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Short communication

Molecular detection of *Babesia rossi* and *Hepatozoon* sp. in African wild dogs (*Lycaon pictus*) in South Africa

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Abstract

Blood specimens from wild dogs ($n = 301$) were obtained from De Wildt Cheetah and Wildlife Centre (Pretoria) and five game reserves (4 in the North-West Province and 1 in Limpopo Province), South Africa. Specimens were screened for *Babesia*, *Theileria*, *Hepatozoon* and *Ehrlichia/Anaplasma* species using PCR and Reverse Line Blot (RLB) assays. Positive results were obtained in 18 (6%) wild dogs. Sixteen specimens were found positive for *Babesia rossi* and two dogs were *Hepatozoon* sp. positive. It appears that these tick-borne pathogens are not widely distributed in wild dog populations.
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Keywords: African wild dog; *Babesia rossi*; *Hepatozoon*; *Lycaon pictus*; RLB; South Africa

1. Introduction

Babesia rossi, originally described from a side-striped jackal (*Canis adustos*) in East Africa (Nuttall, 1910), is the causative agent of canine babesiosis, a major clinical problem in domestic dogs in South Africa (Collett, 2000). Being a vector-specific parasite transmitted by *Haemaphysalis elliptica* (previously lumped with *Haemaphysalis leachi*; Apanaskevich et al., 2007), *B. rossi* occurs only in sub-Saharan Africa (Lewis et al., 1996).

The large piroplasms causing babesiosis in domestic dogs were generally referred to as ''*Babesia canis*'' until Uilenberg et al. (1989) drew attention to the fact that there were three separate, vector-specific taxa involved: *B. canis* (*senso stricto*), transmitted by *Dermacentor reticulatus*; *Babesia vogeli*, transmitted by *Rhipicephalus sanguineus*; and *B. rossi*. Although *B. rossi* is a common infection in dogs in South Africa, *B. vogeli* also occurs (Matjila et al., 2004).

There are pre-1989 accounts in the literature of ''*Babesia canis*'' (*senso lato*) from domestic dogs being transmitted naturally and artificially to African wild dogs (*Lycaon pictus*) and black-backed jackals (*Canis mesomelas*) in South Africa, with no untoward effects (Neitz, 1965; Neitz and Steyn, 1947; Van Heerden, 1980).

Hepatozoon gametocytes are commonly found in blood smears of free-ranging carnivores such as lions (*Panthera leo*), leopards (*Panthera pardus*), cheetahs

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(*Acinonyx jubatus*), spotted hyaenas (*Crocuta crocuta*) and even large-spotted genet (*Genetta tigrina*) (Averbeck et al., 1990; Brocklesby and Vidler, 1963, 1965; Keep, 1970; McCully et al., 1975; Penzhorn et al., 1992). Except for one case, that of a newly introduced cheetah that carried a severe tick burden (Keep, 1970), these were incidental findings. A *Hepatozoon* was identified from swellings on the leg joints of a lion (Maddock et al., 1996). *Hepatozoon*-like parasites have even been described from the tissues of impalas (*Aepyceros melampus*) (Basson et al., 1967; Keep, 1970).

Hepatozoon gametocytes found on blood smears of African carnivores are often called *H. canis*, irrespective of the host, and various named species from other hosts species have been lumped under *H. canis* (Levine, 1988). It has also been suggested that "*Microbesnoitia leoni*", described from the tissues of a lion (Bwangamoi, 1989), is a junior synonym of *H. canis* (Dubey and Bwangamoi, 1994). Clear morphological differences of *Hepatozoon* gametocytes found in cheetahs and wild dogs, respectively, suggest that synonymising many African species with *H. canis* may be erroneous (Peirce et al., 1995).

We endeavoured to ascertain which of the three *Babesia* taxa, as well as other protozoa and rickettsias commonly found in domestic dogs, occurred naturally in wild dog populations in South Africa.

