E.S., du Plessis, J.L., Vogel, S.W., Allsopp, B.A., 1997. Different organisms associated with heartwater as shown by analysis of 16S ribosomal RNA gene sequences. Vet. Parasitol. 71, 283-300.

Allsopp, M.T.E.P., Theron, J., Coetzee, M.L., Dunsterville, M.T., Allsopp, B.A., 1999. The occurrence of *Theileria* and *Cowdria* parasites in African buffalo (*Syncerus caffer*) and their associated *Amblyomma hebraeum* ticks. Onderstepoort J. Vet. Res. 66, 245-249.

Alsaad, K.M., Hassan, S.D., Al-Obaidi, Q.T., 2012. Detection of *Babesia equi* and *Babesia caballi* antibodies in horses and donkeys in Mosul, Iraq. Res. Op. Anim. Vet. Sc. 2, 291-294.

Augustyn, N.J., Bigalke, R.D., 1972. *Anaplasma* infection in a giraffe. Onderstepoort J. Vet. Res 39, 29.

Bekker, C.P.J., Vink, D., Lopes Pereira, C.M., Wapenaar, W., Langa, A., Jongejan, F., 2001. Heartwater (*Cowdria ruminantium* infection) as a cause of postrestocking mortality of goats in Mozambique. Clin. Diag. Lab. Immunol. 8, 843-846.

Bekker, C.P.J., de Vos, S., Taoufik, A., Sparagano, O.A.E., Jongejan, F., 2002. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminatium* in *Amblyomma variegatum* ticks by reverse line blot hybridisation. Vet. Microbiol. 89, 223-238.

Bhoora, R., Franssen, L., Oosthuizen, M.C., Guthrie, A.J., Zweygarth, E., Penzhorn, B.L., Jongejan, F., Collins, N.E., 2009. Sequence heterogeneity in the *18S* rRNA gene within *Theileria equi* and *Babesia caballi* from horses in South Africa. Vet. Parasitol. 159, 112-120.

Bigalke, R.D., 1994. The important role of wildlife in the occurrence of livestock diseases in southern Africa. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 152-163.

Binta, M.G., Losho, T., Allsopp, B.A., Mushi, E.Z., 1998. Isolation of *Theileria taurotragi* and *Theileria mutans* from cattle in Botswana. Vet. Parasitol. 77, 83-91.

Brocklesby, D.W., Vidler, B.O., 1966. Haematozoa found in wild members of the order Artiodactyla in West Africa. Bull. Epiz. Dis. Africa 14, 285-299.

Brothers, P.S., Collins, N.E., Oosthuizen, M.C., Bhoora, R., Troskie, M., Penzhorn, B.L., 2011. Occurrence of blood-borne tick-transmitted parasites in common tsessebe (*Damaliscus lunatus*) antelope in Northern Cape Province, South Africa. Vet. Parasitol. 183, 160-165.

Butler, C.M., Nijhof, A.M., Jongejan, F., van der Kolk, J.H., 2008. *Anaplasma phagocytophilum* infection in horses in the Netherlands. Vet. Rec. 162, 216-218.

Carmichael, I.H., Hobday, E., 1975. Blood parasites of some wild Bovidae in Botswana. Onderstepoort J. Vet. Res. 42, 55-62.

Chochlakis, D., Ioannou, I., Sharif, L., Kokkini, S., Hristophi, N., Dimitriou, T., Tselentis, Y., Psaroulaki, A., 2009. Prevalence of *Anaplasma* sp. in goats and sheep in Cyprus. Vector-Borne Zoon. Dis. 9, 457-463.

Clua, E., Buray, N., Legendre, P., Mourier, J., Planes, S., 2010. Behavioural response of sicklefin lemon sharks *Negaprion acutidens* to underwater feeding for ecotourism purposes. Marine Ecol. Prog. Ser. 414, 257-266.

De Vos, A.J., Bessenger, R., Banting, L.F., 1981. *Theileria* ? *taurotragi*: A probable agent of bovine cerebral theileriosis. Onderstepoort J. Vet. Res. 48, 149-153.

De Waal, D.T., van Heerden, J., 1994. Equine babesiosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 295-304.

Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with descriptions of six new species combinations and designation of *Ehrlichia equi* and "HE agent" as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evolut. Microbiol. 51, 2145-2165.

Du Plessis, J.L., 1990. Increased pathogenicity of an *Ehrlichia*-like agent after passage through *Amblyomma hebraeum*: a preliminary report. Onderstepoort J. Vet. Res. 57, 233-237.

Du Toit, D.J., 2008. The indigenous livestock of Southern Africa. http://www.damarasheep.co.za /files/ParisRoundtable.pdf.

Fernandez, M.H., Vrba, E.S., 2005. A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. Biol. Rev. 80, 269-302.

Geleta, A.R., 2005. Antibody response to *Babesia bigemina* and *Babesia bovis* by vaccinated and unvaccinated cattle in an endemic area of South Africa. M. SC. Thesis, University of Pretoria.

Georges, K., Loria, G.R., Riili S., Greco, A., Caracappa, S., Jongejan, F., Sparagano, O., 2001. Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Vet. Parasitol. 99, 273-286.

Govender, D., Oosthuisen, M.C., Penzhorn, B.L., 2011. Piroplasm parasites of white rhinoceroses (*Ceratotherium simum*) in the Kruger National Park, and their relation to anaemia. J. South African Vet. Ass. 82, 36-40.

Gubbels, J.M., de Vos, A.P., van der Weide, M., Viseras, J., Schouls, L.M., de Vries, E., Jongejan, F., 1999. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridisation. J. Clin. Microbiol. 37, 1782-1789.

Gubbels, M.J., Hong, Y., van der Weide, M., Qi, B., Nijman, I.J., Guangyuan, L., Jongejan, F., 2000. Molecular characterisation of the *Theileria buffeli/orientalis* group. Int. J. Parasitol. 30, 943-952.

Hassanin, A., Douzery, E.J.P., 1999. The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome *b* gene. Mol. Phylogen. Evol. 13, 227-243.

Hea, L., Fenga, H.H., Zhanga, W.J., Zhanga, Q.L., Fanga, R., Wanga, L.X., Tua, P., Zhoub, Y.Q., Zhaoa, J.L., Oosthuizend, M.C., 2011. Occurrence of *Theileria* and *Babesia* species in water buffalo (*Bubalus babalis*, Linnaeus, 1758) in the Hubei province, South China. Vet. Parasitol. 170, 323-326.

Huang, H., Unver, A., Perez, M.J., Orellana, N.G., Rikihisa, Y., 2005. Prevalence and molecular analysis of *Anaplasma platys* in dogs in Lara, Venezuela. Brazilian J. Microbiol. 36, 211-216.

Husson, F., Josse, J., Le, S., Mazet, J., 2012. FactoMineR: Multivariate exploratory data analysis and data mining with R. R package version 1.20, http://CRAN.R-project.org/package=FactoMineR

Inokuma, H., Oyamada, M., Kelly, P.J., Itamoto, K., Okuda, M., Brouqui, P., 2005. Molecular detection of a new *Anaplasma* species closely related to *Anaplasma phagocytophilum* in canine blood from South Africa. J. Clin. Microbiol. 43, 2934-2937.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitol 129, 3-14.

Kamau, J., de Vos, A.J., Playford, M., Salim, B., Kinyanjui, P., Sugimoto, C., 2011. Emergence of new types of Theileria orientalis in Australian cattle and possible cause of theileriosis outbreaks. Parasit. Vect. 4:22.

Kingdon, J., 1989. Bovids, horned ungulates. *In* East African mammals: an atlas of evolution in Africa. Vol. III C and D. Kingdon, J., (ed.). University of Chicago Press, Chicago, pp. 1-746.

Lawrence, J.A., de Vos, A.J., Irvin, A.D., 1994a. *Theileria separata* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 340.

Lawrence, J.A., de Vos, A.J., Irvin, A.D., 1994b. *Theileria taurotragi* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 334-335.

Lawrence, J.A., de Vos, A.J., Irvin, A.D., 1994c. *Theileria mutans* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 336-337.

Löhr, K.F., Meyer, H., 1973. Game anaplasmosis: the isolation of *Anaplasma* organisms from Antelope. Zeitschr. Tropenmed. Parasitol. 24, 192-197.

Löhr, K.F., Ross, J.P., Meyer, H., 1974. Detection in game of fluorescent and agglutination antibodies to intraerythrocytic organisms. Zeitschr. Tropenmed. Parasitol. 25, 217-26.

Mans, B.J., Pienaar, R., Latif, A.A., Potgieter, F.T., 2011. Diversity in the *18S SSU* rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. Parasitol. 138, 766-779.

Martin, H., Brocklesby, D.W., 1960. A new parasite of the eland. Vet. Rec. 72, 331-332.

Matjila, P.T., Penzhorn, B.L., Bekker, C.P.J., Nijhof, A.M., Jongejan, F., 2004. Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa. Vet. Parasitol. 122, 119-125.

Matthee, C.A., Davis, S.K., 2001. Molecular insights into the evolution of the family Bovidae: a nuclear DNA perspective. Mol. Biol. Evol. 18, 1220-1230.

Muhanguzi, D., Matovu, E., Waiswa, C., 2010. Prevalence and characterization of *Theileria* and *Babesia* species in cattle under different husbandry systems in Western Uganda. Int. J. An. Vet. Adv. 2, 51-58.

Murray, M.G., 1984. Grazing antelopes. *In* The encyclopaedia of mammals, MacDonald, D.W., (ed.). Facts on File, New York, pp. 560-569.

Nagore, D., Garcia-Sanmartin, J., Garcia-Perez A.L., Juste, R.A., Hurtado A., 2004. Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in sheep population from Northern Spain. Int. J. Parasitol. 34, 1059-1067.

Neitz, W.O., Du Toit, P.J., 1932. Bovine anaplasmosis. A method of obtaining pure strains of *Anaplasma marginale* and *A. centrale* by transmission through antelopes. 18th Report of the Director of Veterinary Services and Animal Industry, Pretoria, Union of South Africa, pp. 3-20.

Neitz, W.O., 1935. Bovine anaplasmosis. The transmission of *Anaplasma marginale* to black wildebeest (*Connochaetes gnou*). Onderstepoort J. Vet. Sc. An. Ind. 5, 9-11.

Neitz, W.O., 1957. Theileriosis, gonderioses and cytauxzoonoses: A review. Onderstepoort J. Vet. Res. 27, 275-430.

Ngeranwa, J.J.N., Venter, E.H., Penzhorn, B.L., Soi, R.K., Mwanzia, J., Nyongesa, 1998. Characterization of *Anaplasma* isolates from eland (*Taurotragus oryx*): Pathogenicity in cattle and sheep and DNA profiles analysis. Vet. Parasitol. 74, 109-122.

Nijhof, A.M., Penzhorn, B.L., Lynen, G., Mollel, J.O., Morkel, P., Bekker, C.P.J., Jongejan, F., 2003. *Babesia bicornis* sp. nov. and *Theileria bicornis* sp. nov.: Tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). J. Clin. Microbiol. 41, 2249-2254.

Nijhof, A.M., Pillary, V., Steyl, J., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., Penzhorn, B.L., Jongejan, F., 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. J. Clin. Microbiol. 43, 5907-5911.

Oosthuizen, M.C., Zweygarth, E., Collins, N.E., Troskie, M., Penzhorn, B.L., 2008. Identification of a novel *Babesia* sp. from a sable antelope (*Hippotragus niger* Harris, 1838). J. Clin. Microbiol. 46, 2247-2251.

Oosthuizen, M.C., Allsopp, B.A., Troskie, M., Collins, N.E., Penzhorn, B.L., 2009. Identification of novel *Babesia* and *Theileria* species in South African giraffe (*Giraffa camelopardalis*, Linnaeus, 1758) and roan antelope (*Hippotragus equinus*, Desmarest 1804). Vet. Parasitol. 163, 39-46.

Oura, C.A.L., Bishop, R.P., Wampande, E.M., Lubega, G.W., Tait, A., 2004. Application of reverse line blot assay to the study of haemoparasites in cattle in Uganda. Int. J. Parasitol. 34, 603-613.

Penzhorn, B.L., 2006. Babesiosis of wild carnivores and ungulates. Vet. Parasitol. 138, 11-21.

Pfitzer, S., 2009. Occurrence of tick-borne haemoparasites in nyala (Tragelaphus angasii) in KwaZulu-Natal and Eastern Cape Province, South Africa. M. SC. Thesis, University of Pretoria.

Pfitzer, S., Oosthuizen, M.C., Bosman, A.M., Vorster, I., Penzhorn, B.L., 2011. Tick-borne parasites in nyala (Tragelaphus angasii, Gray 1849) from KwaZulu-Natal, South Africa. Vet. Parasitol. 176, 126-131.

Potgieter, F.T., 1979. Epizootiology and control of anaplasmosis in South Africa. J. South African Vet. Ass. 50, 367-372.

Potgieter, F.T., Van Rensburg, L.J., 1987. Tick transmission of *Anaplasma centrale*. Onderstepoort J. Vet. Res. 54, 5-7.

Raup, D.M., Crick, R.E., 1979. Measurement of faunal similarity in paleontology. J. Paleontol. 53, 1213-1227.

Ropiquet, A., Hassanin, A., 2005. Molecular phylogeny of caprines (Bovidae, Antilopinae): the question of their origin and diversification during the Miocene. J. Zool. Syst. Evol. Res. 43, 49-60.

Ros-Garcia, A., M'Ghirbi, Y., Bouattour, A., Hurtado, A., 2011. First detection of *Babesia occultans* in *Hyalomma* ticks from Tunisia. Parasitol. 138, 578-582.

Schnittger, L., Yin, H., Qi, B., Gubbels, M.J., Beyer, D., Niemann, S., Jongejan, F., Ahmed, J.S., 2004. Simultaneous detection and differentiation of *Theileria* and *Babesi*a parasites infecting small ruminants by reverse line blotting. Parasitol. Res. 92, 189-196.

Schouls, L.M., Van De Pol, I., Rijpkema S.G.T., Schot C.S., 1999. Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. J Clin. Microbiol. 37, 2215-2222.

Sparagano, O.A.E., de Vos, A.P., Paoletti, B., Camma, C., de Santis, P., Otranto, D., Giangaspero, A., 2003. Molecular detection of *Anaplasma platys* in dogs using polymerase chain reaction and reverse line blot hybridization. J. Vet. Diag. Investig. 15, 527-534.

Spitalska, E., Riddell, M., Heyne, H., Sparagano, O.A.E., 2005. Prevalence of theileriosis in red hartebeest (*Alcelaphus buselaphus caama*) in Namibia. Parasitol. Res. 97, 77-79.

Skaug, H., Fournier, D., Nielsen, A., 2010. Glmm ADMB-package, http: //127.0.0.1:13596/library/glmmADMB/html/glmmADMB-package.html

Steyl, J.C.A., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., 2012. Theileriosis (Cytauxzoonosis) in roan antelope (*Hippotragus equinus*): Field exposure to infection and identification of potential vectors. Onderstepoort J. Vet. Res. 70, 8 pages.

Stoltsz, W.H., 1989. Theileriosis in South Africa: a brief review. Rev. Scient. Tech. Off. Int. Epiz. 8, 93-102.

Stoltsz, W.H., 1994. Ovine and caprine anaplasmosis. In Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 431-438.

Theiler, G. (1962). The Ixodidae parasites of vertebrates in Africa South of the Sahara. Project S9958. Report to the Director of Veterinary Services, Onderstepoort, South Africa, p. 154-159.

Thomas, S.E., Wilson, D.E., Mason, T.E., 1982. *Babesia, Theileria* and *Anaplasma* spp. infecting sable antelope, *Hippotragus niger* (Harris, 1838) in Southern Africa. Onderstepoort J. Vet. Res. 49, 163-166.

Tonetti, N., Berggoetz, M., Rühle, C., Pretorius, A.M., Gern, L., 2009. Ticks and tick-borne pathogens from wildlife in the Free State province, South Africa. J. Wildl. Dis. 45, 437-446.

Tonnesen, M.H., Penzhorn, B.L., Bryson, N.R., Stoltsz, W.H., Masibigiri, T., 2004. Displacement of *Boophilus decoloratus* by *B. microplus* in the Soutpansberg region, Limpopo province, South Africa. Exp. Appl. Acarol. 32, 199-208.

Uilenberg, G., Schreuder, B.E.C., 1976. Studies on Theileriidae (Sporozoa) in Tanzania. 1. Tick transmission of *Haematoxenus veliferus*. Tropenmed. Parasitol. 27, 106-111.

Uilenberg, G., 1976. Tick-borne livestock diseases and their vectors. 2. Epizootiology of tick-borne diseases. World An. Rev. 17, 8-15.

Uilenberg, G., 1981. Theilerial species of domestic livestock. *In* Advances in the control of theileriosis, Irvin, A.D., Cunningham, M.P., Young, A.S. (eds.). The Hague, Nijhoff, M. pp. 4-37.

Uilenberg, G., 1995. International collaborative research: significance of tick-borne haemoparasitic diseases to world animal health. Vet. Parasitol. 57, 19-41.

Vrba, E.S., 1984. Evolutionary pattern and process in the sister-group Alcelaphini-Aepycerotini (Mammalia: Bovidae). *In* Living fossils, Eldredge, N., Stanley, S.M., (eds.). Springer Verlag, New York, pp. 62-79.

Yin, H., Schnittger, L., Luo, J., Seitzer, U., Ahmed, J.S., 2007. Ovine theileriosis in China: a new look at an old story. Parasitol. Res. 101, 191-195.

Yusufmia, S.B.A.S., Collins, N.E., Nkuna, R., Troskie, M., Van den Bossche, P., Penzhorn, B.L., 2010. Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. J. South African Vet. Ass. 81, 45-49.

Legends to Figures

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).

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).

Fig. 3: Principal component analysis (PCA) on Hellinger transformed data: Infections by 16 pathogen species (163 infections) of 181 hosts belonging to 18 species. **a)** Graph of individuals with confidence ellipse around the group barycenter on the main plane. **b)** Correlation circle of pathogen species on the main plane.

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).

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).

Figure 1

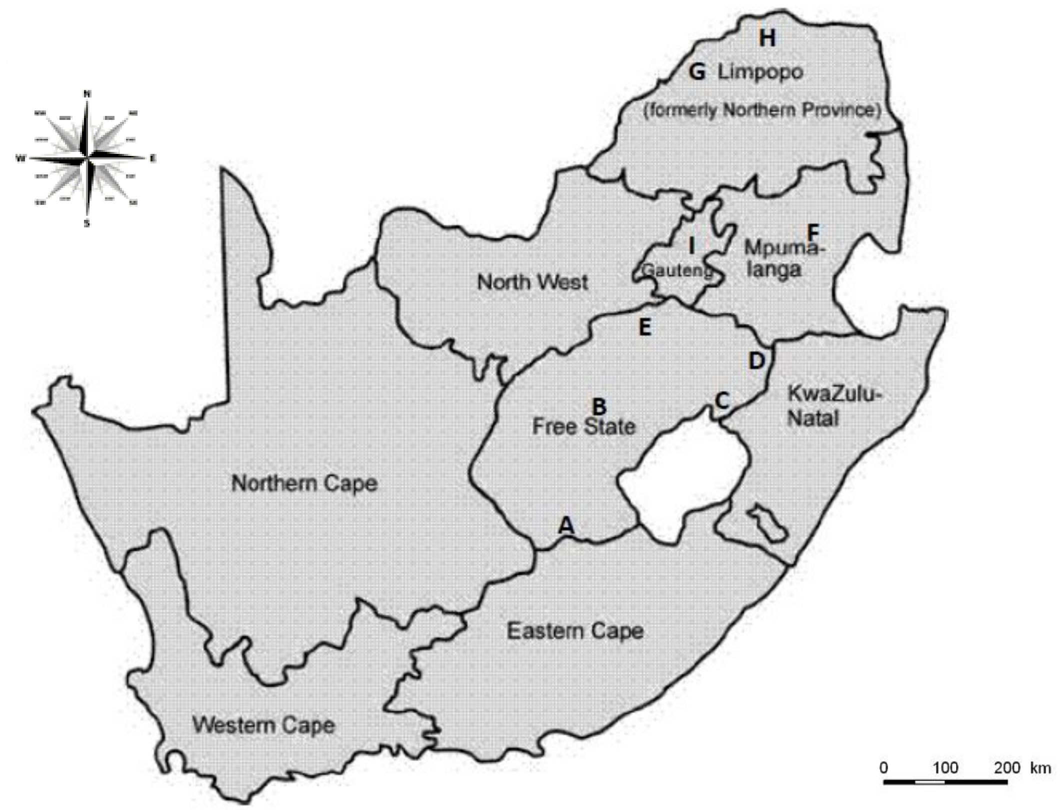
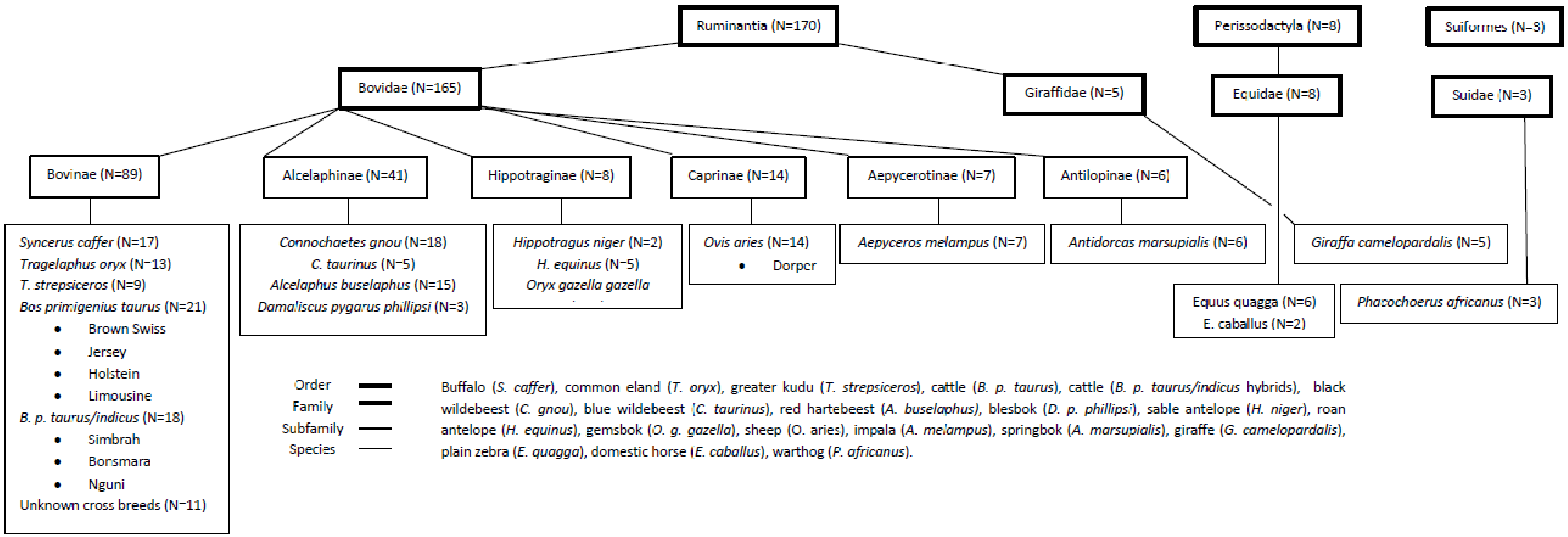

N
W
E
S
H
G Limpopo
(formerly Northern Province)
North West
I
Gauteng
F
Mpuma-
langa
E
D
C
B
Free State
KwaZulu-
Natal
Northern Cape
A
Eastern Cape
Western Cape
0 100 200 km

Figure 2



Buffalo (*S. caffer*), common eland (*T. oryx*), greater kudu (*T. strepsiceros*), cattle (*B. p. taurus*), cattle (*B. p. taurus/indicus* hybrids), black wildebeest (*C. gnou*), blue wildebeest (*C. taurinus*), red hartebeest (*A. buselaphus*), blesbok (*D. p. phillipsi*), sable antelope (*H. niger*), roan antelope (*H. equinus*), gemsbok (*O. g. gazella*), sheep (O. aries), impala (*A. melampus*), springbok (*A. marsupialis*), giraffe (*G. camelopardalis*), plain zebra (*E. quagga*), domestic horse (*E. caballus*), warthog (*P. africanus*).

Figure 3a

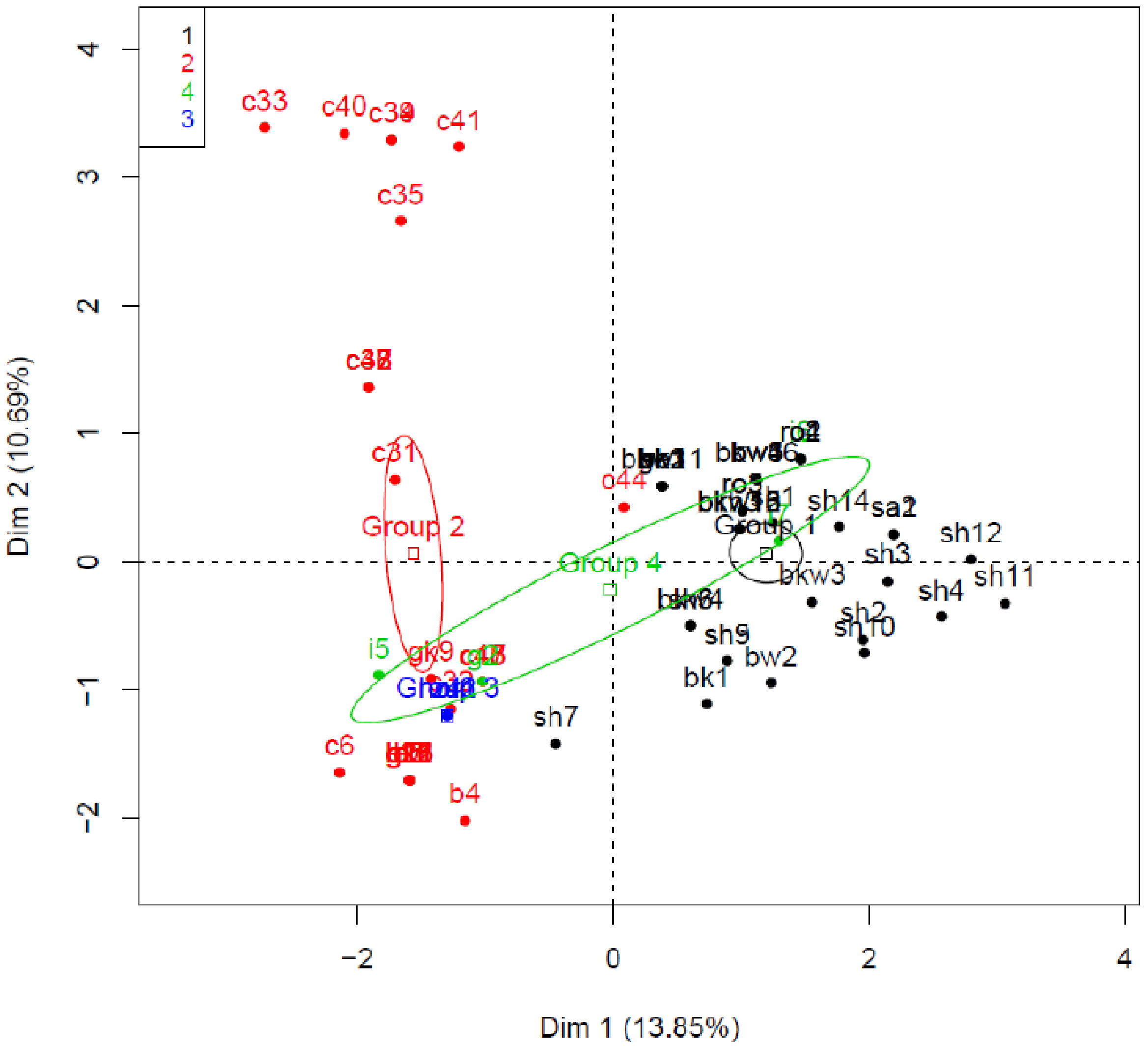

1
2
4
3
c33
c40
c39
c41
c35
c31
Group 2
c44
Group 4
Group 1
Group 3
i5
c6
b4
sh7
sh14
sa2
sh12
sh3
sh11
sh4
bkw3
sh2
sh10
sh9
bw2
bk1
Dim 2 (10.69%)
Dim 1 (13.85%)
4
3
2
1
0
−1
−2
−2
0
2
4

Figure 3b

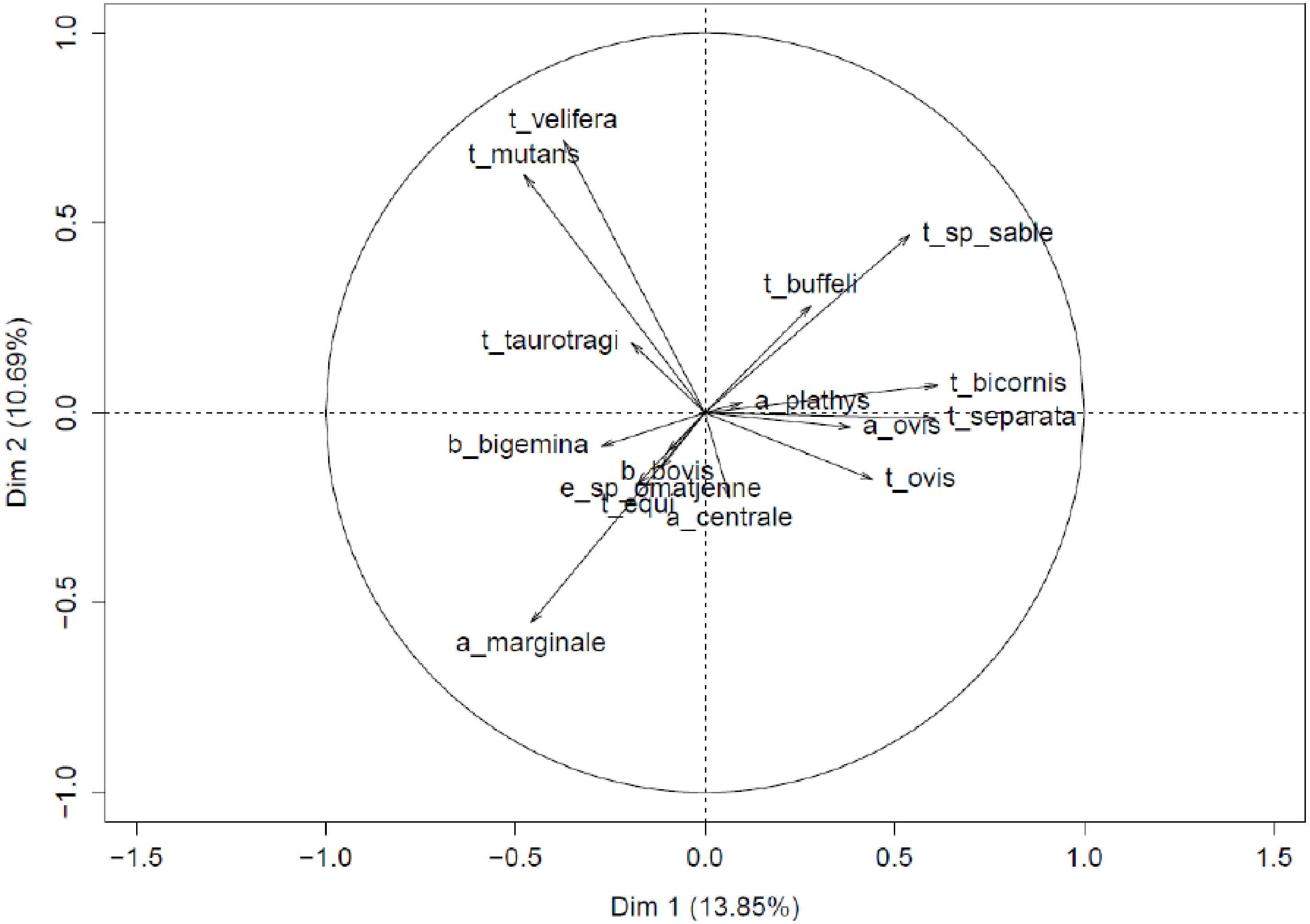

t_velifera
t_mutans
t_sp_sable
t_buffeli
t_taurotragi
a_plathys
t_bicornis
a_ovis
t_separata
b_bigemina
b_bovis
e_sp_omatjenne
t_equi
a_centrale
t_ovis
a_marginale
1.0
0.5
0.0
−0.5
−1.0
Dim 2 (10.69%)
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
Dim 1 (13.85%)

Table 1: Tick-borne pathogens belonging to the genera *Babesia, Theileria, Anaplasma* and *Ehrlichia* in wild (n = 64) and 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

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.

B/T: Includes infections reacting with this probe only, and considered as belonging to the genus *Babesia.*

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. Infection
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**

Locations of the infections identified at species level: a = Tüssen-Die-Riviere; b = Sterkfontein; c = Seekoeivlei, d = Sandveld; e = Pretoria.

B/T: Includes infections reacting with this probe only, and considered as belonging to the genus *Babesia*.

Table 3: Co-infections, constrained to locations, of pathogen species belonging to the genera *Babesia, Theileria, Anaplasma* and *Ehrlichia* involved in co-infections 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. infection/species†
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	0	0	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. infection/species†	39	25	11	16	3	6	1	10	6	2	17	13	4	6	159

*Significant associations (Bonferroni corrected *P* - values < 0.0004) given by the permutation test (Raup and Crick, 1979; modified by Clua et al., 2010).

†Observed among the 181 ruminants.

Supplementary Table S1: Oligonucleotide probes used in this study.

Species	**Probe sequence**	**Reference**
Babesia/Theileria genus-specific	5’-amino-TAATGGTTAATAGGARCRGTTG-3’	Gubbels et al., (1999)
Babesia divergens	5’-amino-GTTAATATTGACTAATGTCGAG-3’	Gubbels et al., (1999)
Babesia bovis	5’-amino-CAGGTTTCGCCTGTATAATTGAG-3’	Gubbels et al., (1999)
Babesia bigemina	5’-amino-CGTTTTTTCCCTTTTGTTGG-3’	Gubbels et al., (1999)
Babesia major	5’-amino-TCCGACTTTGGTTGGTGT-3’	Georges et al., (2001)
Babesia ovis	5’-amino-TGCGCGCGGCCTTTGCGTT-3’	Schnittger et al., (2004)
Babesia bicornis	5’-amino-TTGGTAAATCGCCTTGGT-3’	Nijhof et al., (2003)
Babesia sp. (sable)	5’-amino-GCGTTGACTTTGTGTCTTTAGC-3’	Oosthuizen et al., (2008)
Babesia caballi	5’-amino-GTGTTTATCGCAGACTTTTGT-3’	Butler et al., (2008)
Babesia occultans	5’-amino-GTGTGCCTCTTTTGGCCCATC-3’	Ros-Garcia et al., (2011)
Babesia motasi	5’-amino-ATTGGAGTATTGCGCTTGCTTTTT-3’	Nagore et al., (2004)
Babesia crassa	5’-amino-TTATGGCCCGTTGGCTTAT-3’	Schnittger et al., (2004)
Babesia orientalis	5’-amino-CCTCTTTTGGCCGTCTCACT-3’	Hea et al., (2011)
Babesia gibsoni	5’-amino-CATCCCTCTGGTTAATTTG-3’	From Pfitzer (2009)
Babesia rossi	5’-amino-CGGTTTGTTGCCTTTGTG-3’	Matjila et al., (2004)
Theileria genus-specific	5’-amino-GTTGAATTTCTGCTRCATYGC-3’	Nagore et al., (2004)
Theileria parva	5’-amino-TTCGGGGTCTCTGCATGT-3’	Gubbels et al., (1999)
Theileria taurotragi	5’-amino-TCTTGGCACGTGGCTTTT-3’	Gubbels et al., (1999)
Theileria mutans	5’-amino-CTTGCGTCTCCGAATGTT-3’	Gubbels et al., (1999)
Theileria velifera	5’-amino-CCTATTCTCCTTTACGAGT-3’	Gubbels et al., (1999)
Theileria sp. (greater kudu)	5’-amino-CTGCATTGTTTCTTTCCTTTG-3’	Nijhof et al., (2005)
Theileria buffeli	5’-amino-GGCTTATTTCGGWTTGATTTT-3’	Gubbels et al., (1999)
Theileria bicornis	5’-amino-GCGTTGTGGCTTTTTTCTG-3’	Nijhof et al., (2003)
Theileria sp. (sable)	5’-amino-GCTGCATTGCCTTTTCTCC-3’	Nijhof et al., (2005)
Theileria separata	5’-amino-GGTCGTGGTTTTCCTCGT-3’	Schnittger et al., (2004)
Theileria annulata	5’-amino-CCTCTGGGGTCTGTGCA-3’	Georges et al., (2001)
Theileria lestoquardi	5’-amino-ATTGCTTGTGTCCTC-3’	Schnittger et al., (2004)
Theileria ovis	5’-amino-TTGCTTTTGCTCCTTTACGAG-3’	Schnittger et al., (2004)
Theileria sp. (buffalo)	5’-amino-CAGACGGAGTTTACTTTGT-3’	Oura et al., (2004)
Theileria equi	5’-amino-TTCGTTGACTGCGYTTGG-3’	Butler et al., (2008)
Anaplasma/Ehrlichia genus-specific	5’-amino-GGGGGAAAGATTTATCGCTA-3’	Bekker et al., (2002)
Anaplasma ovis	5’-amino-ACCGTACGCGCAGCTTG-3’	Bekker et al., (2002)
Anaplasma centrale	5’-amino-TCGAACGGACCATACGC-3’	Bekker et al., (2002)
Anaplasma marginale	5’-amino-GACCGTATACGCAGCTTG-3’	Bekker et al., (2002)

Anaplasma bovis	5’-amino-GTAGCTTGCTATGAGAACA-3’	Bekker et al., (2002)
Anaplasma phagocytophilum	5’-amino-TTGCTATAAAGAATAATTAGTGG-3’	From Pfitzer (2009)
Anaplasma phagocytophilum	5’-amino-TTGCTAT**G**AAGAATAATTAGTGG-3’	From Pfitzer (2009)
Anaplasma phagocytophilum a	5’-amino-TTGCTATAAAGAATA**G**TTAGTGG-3’	From Pfitzer (2009)
Anaplasma phagocytophilum	5’-amino-TTGCTATA**G**AGAATAGTTAGTGG-3’	From Pfitzer (2009)
Ehrlichia ruminantium	5’-amino-AGTATCTGTTAGTGGCAG-3’	Bekker et al., (2002)
Ehrlichia sp. (Omatjenne)	5’-amino-CGGATTTTTATCATAGCTTGC-3’	Bekker et al., (2002)

Supplementary Table S2: Panel of co-infections with pathogens belonging to the genera *Babesia, Theileria, Anaplasma* and *Ehrlichia* among 163 infections.

Panel of co-infections/pathogens	*T*. sp. (sable)	*T. separata*	*T. mutans*	*T. velifera*	*T. taurotragi*	*T. buffeli*	*T. bicornis*	*T. ovis*	*T. equi*	*A. ovis*	*A. centrale*	*A. marginale*	*A. platys*	*E.* sp. (Omatjenne)	*B. bigemina*	*B. bovis*	Total	occurrence
1 species	6	3	4	0	1	1	0	1	3	0	1	10	0	4	1	1	36	22.1%
Mixed infections	33	22	6	6	1	16	16	10	0	3	5	3	1	2	3	0	127	77.9%
2 species	6	8	1	1	0	0	1	1	0	0	1	2	0	0	1	0	22	17.3%
3 species	14	7	2	2	0	9	6	2	0	0	2	1	1	0	1	0	47	37%
4 species	8	3	2	2	1	4	5	3	0	2	1	0	0	2	0	0	33	26%
5 species	5	4	1	1	0	3	4	4	0	1	1	0	0	0	1	0	25	19.7%
Total	39	25	10	6	2	17	16	11	3	3	6	13	1	6	4	1	163	100%

4.2 Paper 2

"Protozoan and bacterial pathogens in tick salivary glands in wild and domestic animal environments in South Africa."

M. Berggoetz, M. Schmid, D. Ston, V. Wyss, C. Chevillon, A. M. Pretorius, and L. Gern

In the previous article, tick-borne pathogen infection in wild and domestic ungulates was studied from the perspective of the vertebrate host. In order to better understand the circulation of these pathogens, the infection patterns of ticks collected from 64 wild and 64 domestic animals living in close vicinity, were studied in this second paper. Here, pathogens were detected in tick salivary glands. Infections in salivary glands of ticks originated from a former blood meal taken on a different individual-host than the one on which the ticks were collected (except one-host tick species). Hence, a direct correlation between host infection and tick salivary gland infection cannot be established. By analysing infection in tick salivary glands our aim was to provide information on the exposure of wild and domestic ungulates to tick-borne pathogens. It is important to evaluate the infection patterns in the associated tick species to obtain additional information on the circulation of the target pathogen species in nature. Furthermore, the circulation on tick-borne pathogens between wild and domestic ungulates was rarely studied from the perspectives of the vectors. Thus, in the following manuscript, we aimed at providing information on infection of salivary glands of various tick species living in environments of wild and domestic animals.

Protozoan and bacterial pathogens in tick salivary glands in wild and domestic animal environments in South Africa.

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Submitted to “Ticks and Tick-Borne Diseases”.

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Abstract

A total of 7364 ticks belonging to 13 species were collected from 64 game animals (belonging to 11 species) and 64 livestock animals (cattle and sheep) living in close vicinity at six localities in three South African Provinces (Free State, Mpumalanga and Limpopo). The geographic distribution of all tick species was congruent with the literature except for *Haemaphysalis silacea*. From each infested host, a maximum of 10 males and 10 females of each tick species were dissected to isolate the salivary glands. Salivary glands were screened for tick-borne pathogens using polymerase chain reaction followed by reverse line blotting and sequencing. This approach allowed us to evaluate the exposure of wild and domestic hosts to tick borne pathogens in their respective environments. Among the 2117 examined ticks 329 (15.5%), belonging to eight species, were infected and harboured 397 infections. Among those 57.7% were identified to species level and were assigned to 23 pathogen species of the genera *Babesia, Theileria, Anaplasma* and *Ehrlichia*. In three out of six localities, salivary glands from ticks infesting wild ruminants displayed significantly higher infection prevalence and pathogen mean density than salivary glands from ticks infesting livestock animals. Four piroplasm species (*Theileria bicornis, Babesia* sp. (sable), *Theileria* sp. (giraffe) and *Theileria* sp. (kudu)) were detected for the first time in ticks. The tick species *Rhipicephalus evertsi evertsi, Rhipicephalus (Boophilus) decoloratus, Hyalomma marginatum rufipes, Rhipicephalus appendiculatus,* and *Amblyomma hebraeum* were associated with a broader pathogen range than previously known and thus new vector-pathogen combinations are described. In addition, previously unknown co-infection patterns in tick salivary glands are reported.

Key words: ticks; Tick-borne pathogens; African wildlife; Livestock; Co-infections, *Theileria, Babesia, Ehrlichia, Anaplasma.*

Introduction

Ticks are vectors of a great variety of tick-borne pathogens. In the recent decades, the development of molecular tools increased their number and their variety. The majority of tick-borne pathogen species that circulate between ticks and both game and livestock animals have been reported in the vertebrate hosts they infect and less in the ticks. Indeed, in early studies that aimed to detect tick-borne pathogens in wild African ungulates, animal sera were screened (Neitz and Du Toit 1932; Neitz, 1935; Löhr and Meyer 1973; Löhr et al. 1974; Carmichael and Hobday 1975). More recently, in studies reporting tick-borne pathogens affecting game animals, host blood was examined but not the associated tick species (Nijhof et al., 2003, 2005; Spitalska et al., 2005; Brothers et al., 2011; Oosthuizen et al., 2008, 2009; Pfitzer et al., 2011). In addition, several tick-borne pathogens with known tick vectors display a broader host range than first admitted. An example is *Theileria* sp. (sable) that infects the sable (*Hippotragus niger*) and roan (*H. equinus*) antelope and that have been newly described in red hartebeest (*Alcelaphus buselaphus*) (Spitalska et al., 2005), cattle (Yusufmia et al., 2010) and nyala (*Tragelaphus angassii*) (Pfitzer et al., 2011). For these pathogens, a broader host range could mean that additional unknown vectors associated with a wide host variety might be involved in their transmission. From this point of view the two-host tick *R. e. evertsi,* the three-host ticks *Amblyomma hebraeum* and *R. appendiculatus* as well as the one-host tick *R. (Boophilus) decoloratus* are good candidates. These tick species were recorded from a great variety of host species and are geographically widely distributed in Southern Africa (Walker et al., 2000, 2003).

Therefore, in this study we focused on the presence of *Babesia*, *Theileria*, *Anaplasma* and *Ehrlichia* in the salivary glands of different tick species collected from wild and domestic ruminants in South Africa. Detection of pathogens in salivary glands of ticks allowed us to distinguish pathogens that infected the ticks before they attached to the hosts from those taken up by ticks during their blood meal. Thus, this approach allows evaluate the exposure of wild and domestic ruminants to tick borne pathogens in their respective environments. The presence of a pathogen in salivary glands strongly suggests that it may be transmitted to the host although it is not sufficient to prove the vector role, which would require transmission experiments. The purpose here was a) to evaluate the possible role of ticks in the transmission of recently described pathogen species, b) to verify whether pathogens with broad host range have a broader vector range than currently known, c) to investigate the exposure of game and livestock to tick-borne pathogens in their respective environments and d) to obtain information on co-infections in ticks.

Materials and methods

Study areas

Ticks were collected in 2009 (May to July), 2010, (January to May) and 2011 (April to June) at six localities in three South African Provinces. In the Free State, three provincial nature reserves, Tüssen-Die-Riviere, Willem Pretorius and Sandveld, as well as several livestock farms at their surroundings were investigated (highveld). In the Mpumalanga Province, one game farm and one livestock farm were investigated near Bethal (highveld). Finally, in the Limpopo Province in the Thabazimbi and Lephalale areas (lowveld), five game farms and five livestock farms were investigated.

Tick sampling

At each locality tick samplings were performed on domestic and wild animals within the same week. Ticks were sampled from an equal number of livestock animals living on farms sharing a common border with the reserves or game farms or located in the close surroundings (within the range of 40 km). Ticks were collected from game animals during game capture, culling operations and hunts. Cattle were maintained in holding facilities during sampling and sheep were immobilised by hand in small camps. Hosts were visually examined for ticks, palpation helped to localise specimens attached on flanks, back, belly, neck and legs. The totality of the observed ticks was removed with tweezers. Tick identification was performed according to Matthysse and Colbo (1987), Walker et al. (2000) and Walker et al. (2003). Ticks were pooled per species, host and developmental stage and stocked in alcohol in 50 ml labelled tubes.

Detection and identification of pathogen species in salivary glands

From each individual-host, a maximum of 10 males and 10 females of each tick species was analysed. To distinguish pathogens that infect ticks before they attached to the hosts from those taken up by ticks during their blood meal, each tick was dissected and salivary glands of dissected ticks were analysed for pathogens. Salivary glands were carefully removed with tweezers, special attention was paid to avoid contamination with the midgut, and washed twice in PBS in 96 well plates (Milian®, Geneva, Switzerland). Instruments were sterilised for a few seconds in 5M HCl and 5M NaOH (Aktas et al., 2009) and dried with sterile wipes between each dissection.

DNA from tick salivary glands was extracted using QIAamp® DNA Micro kit (Qiagen, Hombrechtikon, Switzerland) following manufacturer instructions with modifications, tissue lysis buffer and proteinase K were added in the 1.5 ml tubes before the salivary glands. DNA was stored at -20°C.

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* and an approximately 400 bp fragment of the 18S rRNA gene spanning the V4 hypervariable region of the genera *Babesia* and *Theileria* were amplified by PCR (Tonetti et al. 2009). Positive control, included in each run, consisted of DNA of *A. phagocytophilum* (provided by Ana Sofia Santos, Instituto Nacional de Saude, Lisboa, Portugal) and *B. divergens* (provided by Simona Casati, Institut Cantonal de Microbiologie, Bellinzone, Switzerland). PCR products were analysed using Reverse Line Blotting (RLB) (Tonetti et al. 2009). In addition to the original 15 oligonucleotide probes, which are listed in Tonetti et al. (2009) (two genus-specific Babesia/Theileria and Anaplasma/Ehrlichia, and 13 species-specific), 26 probes were added: one genus-specific Theileria spp. (Nagore et al., 2004) and 25 species-specific probes : B. ovis, B. crassa (Schnittger et al., 2004), B. major (Georges et al., 2001), Babesia sp. (sable) (Oosthuizen et al., 2008), B. caballi (Butler et al., 2008), B. occultans (Ros-Garcia et al., 2011), B. orientalis (Hea et al., 2011), B. gibsoni (from Pfizer et al., 2009), B. rossi (Matjila et al., 2004), B. bicornis (Nijhof et al., 2003), B. motasi, Theileria sp. (greater kudu), Theileria sp. (sable) (Nijhof et al., 2005), T. separata, T. lestoquardi, T. ovis (Schnittger et al., 2004), T. buffeli (Gubbels et al., 1999), T. bicornis (Nijhof et al.

2003), Theileria sp. (buffalo) (Oura et al., 2004), T. equi (Butler et al., 2008), T. annulata (Georges et al., 2001), Ehrlichia sp. (Omatjenne) (Bekker et al., 2002) and four A. phagocytophilum (from Pfizer et al. 2009) that replaced the original degenerated probe. Samples reacting only with the Babesia/Theileria probe were considered as *Babesia* spp. since a genus-specific probe was included for the genus *Theileria*. To test for theoretical specificity, oligonucleotide probes 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).

Sequencing

PCR products that reacted only with genus-specific probes Babesia/Theileria, Theileria spp. or Anaplasma/Ehrlichia and did not hybridize with the panel of species-specific probes were sequenced. Prior sequencing PCR products were purified using Wizard® SV and PCR Clean-Up System (Promega, Madison, USA) following the manufacturer instructions except that we eluted with 35 µl rather than 70 µl Nuclease-Free Water. Sequencing was performed by Microsynth AG (Balgach, Switzerland). Sequences were compared and corrected with CLC Sequence Viewer 6 (CLC bio, Aarhus, Denmark) and Bioedit (Tom Hall Ibis Biosciences, Carlsbad). Corrected sequences were compared with available sequences retrieved from GenBank using NCBI Basic Local Alignment Search Tool (BLAST).

Data analysis

Data was 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). A Generalised Linear Mixed Model (GLMM) with negative binominal errors was used to evaluate tick salivary gland infection (taking into account the four main infected tick species) collected from wild and domestic ruminants originating from six localities. In each locality, the significance of the factors LOCATION and HOST TYPE (i.e. ticks infesting wild *vs* domestic ruminants) as well as the LOCATION: HOST TYPE interaction was assessed, *P*-values were considered significant when below 0.05. In order to compute whether associations between pathogen species in co-infections were significant a permutation test proposed by Raup and Crick (1979), adapted to R by Clua et al. (2010) confined to locations was used. Here, *P*-values were considered significant when below 0.0001 (Bonferroni corrected).

Results

Tick sampling

A total of 7364 ticks belonging to 13 species were collected from 13 ruminant species (Table 1). Five tick species contributed for 96.5% of the total sampling: *R. (B.) decoloratus* (*n* = 2611), *R. e. evertsi* (*n* = 2225), *Margaropus winthemi* (*n* = 1085), *A. hebraeum* (*n* = 767) and *H. m. rufipes* (*n* = 417) and 71% of them were collected from wild animals (Table 1). All tick species were detected in areas that were congruent with their known geographic distribution except *Haemaphysalis silacea,* which was recorded at Willem Pretorius game reserve (central Free State) (Supplementary Table S1). The two most abundant tick species were collected at all six sampling sites, *R. (B.) decoloratus* infested nine ruminants species and *R. e. evertsi* all host species.

Infection of tick salivary glands

Among the 2117 examined ticks, 329 (15.5%) were infected and they harboured 397 infections (Table 2). The majority (95%) of the infections were observed in four tick species: *R. e. evertsi* harboured 48.6% of the infections (193/397), *R. (B.) decoloratus* 24.7% (98/397), *A. hebraeum* 11.8% (47/397) and *H. m. rufipes* 9.6% (38/397) (Table 2). Five tick species (*Ixodes rubicundus, M. winthemi, Hae. silacea, Otobius megnini* and *R. zambeziensis*) were free of pathogens. One hundred sixty eight infections were identified at genus level only: 69 *Babesia* spp., 56 *Theileria* spp. and 43 *Anaplasma* or *Ehrlichia* spp. Sequencing of all these samples did not allow further identification. The remaining infections (*n* = 229) (57.7%) were identified to species level and were assigned to 23 pathogen species, all described in South Africa, except *T. annulata* (Table 2). Among them, 46 were identified by sequencing (Table 3). *Theileria* sp. (sable) (*n* = 38; 9.6%), *T. buffeli* (*n* = 37; 9.3%), *T. bicornis* (*n* = 27; 6.8%), *Ehrlichia* sp. (EU191229.1) (*n* = 23; 5.8%) and *T. separata* (*n* = 18; 4.5%) were the most frequent (Table 2).