2. Materials and methods

2.1. Collection of specimens

Blood specimens ($n = 301$) were collected into EDTA Vacutainer® (Franklin Lakes, USA) tubes from the jugular vein of apparently healthy wild dogs (*Lycaon pictus*) from the following areas: De Wildt Cheetah and Wildlife Centre ($n = 227$); Pilanesberg National Park ($n = 18$); Letsile Game Reserve ($n = 9$); Marekele Game Reserve ($n = 5$) and Mafunyane Game Reserve ($n = 5$) in North-West Province, and Kapama Game Reserve ($n = 37$), in Limpopo Province, South Africa. Prior to blood collection, the dogs were immobilised by intramuscular administration of medetomidine (Domitor, Novartis) (total dose 80–90 μg/kg) in combination with ketamine hydrochloride (Anaket-V, Centaur Labs) (total dose 20–25 mg) (Van Heerden et al., 2002). Blood specimens were refrigerated and sent to the Faculty of Veterinary Science, University of Pretoria.

2.2. DNA extraction

DNA was extracted from 200 μl of each blood specimen. The QIAmp blood and tissue extraction kit (Qiagen, Hilden, Germany) was used for DNA extractions, following the manufacturer's protocols.

2.3. PCR

The *Babesia/Theileria/Hepatozoon* PCR was performed with primers RLB-F2 (5′-GAC ACA GGG AGG TAG TGA CAA G-3′) and RLB-R2 (biotin-5′-CTA AGA ATT TCA CCT CTG ACA GT-3′) amplifying a fragment of 460–540 bp from the 18S rRNA gene spanning the V4 region (Gubbels et al., 1999; Matjila et al., 2004). The *Ehrlichia/Anaplasma* PCR was performed with the forward primer Ehr-F (5′-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3′) and Ehr-R (5′-Biotin-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3′) amplifying a fragment of 460–520 bp from the V1 hypervariable region of the 16S SSU rRNA gene (Bekker et al., 2002; Nijhof et al., 2005). The conditions for the PCR included an initial step of 3 min at 42 °C, 10 min at 94 °C, 10 cycles of 94 °C (20 s)–67 °C (30 s)–72 °C (30 s), with lowering of annealing step after every second cycle with 2 °C (touchdown PCR). The reaction was then followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 30 s.

2.4. Reverse line blot hybridisation

RLB was subsequently conducted on amplified products (*Babesia*, *Theileria*, *Hepatozoon*, *Anaplasma* and *Ehrlichia*) as previously described (Matjila et al., 2004). The list of probes and their sequences used for detecting pathogen DNA are listed in Table 1.

3. Results

Eighteen (6.0%) of the 301 specimens, all originating from De Wildt Cheetah and Wildlife Centre, were positive: 16 (5.3%) for *B. rossi* and 2 (0.7%) for *Hepatozoon* sp.

4. Discussion

Previous studies have reported on a fatal case of acute babesiosis in a juvenile African wild dog in the Johannesburg Zoological Gardens (Colly and Nesbit, 1992). On microscopic examination, trophozoites presumed to be "*Babesia canis*" were observed in 2/29 (6.9%) of blood smears from wild dogs in the Kruger National Park (Van Heerden et al., 1995). Additionally, a single piroplasm, possibly *Babesia* sp., was seen on a blood smear from 1/16 wild dogs in Serengeti National

Table 1
List of organisms and their corresponding probe sequences used to detect pathogen DNA