Pathogen distribution among tick species

Nine pathogen species were detected in one tick species only. *T. equi, T. separata*, *A. ovis* and *A. platys* in *R. e. evertsi*, *B. occultans* and *Babesia* sp. (sable) were only detected in *H. m. rufipes,* and *Theileria* sp. (kudu), *Anaplasma centrale* and *Ehrlichia ruminantium* in *R. appendiculatus*, *R. gertrudae* and *A. hebraeum*, respectively (Table 2). Seven pathogens were detected in two tick species. *B. bigemina*, *B. caballi, A. bovis, T. ovis* and *Theileria* sp. (giraffe) were detected in *R. (B.) decoloratus* and *R. e. evertsi*. *T. bicornis* was detected in *R. e. evertsi* and *R. Appendiculatus.* Finally, *T. mutans* were detected in *R. e. evertsi* and *A. hebraeum*. Four pathogen species were identified in three tick species. *Theileria* sp. (sable) was detected in *R. e. evertsi*, *R. (B.) decoloratus* and *A. hebraeum*. *T. taurotragi* was detected in *R. appendiculatus, R. e. evertsi* and *H. m. rufipes*. *A. marginale* was detected in *R. gertrudae*, *R. (B.) decoloratus* and *R. e. evertsi. Ehrlichia* sp. (EU191229.1) was detected in *R. (B.) decoloratus, R. e. evertsi* and *H. m. rufipes*. Finally, *T. buffeli* and *E. ovina* were detected in four tick species: *T. buffeli* was detected in *R. (B.) decoloratus*, *R. e. evertsi, H. m. rufipes* and *R. appendiculatus*, while *E. ovina* was detected in *R. (B.) decoloratus, R. e. evertsi, H. m. rufipes* and *R. gertrudae*.

Salivary gland infection of ticks attached to wild versus domestic ruminants

Ticks that were attached to wild ruminants showed an infection prevalence of 23.3% (227/973) and harboured 284 infections, while ticks attached on domestic ruminants displayed an infection prevalence of 11.5% (102/885) and harboured 113 infections (Table 4a and 4b). The differences were mainly due to *Theileria* spp. (16.1% *vs* 8.4%), *Theileria* sp. (sable) (12.6% *vs* 1.7%), *T. buffeli* (10.2% *vs* 6.7%), *T. bicornis* (7.4% *vs* 5%) and *T. separata* (4.9% *vs* 3.4%) (Table 4a and 4b).

Taking into account the four main infected tick species (*R. (B.) decoloratus, R. e. evertsi, A. hebraeum* and *H. m. rufipes*) salivary gland infection (prevalence of infection and mean density of pathogens) varied significantly among the sampling localities (Table 5). The infection pattern was impacted by the factor LOCATION and HOST TYPE as well as by the interaction of both factors (Glmm analyses $P <$ 0.001 for the factors LOCATION, HOST TYPE and their interactions). In contrast the intensity of infections did not significantly differ among host-types and/or locations at the 5% risk (data not shown). In Sandveld, Thabazimbi and Lephalale infection prevalence and mean density of pathogens were significantly higher in salivary glands of ticks infesting wild than domestic hosts. Similarly, the tick mean density was significantly higher on wild animals than on domestic ones in Sandveld and Thabazimbi (data not shown).

Co-infections

Among infections identified at species level, 93 (44.7%) were co-infections: 57 (61.3%) involved two pathogens, 24 (25.8%) three and 12 (12.9%) four (data not shown). *R. e. evertsi* showed the highest prevalence of co-infections (*n* = 67; 72%), followed by *R. (B.) decoloratus* (*n* = 21; 22.6%), *R. appendiculatus* (*n* = 3; 3.2%) and *H. m. rufipes* (*n* = 2; 2.2%) (data not shown). Six associations involving five pathogen species were significant: *Theileria* sp. (sable) - *T. separata; Theileria* sp. (sable) - *T. bicornis; T. separata - T. bicornis; T. separata - T. buffeli; T. bicornis - T. buffeli* and *T. buffeli - B. caballi* [*P* < 0.0004, permutations test constrained to locations (Raup and Crik, 1979, modified by Clua et al., 2010)] (Supplementary Table S2).

Discussion

In this study, 13 tick species collected from wild and domestic ruminants were analysed by RLB hybridisation using probes allowing identification at the genus and species level. The majority (*n* = 229) of the 397 infections were identified at species level. Among those, 46 infections assigned to seven species were identified by sequencing: *Ehrlichia* sp. (EU191229.1), *E. ovina, Theileria* sp. (giraffe), *A. platys, A. ovis, A. marginale* and *A. bovis*. No species-specific probes were included in the assay for the first four species. The last three species did not react with their specific oligonucleotide probes. For *A. ovis* and *A. marginale* this was probably due to the forward position of the probes on the amplified sequence, while for *A. bovis* it was probably due to one base pair difference on the amplified sequences. The remaining 168 infections were only identified at the genus level, even after sequencing, probably because of the presence of multiple pathogens (Microsynth personal communication) for which no probes were available.

The geographic distribution of all tick and pathogen species was congruent with their known geographic distribution except for *Hae. silacea, T. annulata* and *Ehrlichia* sp. (EU191229.1). The tick *Hae. silacea* was collected at Willem Pretorius game reserve in the centre of the Free State. Its known geographic distribution is the North-Eastern KwaZulu-Natal and the Eastern Cape Province (Norval, 1975, Walker, 1991, Horak et al., 1991, Horak et al., 2007). In Africa *T. annulata* only occurs in Northern Africa (Pipano, 1994). Here, *T. annulata* was detected in salivary glands of four *R. (B.) decoloratus* and two *R. e. evertsi* at Thabazimbi and in the Free State (Willem Pretorius) where they were sharing the same individual hosts. All *T. annulata* infections were identified through hybridisation with RLB-probes designed by Georges et al. (2001). Cross reactivity with pathogens identified in this study can be excluded, whether cross reactivity occurred with an alternative or unknown *Theileria* species remains a possibility. Unfortunately, sequencing of these samples was not successful. Thus, the finding of this economically important cattle parasite in South Africa must be considered with caution. This pathogen is not discussed further, and *Ehrlichia* sp. (EU191229.1) will be discussed elsewhere (Berggoetz et al. in prep).

Among the observed tick-pathogen combinations 17 were congruent with the literature. Among those ten were recorded in the salivary glands of *R. e. evertsi*: *B. bigemina* (Büscher, 1988), *B. caballi* (De Waal and Heerden, 1994), *T. ovis* (Jansen and Neitz, 1956), *T. equi* (De Waal and Potgieter, 1987), *T. separata* (Jansen and Neitz, 1956), *Theileria* sp. (sable) (Steyl et al., 2012), *T. taurotragi* (Lawrence et al., 1994a), *A. marginale* (Potgieter, 1981), *A. ovis* (Kaufman, 1996) and *E. ovina* (Neitz, 1956). The remaining seven known vector-pathogen combinations were recorded in the salivary glands of four tick species: *B. bigemina* (Potgieter and Els, 1977), *T. ovis* (Jansen and Neitz, 1956) and *A. marginale* (Potgieter, 1981) in *R. (B.) decoloratus; T. mutans* (Lawrence et al., 1994a) and *E. ruminantium* (Bezuidenhout et al., 1994) in *A. hebraeum; B. occultans* (Thomas and Mason, 1981) and *T. taurotragi* (Lawrence et al., 1994b) in *H. m. rufipes* and *R. appendiculatus,* respectively. *R. e. evertsi* appears as a very important vector for *T. separata, T. equi* and *Theileria* sp. (sable) in South Africa. Interestingly, *B. bigemina* was detected in the salivary glands of one *R. e. evertsi* adult. The exact role of *R. e. evertsi* in the epidemiology of redwater remains unclear (De Vos and Potgieter, 1994). According to Büscher (1988), only nymphs transmit *B. bigemina* and transovarial transmission does not occur. Further studies are needed to evaluate the role of *R. e. evertsi* adults in the circulation of *B. bigemina.*

To our knowledge, the remaining 23 vector-pathogen combinations were never described before. Among those eleven involved combinations with multiple records at different sites. Three vector-pathogen combinations involved a tick species, *R. gertrudae,* that was never reported as vector (Walker et al., 2000). One third of *R. gertrudae* specimens carried bacterial pathogens, all identified at species level. Four out of six *A. marginale* infections were detected in this tick species suggesting a possible vector role of *R. gertrudae* in the epidemiology of gallsickness. In addition, *E. ovina* and *A. centrale* were each identified in one *R. gertrudae.* One vector-pathogen combination involved *T. bicornis* detected for the first time in ticks and previously reported in rhinoceroses (Nijhof et al., 2003; Govender et al., 2011), cattle (Muhanguzi et all., 2010) and nyala (Pfitzer et al. 2011). It was identified in the salivary glands of 26 *R. e. evertsi* at four localities in the lowveld and the highveld. This strongly suggests that *R. e. evertsi* transmits *T. bicornis* on a large area. Three vector-pathogen combinations involved *T. buffeli* which has recognized vectors in some parts of the world (Gubbels et al., 2000) but to our knowledge, no identified vector in South Africa. *T. buffeli* infections (*n* = 34) were detected in *R. e. evertsi* and *R. (B.) decoloratus,* as well as in two *H. m. rufipes* at sites situated in the lowveld and highveld. This suggests that *R. e. evertsi* and *R. (B.) decoloratus* play a role in the maintenance of this parasite in South Africa. Three vector-pathogen combinations involved *A. bovis* and *Theileria* sp. (sable)) identified in salivary glands of tick species that were suspected vectors. *A. bovis* was reported by Tonetti et al. (2009) in entire *R. e. evertsi* ticks. Here, *A. bovis* was detected in salivary glands of 11 *R. e. evertsi* collected at three localities. Our observation corroborates previous report and supports that *R. e. evertsi* may act as vector for *A. bovis*. The detection of *Theileria* sp. (sable) in salivary glands of nine *R. (B.) decoloratus* at three sites and of five *A. hebraeum* at two of these sites indicates that these ticks may act as vectors in addition to *R. e. evertsi* and *R. appendiculatus* (Steyl et al. 2012). The observation of *Theileria* sp. (sable) in these two additional ticks contributes to explain the broad host range of *Theileria* sp. (sable) (Spitalska et al., 2005; Yusufmia et al., 2010; Pfitzer et al., 2011; Steyl et al., 2012). *B. caballi* has a known vector in South Africa but it was observed in one new vector-pathogen combinations, involving a tick species known to transmit other pathogens. We detected *B. caballi* in the salivary glands of seven *R. (B.) decoloratus* at three locations suggesting that this tick may be an additional vector to *R. e. evertsi* (De Wall et al., 1988).

The remaining 12 vector-pathogen combinations involved only one or two records. Among those, five were previously suspected. This was the case for *T. mutans* in *R. e. evertsi* (de Vos and Ross, 1981), *E. ovina* in *R. (B.) decoloratus* (Schulz 1940) and in *H. m. rufipes* (*Hyalomma* spp., Schulz 1940) and *T. taurotragi* in *H. m. rufipes* (Binta et al., 1998). In addition, *Babesia* sp. (sable) (Oosthuizen et al., 2008) that was identified in the salivary glands of *H. m. rufipes* is genetically very close to *B. occultans* (Oosthuizen et al., 2008) which is transmitted by *H. m. rufipes* (Thomas and Mason, 1981). Thus our observation is not so surprising. The remaining seven vector-pathogen combinations were unknown. *T. bicornis* was detected in one *R. appendiculatus*. *A. bovis* was detected in one *R. (B.) decoloratus,* its vector in South Africa is *R. appendiculatus* (Scott, 1994). *A. platys* which is generally associated with canine hosts (Huang et al., 2005) and *R. sanguineus* (Hoskins et al., 1991) was detected in one *R. e. evertsi*. *Theileria* sp. (giraffe) which was only known in giraffe (Oosthuizen et al., 2009) was detected in one *R. e. evertsi* and one *R. (B) decoloratus.* Finally, *Theileria* sp. (kudu) a pathogen of greater kudu (Nijhof et al., 2005) and nyala (*T. angassii*) (Pfitzer et al., 2011) and *T. buffeli* were identified in salivary glands *R. appendiculatus.* These 12 vector-pathogen combinations need further records for confirmation for the vector role of these ticks.

More than 70% of the collected tick species were attached to wild ruminants. Differences in infestation between wild and domestic hosts can be explained by a lower exposure to ticks of domestic animals that were treated with acaricides on a regular basis. Furthermore, domestic animals graze in managed areas restricted to several hectares while wild animal habitats are much larger and non-managed. Salivary glands of ticks that were attached to wild ruminants displayed significantly higher infection prevalences and pathogen mean density than salivary glands of ticks attached to domestic ones at three localities. The pathogens that mainly contributed to the significant difference between wild and domestic animals were theilerial species transmitted only transstadially by the two-host tick *R. e. evertsi. R. e. evertsi* populations in reserves have a greater variety of hosts than those living on farms. This allows them to accumulate pathogen species originating from various hosts. This could be one explanation for the differences between salivary gland infections of ticks associated to wild and domestic ruminant environments. Another one could be that acaricide treatments applied on domestic animals could affect the tick population structure (e.g., proportions between life stages) and therefore reduce vertical transmission and pathogen load in tick population. Finally, differences in susceptibility to acaricides of infected ticks and non-infected ticks could reduce the proportion of infected ticks in the environment.

Here, 72% of co-infections were observed in the two-host tick *R. e. evertsi* and only 22.6% in the one-host tick *R. (B.) decoloratus*, despite the fact that the one-host tick displayed slightly higher infection prevalence and pathogen mean density. Tick life cycles with two and three hosts increase the chance of co-infections. Among the 529 (23 x 23) possibilities of co-infections, six pathogen associations involving four *Theileria* species and one *Babesia* species were significant. Although co-infections in *Ixodes* ticks involving *Borrelia* spp., *Babesia* spp., *Anaplasma* spp. and *Rickettsia* spp. were described in North America (for example, Swanson et al., 2006) and Europe (for example, Lommano et al., 2012), much less is known on co-infections in ticks in Southern Africa. Here, significant associations involved the most frequent pathogen species. This could indicate that associations are linked to pathogen frequency. However, the most frequent associations (*Theileria* sp. (sable) - *T. separata, Theileria* sp. (sable) - *T. bicornis* and *T. separata - T. bicornis*) were the same as those observed in the blood of hosts exposed to these ticks (Berggoetz et al., in prep.). This suggests that the associated species take advantage of their reciprocal presence. Future studies will have to understand the mechanisms of co-infections in ticks and evaluate their impact on wild and domestic ruminants.

The present study showed that many examined tick species were associated with a much broader pathogen range than previously known. *R. e. evertsi* and *R. (B.) decoloratus* are mainly concerned and appear as important vectors in South Africa. Pathogen species like *T. bicornis, Theileria* sp. (giraffe), *Theileria* sp. (kudu) and *Babesia* sp. (sable) were detected for the first time in ticks, more precisely in salivary glands, which suggests their vector role. Furthermore, the present observations showed that ticks from wild animal environments generally harboured more infections than ticks from domestic animal environments. This indicates that wild ruminants are more exposed to tick borne pathogens, which can probably be explained by the fact that they live in an uncontrolled environment with a higher host species richness than in the managed land of domestic ruminants. Our results also shed lights on the high frequency of co-infections in ticks.

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References

Aktas, M., Altay, K., Dumanli, N., Kalkan, A., 2009. Molecular detection and identification of *Ehrlichia* and *Anaplasma* species in Ixodid ticks. Parasitol. Res. 104, 1243-1248.

Bekker, C.P.J., de Vos, S., Taoufik, A., Sparagano, O.A.E., Jongejan, F., 2002. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminatium* in *Amblyomma variegatum* ticks by reverse line blot hybridisation. Vet. Microbiol. 89, 223-238.

Bezuidenhout, J.D., Prozesky, L., du Plessis, J.L., van Amstel, S.R., 1994. Heartwater. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 351-370.

Binta, M.G., Losho, T., Allsopp, B.A., Mushi, E.Z., 1998. Isolation of *Theileria taurotragi* and *Theileria mutans* from cattle in Botswana. Vet. Parasitol. 77, 83-91.

Brothers, P.S., Collins, N.E., Oosthuizen, M.C., Bhoora, R., Troskie, M., Penzhorn, B.L., 2011. Occurrence of blood-borne tick-transmitted parasites in common tsessebe (*Damaliscus lunatus*) antelope in Northern Cape Province, South Africa. Vet. Parasitol. 183, 160-165.

Büscher, G., 1988. The infection of various tick species with *Babesia bigemina*, its transmission and identification. Parasitol. Res. 74, 324-30.

Butler, C.M., Nijhof, A.M., Jongejan, F., van der Kolk, J.H., 2008. *Anaplasma phagocytophilum* infection in horses in the Netherlands. Vet. Rec. 162, 216-218.

Carmichael, I.H., Hobday, E., 1975. Blood parasites of some wild Bovidae in Botswana. Onderstepoort J. Vet. Res. 42, 55-62.

Clua, E., Buray, N., Legendre, P., Mourier, J., Planes, S., 2010. Behavioural response of sicklefin lemon sharks *Negaprion acutidens* to underwater feeding for ecotourism purposes. Marine Ecol. Prog. Series 414, 257-266.

De Vos, A.J., Roos, J.A., 1981. Observations on the transmission of *Theileria mutans* in South Africa. Onderstepoort J. Vet. Res. 48, 1-6.

De Vos, A.J., Potgieter, F.T., 1994. Bovine babesiosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 278-294.

De Waal, D.T., Potgieter, F.T., 1987. The transstadial transmission of *Babesia caballi* by *Rhipicephalus evertsi evertsi*. Onderstepoort J. Vet. Res. 54, 655-6.

De Waal, D.T., Van Heerden, J., Van den Berg, S.S., Stegmann, G.F., Potgieter, F.T., 1988. Isolation of pure *Babesia equi* and *Babesia caballi* organisms in splenectomized horses from endemic areas in South Africa. Onderstepoort J. Vet. Res. 55, 33-35.

De Waal, D.T., van Heerden, J., 1994. Equine babesiosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 295-304.

Georges, K., Loria, G.R., Riili S., Greco, A., Caracappa, S., Jongejan, F., Sparagano, O., 2001. Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Vet. Parasitol. 99, 273-286.

Govender, D., Oosthuisen, M.C., Penzhorn, B.L., 2011. Piroplasm parasites of white rhinoceroses (*Ceratotherium simum*) in the Kriger National Park, and their relation to anaemia. J. South African Vet. Ass. 82, 36-40.

Gubbels, J.M., de Vos, A.P., van der Weide, M., Viseras, J., Schouls, L.M., de Vries, E. and Jongejan, F., 1999. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridisation. J. Clin. Microbiol. 37, 1782-1789.

Gubbels, M.J., Hong, Y., van der Weide, M., Qi, B., Nijman, I.J., Guangyuan, L., Jongejan, F., 2000. Molecular characterisation of the *Theileria buffeli/orientalis* group. Int. J. Parasitol. 30, 943-952.

Hea, L., Fenga, H.H., Zhanga, W.J., Zhanga, Q.L., Fanga, R., Wanga, L.X., Tua, P., Zhoub, Y.Q., Zhaoa, J.L., Oosthuizend, M.C., 2011. Occurrence of *Theileria* and *Babesia* species in water buffalo

(*Bubalus babalis*, Linnaeus, 1758) in the Hubei province, South China. Vet. Parasitol. 170, 323-326.

Horak, I.G., Williams, E.J., Van Schalkwyk, P.C., 1991. Parasites of domestic and wild animals in South Africa. XXV. Ixodid ticks on sheep in the north-eastern Orange Free State and in the eastern Cape Province. Onderstepoort J. Vet. Res. 58, 115-123.

Horak, I.G., Golezardy, H., Uys, A.C., 2007. Ticks associated with the three largest wild ruminants species in southern Africa. Onderstepoort J. Vet. Res. 74, 231-242.

Hoskins, J.D., 1991. Ehrlichial diseases of dogs: diagnosis and treatments. Canine Pract. 16, 13-21.

Huang, H., Unver, A., Perez, M.J., Orellana, N.G., Rikihisa, Y., 2005. Prevalence and molecular analysis of *Anaplasma platys* in dogs in Lara, Venezuela. Brazilian J. Microbiol. 36, 211-216.

Husson, F., Josse, J., Le, S., Mazet, J., 2012. FactoMineR: Multivariate exploratory data analysis and data mining with R. R package version 1.20, http://CRAN.R-project.org/package=FactoMineR

Jansen, B.C., Neitz, W.O., 1956. The experimental transmission of *Theileria ovis* by *Rhipicephalus evertsi*. Onderstepoort J. Vet. Res. 27, 3-6.

Kaufmann, J., 1996. Parasitic infections of domestic animals: a diagnostic manual. Birkhäuser Verlag, Basel, Schweiz, p. 189.

Lawrence, J.A., de Vos, A.J., Irvin, A.D., 1994a. *Theileria mutans* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 336-337.

Lawrence, J.A., de Vos, A.J., Irvin, A.D., 1994b. *Theileria taurotragi* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 334-335.

Löhr, K.F., Meyer, H., 1973. Game anaplasmosis: the isolation of *Anaplasma* organisms from Antelope. Zeitsch. Tropenmed. Parasitol. 24, 192-197.

Löhr, K.F., Ross, J.P., Meyer, H., 1974. Detection in game of fluorescent and agglutination antibodies to intraerythrocytic organisms. Zeitsch. Tropenmed. Parasitol. 25, 217-26.

Lommano, E., Bertaiola, L., Dupasquier, C., Gern, L., 2012. Infections and coinfections of questing *Ixodes ricinus* ticks by emerging zoonotic pathogens in western Switzerland. Appl. Environm. Microbiol. 78, 4606-4612.

Matthysse, J.G., Colbo, M.H., 1987. The Ixodid ticks of Uganda: together with species pertinent to Uganda because of their present distribution. Entomological Society of America, College Park, Maryland.

Matjila, P.T., Penzhorn, B.L., Bekker, C.P.J., Nijhof, A.M., Jongejan, F., 2004. Confirmation of the occurrence of *Babesia canis vogeli* in domestic dogs in South Africa. Vet. Parasitol.122, 119-125.

Muhanguzi, D., Matovu, E., Waiswa, C., 2010. Prevalence and characterization of *Theileria* and *Babesia* species in cattle under different husbandry systems in Western Uganda. Int. J. An. Vet. Adv. 2, 51-58.

Nagore, D., Garcia-Sanmartin, J., Garcia-Perez A.L., Juste, R.A., Hurtado A., 2004. Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in sheep population from Northern Spain. Int. J. Parasitol. 34, 1059-1067.

Neitz, W.O., Du Toit, P.J., 1932. Bovine anaplasmosis. A method of obtaining pure strains of *Anaplasma marginale* and *A. centrale* by transmission through antelopes. 18th Report of the Director of Veterinary Services and Animal Industry, Pretoria, Union of South Africa, pp. 3-20.

Neitz, W.O., 1935. Bovine anaplasmosis. The transmission of *Anaplasma marginale* to black wildebeest (*Connochaetes gnou*). Onderstepoort J. Vet. Res. Sc.An. Ind. 5, 9-11.

Neitz, W.O., 1956. A consolidation of our knowledge of the transmission of tick-borne diseases. Onderstepoort J. Vet. Res. 27, 115-63.

Nijhof, A.M., Penzhorn, B.L., Lynen, G., Mollel, J.O., Morkel, P., Bekker, C.P.J., Jongejan, F., 2003. *Babesia bicornis* sp. nov. and *Theileria bicornis* sp. nov.: Tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). J. Clin. Microbiol. 41, 2249-2254.

Nijhof, A.M., Pillary, V., Steyl, J., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., Penzhorn, B.L., Jongejan, F., 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. J. Clin. Microbiol. 43, 5907-5911.

Norval, R.A.I., 1975. Studies on the ecology of *Haemaphysalis silacea* Robinson 1912 (Acarina: Ixodidae). J. Parasitol. 61, 730-736.

Oosthuizen, M.C., Zweygarth, E., Collins, N.E., Troskie, M., Penzhorn, B.L., 2008. Identification of a novel *Babesia* sp. from a sable antelope (*Hippotragus niger* Harris, 1838). J. Clin. Microbiol. 46, 2247-2251.

Oosthuizen, M.C., Allsopp, B.A., Troskie, M., Collins, N.E., Penzhorn, B.L., 2009. Identification of novel *Babesia* and *Theileria* species in South African giraffe (*Giraffa camelopardalis*, Linnaeus, 1758) and roan antelope (*Hippotragus equinus*, Desmarest 1804). Vet. Parasitol. 163, 39-46.

Oura, C.A.L., Bishop, R.P., Wampande, E.M., Lubega, G.W., Tait, A., 2004. Application of reverse line blot assay to the study of haemoparasites in cattle in Uganda. Int. J. Parasitol. 34, 603-613.

Pfitzer, S., 2009. Occurrence of tick-borne haemoparasites in nyala (*Tragelaphus angasii*) in KwaZulu-Natal and Eastern Cape Province, South Africa. M. SC. Thesis, University of Pretoria.

Pfitzer, S., Oosthuizen, M.C., Bosman, A.M., Vorster, I., Penzhorn, B.L., 2011. Tick-borne parasites in nyala (*Tragelaphus angasii*, Gray 1849) from KwaZulu-Natal, South Africa. Vet. Parasitol. 176, 126-131.

Pipano, E., 1994. *Theileria annulata* theileriosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 341-348.

Potgieter, F.T., Els, H.J., 1977. Light and electron microscopic observations on the development of *Babesia bigemina* in larvae, nymphae and non-replete females of *Boophilus decoloratus*. Onderstepoort J. Vet. Res. 44, 213-232.

Potgieter, F.T., 1981. Tick transmission of anaplasmosis in South Africa. *In* Proceedings of the International Conference on Tick Biology and Control, 27-29 January 1981, Grahamstown, South Africa 53-56.

Raup, D.M., Crick, R.E., 1979. Measurement of faunal similarity in paleontology. J. Paleontol. 53, 1213-1227.

Ros-Garcia, A., M'Ghirbi, Y., Bouattour, A. and Hurtado, A., 2011. First detection of *Babesia occultans* in *Hyalomma* ticks from Tunisia. Parasitol. 138, 578-582.

Schnittger, L., Yin, H., Qi, B., Gubbels, M.J., Beyer, D., Niemann, S., Jongejan, F., Ahmed, J.S., 2004. Simultaneous detection and differentiation of *Theileria* and *Babesia* parasites infecting small ruminants by reverse line blotting. Parasitol. Res. 92, 189-196.