Anaplasma centrale	TCG AAC GGA CCA TAC GC
Anaplasma marginale	GAC CGT ATA CGC AGC TTG
Anaplasma ovis	ACC GTA CGC GCA GCT TG
Anaplasma phagocytophilum 1	TTG CTA TAA AGA ATA ATT AGT GG
Anaplasma phagocytophilum 3	TTG CTA TGA AGA ATA ATT AGT GG
Anaplasma phagocytophilum 5	TTG CTA TAA AGA ATA GTT AGT GG
Anaplasma phagocytophilum 7	TTG CTA TAG AGA ATA GTT AGT GG
Ehrlichia/Anaplasma catch-all	GGG GGA AAG ATT TAT CGC TA
Ehrlichia canis/Ehrlichia ovina	TCT GGC TAT AGG AAA TTG TTA
Ehrlichia chaffeensis	ACC TTT TGG TTA TAA ATA ATT GTT
Ehrlichia ruminantium	AGT ATC TGT TAG TGG CAG
Ehrlichia sp. (*Omatjenne*)	CGG ATT TTT ATC ATA GCT TGC
Hepatozoon catch-all	GCT TTG TAA TTG GAA TGA TAG A
Theileria/Babesia catch-all	TAA TGG TTA ATA GGA RCR GTT G
Theileria annae	CCG AAC GTA ATT TTA TTG ATT TG
Theileria annulata	CCT CTG GGG TCT GTG CA
Theileria bicornis	GCG TTG TGG CTT TTT TCT G
Theileria buffeli	GGC TTA TTT CGG WTT GAT TTT
Theileria catch-all	ATT AGA GTG CTC AAA GCA GGC
Theileria equi	TTC GTT GAC TGC GYT TGG
Theileria parva	GGA CGG AGT TCG CTT TG
Theileria sp. (*buffalo*)	CAG ACG GAG TTT ACT TTG T
Theileria sp. (*duiker*)	CAT TTT GGT TAT TGC ATT GTG G
Theileria sp. (*kudu*)	CTG CAT TGT TTC TTT CCT TTG
Theileria sp. (*sable*)	GCT GCA TTG CCT TTT CTC C
Theileria taurotragi	TCT TGG CAC GTG GCT TTT
Theileria velifera	CCT ATT CTC CTT TAC GAG T
Babesia bigemina	CGT TTT TTC CCT TTT GTT GG
Babesia bovis	CAG GTT TCG CCT GTA TAA TTG AG
Babesia caballi	GTG TTT ATC GCA GAC TTT TGT
Babesia canis	TGC GTT GAC CGT TTG AC
Babesia canis 2	TGG TTG GTT ATT TCG TTT TCG
Babesia catch-all 1	ATT AGA GTG TTT CAA GCA GAC
Babesia catch-all 2	ACT AGA GTG TTT CAA ACA GGC
Babesia felis	TTA TGC GTT TTC CGA CTG GC
Babesia gibsoni Japan	TAC TTG CCT TGT CTG GTT T
Babesia gibsoni USA	CAT CCC TCT GGT TAA TTT G
Babesia microti	GRC TTG GCA TCW TCT GGA
Babesia ovis	TGC GCG CGG CCT TTG CGT T
Babesia rossi	CGG TTT GTT GCC TTT GTG
Babesia vogeli	AGC GTG TTC GAG TTT GCC

Park, Tanzania (Peirce et al., 1995). This study is the first report of molecular detection and characterization of *Babesia* parasites in African wild dogs. *B. rossi* appears to be the species infecting wild dogs and this study confirms previous studies that prevalence is fairly low in wild dog populations. Clinical babesiosis has not been reported from African wild dogs in any of the populations sampled. *B. rossi* infections cause a severe to fatal disease in domestic dogs (Jacobson, 2006). It is still not clear whether there is any clinical significance of *B. rossi* infections in wild dogs as there is in domestic dogs. The implication is that *B. rossi* might be a natural parasite of wild dogs.

Haemaphysalis elliptica, the only known vector of *B. rossi*, has been recorded from domestic and wild dogs (Horak, 1995; Van Heerden, 1986; Van Heerden et al., 1995). In as far as we can ascertain, there is no physical contact between domestic and wild dogs in South Africa. The fact that wild dogs are endangered and a protected species, makes them extremely valuable. Proper care is taken in avoiding contact between domestic and wild dogs. The only known case of a fatal acute babesiosis in a wild dog juvenile was, however, reported to have been a vaccine related entity, since the acute severe disease occurred 12 days after vaccination. Furthermore, the authors stated that there might have

been a possible tick transmission since there was a potential for an excessive tick burden in the wild dog enclosure, because of dog-walking activities along the zoo perimeter (Colly and Nesbit, 1992). The risk implications of transmitting parasites from one dog species to the other, however, remain low.

Previous studies have found *Hepatozoon* gametocytes in 26/29 (89.7%) of blood smears from wild dogs in the Kruger National Park (Van Heerden et al., 1995). Peirce et al. (1995) found *Hepatozoon* gametocytes in 13/16 (81.5%) wild dogs in the Serengeti. Our study uses molecular techniques to confirm *Hepatozoon* infections in wild dogs. The low prevalence of *Hepatozoon* sp. reported here contrasts with these findings. The low prevalence reported here may be as a result of strict tick-control practised in the two captive populations (De Wildt and Kapama), where most of our specimens originated. These populations are under strict veterinary care and it would appear that strict tick-control measures can effectively reduce *Hepatozoon* burdens in captive wild dog populations.

Based on our current results, it would appear that these tick-borne pathogens are not widely distributed in wild dog populations. Furthermore, since we could not associate parasite infections to clinical disease, we are of the opinion that clinical importance of *Babesia* and/or *Hepatozoon* infections in wild dogs is insignificant.

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