Schulz, K., 1940. A rickettsiosis new to South Africa. Onderstepoort J. Vet. Sc. An. Ind. 13, 287-289.

Scott, G.R., 1994. Lesser-known rickettsias infecting livestock. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 371-377.

Skaug, H., Fournier, D., Nielsen, A., 2010. Glmm ADMB-package, http: //127.0.0.1:13596/library/glmmADMB/html/glmmADMB-package.html

Spitalska, E., Riddell, M., Heyne, H., Sparagano, O.A.E., 2005. Prevalence of theileriosis in red hartebeest (*Alcelaphus buselaphus caama*) in Namibia. Parasitol. Res. 97, 77-79.

Steyl, J.C.A., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., 2012. *Theileriosis (Cytauxzoonosis)* in roan antelope (*Hippotragus equinus*): Field exposure to infection and identification of potential vectors. Onderstepoort J. Vet. Res. 70, 1-8.

Swanson, S.J., Neitzel, D., Reed, K.D., Belongia, E.A., 2006. Coinfections acquired from *Ixodes* ticks. Clin. Microbiol. Rev. 19, 708-727.

Thomas, S.E., Mason, T.E., 1981. Isolation and transmission of an unidentified *Babesia* sp. infective for cattle. Onderstepoort J. Vet. Res. 48, 155-158.

Tonetti, N., Berggoetz, M., Rühle, C., Pretorius, A.M., Gern, L., 2009. Ticks and tick-borne pathogens from wildlife in the Free State province, South Africa. J. Wildl. Dis. 45, 437-446.

Walker, J.B., 1991. A review of the ixodid ticks (Acari, Ixodidae) occurring in Southern Africa. Onderstepoort J. Vet. Res. 58, 81-105.

Walker, J.B., Keirans, J.E., Horak, I.G., (eds.), 2000. The genus *Rhipicephalus* (Acari, Ixodidae). Cambridge University Press, Cambridge, UK.

Walker, A.R., Bouattour, A., Camicas, J.-L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., (eds.), 2003. Ticks of domestic animals in Africa: a guide to identification of species. Bioscience reports: Edinburgh.

Yusufmia, S.B.A.S., Collins, N.E., Nkuna, R., Troskie, M., Van den Bossche, P., Penzhorn, B.L., 2010. Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. J. South African Vet. Ass. 81, 45-49.

Table 1: Host infestation (*n* = 128) by 13 tick species in six localities in South Africa.

Tick **(bold)** and host species	Hosts	Number of ticks				
	No. Infested / examined	Larvae	Nymphs	Males	Females	Total
R. (B.) decoloratus						
Common eland (*Tragelaphus oryx*)	1/9 (11%)	9	91	135	97	332
Greater kudu (*T. strepsiceros*)	3/8 (38%)	1	59	35	35	130
Impala (*Aepyceros melampus*)	2/7 (29%)	213	483	434	207	1337
Southern giraffe (*Giraffa camelopardalis*)	5/5 (100%)	0	0	6	23	29
Blue wildebeest (*Connochaetes taurinus*)	4/5 (80%)	8	10	40	53	111
Sable antelope (*Hippotragus niger*)	2/2 (100%)	0	14	37	45	96
Gemsbok (*Oryx gazella gazella*)	1/1 (100%)	33	52	72	56	213
Cattle (*Bos primigenius taurus / B. p. taurus / indicus*)	17/50 (34%)	0	21	136	204	361
Sheep (*Ovis aries*)	2/14 (14%)	0	0	0	2	2
Total	37/101 (37%)	264	730	895	722	2611
R. e. evertsi						
African buffalo (*Syncerus caffer*)	14/15 (93%)	0	0	330	112	442
Common eland (*T. oryx*)	9/9 (100%)	0	2	262	27	291
Greater kudu (*T. strepsiceros*)	4/8 (50%)	7	24	7	0	38
Impala (*A. melampus*)	3/7 (43%)	0	49	11	2	62
Blue wildebeest (*C. taurinus*)	5/5 (100%)	0	0	48	38	86
Springbok (*Antidorcas marsupialis*)	4/6 (67%)	0	0	6	5	11
Southern giraffe (*G. camelopardalis*)	4/5 (80%)	0	0	15	12	27
Blesbok (*Damaliscus pygarus phillipsi*)	1/3 (33%)	3	5	0	0	8
Black wildebeest (*C. gnou*)	2/3 (67%)	0	0	3	3	6
Sable antelope (*H. niger*)	2/2 (100%)	0	0	9	1	10
Gemsbok (*O. g. gazella*)	1/1 (100%)	0	0	26	5	31
Cattle (*B. p. taurus / B. p. t. / indicus*)	49/50 (98%)	189	424	431	153	1197
Sheep (*O. aries*)	5/14 (36%)	0	0	14	2	16
Total	101/128 (79%)	199	504	1162	360	2225
M. winthemi						
Common eland (*T. oryx*)	6/9 (67%)	11	505	287	234	1037
Greater kudu (*T. strepsiceros*)	1/8 (13%)	0	0	0	9	9
Gemsbok (*O. g. gazella*)	1/1 (100%)	1	0	0	27	28
Cattle (*B. p. taurus / B. p. t. / indicus*)	3/50 (6%)	0	0	0	11	11
Total	11/68 (16%)	12	505	287	281	1085
A. hebraeum						
Southern giraffe (*G. camelopardalis*)	5/5 (100%)	0	0	307	117	424
Cattle (*B. p. taurus / B. p. t. / indicus*)	10/50 (20%)	0	0	229	114	343
Total	15/55 (27%)	0	0	536	231	767
H. m. rufipes						
Buffalo (*S. caffer*)	8/15 (53%)	0	0	13	8	21
Common eland (*T. oryx*)	9/9 (100%)	0	0	215	14	229
Southern giraffe (*G. camelopardalis*)	4/5 (80%)	0	0	19	5	24
Blue wildebeest (*C. taurinus*)	3/5 (60%)	0	0	4	7	11
Sable antelope (*H. niger*)	1/2 (50%)	0	0	1	0	1
Cattle (*B. p. taurus / B. p. t. / indicus*)	28/50 (56%)	0	0	80	49	129
Sheep (*O. aries*)	2/14 (14%)	0	0	1	1	2
Total	55/100 (55%)	0	0	333	84	417
I. rubicundus						
Common eland (*T. oryx*)	4/9 (44%)	0	0	22	32	54
Greater kudu (*T. strepsiceros*)	5/8 (63%)	0	0	16	34	50
Springbok (*A. marsupialis*)	5/6 (83%)	0	0	9	11	20
Cattle (*B. p. taurus / B. p. t. / indicus*)	4/50 (8%)	0	0	7	21	28
Sheep (*O. aries*)	2/14 (14%)	0	0	1	1	2
Total	20/87 (23%)	0	0	55	99	154
R. appendiculatus						
Impala (*A. melampus*)	1/7 (14%)	0	0	4	0	4
Southern giraffe (*G. camelopardalis*)	1/5 (20%)	0	0	9	4	13
Cattle (*B. p. taurus / B. p. t. / indicus*)	3/50 (6%)	0	0	19	2	21
Total	5/62 (8%)	0	0	32	6	38
R. gertrudae						
African buffalo (*S. caffer*)	1/15 (7%)	0	0	21	1	22
Common eland (*T. oryx*)	1/9 (11%)	0	0	8	0	8
Total	2/24 (8%)	0	0	29	1	30
R. warburtoni						
African Buffalo (*S. caffer*)	1/15 (7%)	0	0	1	0	1
Common eland (*T. oryx*)	2/9 (22%)	0	0	13	1	14
Total	3/24 (12%)	0	0	14	1	15
H. silacea						
African buffalo (*S. caffer*)	2/15 (13%)	0	0	0	8	8
Total	2/15 (13%)	0	0	0	8	8
R. (B.) microplus						
Cattle (*B. p. taurus / B. p. t. / indicus*)	1/50 (2%)	0	3	1	3	7
O. megnini						
Cattle (*B. p. taurus / B. p. t. / indicus*)	2/50 (4%)	0	5	0	0	5
Total	2/50 (4%)	0	5	0	0	5
R. zambeziensis						
Impala (*A. melampus*)	1/7 (14%)	0	0	2	0	2
Total	/	475	1747	3346	1796	7364

Table 2: Infection of 8 out of 13 tick species by 23 pathogen species collected from 64 wild and 64 domestic ruminants in South Africa.

Pathogens / Ticks	*R. (B.)decoloratus*	*R. e. evertsi*	*A. hebraeum*	*H.m.rufipes*	*R. appendiculatus*	*R. gertrudae*	*R. (B.)microplus*	*R. warburtoni*	*I. rubicundus*	*M. winthemi*	*H. silacea*	*O. megnini*	*R. zambeziensis*	% of pathogen species*^{4}
B/T genus†	24	30	6	6	3	0	0	0	0	0	0	0	0	69 (17.4%)
B. occultans	0	0	0	$2^{a,f}$	0	0	0	0	0	0	0	0	0	2 (0.5%)
B. bigemina	$5^{b,c,e,f}$	1^{f}	0	0	0	0	0	0	0	0	0	0	0	6 (1.5%)
B. caballi	**7**^{b,c,e}	$5^{b,c}$	0	0	0	0	0	0	0	0	0	0	0	12 (3%)
B. sp. sable	0	0	0	**1**^{b}	0	0	0	0	0	0	0	0	0	1 (0.3%)
T. spp.	15	23	13	2	3	0	0	0	0	0	0	0	0	56 (14.1%)
T. bicornis	0	**26**^{a,b,c,e}	0	0	**1**^{e}	0	0	0	0	0	0	0	0	27 (6.8%)
T. ovis	2^{e}	$2^{a,c}$	0	0	0	0	0	0	0	0	0	0	0	4 (1%)
T. equi	0	$14^{a,b,c,e,f}$	0	0	0	0	0	0	0	0	0	0	0	14 (3.5%)
T. separata	0	$18^{b,c,d,e}$	0	0	0	0	0	0	0	0	0	0	0	18 (4.5%)
T. sp. (sable)	**9**^{c,e,f}	$24^{b,c,d,e}$	**5**^{e,f}	0	0	0	0	0	0	0	0	0	0	38 (9.6%)
T. buffeli	**17**^{b,c,e,f}	**17**^{b,c,e,f}	0	**2**^{a,f}	**1**^{e}	0	0	0	0	0	0	0	0	37 (9.3%)
T. taurotragi	0	1^{f}	0	**1**^{b}	2^{e}	0	0	0	0	0	0	0	0	4 (1%)
T. annulata	$4^{b,e}$	$2^{b,e}$	0	0	0	0	0	0	0	0	0	0	0	6 (1.5%)
T. sp. (kudu)	0	0	0	0	**1**^{e}	0	0	0	0	0	0	0	0	1 (0.3%)
T. mutans	0	**1**^{f}	1^{f}	0	0	0	0	0	0	0	0	0	0	2 (0.5%)
T. sp. (giraffe)	**1**^{f}	**1**^{f}	0	0	0	0	0	0	0	0	0	0	0	2 (0.5%)
A/E genus	3	7	18	11	0	0	1	3	0	0	0	0	0	43 (10.8%)
A. bovis	**1**^{b}	**11**^{a,b,c}	0	0	0	0	0	0	0	0	0	0	0	12 (3%)
A. centrale	0	0	0	0	0	**1**^{a}	0	0	0	0	0	0	0	1 (0.3%)
A. marginale	1^{d}	1^{c}	0	0	0	**4**^{a}	0	0	0	0	0	0	0	6 (1.5%)
A. ovis	0	$3^{c,f}$	0	0	0	0	0	0	0	0	0	0	0	3 (0.8%)
E. sp. (EU191229.1)	$8^{b,e}$	$3^{c,f}$	0	$12^{a,c}$	0	0	0	0	0	0	0	0	0	23 (5.8%)
E. ovina	**1**^{c}	$2^{a,c}$	0	**1**^{a}	0	**1**^{a}	0	0	0	0	0	0	0	5 (1.3%)
E. ruminantium	0	0	4^{f}	0	0	0	0	0	0	0	0	0	0	4 (1%)
A. platys	0	**1**^{a}	0	0	0	0	0	0	0	0	0	0	0	1 (0.3%)
Prevalence of infection*^{1}	84/395 (21.3%)	147/909 (16.2%)	42/233 (18%)	37/259 (14.3%)	9/26 (34.6%)	6/18 (33.3%)	1/4 (25%)	3/14 (21.4%)	0/131	0/116	0/7	0/4	0/1	329/2117 (15.5%)
Pathogen mean density*^{2}	98/395 (0.25)	193/909 (0.21)	47/233 (0.20)	38/259 (0.15)	11/26 (0.42)	6/18 (0.33)	1/4 (0.25)	3/14 (0.21)	0/131	0/116	0/7	0/4	0/1	397/2117 (0.19)
Intensity of infection*^{3}	98/84 (1.17)	193/147 (1.31)	47/42 (1.12)	38/37 (1.03)	11/9 (1.22)	6/6 (1)	1/1 (1)	3/3 (1)	0	0	0	0	0	397/329 (1.21)

*^{1} No. of infected ticks (data not shown) / no. of tested ticks; *^{2} no. of infections / no. of tested ticks; *^{3} no. of infections / no. of infected ticks; *^{4} % of the given pathogen species among the 397 infections.

†*B/T*: Includes infections reacting with this probe only, and considered as belonging to the genus *Babesia*.

Locations of the infections identified at species level: a = Tüssen-Die-Riviere; b = Willem Pretorius, c = Sandveld; d = Bethal; e = Thabazimbi; f = Lephalale.

Table 3: Pathogen species identified from tick salivary glands by sequencing.

Identified sequence	Homology with	% of homology	Species name	Identified from
KF414714	EU191229.1	100%	unamed (*E*. sp.)	*H.m.rufipes* (n = 12)
KF414714	EU191229.1	100%	unamed (*E*. sp.)	*R. (B.) decoloratus* (n = 8)
KF414714	EU191229.1	100%	unamed (*E*. sp.)	*R. e. evertsi* (n = 3)
KF414715	AF318946.1	99%	*E. ovina*	*R. e. evertsi* (n = 2)
KF414715	AF318946.1	99%	*E. ovina*	*H.m.rufipes* (n = 1)
KF414715	AF318946.1	99%	*E. ovina*	*R. gertrudae* (n = 1)
KF414715	AF318946.1	99%	*E. ovina*	*R. (B.) decoloratus* (n = 1)
KF414716	FJ155997.1	100%	*A. bovis*	*R. e. evertsi* (n = 11)
KF414717	EU191231.1	100%	*A. ovis*	*R. e. evertsi* (n = 3)
KF414718	FJ155998.1	99%	*A. marginale*	*R. gertrudae* (n = 1)
KF414719	AY040853.1	99%	*A. platys*	*R. e. evertsi* (n = 1)
KF414720	FJ213583.1	100%	*T.* sp. (giraffe)	*R. e. evertsi* (n = 1)
KF414720	FJ213583.1	100%	*T.* sp. (giraffe)	*R. (B.) decoloratus* (n = 1)

Table 4a: Pathogens identified in seven tick species collected from 64 wild ruminants.

Pathogens / Ticks	*R. (B.) decoloratus*	*R. e. evertsi*	*A. hebraeum*	*H.m.rufipes*	*R. appendiculatus*	*R. gertrudae*	*R. warburtoni*	% of pathogen species*[4]
B/T genus†	16	21	2	1	3	0	0	43 (15.1%)
B. occultans	0	0	0	1	0	0	0	1 (0.4%)
B. bigemina	2	0	0	0	0	0	0	2 (0.7%)
B. caballi	6	5	0	0	0	0	0	11 (3.9%)
T. spp.	14	19	9	1	3	0	0	46 (16.1%)
T. bicornis	0	20	0	0	1	0	0	21 (7.4%)
T. ovis	2	0	0	0	0	0	0	2 (0.7%)
T. equi	0	14	0	0	0	0	0	14 (4.9%)
T. separata	0	14	0	0	0	0	0	14 (4.9%)
T. sp. (sable)	9	23	4	0	0	0	0	36 (12.6%)
T. buffeli	14	14	0	1	0	0	0	29 (10.2%)
T. taurotragi	0	1	0	1	0	0	0	2 (0.7%)
T. annulata	3	1	0	0	0	0	0	4 (1.4%)
T. sp. (giraffe)	1	1	0	0	0	0	0	2 (0.7%)
A/E genus	2	7	9	5	0	0	3	26 (9.1%)
A. bovis	0	8	0	0	0	0	0	8 (2.8%)
A. centrale	0	0	0	0	0	1	0	1 (0.4%)
A. marginale	0	0	0	0	0	4	0	4 (1.4%)
E. sp. (EU191229.1)	2	1	0	11	0	0	0	14 (4.9%)
E. ovina	1	0	0	0	0	1	0	2 (0.7%)
E. ruminantium	0	0	2	0	0	0	0	2 (0.7%)
Prevalence of infection*[1]	61/235 (26%)	108/474 (22.78%)	22/91 (24.18%)	20/129 (15.5%)	7/12 (58.33%)	6/18 (33.33%)	3/14 (21.43%)	227/973 (23.33%)
Pathogen mean density*[2]	72/235 (0.31)	149/474 (0.31)	26/91 (0.29)	21/129 (0.16)	7/12 (0.58)	6/18 (0.33)	3/14 (0.21)	284/973 (0.29)
Intensity of infection*[3]	72/61 (1.18)	149/108 (1.38)	26/22 (1.18)	21/20 (1.05)	7/7(1)	6/6 (1)	3/3(1)	284/227 (1.25)

Table 4b: Pathogens identified in six tick species collected from 64 domestic ruminants.

Pathogens / Ticks	*R. (B.)decoloratus*	*R. e. evertsi*	*A. hebraeum*	*H.m.rufipes*	*R. appendiculatus*	*R. (B.)microplus*	% of pathogen species*[4]
B/T genus†	8	9	4	5	0	0	26 (21.8%)
B. occultans	0	0	0	1	0	0	1 (0.8%)
B. bigemina	3	1	0	0	0	0	4 (3.4%)
B. caballi	1	0	0	0	0	0	1 (0.8%)
B . sp. (sable)	0	0	0	1	0	0	1 (0.8%)
T . spp.	1	4	4	1	0	0	10 (8.4%)
T. bicornis	0	6	0	0	0	0	6 (5%)
T. ovis	0	2	0	0	0	0	2 (1.7%)
T. separata	0	4	0	0	0	0	4 (3.4%)
T . sp. (sable)	0	1	1	0	0	0	2 (1.7%)
T. buffeli	3	3	0	1	1	0	8 (6.7%)
T. taurotragi	0	0	0	0	2	0	2 (1.7%)
T. annulata	1	1	0	0	0	0	2 (1.7%)
T . sp. (kudu)	0	0	0	0	1	0	1 (0.8%)
T. mutans	0	1	1	0	0	0	2 (1.7%)
A/E genus	1	0	9	6	0	1	17 (14.3%)
A. bovis	1	3	0	0	0	0	4 (3.4%)
A. marginale	1	1	0	0	0	0	2 (1.7%)
A. ovis	0	3	0	0	0	0	3 (2.5%)
E . sp (EU191229.1)	6	2	0	1	0	0	9 (7.6%)
E. ovina	0	2	0	1	0	0	3 (2.5%)
E. ruminantium	0	0	2	0	0	0	2 (1.7%)
A. platys	0	1	0	0	0	0	1 (0.8%)
Prevalence of infection*[1]	23/160 (14.4%)	39/435 (9%)	20/142 (14.1%)	17/130 (13.1%)	2/14 (14.3%)	1/4 (25%)	102/885 (11.5%)
Pathogen mean density*[2]	26/160 (0.16)	44 /435 (0.10)	21/142 (0.15)	17/130 (0.13)	4/14 (0.29)	1/4 (0.25)	113/885 (0.13)
Intensity of infection*[3]	26/23 (1.13)	44/39 (1.13)	21/20 (1.05)	17/17 (1)	4/2 (2)	1/1 (1)	113/102 (1.11)

*[1] No. of infected ticks (data not shown) / no. of tested ticks; *[2] no. of infections / no. of tested ticks; *[3] no. of infections / no. of infected ticks; *[4] % of the given pathogen species among the 284 infections.

†*B/T*: Includes infections reacting with this probe only, and considered as belonging to the genus *Babesia*.

Table 5: Within localities comparison of tick infection prevalences and tick infection mean densities considering the four main infected tick species (*R. (B.) decoloratus, R. e. evertsi, A. hebraeum* and *H. m. rufipes*).

Location	Tick infection prevalence			Tick pathogen mean density		
	No. infected / tested	z values	*P* values	Mean infection / tick	z values	*P* values
Sandveld (w)	77/343 (22.45%)	4.02	< 0.0001	110/343 (0.32)	5.09	< 0.0001
Sandveld (d)	24/251 (9.56%)			25/251 (0.10)		
Willem Pretorius (w)	14/137 (10.22%)	0.1	0.91	18/137 (0.13)	0.28	0.78
Willem Pretorius (d)	25/254 (9.84%)			29/254 (0.11)		
Tüssen-Die-Riviere (w)	32/183 (17.49%)	-0.55	0.58	33/183 (0.18)	-0.09	0.93
Tüssen-Die-Riviere (d)	21/108 (19.44%)			20/108 (0.19)		
Bethal (w)	2/3 (66.67%)	1.63	0.1	2/3 (0.67)	2.41	0.016
Bethal (d)	3/62 (4.84%)			4/62 (0.06)		
Thabazimbi (w)	41/78 (52.56%)	4.51	< 0.0001	50/78 (0.64)	4.01	< 0.0001
Thabazimbi (d)	10/80 (12.50%)			14/80 (0.18)		
Lephalale (w)	59/228 (25.88%)	2.43	0.01	71/228 (0.31)	2.54	0.01
Lephalale (d)	21/131 (16.3%)			21/131 (0.16)		

P-values are considered significant when below or equal 0.05.

Supplement material 1: Geographic distribution of various tick species in South Africa.

Tick (bold) and location	Number of ticks				
	Larvae	Nymphs	Males	Females	Total
R. (B.) decoloratus					
Willem Pretorius	0	9	25	65	99
Tüssen-Die-Riviere	0	0	0	1	1
Sandveld	51	216	364	330	961
Bethal	0	3	10	17	30
Thabazimbi	213	488	451	231	1383
Lephalale	0	14	45	78	137
Total	264	730	895	722	2611
R. e. evertsi					
Willem Pretorius	0	0	328	111	439
Tüssen-Die-Riviere	181	373	239	48	841
Sandveld	15	73	490	152	730
Bethal	3	7	37	24	71
Thabazimbi	0	51	17	6	74
Lephalale	0	0	51	19	70
Total	199	504	1162	360	2225
M. winthemi					
Tüssen-Die-Riviere	7	505	286	213	1011
Sandveld	5	0	1	68	74
Total	12	505	287	281	1085
A. hebraeum					
Thabazimbi	0	0	82	64	146
Lephalale	0	0	454	167	621
Total	0	0	536	231	767
H. m. rufipes					
Willem Pretorius	0	0	45	38	83
Tüssen-Die-Riviere	0	0	206	18	224
Sandveld	0	0	53	19	72
Bethal	0	0	4	2	6
Lephalale	0	0	25	7	32
Total	0	0	333	84	417
I. rubicundus					
Tüssen-Die-Riviere	0	0	55	99	154
Rhipicephalus appendiculatus					
Thabazimbi	0	0	23	2	25
Lephalale	0	0	9	4	13
Total	0	0	32	6	38
R. gertrudae					
Tüssen-Die-Riviere	0	0	22	0	22
Sandveld	0	0	5	1	6
Willem Pretorius	0	0	2	0	2
Total	0	0	29	1	30
R. warburtoni					
Tüssen-Die-Riviere	0	0	14	1	15
Haemaphysalis silacea					
Willem Pretorius	0	0	0	8	8
R. (B.) microplus					
Bethal	0	3	1	3	7
O. megnini					
Tüssen-Die-Riviere	0	5	0	0	5
R. zambeziensis					
Thabazimbi	0	0	2	0	2
Total	475	1747	3346	1796	7364

Supplement material 2: Co-infections, constrained to location, of 12 out of 23 pathogen species observed in 4/8 infected tick species collected from 64 wild and 64 domestic ruminants.

Pathogens	*T.* sp. (sable)	*T. separata*	*T. bicornis*	*T. buffeli*	*T. taurotragi*	*T.* sp. (kudu)	*T. equi*	*T. annulata*	*B. occultans*	*B. caballi*	*A. bovis*	*E.* sp. (EU191229.1)	No. of infection/species†
T. sp. (sable)	/	**9***	**9***	6	0	0	0	0	0	0	0	0	38
T. separata	**9***	/	**9***	**9***	0	0	1	0	0	0	0	0	18
T. bicornis	**9***	**9***	/	**7***	0	0	0	0	0	0	1	0	27
T. buffeli	6	**9***	**7***	/	2	1	0	0	1	**2***	0	1	37
T. taurotragi	0	0	0	2	/	1	0	0	0	0	0	0	4
T. sp. (kudu)	0	0	0	1	1	/	0	0	0	0	0	0	1
T. equi	0	1	0	0	0	0	/	1	0	0	0	1	14
T. annulata	0	0	0	0	0	0	1	/	0	0	0	0	6
B. occultans	0	0	0	1	0	0	0	0	/	0	0	0	2
B. caballi	0	0	0	**2***	0	0	0	0	0	/	0	1	12
A. bovis	0	0	1	0	0	0	0	0	0	0	/	0	12
No. of infection/species†	38	18	27	37	4	1	14	6	2	12	12	23	194

*Significant associations (Bonferroni corrected *P* - values < 0.0001) computed trough random permutations (Raup & Crick, 1979; modified by Clua et al., 2010).

† Total no. of infections of the 12 pathogen species involved in co-infections (data shown in Table 2).

5 Discussion

In this study, blood samples from wild and domestic hosts as well as their associated tick species were screened for tick-borne pathogens. This was done in order to evaluate the exposure of these animals to tick-borne diseases and it allowed shedding more light on the pathogen exchange at the wildlife-livestock interface. A prerequisite to achieve this goal was to work with a broad variety of ungulate host species (n = 18). Indeed, it allowed clarifying the host range and vector range of numerous pathogen species (Appendix D). This gave a novel perspective of the circulation of some of these pathogens in nature involving wild and domestic animals. In addition, it enabled the description of associations between host and pathogen species using PCA. These associations revealed infection patterns among host groups defining transmission pathways between given host species (Appendix E), among and between domestic and wild ungulates. Both of these aspects contribute to a better understanding of the interplay existing between the pathogens and their wild and domestic hosts.

However, a dataset composed of a broad variety of host species (mainly wildlife species) has the disadvantage of being not homogenous. In fact, in the data set consisting of 64 wild and 64 domestic animals living in close vicinity, 11 wildlife species were compared with only two livestock species (cattle and sheep). An alternative way of sampling would have been to examine equal numbers of related wild and domestic species (e.g., cattle *vs* African buffalo and/or sheep and goats *vs* blue wildebeest and red hartebeest). This would have required an important amount of individuals per species to allow statistical analysis. Such a dataset would have given an accurate comparison of wild and domestic animals. But it would not have allowed describing as much new host-pathogen and vector-pathogen relations as presently achieved. Furthermore, the observation that transmission of the majority of tick-borne pathogens occurs mainly among animals belonging to defined groups would not have been possible or would not have benefit from enough different pathogen and host species for statistical analysis. In addition, practical aspects would have made an alternative sampling difficult, since sampling of wild animals depends on the activity of game capture teams, culling teams and hunters. Thus, in this context, host species and numbers of individuals per species could, usually, not been freely chosen as a function of our scientific interest. In some few cases it would have been, theoretically, possible to sample more individuals for a given wildlife species (e.g., African buffalo during game capture). In such cases, the main hindrance to an alternative sampling (requiring more individuals from a single species) would have been the time (e.g., high number of animals to examine in a very short amount of time).

The dependence to game capture teams, culling teams and hunters also highly influenced our choice and numbers of localities in which animals were sampled as well as the sampling months. Multiple sample sites (n = 9) as used in the present study have the advantage to increase host, pathogen and tick diversity. On the other hand, it complicates the statistical analysis since comparisons between wild and domestic animals must be restricted per location. Over the three sampling years, parts of summer, autumn and winter were covered (January to July), although sampling occurred mainly in autumn. This allowed increase the number of collected tick species. Indeed, sampling during end of summer allows collecting high numbers of adults belonging to species such as *A. hebraeum, H. m. rufipes* and *R. (B.) decoloratus*. While sampling into winter allowed collecting species such as *I. rubicundus* and *M. winthemi* which occur only at that time of the year.

Finally, the dependence to the former mentioned teams also influenced tick sampling on hosts. Indeed, sampling must not only be achieved in a short period of time during game capture but, usually, also when working with the culling team and hunters. Animals which had been shot needed to be quickly brought to the butchery, thus sampling was also limited in time during these activities. An exhaustive evaluation of the total tick burden, as done for example by Horak et al. (1992) where animal skins were examined, was not possible in the present study. Here, data gathered concerning infestation of wild animals must be seen as a qualitative and quantitative indication of the total tick burden. Similarly, tick sampling on livestock animals probably also does not reflect the total tick burden. It was done exclusively on live animals immobilised in holding facilities (cattle) or by hand which was the case for sheep. Given that tick sampling was undertaken in the same manner on wild and domestic animals, this allows comparison in infestation observed between wild and domestic hosts and should reflect the situation. However, our interest was given more to host and tick infections, than host infestation. Indications on tick burdens are mentioned as additional information to allow a better understanding of the difference in tick infection between ticks attached to wild animals and ticks attached to domestic animals.

We chose to store the blood samples on FTA Classic Cards rather than to keep the blood in EDTA tubes. FTA cards present the advantage to protect the nucleic acids from oxidation, UV damage and microbial attack during the sampling campaign and did not require to be kept frozen. Furthermore, infectious pathogens were inactivated upon contact with the FTA matrix allowing safe shipment of the samples to Switzerland.

The location of the pathogens in the adult tick provides different information. a) Pathogens located in the tick midgut have been acquired during the blood meal taken on the host on which the tick is collected and will not be transmitted to the host during the same life stage. b) Pathogens located in salivary glands have been acquired during a former blood meal, taken by a different life stage and will, usually, be transmitted to the individual host on which the tick was collected. In the present study we chose to detect pathogen DNA from the tick salivary glands in order to provide information on exposure to various pathogen species of both wild and domestic ungulates. Different situations must be distinguished depending on the life cycle of the tick species. In one-host tick species (e.g., *R. (B.) decoloratus*) the individual host on which the tick was attached can be the source of the infection identified in the salivary glands. Unlike, in two and three-host tick species the individual host on which the tick was attached cannot be the source of the infection identified in the salivary glands. In fact, adults feed on a different individual host or even on a different host species as the immature stages. In the two-host tick species *R. e. evertsi* and the three-host tick species *R. appendiculatus* and *R. zambeziensis* the hosts of the adults might belong to the same species than the host of the immature stages. Whereas, in tick species like *R. gertrudae* and *H. m. rufipes* the host of the adults belong to a different host species as the host of the immature stages. The latter use small rodents (*R. gertrudae*), hares and ground-frequenting birds (*H. m. rufipes*) as hosts but will normally not be found on large ruminant species like the adults. In our context, no direct correlations (except for the one-host tick species *R. (B.) decoloratus*) can be made between the infections detected in host blood and those detected in salivary glands of ticks collected on the same host. It would have been possible to roughly estimate the capacity of the ticks to acquire novel infections from their individual hosts by analysing the tick midgut separately, in addition to the salivary glands. Such an approach would have allowed providing more information on the direct pathogen exchange occurring at the host-vector interface. Unfortunately, due to lack of time we chose to analyse the salivary glands only.

The RLB methodology was chosen for tick-borne pathogen detection in host blood and tick salivary glands. This technique was conceived for the detection of *Ehrlichia* (Schouls et al., 1999), *Babesia, Theileria* (Gubbels et al., 1999) and *Anaplasma* species (Georges et al., 2001) more than ten years ago. RLB has the advantage to test simultaneously up to 45 samples for a maximum of 45 different pathogen species in a single assay, while keeping a high sensitivity level and being relatively cost effective. Gubbels et al. (1999) determined that their RLB assay was able to detect a parasitemia level of 10^{-3}%. Its broad detection range makes RLB very useful in epidemiological surveys of tick borne diseases compared to alternative techniques. Indeed, other standard molecular techniques able to detect multiple tick-borne pathogen species such as multiplex PCR (Figueroa et al., 1993; Sirigireddy and Ganta, 2005; Lew and Jorgensen, 2005; Iseki et al., 2007), microarrays (Houck et al., 2011) and PCR-RFLP (Caccio et al., 2000) are less efficient than RLB in regards to the number of species and samples which can be detected in a single assay. Using multiplex PCR, three pathogen species (*B. bigemina, B. bovis* and *A. marginale*) were detected simultaneously (Lew and Jorgensen, 2005), up to three *Borrelia* genospecies with low-density microarrays (Houck et al., 2011) and nine piroplasm species with PCR-RFLP (Caccio et al., 2000). According to Houck et al. (2011) microarrays have, theoretically, the potential to detect much more than three tick-borne pathogen species in a single assay. However, according to these authors, the sensitivity of this approach would be a great limitation. Nevertheless, in the last few years new sequencing technologies able to identify the entire microorganism load present in a given environment (e.g., soils, blood samples, vectors) were developed (Liu et al., 2012). From a technical point of view such next-generation sequencing systems could have been an excellent alternative to the RLB technique. The first commercially successful next generation sequencing system is the 454 system from Roche (Liu et al., 2012). Its first version appeared in 2005, and was further developed until 2009, which is still the current version. In this methodology each different sequence is individually amplified (clonal amplification) and read by the sequencer (http://www.roche.com/mrt_091120_mcleod.pdf).

Such a technique would have allowed identifying each tick-borne pathogen to species level without depending on specific RLB-probes. This would have been a great advantage in a field study since it is difficult to evaluate which pathogen species will be present in host blood and ticks. While in RLB assays pathogen species for which no specific probes are available can only be identified to genus level. But, in 2009 at the beginning of the present study, the extremely high cost (Liu et al., 2012) of the brand-new 454 sequencing technology would have been a hindrance to its use.

In the present study, the RLB methodology followed by Sanger cycle sequencing (for samples which reacted with genus-specific probes only) allowed identifying 392 infections assigned to 25 pathogen species in host blood and tick salivary glands. The majority were *Theileria* species (*n* = 12) followed by *Babesia* species (*n* = 5), *Anaplasma* species (*n* = 5) and *Ehrlichia* species (*n* = 3). Among the infections identified to species level, 163 (77.6%) (16 pathogen species) were detected in host blood and 229 (57.7%) (23 pathogen species) in tick salivary glands. The majority of the species detected in host blood and ticks were identified by hybridisation with RLB-probes. However, 53 infections (identified in tick salivary glands and host blood) assigned to seven species were identified through sequencing: *E.* sp. (EU191229.1), *E. ovina, Theileria* sp. (giraffe), *A. platys, A. bovis, A. ovis* and *A. marginale*. The last three species did not react with their specific oligonucleotide probes included in the assay. For *A. ovis* and *A. marginale* absence of hybridisation was due to the forward position of the probes on the amplified sequence, while for *A. bovis* it was due to one base pair difference on the amplified sequences. In addition, 215 infections could only be identified to the genus level, 168 (42.3%) in tick salivary glands and 47 (22.4%) in host blood. These infections could not be identified to species level even after sequencing. Discussions with the Microsynth technical service had revealed that it is most probably due to multiple pathogen species present in the samples. This does not allow a proper read of the sequencer, given that all sequences were amplified with the same primer set and thus have the same lengths. This also means that they could not be separated by electrophoresis prior sequencing. In fact, *Ehrlichia* and *Anaplasma* species were amplified with the same primer set designed by Schouls et al. (1999) and modified by Bekker et al. (2002). Similarly, *Babesia* and *Theileria* species were amplified together with the primer set from Georges et al. (2001). Specific primer sets able to amplify the four genera separately would have allowed sequencing the obtained PCR products separately. A literature review of such specific primer sets has revealed that most of them amplify *Anaplasma* and *Ehrlichia* species simultaneously (Liz et al., 2002; Kawahara et al., 2006; Lobanov et al., 2011) or *Babesia* and *Theileria* species simultaneously (Kjemtrup et al., 2000a; Kjemtrup et al., 2000b; Luo et al., 2005; Bhoora et al., 2009; Oosthuizen et al., 2009). This was confirmed by using the primer BLAST on the NCBI international data bank. Indeed, over the last decades these four genera have undergone major reorganisations in which *Babesia* species were reclassified as *Theileria* species, *Ehrlichia* species as *Anaplasma* species and vice-versa (Mehlhorn and Schein, 1998; Dumler et al., 2001; Schnittger et al., 2012). This attests their close phylogenetic relations and explains the difficulties to amplify them separately. Other primer sets are able to amplify a selection of given species from a given genera. As for example the primer set from Hilpertshauser et al. (2006) targeting various *Babesia* species and the one from Casati et al. (2006) targeting *Babesia* species occurring in Switzerland. These primer sets were virtually tested with the use of the primer BLAST on the NCBI international data bank. Their amplification range appeared to be too narrow for the detection of the target African species and not specific enough since *Theileria* species occurring in South Africa would also have been amplified. Attempts to design new primer sets able to amplify the four genera separately failed. This is most probably due to their close phylogenetic relations as mentioned above. Cloning each sample, which had reacted with a genus specific probe only, would have been an alternative. But given that 215 samples were concerned, it would have been beyond the financial resources of this project.

Cross-reactivity between species-specific oligonucleotide probes in RLB assays had been reported in some studies. For example, Nagore et al. (2004) reported that the T. lestoquardi and the T. annulata probes can cross-react and Zeng et al. (2007) reported cross reactivity between RLB specific-probes used to detect fungal species. In the present study cross-reactivity was virtually tested using BLAST, which should exclude cross-reactions. However, the reported cross-reactivity between the T. lestoquardi and T. annulata probes, two species which are not known to occur in South Africa, must be considered. Indeed, *T. annulata* was detected in this study whether it was confused with an unknown *Theileria* species or with *T.*

lestoquardi cannot be excluded even if *T. lestoquardi* is theoretically not supposed to occur in South Africa.

Among the 26 pathogens identified to species level, 13 were common to both, host blood and tick salivary glands: *B. bigemina, T. bicornis, T. ovis, T. equi, T. separata, Theileria* sp. (sable), *T. buffeli, T. taurotragi, T. mutans, A. centrale, A. marginale, A. ovis* and *A. platys*. All of them, except *A. ovis*, were associated to previously unknown host species and/or unknown vector species. This allowed clarifying host and vector ranges of these pathogen species. These new host-pathogen combinations allowed studying associations between host and pathogen species, this defined transmission pathways between given host species, among and between wild and domestic animals. In addition, observations of co-infections patterns described in host blood and tick salivary glands revealed that 77.9% and 44.7% of the infections in host blood and ticks, respectively, involved more than one pathogen species. Finally, new vector-pathogen combinations contributed to estimate the importance of a given tick species for the transmission of a given pathogen species. All these aspects shed new light on the circulation of these pathogens in nature at the interface of wild and domestic ungulates.

For example, *Theileria* sp. (sable), the most frequently detected species in host blood and in tick salivary glands, was identified in 11 host species and three tick species. Its previously unknown relations with sheep (Caprinae), black wildebeest (Alcelaphinae), springbok (Antilopinae) and gemsbok (Hippotraginae) together with previously known host relations with red hartebeest (Spitalska et al., 2005), blue wildebeest, blesbok (Alcelaphinae) (Steyl et al., 2012), sable antelope and roan antelope (Hippotraginae) (Nijhof et al., 2005) allowed the assumption that *Theileria* sp. (sable) mainly circulates among these closely related host species. This stands in agreement with Yin et al. (2007) observations showing that *Theileria* sp. (sable) is closely related to *T. separata* a parasite known to infect domestic Caprinae. The PCA model showed that *Theileria* sp. (sable) is significantly associated to *T. separata, T. bicornis, T. ovis* and *A. ovis* and that these five pathogens are significantly associated to the same phylogenetically related hosts. Interestingly, *Theileria* sp. (sable) was observed in significant co-infections with *T. separata* and *T. bicornis* in both host blood and in the salivary glands of its vector *R. e. evertsi*. In addition to its known vectors *R. e. evertsi* and *R.*

appendiculatus (Steyl et al., 2012) *Theileria* sp. (sable) was isolated from the salivary glands of *R. (B.) decoloratus* and *A. hebraeum* which contributes to explain its very broad host range. The fact that 63% of the observed *Theileria* sp. (sable) records, from salivary glands, were observed in *R. e. evertsi* suggests that this tick plays an important role in the transmission of this parasite in South Africa. Considering these different observations together, *Theileria* sp. (sable) which was seen as a specific parasite of sable and roan antelopes, a few years ago, appears now under a different light. Indeed, *Theileria* sp. (sable) displays a very broad host range, mainly among sheep related host species and is transmitted by at least four tick species. Thus, in terms of exchange at the livestock-wildlife interface this pathogen species mainly concerns sheep and wild members of the Alcelaphinae, Hippotraginae and Antilopinae. Whether sheep can suffer from *Theileria* sp. (sable) infections remains unknown. *Theileria* sp. (sable) seems to be composed of different genotypes as observed by Mans et al. (2011) explaining the few observations of this parasite in non Caprinae-related host species. This could indicate that speciation is still in process.

As discussed above a direct correlation between the infections detected in host blood and those detected in tick salivary glands cannot be established for tick species displaying a two or three-host life cycle. Inversely, pathogen species, without transovarial transmission, detected in salivary glands of ticks displaying a one-host life cycle should have been acquired on the individual host on which the tick was attached. Indeed, for the one-host tick *R. (B.) decoloratus*, the majority of the observed infections were acquired on the individual host on which the tick was collected. Among the 56 infections identified to species level in *R. (B.) decoloratus* salivary glands 22 infections belonged to *B. caballi, T. annulata, Theileria* sp. (giraffe), *A. bovis, E.* sp. (EU191229.1) and *E. ovina*. These species were not detected in the blood of the examined hosts in the present study, hence they will not be considered further. Among the remaining 34 infections nine were recorded from the same host species on which *R. (B.) decoloratus* was attached and 22 from the same individual-host (data not shown). Two records which were not recorded from the same host species were *B. bigemina* infections and thus could have been acquired on a former host since transovarial transmission occurs. Infections which were not acquired on the individual host on which the tick was collected must have been acquired elsewhere. Chevillon et al. (2007) showed that *R.*

(B.) microplus ticks probably move among cows within a herd during their parasitic life time despite the fact that *R. (B.) microplus* displays a one-host life cycle.

When we compared pathogen load in ticks living in the environment of wild and domestic animals, we observed that salivary glands of ticks attached to wild animals had significantly higher infection prevalences and pathogen mean densities than those attached to domestic animals. This means that wild animals were more exposed to tick borne-pathogens than livestock animals. When we compared pathogen load in the blood of wild and domestic ungulates living in close vicinity we observed no significant differences between animal types (wild *vs* domestic), despite the fact that wild animals were more exposed to tick-borne pathogens. This clearly demonstrates that wild ungulates are more refractory to tick-borne pathogens than domestic ones.

Future studies on the circulation of tick-borne pathogens between game and livestock animals should include: a) Descriptions of the different genotypes of pathogen species like *Theileria* sp. (sable), *T. buffeli, T. bicornis, Ehrlichia* sp. (Omatjenne), *A. platys*, *A. marginale* and *A. centrale* in order to understand their occurrence in different non related host groups. b) Transmission experiments to confirm the vector status of the ticks presently involved in new tick-pathogen combinations. c) Analysis of the tick midgut for pathogens, in addition to salivary glands, for a better understanding of the pathogen exchange at the host vector interface.

6 Conclusion

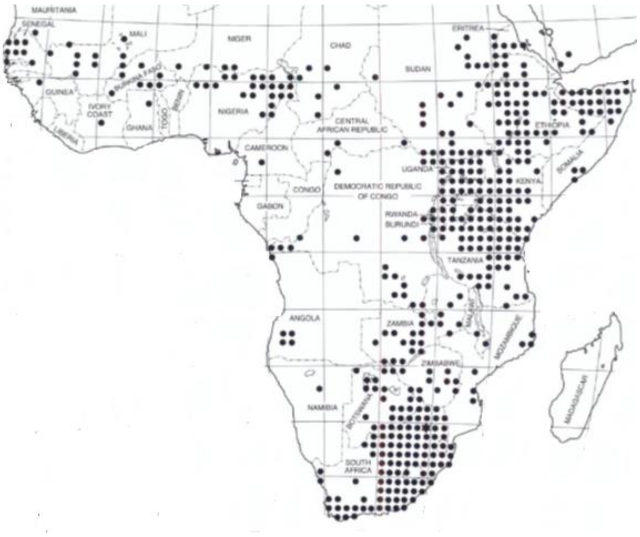
To conclude, this study showed that livestock and game animals are infected by the same pathogen species. Infection prevalence and intensity of infection displayed similar non-significant values between animal types (wild and domestic) despite that game animals were more exposed to tick-borne pathogens and thus appeared more refractory to infections. However, pathogen species were not homogeneously distributed among host species. This led to the description of infection patterns defining genetically-related host groups. These host groups represent the most privileged transmission routes of tick-borne pathogen species among ungulates species.

Furthermore, this study highlights a broader host range for ten pathogen species which were involved in 30 new host-pathogen combinations. Similarly, a broader vector range is described for 14 pathogen species which were involved in 23 new vector-pathogen combinations. Among those, recently described pathogens like *T. bicornis, Theileria* sp. (giraffe), *Theileria* sp. (kudu) and *Babesia* sp. (sable) are described for the first time in ticks. The tick species *R. e. evertsi* and *R. (B.) decoloratus* are mainly involved in these new vector-pathogen combinations and thus appear as important vectors in South Africa. The observation that the most frequent significant co-infection patterns were the same in host blood and tick salivary glands suggests that the associated species take advantage of their reciprocal presence. These different aspects involving host-pathogen, vector-pathogen and pathogen-pathogen relations shed new light on the circulation of these tick-borne pathogens naturally achieved in nature.

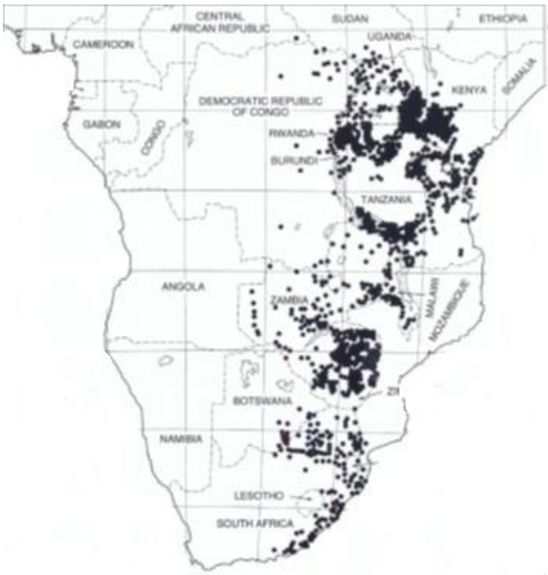
Results from this study can lead to practical applications in game management and in tick-borne disease diagnostic in game and livestock animals. In fact, our observation suggesting that transmission of tick-borne pathogen species remains mainly restricted to genetically-related host-species (Appendix E) allows to evaluate the transmission risk between given wildlife communities and given livestock animals. Such knowledge is important, especially in the southern African context where formerly established game-proof fences are currently removed for the development of Transfrontier Conservation Areas aiming at connecting different National Parks. This leads to more contacts between wild and domestic ungulates (Ferguson and Hanks, 2012) and requires a mixed economy which associates wildlife and agriculture in the concerned areas. Furthermore, this knowledge can also find applications on farms where game and livestock animals are bred in close vicinity, which is quite common in southern Africa. Indeed, game and livestock animals can be chosen in function of their phylogenetic status in order to reduce tick-borne pathogen transmission between game and livestock animals. Finally, the new pathogen-host and pathogen-vector relations (Appendix D) make the diagnostic of disease outbreaks caused by novel tick-borne pathogens easier.

7 Appendix

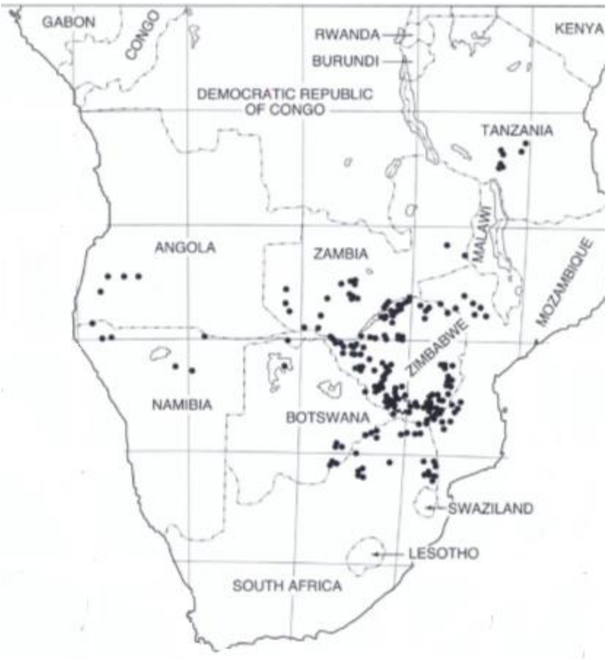
7.1 Appendix A: Geographic distribution of tick species



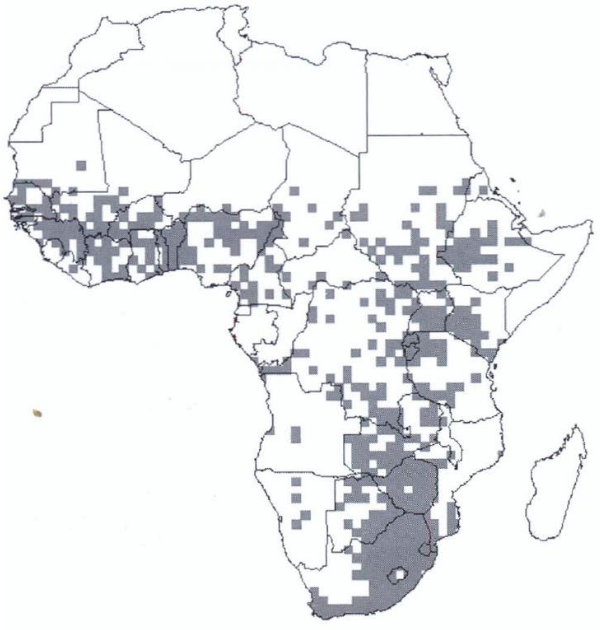
A1: Distribution of *Rhipicephalus evertsi evertsi* (Walker et al., 2000).



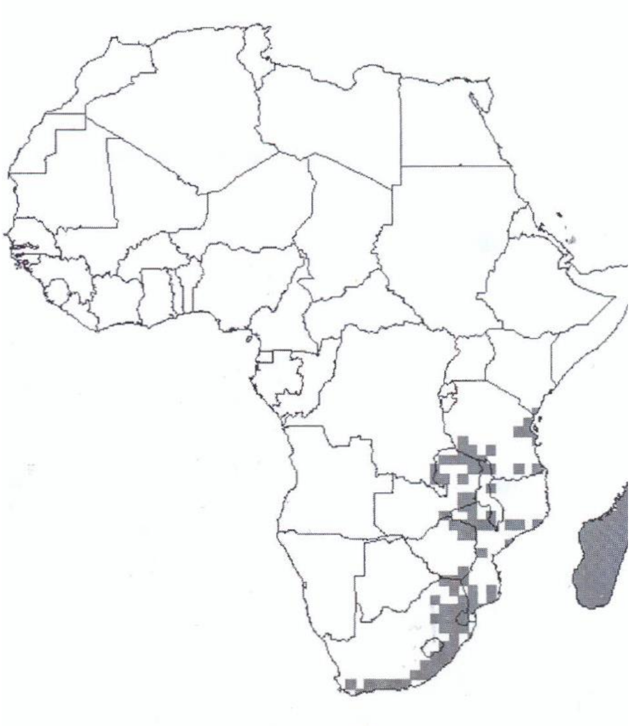
A2: Distribution of *Rhipicephalus appendiculatus* (Walker et al., 2000).

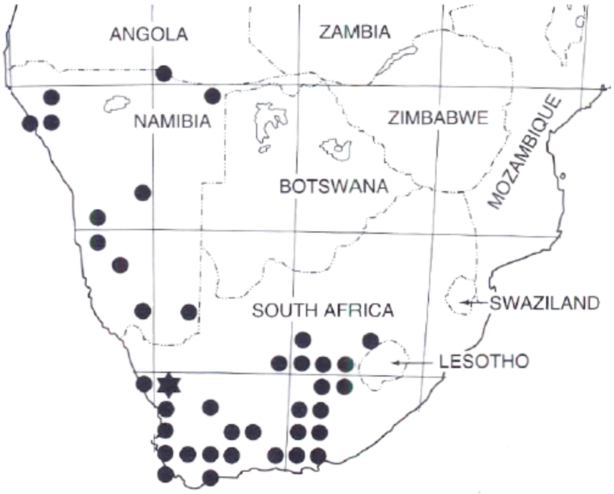


A3: Distribution of *Rhipicephalus zambeziensis* (Walker et al., 2000).



A4: Distribution of *Rhipicephalus (Boophilus) decoloratus* (Walker et al., 2003).



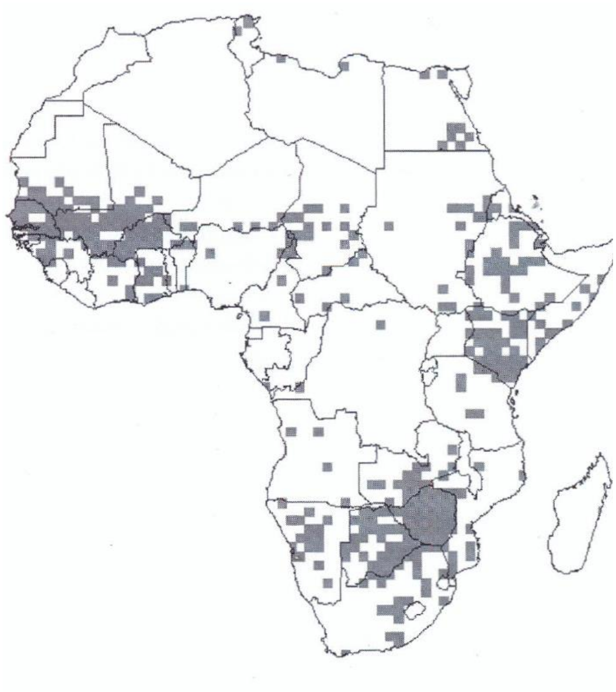


A5: Distribution of *Rhipicephalus (Boophilus) microplus* (Walker et al., 2003).

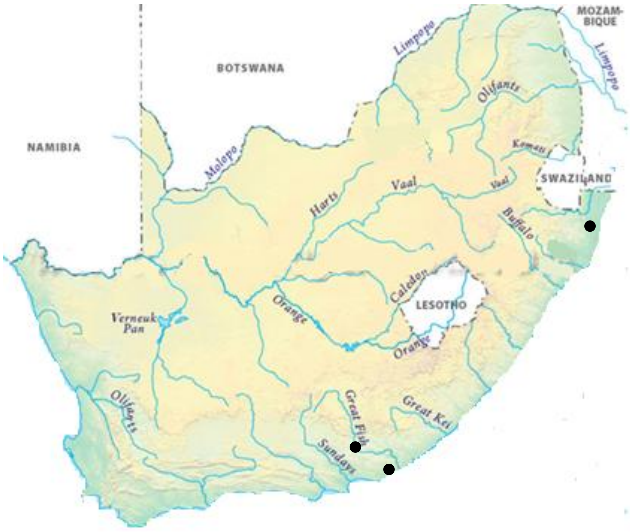
A6: Distribution of *Rhipicephalus gertrudae* (Walker et al., 2000).

A7: Distribution of *Rhipicephalus warburtoni* (Walker et al.,2000).

A8: Distribution of *Amblyomma hebraeum* (Walker et al., 2003).



A9: Distribution of *Hyalomma marginatum rufipes* (Walker et al., 2003).



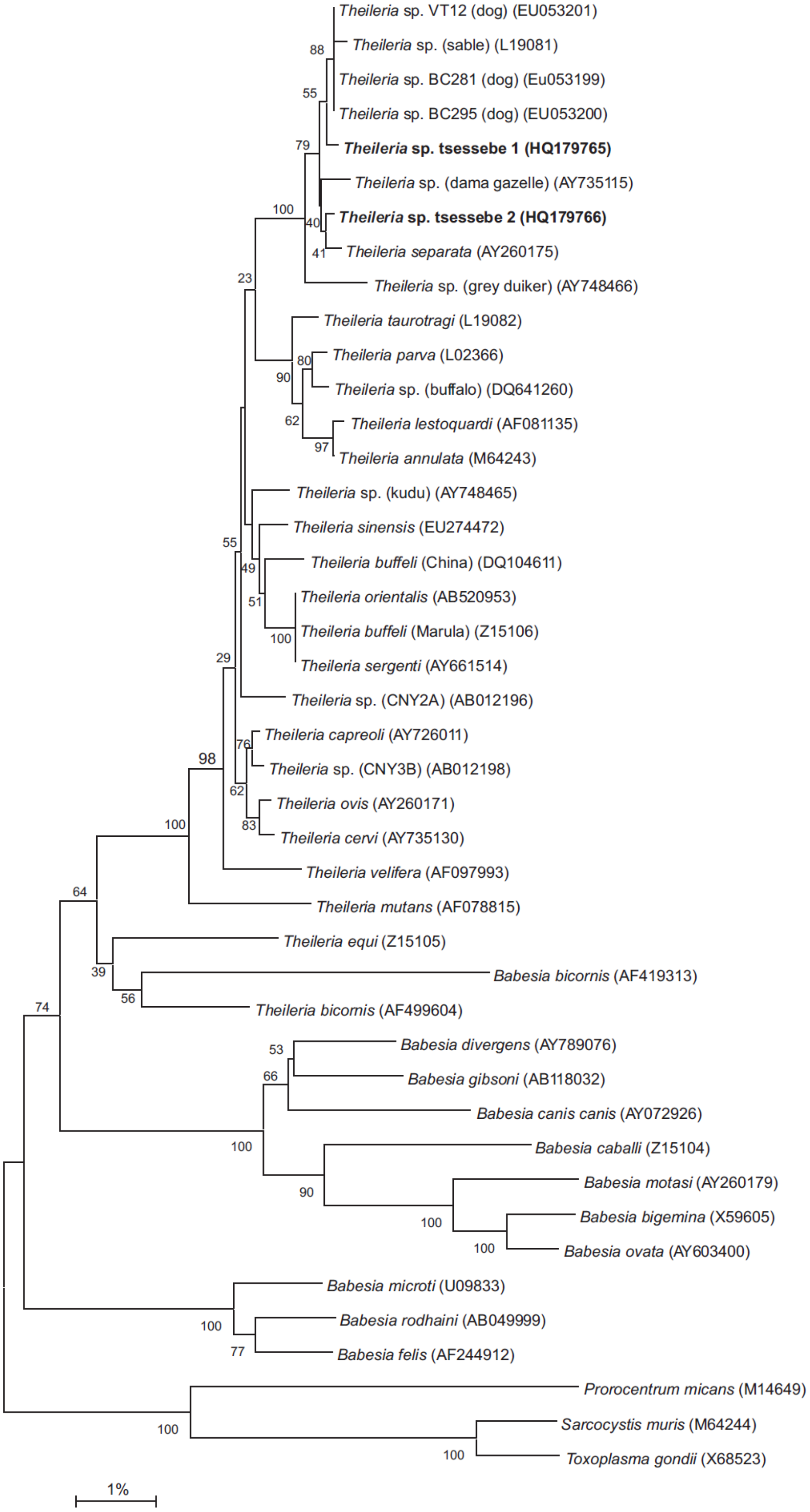
A10: Distribution of *Haemaphysalis silacea* (Horak et al., 2007) (http://www.mapsofworld.com/south-africa/river-map.html. Modified).

A11: Distribution of *Ixodes rubicundus* (Walker et al., 2003).

A12: Distribution of *Margaropus winthemi* (Walker et al., 2003).

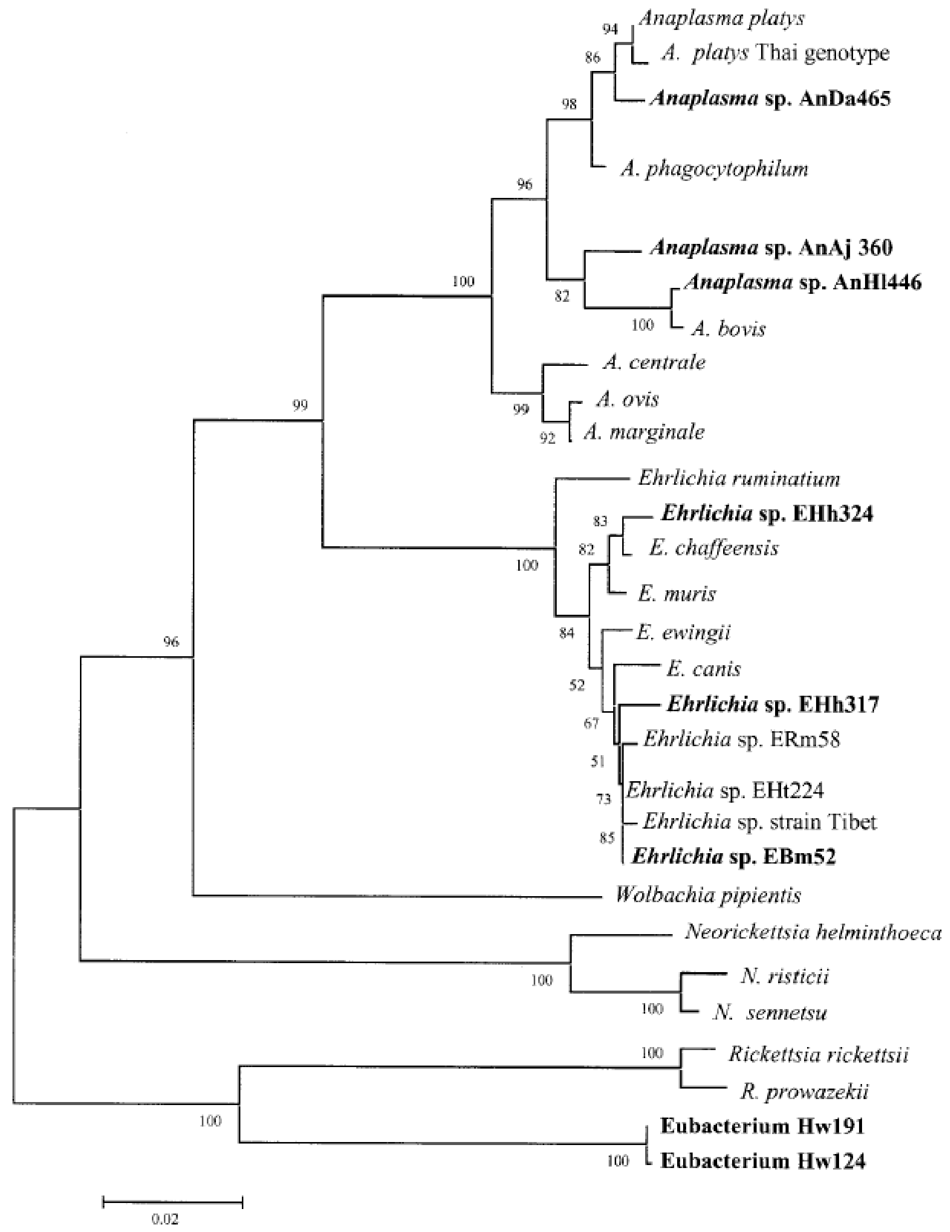
A13: Distribution of *Otobius megnini*
(Walker et al., 2003).

7.2 Appendix B: Phylogenetic relationships of *Babesia* and *Theileria* spp.



From Brothers et al. (2011)

7.3 Appendix C: Phylogenetic relationships of *Anaplasma* and *Ehrlichia* spp.

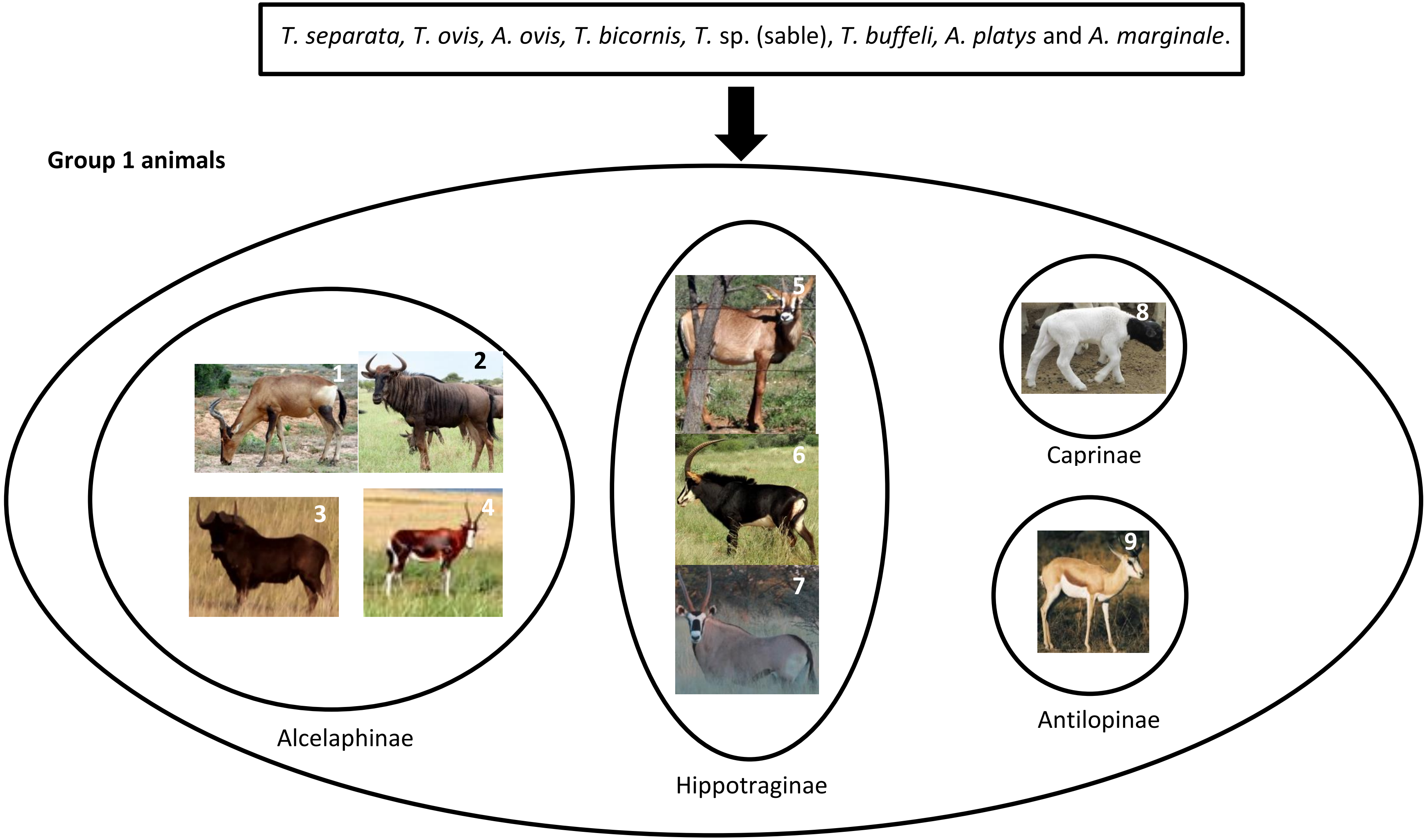


From Parola et al. (2003)

7.4 Appendix D: Novel pathogen-host and pathogen-vector combinations.

New pathogen-host relations (*n* = 30)	Pathogen species	New pathogen-vector relations (*n* = 23)
Sheep, sable antelope, roan antelope, black wildebeest, impala	***T. bicornis***	*R. evertsi evertsi, R. appendiculatus*
Sheep, black wildebeest, springbok, gemsbok, impala	***T.* sp. (sable)**	*R. (B.) decoloratus, A. hebraeum*
Sable antelope, roan antelope, black wildebeest, blue wildebeest, kudu, impala	***T. buffeli***	*R. (B.) decoloratus, R. evertsi evertsi, R. appendiculatus, A. hebraeum*
Impala	***A. centrale***	*R. gertrudae*
Kudu	***A. marginale***	*R. gertrudae*
Sheep	***A. platys***	*R. evertsi evertsi*
Sable antelope, black wildebeest, blue wildebeest, springbok, red hartebeest	***T. separata***	
Sable antelope, blue wildebeest, blesbok	***T. ovis***	
Kudu	***B. bigemina***	
Roan antelope, giraffe	***E.* sp. (Omatjenne)**	
	B. caballi	*R. (B.) decoloratus*
	***B.* sp. (sable)**	*H. m. rufipes*
	T. taurotragi	*H. m. rufipes*
	***T.* sp. (kudu)**	*R. appendiculatus*
	T. mutans	*R. evertsi evertsi*
	***T.* sp. (giraffe)**	*R. (B.) decoloratus, R. evertsi evertsi*
	A. bovis	*R. (B.) decoloratus, R. evertsi evertsi*
	E. ovina	*R. (B.) decoloratus, H. m. rufipes, R. gertrudae*

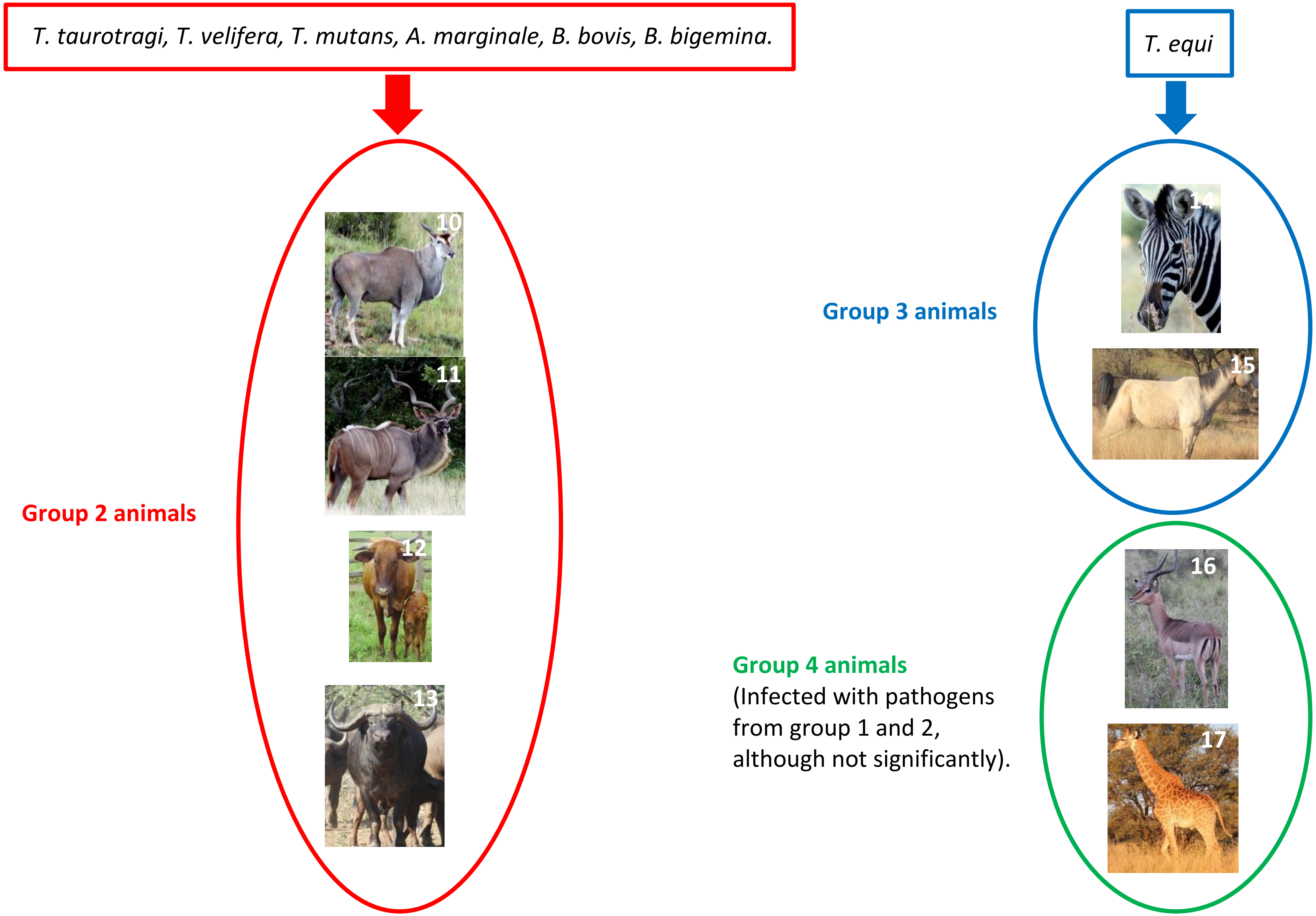
7.5 Appendix E: Exposure of host species to pathogen species.



T. separata, T. ovis, A. ovis, T. bicornis, T. sp. (sable), *T. buffeli, A. platys* and *A. marginale*.

Group 1 animals

1, Red hartebeest; 2, blue wildebeest; 3, black wildebeest; 4, blesbok; 5, roan antelope; 6, sable antelope; 7, gemsbok; 8, sheep; 9, springbok.



T. taurotragi, T. velifera, T. mutans, A. marginale, B. bovis, B. bigemina.

T. equi

Group 2 animals

Group 3 animals

Group 4 animals
(Infected with pathogens
from group 1 and 2,
although not significantly).

10, Common eland; 11, greater kudu; 12, cattle; 13, African buffalo; 14, Plain zebra; 15, horse; 16, impala, 17, southern giraffe.

8 References

Aktas, M., Altay, K., Dumanli, N., Kalkan, A. (2009). Molecular detection and identification of *Ehrlichia* and *Anaplasma* species in ixodid ticks. Parasitology research **104:** 1243-1248.

Aktas, M., Altay, K., Dumanli, N. (2011). Molecular detection and identification of *Anaplasma* and *Ehrlichia* species in cattle from Turkey. Ticks and Tick-Borne Diseases **2:** 62-65.

Aktas, M., Altay, K., Ozubek, S., Dumanli, N. (2012). A survey of Ixodid ticks feeding on cattle and prevalence of tick-borne pathogens in the Black Sea region of Turkey. Veterinary Parasitology **187:** 567-571.

Allsopp, M.T.E.P., Cavalier-Smith, T., De Waal, D.T., Allsopp, B.A. (1994). Phylogeny and evolution of the piroplasms. Parasitology **108:** 147-152.

Allsopp, M.T.E.P., Visser, E.S., du Plessis, J.L., Vogel, S.W., Allsopp, B.A. (1997). Different organisms associated with heartwater as shown by analysis of 16S ribosomal RNA gene sequences. Veterinary Parasitology **71:** 283-300.

Allsopp, M.T.E.P., Theron, J., Coetzee, M.L., Dunsterville, M.T., Allsopp, B.A. (1999). The occurrence of *Theileria* and *Cowdria* parasites in African buffalo (*Syncerus caffer*) and their associated *Amblyomma hebraeum* ticks. Onderstepoort Journal of Veterinary Research **66:** 245-249.

Allsopp, M.T.E. and Allsopp, B.A. (2001). Novel *Ehrlichia* genotype detected in dogs in South Africa. Journal of Clinical Microbiology **39:** 4204-4207.

Alsaad, K.M., Hassan, S.D., Al-Obaidi, Q.T. (2012). Detection of *Babesia equi* and *Babesia caballi* antibodies in horses and donkeys in Mosul, Iraq. Research Opinion in Animal and Veterinary Sciences **2:** 291-294.

Altay, K., Dumanli, N., Holman, P.J., Aktas, M. (2005). Detection of *Theileria ovis* in naturally infected sheep by nested PCR. Veterinary Parasitology **127:** 99-104.

Apanaskevich, D.A. and Horak, I.G. (2008). Two new species of African *Haemaphysalis* Ticks (Acari: Ixodidae), Carnivore Parasites of the *H. (Rhipistoma) leachi* Group. Journal for Parasitology **94:** 594-607.

Barker, S.C. and Murrell, A. (2004). Systematics and evolution of ticks with a list of valid genus and species names. Parasitology **129:** 15-36.

Basson, P.A., McCully, R.M., Kruger, S.P., Van Niekerk, J.W., Young, E., De Vos, V. (1970). Parasitic and other diseases of the African buffalo in the Kruger National Park. Onderstepoort Journal of Veterinary Research **37:** 11-28.

Bekker, C.P.J., Vink, D., Lopes Pereira, C.M., Wapenaar, W., Langa, A., Jongejan, F. (2001). Heartwater (Cowdria ruminantium infection) as a cause of postrestocking mortality of goats in Mozambique. Clinical and Diagnostic Laboratory Immunology **8:** 843-846.

Bekker, C.P.J., de Vos, S., Taoufik, A., Sparagano, O.A.E., Jongejan, F. (2002). Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminatium* in *Amblyomma variegatum* ticks by reverse line blot hybridisation. Veterinary Microbiology **89:** 223-238.

Bell-Sakyi, L., Koneya, B.M., Dogbeya, O., Walker, A.R. (2004). Incidence and prevalence of tick-borne haemoparasites in domestic ruminants in Ghana. Veterinary Parasitology **124:** 25-42.

Bengis, R.G. and Erasmus, J.M. (1988). Wildlife diseases in South Africa: A review. Revue Scientifique et technique de l'Office International des Epizooties **7:** 807-821.

Berger, J. (1979). *Theileria velifera* demonstrated in cattle in the Eastern Cape Province of the Republic of South Africa. Journal of the South African Veterinary Association **50:** 45-46.

Bevan, L.E.W. (1912). Anaplasmosis of sheep. Veterinary Journal **68:** 400-401.

Bezuidenhout, J.D., Prozesky, L., du Plessis, J.L., van Amstel, S.R. (1994). Heartwater. . *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 351-370.

Bhoora, R., Franssen, L., Oosthuizen, M.C., Guthrie, A.J., Zweygarth, E., Penzhorn, B.L., Jongejan, F., Collins, N.E. (2009). Sequence heterogeneity in the 18S rRNA gene within Theileria equi and Babesia caballi from horses in South Africa. Veterinary Parasitology **159:** 112-120.

Bigalke, R.D., Keep, M.E., Keep, P.J., Schoeman, J.H. (1970). A large *Babesia* sp. and a *Theileria*-like Piroplasm of the square-lipped rhinoceros. Journal of the South African Veterinary and Medical Association **41:** 292-294.

Bigalke, R.D., De Vos, A.J., Barrowman, P.R. (1976). The control of some tick-borne diseases in South Africa. Bulletin de l'Office International des Epizooties **86:** 89-100.

Bigalke, R.D. (1994). The important role of wildlife in the occurrence of livestock diseases in southern Africa. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 152-163.

Binta, M.G., Losho, T., Allsopp, B.A., Mushi, E.Z. (1998). Isolation of *Theileria taurotragi* and *Theileria mutans* from cattle in Botswana. Veterinary Parasitology **77:** 83-91.

Bishop, R., Musoke, A., Morzaria, S., Gardner, M., Nene, V. (2004). Theileria: intracellular protozoan parasites of wild and domestic ruminants transmitted by Ixodid ticks. Parasitology **129:** 271-283.

Bock, R., Jackson, L., De Vos, A., Jorgensen, W. (2004). Babesiosis of cattle. Parasitology **129:** 247-269.

Brain, C. and Bohrmann, R. (1992). Tick infestation of baboons (*Papio ursinus*) in the Namib desert. Journal of Wildlife Diseases **28:** 188-91.

Bredenkamp, G. and van Rooyen, N. (1998a). Moist Cool Highveld Grassland. In: Vegetation of South Africa, Lesotho and Swaziland. Eds. Low, A.B. & Rebelo, A.G. Pretoria: Department of Environmental Affairs and Tourism.

Bredenkamp, G. and van Rooyen, N. (1998b). Moist Clay Highveld Grassland. In: Vegetation of South Africa, Lesotho and Swaziland. Eds. Low, A.B. & Rebelo, A.G. Pretoria: Department of Environmental Affairs and Tourism.

Brocklesby, D.W., Vidler, B.O. (1965). Some parasites of East African wild animals. East African Wildlife Journal **3:** 120-122.

Brocklesby, D.W., Vidler, B.O. (1966). Haematozoa found in wild members of the order Artiodactyla in East Africa. Bulletin of Epizootic Diseases of Africa **14:** 285-299.

Brocklesby, D.W. (1967). A *Babesia* species of the black rhinoceros. The Veterinary Record **80:** 484.

Brothers, P.S., Collins, N.E., Oosthuizen, M.C., Bhoora, R., Troskie, M., Penzhorn, B.L. (2011). Occurrence of blood-borne tick-transmitted parasites in common tsessebe *(Damaliscus lunatus)* antelope in Northern Cape Province, South Africa. Veterinary Parasitology **183:** 160-165.

Büscher, G. (1988). The infection of various tick species with *Babesia bigemina*, its transmission and identification. Parasitology Research **74:** 324-30.

Butler, C.M., Nijhof, A.M., Jongejan, F., van der Kolk. (2008). *Anaplasma phagocytophilum* infection in horses in the Netherlands. The Veterinary Record **162:** 216-218.

Caccio, S., Camma, C., Onuma, M., Severini, C. (2000). The β-tubulin gene of *Babesia* and *Theileria* parasites is an informative marker for species discrimination. International Journal for Parasitology **30:** 1181-1185.

Carmichael, I.H., Hobday, E. (1975). Blood parasites of some wild Bovidae in Botswana. Onderstepoort Journal for Veterinary Research **42:** 55-62.

Casati, S., Sager, H., Gern, L., Piffaretti, J.C. (2006). Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. Annals of Agricultural and Environmental Medicine **13:** 65-75.

Chevillon, C., Kofi, B. B., Barré, N., Durand, P., Arnathau, C., de Meeûs, T. (2007). Direct and indirect inferences on parasite mating and gene transmission patterns pangamy in the cattle tick *Rhipicephalus (Boophilus) microplus*. Infection, Genetics and Evolution **7:** 298-304.

Chochlakis, D., Ioannou, I., Sharif, L., Kokkini, S., Hristophi, N., Dimitriou, T., Tselentis, Y., Psaroulaki, A. (2009). Prevalence of Anaplasma sp. in goats and sheep in Cyprus. Vector-Borne and Zoonotic Diseases **9:** 457-463.

Cossio-Bayugar, R., Pillars, R., Schlater, J., Holman, P.J. (2002). *Theileria buffeli* infection of a Michigan cow confirmed by small subunit ribosomal RNA gene analysis. Veterinary Parasitology **105:** 105-110.

De Kock, G., Van Heerden, C.J., Du Toit, R., Neitz, W.O. (1937). Bovine theileriosis in South Africa with special reference to *Theileria mutans.* Onderstepoort Journal of Veterinary Science and Animal Industry **8:** 9-125.

De Matos, C. (2008). Species composition and geographic distribution of ticks infesting cattle, goats and dogs in Maputo Province, Mozambique. M. SC. Thesis, University of Pretoria.

De Vos, A.J., (1981). *Rhipicephalus appendiculatus*: cause and vector of diseases in Africa. Journal of the South African Veterinary Association **52:** 315-22.

De Vos A.J. and Roos, J.A. (1981a). The isolation of *Theileria taurotragi* in South Africa. Onderstepoort Journal of Veterinary Research **48:** 149-153.

De Vos, A.J. and Roos, J.A. (1981b). Observation on the transmission of *Theileria mutans* in South Africa. Onderstepoort Journal of Veterinary Research **48:** 1-6.

De Vos, A., Potgieter, F.T., de Waal, D.T., van Heerden, J. (1994). Babesiosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, p. 277.

De Vos, A.J., Potgieter, F.T. (1994). Bovine babesiosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 278-294.

De Waal, D.T., Potgieter, F.T. (1987). The transstadial transmission of *Babesia caballi* by *Rhipicephalus evertsi evertsi*. Onderstepoort Journal of Veterinary Research **54:** 655-6.

De Waal, D.T., van Heerden, J., van den Berg, S.S., Stegmann, G.F., Potgieter, F.T. (1988). Isolation of pure *Babesia equi* and *Babesia caballi* organisms in splenectomized horses from endemic areas in South Africa. Onderstepoort Journal of Veterinary Research **55:** 33-35.

De Waal, D.T., van Heerden, J. (1994). Equine babesiosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 295-304.

De Waal, D.T. (2000). Anaplasmosis control and diagnosis in South Africa. Annals of the New York Academy of Sciences **916:** 474-83.

Di Domizio, G. (1919). Dell' *Anaplasma marginale*. (corpi di jolly nel sangue anemico. Forme anaplasmatiche di piroplasmi). Clinica Veterinaria e Rassegna di Polizia Sanitaria di Igiena **42:** 203-311.

Dolan, T.T. (1989). Theileriasis: A comprehensive review. Revue scientifique et technique-Office international des Epizooties **8:** 11-36.

Donatien, A. and Lestoquard, F. (1935). Existence en Algérie d'une Rickettsia du chien. Bulletin de la Société de Pathologie Exotique **28:** 418-419.

Donatien, A. and Lestoquard, F. (1936). *Rickettsia bovis,* nouvelle espèce pathogène pour le bœuf. Bulletin de la Société de Pathologie Exotique **29 :** 1057-1061.

D'Oliveira, C., van der Weide, M., Jacquiet, P., Jongejan, F. (1997). Detection of *Theileria annulata* by the PCR in ticks (Acari: Ixodidae) collected from cattle in Mauritania. Experimental and Applied Acarology **21:** 279-291.

Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R. (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with descriptions of six new species combinations and designation of Ehrlichia equi and "HE agent" as subjective synonyms of Ehrlichia phagocytophila. International Journal of Systematics and Evolutionary Microbiology **51:** 2145-2165.

Du Plessis, J.L. (1990). Increased pathogenicity of an Ehrlichia-like agent after passage through *Amblyomma hebraeum*: a preliminary report. Onderstepoort Journal of Veterinary Research **57:** 233-237.

Du Preez, P.J., Marneweck, G.C. (1996). Seekoeivlei Nature Reserve South Africa, information sheet for the site designated to the List of Wetlands of International Importance in terms of the Conservation on Wetlands of International Importance especially as Waterfowl Habitat. Pretoria: Department of Environmental Affairs and Tourism.

Du Toit, D.J. (2008). The indigenous livestock of Southern Africa. http://www.damarasheep.co.za /files/ParisRoundtable.pdf.

Enigk, K. (1942). Die Empfänglichkeit der Elenantilope für *Anaplasma ovis* und *Eperythrozoon ovis*. Deutsche Tropenmedizinische Zeitschrift **46:** 48-52.

Estrada-Pena, A., Bouattour, A., Camicas, J.-L., Guglielmone, A., Horak, I., Jongejan, F., Latif, A., Pegram, R., Walker, A.R. (2006). The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. Experimental and Applied Acarology **38:** 219-235.

FAO (2005). Livestock sector brief, South Africa. Food and Agriculture Organization of the United Nations. Livestock Information, Sector Analysis and Policy Branch AGAL, pp. 1-15.

Ferguson, K. and Hanks, J. (2012). The effect of protected area and veterinary fencing on wildlife conservation in southern Africa. Parks **18:** 49-60.

Fernandez, G.P., Gonzalez, C.V., Alarcon, V.J. (2006). Detection of *Theileria annulata* in the parasitic Ixodids of the Spanish fightingbull by the nested-PCR technique. Revista Ibérica de Parasitologia **66:** 13-16.

Figueroa, J.V., Chieves, L.P., Johnson, G.S., Buening, G.M. (1993). Multiplex polymerase chain reaction based assay for the detection of *Babesia bigemina, Babesia bovis,* and *Anaplasma marginale* DNA in bovine blood. Veterinary Parasitology **50:** 69-81.

Fivaz, B.H., Norval, R.A.I, Lawrence, J.A. (1989). Transmission of *Theileria parva bovis* (Boleni strain) to cattle resistant to the brown ear tick *Rhipicephalus appendiculatus* (Neumann). Tropical Animal Health and Production **21:** 129-34.

Flach, E.J., Woodford, J.D., Morzaria, S.P., Dolan, T.T., Hambwana, I. (1990). Identification of *Babesia bovis* and *Cowdria ruminantium* on the island of Unguja, Zanzibar. The Veterinary Record **126:** 57-59.

Folkers, C., Kuil, H. (1967). Blood parasites in cattle, sheep and goats in northern Nigeria. Bulletin of epizootic Diseases of Africa **15:** 121-123.

Fourie, L.J. and Horak, I.G. (1991). The seasonal activity of adult Ixodid ticks on Angora goats in the south western Orange Free State. Journal of the South African Veterinary Association **62:** 104-6.

Fourie, L.J., Horak, I.G., Marais, L. (1988). An undescribed *Rhipicephalus* species associated with field paralysis of Angora goats. Journal of the South African Veterinary Association **59:** 47-9.

Fourie, L.J., Horak, I.G., Van Zyl, J.M. (1991). Sites of attachment and intraspecific infestation densities of the brown paralysis tick (*Rhipicephalus punctatus*) on Angora goats. Experimental and Applied Acarology **12:** 243-9.

Fourie, L.J., Horak, I.G., Van den Heever, J.J. (1992). The relative host status of rock elephant shrews *Elephantulus myurus* and Namaqua rock mice *Aethomys namaquensis* for economically important ticks. South African Journal of Zoology **27:** 108-14.

Fourie, L.J., Kok, D.J., Heyne, H. (1996). Adult Ixodid ticks on two cattle breeds in the southwestern Free State, and their seasonal dynamics. Onderstepoort Journal of Veterinary Research **63:** 19-23.

Friedhoff, K.T. and Smith, R.D. (1981). Transmission of *Babesia* by ticks: *in* Babesiosis Ristic, M. and Keirans, J.P. (eds). Academic Press, New York, pp. 268-321.

Geleta, A.R. (2005). Antibody response to *Babesia bigemina* and *Babesia bovis* by vaccinated and unvaccinated cattle in an endemic area of South Africa. M. Sc. thesis, University of Pretoria.

Georges, K., Loria, G.R., Riili S., Greco, A., Caracappa, S., Jongejan, F., Saragano, O. (2001). Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Veterinary Parasitology **99:** 273-286.

Goethert, H.K. and Telford, S.R. (2003). Enzootic transmission of *Anaplasma bovis* in Nantucket cottontail rabbits. Journal of Clinical Microbiology **41:** 3744-3747.

Govender, D., Oosthuizen, M.C., Penzhorn, B.L. (2011). Piroplasm parasites of white rhinoceroses (*Ceratotherium simum*) in the Kruger National Park, and their relation to anaemia. Journal of the South African Veterinary Association **82:** 36-40.

Grey, J.S. and de Vos, A.J. (1981). Studies on a Bovine *Babesia* transmitted by *Hyalomma marginatum rufipes* Koch, 1844. Onderstepoort Journal of Veterinary Research **48:** 215-223.

Grootenhuis, J.G. and Young, A.S. (1980). The importance of wildlife in the epidemiology of theileriosis. *In* Wildlife Disease Research and Economic Development, Karstad, L., Nestel, B., Grahman, M. (eds). Proceedings of a workshop held in Kabete, Kenya, 8 and 9 September 1980, pp. 33-39.

Grootenhuis, J.G., Morrison, W.I., Karstad, L., Sayer, P.D., Young, A.S., Murray, M., Haller, R.D. (1980). Fatal theileriosis in eland (*Taurotragus oryx*): Pathology of natural and experimental cases. Research in Veterinary Science **29:** 219-229.

Gubbels, J.M., de Vos, A.P., van der Weide, M., Viseras, J., Schouls, L.M., de Vries, E. and Jongejan, F. (1999). Simultaneous Detection of Bovine *Theileria* and *Babesia* Species by Reverse Line Blot Hybridisation. Journal of Clinical Microbiology **37:** 1782-1789.

Gubbels, M.-J., Hong, Y., van der Weide, M., Qi, B., Nijman, I.J., Guangyuan, L., Jongejan, F. (2000). Molecular characterization of the *Theileria buffeli/orientalis* group. International Journal for Parasitology **30:** 943-952.

Gueye, A., Mbengue, Mb., Diouf, A. (1987a). Présence de *Theileria velifera* au Sénégal. Revue d’élevage et de médecine vétérinaire des pays tropicaux **40:** 117-118.

Gueye, A., Camicas, J.L., Diouf, A., Mbengue, M.B. (1987b). Tiques et hémoparasitoses du bétail au Sénégal. II. La zone sahélienne. Revue d’élevage et de médecine vétérinaire des pays tropicaux **40:** 119-125.

Gueye, A., Mbengue, Mb., Diouf, A. (1994). Tiques et hémoparasitoses du bétail au Sénégal. VI. La zone soudano-sahélienne. Revue d’élevage et de médecine vétérinaire des pays tropicaux **47:** 39-46.

Gummow, B., de Wet, C.S., de Waal, D.T- (1996). A ser-epidemiological survey of equine piroplasmosis in the Northern and Eastern Cape Province of South Africa. Journal of the South African Veterinary Association **67:** 204-208.

Habela, M., Rol, J.A., Boticario, D., Solano, A., Navarrete, I. (1993). Transmision experimental de *Theileria annulata* por *Dermacentor marginatus.* Acta Parasitologica Portuguesa **1:** 7.

Han, J.-I., Jang, H.-J., Lee, S.-J., Na, K.-J. (2009). High prevalence of *Theileria* sp. in wild Chinese water Deer (*Hydropotes inermis argyropus*) in South Korea. Veterinary Parasitology **164:** 311-314.

Hea, L., Fenga, H.H., Zhanga, W.J., Zhanga, Q.L., Fanga, R., Wanga, L.X., Tua, P., Zhoub, Y.Q., Zhaoa, J.L., Oosthuizen, M.C. (2011). Occurrence of *Theileria* and *Babesia* species in water buffalo (*Bubalus babalis,* Linnaeus, 1758) in the Hubei province, South China. Veterinary Parasitology **170:** 323-326.

Heidarpour Bami, M., Khazraiinia, P., Haddadzadeh, H.R., Kazemi, B. (2010). Identification of *Theileria* species in sheep in the eastern half of Iran using nested PCR-RFLP and microscopic techniques. Iranian Journal of Veterinary Research **11:** 262-266.

Henning, M.W. (1956). Animal Diseases in South Africa, 3rd edn. Pretoria: Central News Agency Ltd.

Hilpertshauser, H., Deplazes, P., Schnyder, M., Gern, L., Mathis, A. (2006). *Babesia* spp. identified by PCR in ticks collected from domestic and wild ruminants in southern Switzerland. Applied and Environmental Microbiology **72:** 6503-6507.

Hlatshwayo, M., Mbati, P.A., Dipeolu, O.O. (2000). An epidemiological survey of ticks (Acari: Ixodidae) infesting cattle in the eastern Free State Province of South Africa: attachment preferences. Journal of Protozoology Research **10:** 98-105.

Horak, I.G., Williams, E.J., Van Schalkwyk, P.C. (1991). Parasites of domestic and wild animals in South Africa. XXV. Ixodid ticks on sheep in the north-eastern Orange Free State and in the eastern Cape Province. Onderstepoort Journal Veterinary Research **58:** 115-123.

Horak, I.G., Boomker, J., Spickett, A.M., De Vos, V. (1992). Parasites of domestic and wild animals in South Africa. XXX. Ectoparasites of kudus in the eastern Transvaal Lowveld and the Eastern Cape Province. Onderstepoort Journal of Veterinary Research **59:** 259-273.

Horak, I.G., Braack, L.E.O., Fourie, L.J., Walker, J.B. (2000). Parasites of domestic and wild animals in South Africa. XXXVIII. Ixodid ticks collected from 23 wild Carnivore species. Onderstepoort Journal of Veterinary Research **67:** 239-250.

Horak, I.G., Golezardy, H., Uys, A.C. (2007). Ticks associated with the three largest wild ruminants species in southern Africa. Onderstepoort Journal of Veterinary Research **74:** 231-242.

Horak, I.G. (2009). A century of tick taxonomy in South Africa. Onderstepoort Journal of Veterinary Research **76:** 69-74.

Horak, I.G., Heyne, H., Donkin, E.F. (2010). Parasites of domestic and wild animals in South Africa. XLVIII. Ticks (Acari: Ixodidae) infesting domestic cats and wild Felids in Southern Africa. Onderstepoort Journal of Veterinary Research **77:** 1-7.

Hoskins, J.D. (1991). Ehrlichial diseases of dogs: diagnosis and treatments. Canine Practice **16:** 13-21.

Houck, J.A., Hojgaard, A., Piesman, J., Kuchta, R.D. (2011). Low-density microarrays for the detection of *Borrelia burgdorferi* s.s. (the Lyme diseases spirochete) in nymphal *Ixodes scapularis*. Ticks and Tick-borne Diseases **2:** 27-36.

Husson, F., Josse, J., Le, S., Mazet, J. (2012). FactoMineR: Multivariate exploratory data analysis and data mining with R. R package version 1.20, http://CRAN.R-project.org/package=FactoMineR

Howell, C.J., Walker, J.B., Nevill, E.M. (1978). Ticks, mites and insects infesting domestic animals in South Africa. Part 1. Descriptions and biology. Department of Agriculture Technical Services, Republic of South Africa, Science Bulletin no. 393.

Huang, H., Unver, A., Perez, M.J., Orellana, N.G., Rikihisa, Y. (2005). Prevalence and molecular analysis of Anaplasma platys in dogs in Lara, Venezuela. Brazilian Journal of Microbiology **36:** 211-216.

Ica, A., Vatansever, Z., Yildirim, A., Duzulu, O., Inci, A. (2007). Detection of *Theileria* and *Theileria* species in ticks collected from cattle. Veterinary Parasitology **148:** 156-160.

Illgner, P. (2004). Environmental impact assessment for the proposed Matimba-Witkop No. 2400 kv transmission line, Limpopo Province. Rhodes University.

Inokuma, H., Oyamada, M., Kelly, P.J., Itamoto, K., Okuda, M., Brouqui, P. (2005). Molecular detection of a new Anaplasma species closely related to Anaplasma phagocytophilum in canine blood from South Africa. Journal of Clinical Microbiology **43:** 2934-2937.

Iseki, H., Alhassan, A., Ohta, N., Thekisoe, O.M.M., Yokoyama, N., Inoue, N., Nambota, A., Yasuda, J., Igarashi, I. (2007). Development of multiplex loop-mediated isothermal amplification (mLAMP) method for the simultaneous detection of bovine *Babesia* parasite. Journal of Microbiological Methods **71:** 281-287.

Ismail, N., Bloch, K.C., McBride, J.W. (2010). Human ehrlichiosis and anaplasmosis. The Journal of Laboratory and Clinical Medicine **30:** 261-292.

Jacquiet, P., Colas, F., Cheikh, D., Thiam, E., Ly, B.A. (1994). Epidémiologie descriptive de la theileriose bovine à *Theileria annulata* en Mauritanie, Afrique de l'Ouest sub-saharienne. Revue d'élevage et de médecine vétérinaire des pays tropicaux **47:** 147-155.

Jansen, B.C., Neitz, W.O. (1956). The experimental transmission of *Theileria ovis* by *Rhipicephalus evertsi evertsi*. Onderstepoort Journal of Veterinary Research **27:** 3-6.

Jongejan, F., Uilenberg, G. (2004). The global importance of ticks. Parasitology **129:** 3-14.

Jordaan, H.L. (2010). Behavioural-Ecology of the White Rhinoceros (*Ceratotherium simum*) in the Willem Pretorius Game Reserve. M. Sc. thesis, University of South Africa.

Kamau, J., de Vos, A.J., Playford, M., Salim, B., Kinyanjui, P., Sugimoto, C. (2011). Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. Parasites and Vectors **4:**22.

Karbe, E., Grootenhuis, J.G., Kelly, S., Karstad, L. (1979). Experiments on the *Babesia bigemina* carrier state in East African buffalo and eland. Zeitschrift für Tropenmedizin und Parasitologie **30:** 313-317.

Katz, J., Dewald, R., Nicholson, J. (2000). Procedurally similar competitive immunoassay systems for serodiagnosis of *Babesia equi, Babesia caballi, Trypanosoma equiperdum* and *Burkholderia mallei* infection in horses. Journal of Veterinary Diagnostic Investigation **12:** 123-126.

Kaufhold A., Podbieldski, A., Baumgarten, G., Blokpoel, M., Top, J. and Schouls, L. (1994). Rapid typing of group A streptococci by the use of DNA amplification and non-radioactive allele-specific oligonucleotide probes. FEMS Microbiol letters **119:** 19-26.

Kaufmann, J. (1996). Parasitic infections of domestic animals: a diagnostic manual. Birkhäuser Verlag, Basel, Schweiz, p. 189.

Kawahara, M., Rikihisa, Y., Lin, Q., Isogai, E., Tahara, K., Itagaki, A., Hiramitsu, Y., Tajima, T. (2006). Novel genetic variants of *Anaplasma phagocytophilum, Anaplasma bovis, Anaplasma centrale,* and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. Applied and Environmental Microbiology **72:** 1102-1109.

Keirans, J.E. and Pound J.M. (2003). An annotated bibliography of the spinose ear tick, *Otobius megnini* (Dugès, 1883) (Acari: Ixodida: Argasidae) 1883-2000. Systematic and Applied Acarology Special Publications **13:** 1-68.

Kietzman, A. (1998). Development of the WPGR management plan. M. Sc. thesis, University of the Free State, Bloemfontein.

Kjemtrup, A.M., Thomford, J., Robinson, T., Conrad, P.A. (2000a). Phylogenetic relationships of human and wildlife piroplasm isolates in the western United States inferred from the 18S nuclear small subunit RNA gene. Parasitology **120:** 487-493.

Kjemtrup, A.M., Kocan, A.A., Whithworth, L., Meinkoth, J., Birkenheuer, A.J., Cummings, J., Boudtreaux, M.K., Stockham, S.L., Irizarry-Rovira, A., Conrad, P.A. (2000b). There are at least three genetically distinct small piroplasms from dogs. International Journal for Parasitology **30:** 1501-1505.

Knowles, D. (1996). Equine babesiosis (piroplasmosis): a problem in the international movement of horses. British Veterinary Journal **152:** 123-126.

Kok, J.B., D'Oliveira, C., Jongejan, F. (1993). Detection of the protozoan parasite *Theileria annulata* in *Hyalomma* ticks by the polymerase chain reaction. Experimental and Applied Acarology **17:** 839-846.

Krecek, R.C., Boomker, J., Penzhorn, B.L., Scheepers, L. (1990). Internal Parasites of Giraffes (*Giraffa camelopardalis angolensis*) from Etosha National Park, Namibia. Journal of Wildlife Diseases **26:** 395-397.

Kroger, M. (1981). Characteristics of types and breeds of cattle in the tropics. *In*: Diseases of cattle in the tropics, Ristic, M. and McIntyre, I. (eds.). The Hague, Boston, London: Martinus Nijhoff Publishers.

Latif, B.M., Hawa, N.J., Bakir, F.A. (1977). Incidence of malignant theileriosis (*Theileria hirci*) of sheep in Iraq. Journal of Veterinary Medical Science **1:** 124-128.

Latif, A.A., Walker, A.R., 2004. Ticks of veterinary and medical importance, Africa. ICTTD-2, CD-Rom.

Lawrence, J.A. and Mackenzie, P.K.I. (1980). Isolation of a non-pathogenic *Theileria* of cattle transmitted by *Rhipicephalus appendiculatus*. Zimbabwe Veterinary Journal **11:** 27-35.

Lawrence, J.A., Norval, R.A.I., Uilenberg, G. (1983). *Rhipicephalus zambeziensis* as a vector of bovine Theileriae. Tropical Animal Health and Production **15:** 39-42.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. (1994a). East Coast fever. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 309-325.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. (1994b). Corridor disease. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 326-328.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. (1994c). *Theileria taurotragi* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 334-335.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. (1994d). *Theileria mutans* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 336-337.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. (1994e). *Theileria velifera* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 338-339.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. (1994f). *Theileria separata* infection. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, p. 340.

Leeflang, P. and Ilemobade, A.A. (1977). Tick-Borne diseases of domestic animals in northern Nigeria. II. Research summary, 1964 to 1976. Tropical Animal Health and Production **9:** 211-218.

Lestoquard, F. (1924). Deuxième note sur les piroplasmosis du mouton en Algérie. L'Anaplasmose: *Anaplasma ovis* nov. sp. Bulletin de la Société de Pathologie Exotique **17:** 784-787.

Levine, N.D. (1971). Taxonomy of the piroplasms. Transactions of the American Microscopical Society **90:** 2-33.

Lew, A. and Jorgensen, W. (2005). Molecular approaches to detect and study the organisms causing bovine tick borne diseases: babesiosis and anaplasmosis. African Journal of Biotechnology **4:** 292-302.

Li, Y., Guan, G., Liu, A., Peng, Y., Luo, J., Yin, H. (2010). Experimental transmission of *Theileria ovis* by *Hyalomma anatolicum anatolicum*. Parasitology Research **106:** 991-994.

Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., Lin, D., Lu, L., Law, M. (2012). Comparison of Next-Generation sequencing systems. Journal of Biomedicine and Biotechnology ID 251364, 11 pages doi:10.1155/2012/251364.

Liz, J.S., Sumner, J.W., Pfister, K., Brossard, M. (2002). PCR detection and serological evidence of granulocytic Ehrlichial infection in roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). Journal of Clinical Microbiology **40:** 892-897.

Lobanov, V.A., Gajadhar, A. A., Al-Adhami, B., Schwantje, H.M. (2011). Molecular study of free-ranging mule deer and white-tailed deer from British Columbia, Canada, for evidence of *Anaplasma* spp. and *Ehrlichia* spp. Transboundary and Emerging Diseases **59:** 233-43.

Löhr, K.F., Meyer, H. (1973). Game anaplasmosis: the isolation of Anaplasma organisms from Antelope. Zeitschrift für Tropenmedizin und Parasitologie **24:** 192-197.

Löhr, K.F., Ross, J.P., Meyer, H. (1974). Detection in game of fluorescent and agglutination antibodies to intraerythrocytic organisms. Zeitschrift für Tropenmedizin und Parasitologie **25:** 217-26.

Lounsbury, C.P. (1906). Tick and African Coast fever. Agricultural J. Cape Good Hope **28:** 634-54.

Low, A.B. & Rebelo, A.G. (1998). Vegetation of South Africa, Lesotho and Swaziland. Pretoria: Department of Environmental Affairs and Tourism.

Luo, J., Yin, H., Liu, Z., Yang, D., Guan, G., Liu, A., Ma, M., Dang, S., Lu, B., Sun, C., Bai, Q., Lu, W., Chen, P. (2005). Molecular phylogenetic studies on an unnamed *Babesia* sp. based on small subunit ribosomal RNA gene sequences. Veterinary Parasitology **133:** 1-6.

Mans, B.J., Pienaar, R., Latif, A.A., Potgieter, F.T. (2011). Diversity in the *18S SSU* rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. Parasitology **138:** 766-779.

Mark, K.K. (2010). Equine piroplasmosis in Greece. Veterinary Parasitology **169:** 73-278. Martin, H. and Brocklesby, D.W. (1960). A new parasite of the eland. The Veterinary Record **72:** 331-332.

Marcelino, I., de Almeida, A.M., Ventosa, M., Pruneau, L., Coelho, A.V. (2012). Tick-borne diseases in cattle: application of proteomics to develop new generation vaccines. Journal of Proteomics **75:** 4232-4250.

Martins, T.M., Neves, L., Pedro, O., Fafetine, J. M., Do Rosario, V. E. and Domingos, A. (2010). Molecular detection of *Babesia* spp. and other haemoparasitic infections of cattle in Maputo Province, Mozambique. Parasitology **137:** 939-946.

Matjila, P.T., Penzhorn, B.L., Bekker, C.P.J., Nijhof, A.M., Jongejan, F. (2004). Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa. Veterinary Patasitology **122:** 119-125.

Matthysse, J.G., and Colbo, M.H. (1987). The ixodid ticks of Uganda: Together with species pertinent to Uganda because of their present known distribution. Maryland: Entomological Society of America, College Park.

McCully, R.M., Keep, M.E., Basson, P.A. (1970). Cytauxzoonosis in a giraffe [*Giraffa camelopardalis* (Linnaeus, 1758)] in Zululand. Onderstepoort Journal of Veterinary Research **37:** 7-10.

McInnes, E.F., Stewart, C.G., Penzhorn, B.L., Meltzer, D.G. (1991). An outbreak of babesiosis in imported sable antelope (*Hippotragus niger*). Journal of the South African Veterinary Association **62:** 30-2.

Mehlhorn, H., Schein, E. (1998). Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi* Mehlhorn, Schein 1988. Parasitology research **84:** 467-475.

Muhanguzi, D., Matovu, E., Waiswa, C. (2010). Prevalence and characterization of *Theileria* and *Babesia* species in cattle under different husbandry systems in Western Uganda. International Journal of Animal and Veterinary Advances 2: 51-58.

Müller, D.B. (1986). Plantekologie van die Willem Pretorius Wildtuim. PhD thesis, University of the Orange Free State, Bloemfontein.

Murrell, A. and Barker, S.C. (2003). Synonyms of *Boophilus* Curtice, 1891 with *Rhipicephalus* Koch, 1844 (Acari: Ixodidae). Systematic Parasitology **56:** 169-172.

Nagore, D., Garcia-Sanmartin, J., Garcia-Perez A.L., Juste, R.A., Hurtado A. (2004). Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in sheep population from Northern Spain. International Journal for Parasitology **34:** 1059-1067.

Nava, S., Guglielmone, A.A., Mangold, A.J. (2009). An overview of systematics and evolution of ticks. Frontiers in Biosciences **14:** 2857-77.

Neitz, W.O., Du Toit, P.J. (1932). Bovine anaplasmosis. A method of obtaining pure strains of Anaplasma marginale and A. centrale by transmission through antelopes. 18th Report of the Director of Veterinary Services and Animal Industry, Pretoria, Union of South Africa, pp. 3-20.

Neitz, W.O. (1933). Blood parasites of game in Zululand. Further Report. Onderstepoort Journal of Veterinary Science and Animal Industry **1:** 411-417.

Neitz, W.O. (1935). Bovine anaplasmosis. The transmission of *Anaplasma marginale* to black wildebeest (*Connochaetes gnou*). Onderstepoort Journal of Veterinary Science and Animal Industry **5:** 9-11.

Neitz, W.O. (1941). The occurrence of *Babesia bovis* in South Africa. Journal of the South African Veterinary Medical Association **12:** 62-66.

Neitz, W.O. (1956). A consolidation of our knowledge of the transmission of tick-borne diseases. Onderstepoort Journal of Veterinary Research **27:** 115-63.

Neitz, W.O. (1957). Theileriosis, gonderioses and cytauxzoonoses: A review. Onderstepoort Journal of Veterinary Research **27:** 275-430.

Neitz, W.O. (1968). *Ehrlichia ovina* infection. Bulletin de l'Office International des Epizooties **70:** 337-340.

Nestel, B. (1980). Foreword. *In*: Wildlife diseases research and economic development. Proceedings of a workshop held in Kabete, Kenya, 8 and 9 September, pp. 5-6.

Niemand, L. (2009). Proposed Eskom landfill site, Lephalale, Limpopo Province, ecological assessment (final report). Pretoria: Pachnoda consulting.

Nijhof, A.M., Penzhorn, B.L., Lynen, G., Mollel, J.O., Morkel, P., Bekker, C.P.J. and Jongejan, F. (2003). *Babesia bicornis* sp. Nov. and *Theileria bicornis* sp. Nov.: Tick-borne parasites associated with mortality in the Black Rhinoceros *(Diceros bicornis)*. Journal Of Clinical Microbiology **41:** 2249-2254.

Nijhof, A.M., Pillay, V., Steyl, J., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., Penzhorn, B.L. and Jongejan, F. (2005). Molecular Characterisation of *Theileria* Species Associated with Mortality in four Species of African Antelopes. Journal of Clinical Microbiology **43:** 5907-5911.

Norval, R.A.I. (1975). Studies on the ecology of *Haemaphysalis silacea* Robinson 1912 (Acarina: Ixodidae). The Journal for Parasitology **61:** 730-736.

Norval, R.A.I. (1979). Tick infestations and tick-borne diseases in Zimbabwe Rhodesia. Journal of the South African Veterinary Association **50:** 289-92.

Norval, R.A.I., Perry, B.D., Young, A.S. (1992). The Epidemiology of Theileriosis in Africa. San Diego: Academic Press, p. 41.

Norval, R.A.I. (1994). Vectors: Ticks. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 3-24.

Oberem, P.T. and Bezuidenhout, J.D. (1987). Heartwater in hosts other than domestic ruminants. Onderstepoort Journal of Veterinary Research **54:** 271-275.

Oosthuizen, M.C., Zweygarth, E., Collins, N.E., Troskie, M. and Penzhorn, B.L. (2008). Identification of a Novel *Babesia* sp. From a Sable Antelope *(Hippotragus niger* Harris, 1838*)*. Journal of Clinical Microbiology **46:** 2247-2251.

Oosthuizen, M.C., Allsopp, B.A., Troskie, M., Collins, N.E., Penzhorn, B.L. (2009). Identification of novel *Babesia* and *Theileria* species in South African giraffe *(Giraffa camelopardalis,* Linnaeus, 1758) and roan antelope *(Hippotragus equinus,* Desmarest 1804). Veterinary Parasitology **163:** 39-46.

Oura, C.A.L., Bishop, R.P., Wampande, E.M., Lubega, G.W., Tait, A. (2004). Application of reverse line blot assay to the study of haemoparasites in cattle in Uganda. International Journal for Parasitology **34:** 603-613.

Parola, P., Cornet, J.-P., Sanogo, Y.o., Miller, R.S., Van Thien, H.V., Gonzalez, J.-P., Raoult, D., Telford, S.R., Wongsrichanalai, c. (2003). Detection of *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., and other Eubacteria in ticks from the Thai-Myanmar border and Vietnam. Journal for Clinacal Microbiology **41:** 1600-1608.

Penzhorn, B.L., Oosthuizen, M.C., Bosman, A.-M., Kilian, J.W., Horak, I.G. (2008). Black rhinoceros (Diceros bicornis) populations in Northwestern Namibia are apparently not infected with piroplasms. Journal of Wildlife Diseases **44:** 1032-1035.

Peter, T.F., Anderson, E.C., Burridge, J., Mahan, M. (1998). Demonstration of carrier state for Cowdria ruminatium in wild ruminants from Africa. Journal of Wildlife Diseases **34:** 567-575.

Pfitzer, S. 2009. Occurrence of tick-borne haemoparasites in nyala (*Tragelaphus angasii*) in KwaZulu-Natal and Eastern Cape Province, South Africa. M. SC. Thesis, University of Pretoria.

Pfitzer, S., Oosthuizen, M.C., Bosman, A.-M., Vorster, I., Penzhorn, B.L. (2011). Tick-borne blood parasites in nyala (*Tragelaphus angasii,* Gray 1849) from KwaZulu-Natal, South Africa. Veterinary Parasitology **176:** 126-131.

Pipano, E. (1966). Piroplasmosis-a review. Refuah Veterinarith **23:** 52-54.

Pipano, E. (1994). *Theileria annulata* theileriosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 341-348.

Potgieter, F.T. and Van Rensburg, L. (1987). Tick transmission of *Anaplasma centrale.* Onderstepoort Journal of Veterinary Research **54:** 5-7.

Potgieter, F.T. (1979). Epizootiology and control of anaplasmosis in South Africa. Journal of the South African Veterinary Association **50:** 367-372.

Potgieter, F.T. (1981). Tick transmission of anaplasmosis in South Africa. *In* Proceedings of the International Conference on Tick Biology and Control, 27-29 January 1981, Grahamstown, South Africa 53-56.

Potgieter, F.T. and Stoltsz, W.H. (1994). Anaplasmosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, p. 407.

Provost, A. and Bezuidenhout, J.D. (1987). The historical background and global importance of heartwater. Onderstepoort Journal of Veterinary Research **54:** 165-169.

Purnell, R.E., Branagan, D., Brown, C.G.D. (1970). Attempted transmission of some piroplasms by Rhipicephalid ticks. Tropical Animal Health and Production **2:** 146-150.

Purnell, R.E. (1980). Tick-borne diseases as a barrier to efficient land use. Outlook on Agriculture, **10:** 230-234.

Rar, V. and Golovljova, I. (2011). Anaplasma, Ehrlichia, and “Candidatus Neoehrlichia” bacteria: Pathogenicity biodiversity and molecular genetic characteristics, a review. Infection, Genetics and Evolution **11:** 1842-1861.

Rafyi, A. and Maghami, G. (1966). Contribution à l’étude de quelques parasites du sang du mouton et de la chèvre en Ran et dans les pays voisins. Bulletin de l’Office International des Epizooties **65:** 1769-1783.

Rastegaieff, E.F. (1933). Zur Frage der Überträger der Scharfpiroplasmosen in Aserbaidschan (Transkaukasien). Archiv für Wissenschaftliche und Praktische Tierheilkunde **67:** 176-186.

Rastegaieff, E.F. (1935). Un nouveau vecteur dans la transmission des haemoparasites des animaux domestiques: *Ornithodorus lahorensis,* Neumann 1908. Annales de l’Institut Pasteur **54:** 250-258.

Rastegaieff, E.F. (1937). *Dermacentor silvarum,* vecteur des haemoparasites du mouton: *Anaplasma ovis* et *Theileria recondita*. Bulletin de la Société de Pathologie Exotique **30:** 479-480.

Rikihisa, Y. (2010). *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*: subversive manipulations of host cells. Nature **8:** 328-339.

Robinson, P.M. (1982). *Theileria annulata* and its transmission: A review. Tropical Animal Health and Production **14:** 3-12.

Ros-Garcia, A., M'Ghirbi, Y., Bouattour, A. and Hurtado, A. (2011). First detection of *Babesia occultans* in Hyalomma ticks from Tunisia. Parasitology **138:** 578-582.

Rühle, C. (2008). Study of the epidemiology of ticks and tick-borne diseases in cattle in the South of the Ivory Coast using reverse line blot-PCR. PhD thesis, University of Neuchâtel, Neuchâtel.

Ryff, J.F., Weibel, J.L., Thomas, G.M. (1964). Relationship of ovine to bovine anaplasmosis. Cornell Veterinarian **54:** 407-414.

Rymaszewska, A. and Grenda, S. (2008). Bacteria of the genus *Anaplasma* – characteristics of *Anaplasma* and their vectors: a review. Veterinary Medicina **53:** 573-584.

Sashika, M., Abe G., Matsumoto, K., Inokuma, H. (2011). Molecular survey of *Anaplasma* and *Ehrlichia* infections of feral raccoons (*Procyon lotor*) in Hokkaido, Japan. Vector-Borne and Zoonotic Diseases **11:** 349-354.

Schnittger, L., Yin, H., Qi, B., Gubbels, M.J., Beyer, D., Niemann, S., Jongejan, F., Ahmed, J.S. (2004). Simultaneous detection and differentiation of *Theileria* and *Babesia* parasites infecting small ruminants by reverse line blotting. Parasitology Research **92:** 189-196.

Schnittger, L., Rodriguez, A.E., Florin-Christensen, M., Morrison, D.A. (2012). *Babesia:* A world emerging. Infection, Genetics and Evolution **12:** 1788-1809.

Schouls, L.M., Van De Pol, I., Rijpkema S.G.T. and Schot C.S. (1999). Detection and Identification of *Ehrlichia, Borrelia burgdorferi* Sensu Lato, and *Bartonella* Species in Dutch *Ixodes ricinus* Ticks. Journal of Clinical Microbiology **37:** 2215-2222.

Schulz, K. (1940). A rickettsiosis new to South Africa. Onderstepoort Journal of Veterinary Sciences and Animal Industry **13:** 287-289.

Scott, G.R. (1994). Lesser-known rickettsias infecting livestock. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 371-377.

Seneviratna, P. and Jainudeen, M.R. (1967). The presence of *Ehrlichia ovina*-like organisms in the mononuclear cells in Ceylon. Ceylon Veterinary Journal **15:** 141.

Simuunza, M., Weir, W., Courcier, E., Tait, A., Shiels, B. (2011). Epidemiological analysis of tick-borne diseases in Zambia. Veterinary Parasitology **175:** 331-342.

Sirigireddy, K. R. and Ganta, R.R. (2005). Multiplex detection of *Ehrlichia* and *Anaplasma* species pathogens in peripheral blood by real-time reverse transcriptase-polymerase chain reaction. Journal of Molecular Diagnostics **7:** 308- 316.

Skaug, H., Fournier, D., Nielsen, A. (2010). Glmm ADMB-package, http: //127.0.0.1:13596/library/glmmADMB/html/glmmADMB-package.html

Sluyter, F.J. (2001). Traceability of Equidae: a population in motion. Revue Scientifique et Technique **20:** 550-509.

Smith, K., Brocklesby, D.W., Bland, P., Purnell, R.E., Brown, C.G.D., Payne, R.C. (1974). The fine structure of intra-erythrocytic stages of *Theileria gorgonis* and a strain of *Anaplasma marginale* isolated from wildebeest (*Connochaetes taurinus*). Zeitschrift für Tropenmedizin und Parasitologie **25:** 293-300.

Spitalska, E., Riddell, M., Heyne, H., Sparagano, O.A.E. (2005). Prevalence of theileriosis in red hartebeest (*Alcelaphus buselaphus caama*) in Namibia. Parasitology Research **97:** 77-79.

Splitter, E.J., Anthony, H.D., Twiehaus, M.J. (1956). *Anaplasma ovis* in the USA. Experimental studies with sheep and goats. American Journal of Veterinary Research **17:** 487-491.

Steyl, J.C.A., Prozesky, L., Stoltsz, W.H., Lawrence, J.A. (2012). *Theileriosis (Cytauxzoonosis)* in roan antelope (*Hippotragus equinus*): Field exposure to infection and identification of potential vectors. Onderstepoort Journal of Veterinary Research **70:** 1-8.

Stiller, D., Goff, W.L., Shompolse, S.P., Johnson, L.W., Gorham, J.R., Micguire, T.C. (1989). *Dermacentor andersoni* Stiles: A natural vector of *Anaplasma ovis* Lestoquard on sheep in Idaho. Proceedings of the Eighth National Hemoparasite Diseases Conference, 10-12 April 1989, St. Louis, Missouri.

Stoenner, H.G. (1980). Rickettsiosis of domestic animals not transmissible to man. CRC handbook series in zoonosis. Section A: bacterial, rickettsial, and mycotic diseases. Volume II. Steele, J.H. (Editor-in-Chief). pp. 353-355.

Stoltsz, W.H. (1989). Theileriosis in South Africa: a brief review. Revue scientifique et technique- Office international des Epizooties **8:** 93-102.

Stoltsz, W., H. and Dunsterville, M.T. (1992). In vitro establishment and cultivation of a *Cytauxzoon* sp. (*Theileria* sp.) from a sable antelope (*Hippotragus niger,* Harris 1838). Journal of the South African Veterinary Association **63:** 182. (Abstract).

Stoltsz, W.H. (1994). Ovine and caprine anaplasmosis. *In* Infectious diseases of livestock, with special reference to Southern Africa, Coetzer, J.A.W., Thomson, G.R., Tustin, R.C., (eds.). Oxford University Press, Oxford, UK, pp. 431-438.

Sutherst, R.W. (1987). The dynamics of hybrid zones between tick (Acari) species. International Journal for Parasitology **17:** 921-926.

Taoufik, A., Nijhof, A., Hamidjaja, R., Jongejan, F., Pillay, V., Sonnevelt, M., de Boer, M. (2004). Reverse line blot hybridisation in the detection of tick-borne diseases. Maarsen: Isogen life Science.

Taylor, W.A., Skinner, J.D., Krecek, R.C. (2005). Seasonal body condition indices of mountain reedbuck *Redunca fulvorufula* in two areas of South African Highveld: The Grassland and Karoo biomes. South African Journal of Animal Science **35:** 19-29.

Theiler, A. (1906). *Piroplasma mutans* (n. spec.) of South African cattle. Journal of Comparative Pathology and Therapeutics **19:** 292-300.

Theiler, A. (1907). Further notes on *Piroplasma mutans*, a new species of piroplasm in South African cattle. Journal of Comparative Pathology and Therapeutics **20:** 1-18.

Theiler, A., (1909). Transmission des spirilles et des piroplasms par différentes espèces de tiques. Bulletin de la Société de Pathologie Exotique **2:** 293-294.

Theiler, A. (1910). *Anaplasma marginale* (Gen. spec. Nova.): The marginal points in the blood of cattle suffering from a specific disease. Report of the Government Veterinary Bacteriologist of the Transvaal. 7-64.

Theiler, A. (1911). Further investigations into anaplasmosis of South African cattle. First Report of the Director of Veterinary Research, Union of South Africa. 7-46.

Theiler, G. (1962). The Ixodidae parasites of vertebrates in Africa South of the Sahara. Project S9958. Report to the Director of Veterinary Services, Onderstepoort, South Africa, p. 154-159.

Thomas, S.E., Mason, T.E. (1981). Isolation and transmission of an unidentified *Babesia* sp. infective for cattle. Onderstepoort Journal for Veterinary Research **48:** 155-158.

Thomas, S.E., Wilson, D.E., Mason, T.E. (1982). *Babesia, Theileria* and *Anaplasma* spp. infecting sable antelope, *Hippotragus niger* (Harris, 1838) in Southern Africa. Onderstepoort Journal of Veterinary Research **49:** 163-166.

Todd, J.L. and Wolbach, S.B. (1912). Parasitic protozoa from the Gambia. Second report of the expedition of the Liverpool School of Tropical Medicine to the Gambia, 1911. The Journal of Medical Research **26:** 195-218.

Tonetti, N., Berggoetz, M., Rühle, C., Pretorius, A.M., Gern, L. (2009). Ticks and tick-borne pathogens from wildlife in the Free State Province, South Africa. Journal of wildlife Diseases **45:** 437-446.

Tonnesen, M.H., Penzhorn, B.L., Bryson, N.R., Stoltsz, W.H., Masibigiri, T. (2004). Displacement of *Boophilus decoloratus* by *Boophilus microplus* in the Soutpansberg region, Limpopo Province, South Africa. Experimental and Applied Acarology **32:** 199-208.

Uilenberg, G., (1964). *Haematoxenus veliferus*, n.g., n. sp., parasite *incertae sedis* du sang de bovin à Madagascar. Revue d'Elevage et de Médecine Vétérinaire des pays Tropicaux **17:** 655-662.

Uilenberg, G. (1970). Existence d'*Haematoxenus veliferus* (Sporozoa, Theileriidae) en Afrique centrale. Présence d'*Haematoxenus* sp. chez le buffle africain. Revue d'Elevage et de Médecine Vétérinaire des pays Tropicaux **23:** 455-456.

Uilenberg, G., 1976. Tick-borne livestock diseases and their vectors. 2. Epizootiology of tick-borne diseases. World Animal Review **17:** 8-15.

Uilenberg, G. and Schreuder, B.E.C. (1976). Studies on Theileriidae (Sporozoa) in Tanzania. 1. Tick transmission of *Haematoxenus veliferus*. Tropenmedizin und Parasitologie **27:** 106-111.

Uilenberg, G., Schreuder, B.E., Mpangala, C., Tondeur, W. (1977). Studies on *Theileriidae* (Sporozoa) in Tanzania IX. Unidentified bovine *Theileriae*. Zeitschrift für Tropenmedizin und Parasitologie **28:** 494-8.

Uilenberg, G. (1981). Theilerial species of domestic livestock. *In* Advances in the control of theileriosis, Irvin, A.D., Cunningham, M.P., Young, A.S. (eds.). The Hague, Nijhof, M. pp. 4-37.

Uilenberg, G. (1983). Acquisitions nouvelles dans la connaissance du rôle vecteur de tiques du genre *Amblyomma* (Ixodidae). Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux **36:** 61-66.

Uilenberg, G. (1995). International collaborative research: Significance of tick-borne haemoparasitic diseases to world animal health. Veterinary Parasitology **57:** 19-41.

Uilenberg, G. (1997). General review of tick-borne diseases of sheep and goats world-wide. Parasitologia **39:** 161-165.

Van Rooyen, N. & Bredenkamp, G. (1998a). Kimberly Thorn Bushveld. In: Vegetation of South Africa, Lesotho and Swaziland. Eds. Low, A.B. & Rebelo, A.G. Pretoria: Department of Environmental Affairs and Tourism.

Van Rooyen, N. and Bredenkamp, G. (1998b). Waterberg Moist Mountain Bushveld. In: Vegetation of South Africa, Lesotho and Swaziland. Eds. Low, A.B. & Rebelo, A.G. Pretoria: Department of Environmental Affairs and Tourism.

Van Rooyen, N. and Bredenkamp, G. (1998c). Sweet Bushveld. In: Vegetation of South Africa, Lesotho and Swaziland. Eds. Low, A.B. & Rebelo, A.G. Pretoria: Department of Environmental Affairs and Tourism.

Van Valen, L. (1973). "A new evolutionary law". Evolutionary Theory **1:** 1-30.

Verbeeck, W.A. (1971). Cattle. *In*: Standard Encyclopaedia of southern Africa, Potgieter, D.J., Du Plessis, P.C., Heimstra, L.W., (eds.). South Africa: National Commercial Printers.

Vrahimis, S. (1991). Activity patterns of Black Wildebeest (*Connochaetes gnou* Zimmerman, 1780) in the Orange Free State. M. SC. thesis, University of the Orange Free State.

Walker, J.B. (1991). A review of the Ixodid ticks (Acari, Ixodidae) occurring in Southern Africa. Onderstepoort Journal for Veterinary Research **58:** 81-105.

Walker, J.B, Keirans, J.E., and Horak, I.G. (2000). The genus *Rhipicephalus* (Acari, Ixodidae): A guide to the brown ticks of the world. Cambridge, UK: Cambridge University Press.

Walker, A.R., Bouattour, A., Camicas, J.-L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., and Preston, P.M. (2003). Ticks of domestic animals in Africa: A guide to identification of species. Edinburgh, UK: Bioscience Reports.

Watson, J.P. (2006). Check list of the mammals of Tüssen-die-Riviere Provincial Nature Reserve, Free State Province, South Africa. Koedoe **49:** 11-117.

Wilson, D.E., Bartsch, R.C., Bigalke, R.D., Thomas, S.E. (1974). Observation on mortality rates and diseases in roan and sable antelope on nature reserves in the Transvaal. Journal of the South African Wildlife Management Association **4:** 203-206.

Winterbach, H.E.K. (1999). Habitat utilization, activity patterns and management of Cape buffalo in the Willem Pretorius Game Reserve. M. Sc. thesis, University of Pretoria.

Yin, H., Schnittger, L., Luo, J., Seitzer, U., Ahmed, J.S. (2007). Ovine theileriosis in China: a new look at an old story. Parasitology Research **101:** 191-195.

Young, A.S., Brown, C.G.D., Burridge, M.J., Cunningham, M.P., Payne, R.C. (1977). Establishment of an experimental field population of *Theileria lawrencei*-infected ticks maintained by African buffalo (*Syncerus caffer*). Journal for Parasitology **63:** 903-907.

Young, A.S., Purnell, R.E., Payne, R.C., Brown, C.G.D., Kanhai, G.K. (1978). Studies on the transmission and course of infection of a Kenyan strain of *Theileria mutans*. Parasitology **76:** 99-115.

Young, A.S., Groocock, C.M., Kariuki, D.P. (1988). Integrated control of ticks and tick-borne diseases of cattle in Africa. Parasitology **96:** 403-432.

Yusufmia, S.B.A.S., Collins, N.E., Nkuna, R., Troskie, M., Van den Bossche, P., Penzhorn, B.L. (2010). Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. Journal of the South African Veterinary Association **81:** 45-49.

Zeng, X., Kong, F., Halliday, C., Chen, C., Lau, A., Playford, G., Sorrell, T.C. (2007). Reverse Line Blot Hybridization assay for identification of medically important fungi from culture and clinical specimens. Journal of Clinical Microbiology **45:** 2872-2880.

Zimmermann, D.E. (2009). The occurrence of piroplasms in various South African black rhinoceros (*Diceros bicornis*) populations. M. Sc. thesis, University of Pretoria.

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