Black-backed jackals (*Canis mesomelas*) from semi-arid rangelands in South Africa harbour *Hepatozoon canis* and a *Theileria* species but apparently not *Babesia rossi*

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Highlights

•This is the second study to examine vector-borne pathogens in black-backed jackal.
•Black-backed jackals host multiple pathogens also observed in domestic dogs.
•This population shows no evidence of infection with *Babesia rossi.*

Abstract

Despite the importance of disease as a wildlife management challenge in South Africa, baseline data on the epidemiology of pathogens occurring in free-ranging species has received little attention to date. Black-backed jackals (*Canis mesomelas*) are a wide-ranging, abundant carnivore with substantial economic importance due to their role in livestock depredation. They are known reservoirs hosts of *Babesia rossi*, a virulent pathogen in domestic dogs in sub-Saharan Africa. We investigated the prevalence and diversity of tick-borne pathogens (TPBs) including *Babesia*, *Theileria*, *Hepatozoon*, *Ehrlichia* and *Anaplasma* species, together with host-attached tick diversity, in a black-backed jackal population from the semi-arid Central Karoo, a small-livestock farming region in South Africa. Using reverse line blot hybridisation, we screened 43 blood samples and sequenced the 18S rRNA gene from positive samples to confirm and characterise pathogen identity using a phylogenetic framework. *Hepatozoon canis*, a ubiquitous pathogen of domestic and wild canids globally, was observed in 47% of jackals, while a *Theileria* sp. most similar to *T. ovis*, a piroplasm found in small livestock, was observed in 5% of jackals. No *Babesia*, *Ehrlichia* or *Anaplasma* species were identified, although a *Sarcocystis* sp. sequence was isolated from one jackal. Host-attached ticks (n = 20) comprised three species, *Amblyomma marmoreum*, *Haemaphysalis elliptica/zumpti* and *Ixodes rubicundus,* commonly known ticks in the region. In summary, prevalence of TBPs in black-backed jackals from this semi-arid rangeland region was lower than in jackal populations in more mesic regions. These jackals were apparently not infected with *B. rossi*. While this study is one of the first investigations into the epidemiology of TBPs infecting jackals and adds to the sparse literature, further studies which span landscape uses, climate conditions and seasonality are encouraged.

Keywords: Black-backed jackals; *Babesia rossi; Canis mesomelas; Hepatozoon canis;* Reverse Line Blot Hybridisation; Sequencing; South Africa; *Theileria ovis*

Abbreviations

AICc, Akaike's Information Criterion corrected for small sample size
BLASTn, Basic Local Alignment Search Tool for nucleotide sequences
EDTA, Ethylenediaminetetraacetic acid
CI, Confidence Interval
TBP, Tick-Borne Pathogen
RLB, Reverse Line Blot
mPCR, Multiplex Polymerase Chain Reaction
rRNA, Ribosomal ribonucleic acid
DNA, Deoxyribonucleic acid
LB, Luria Broth

1. Introduction

Black-backed jackals (hereafter, 'jackals') are common across their range in east and southern Africa (Macdonald et al., 2004), exhibiting impressive adaptability to modified landscapes and bringing them into close contact with humans and domestic animals (Loveridge and Macdonald, 2001; Macdonald et al., 2004). Given their relatively high population densities, large dispersal distances, and wide species range, the epidemiological role of jackals in the spread and maintenance of relevant diseases warrants consideration. It was recently demonstrated that jackals are natural hosts of *Babesia rossi*, an important pathogen of domestic dogs in sub-Saharan Africa (Penzhorn et al., 2017). Nevertheless, evidence of how jackals contribute to the transmission of multi-host carnivore pathogens, and whether humans, livestock and other wildlife species are influenced by jackal disease dynamics remains scarce (Bellan et al., 2012). Considering the potential for comparison with extensive existing literature on wild canids from other parts of the globe, e.g. the grey wolf (*Canis lupus*) (Hodžić et al., 2020), red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) (Alexander et al., 1994; Wolfe et al., 2001; Duscher et al., 2013; Farkas et al., 2014; Cardoso et al., 2015), jackals present themselves as useful sentinels of wider ecosystem health.

Owing to their importance as rabies vectors, previous studies on black-backed jackals have primarily focussed on disease (Courtin et al., 2000; Bellan et al., 2012); little research on Tick-Borne Pathogens (TBPs) has been carried out on the species. Jackals have been surveyed for certain pathogens of zoonotic or conservation interest, particularly those found in co-occurring domestic dog populations (Alexander et al., 1994; Bellan et al., 2012). To date, jackals are known to host canine adenovirus (Spencer et al., 1999), canine distemper virus, canine parvovirus and African horse sickness virus (Alexander et al., 1994) and are susceptible to anthrax (Lindeque and Turnbull, 1994). Other known pathogens include various trematodes, cestodes (see Walton and Joly, 2003) and protozoans, such *B. rossi* (previously referred to as *B. canis* sensu lato or *B. canis rossi*) (Penzhorn et al., 2017) and *Hepatozoon canis* (Penzhorn et al., 2018), as well as the tissue-cyst-forming coccidians *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. (Seltmann et al., 2020; Wesemeier et al., 1995). Tick-transmitted bacteria (family Anaplasmataceae) also infect jackals, including *Ehrlichia canis* (Price and Karstad, 1980; Van Heerden, 1979) and the zoonotic *Anaplasma phagocytophilum* (Penzhorn et al., 2018).

Ticks are among the most important arthropod disease vectors globally, transmitting a greater diversity of pathogens than any other arthropod vector (Madder et al., 2013). In South Africa, extensive research has been conducted on tick ecology (Golezardy et al., 2016; Golezardy and Horak, 2007; Horak et al., 2010; Matthee et al., 2010; Tonetti et al., 2009), thus providing a valuable framework for comparison with new research. *Haemaphysalis elliptica*, the only confirmed vector of *B. rossi*, is the most prevalent tick in jackal populations in the more mesic eastern and north-eastern parts of South Africa (Penzhorn et al., 2020).

In the Central Karoo, jackals are keystone species in the systems in which they persist, and are of economic importance as a result of their affinity for depredation of small livestock on farmlands across southern Africa (Beinart, 1998; Bergman et al., 2013; Drouilly et al., 2018). Knowledge of the ecology of this species is critical to our understanding of ecology in human-modified landscapes. Here we use a combination of molecular techniques to determine tick-borne pathogen prevalence and diversity, and report on host-attached tick diversity in jackals living in the Central Karoo rangelands of South Africa. Detection procedures (mPCR/RLB and conventional PCR screening) for TBPs of zoonotic and/or veterinary concern have already been developed for use in closely-related host species (see Bosman et al., 2007; Matjila et al., 2008), and while there is an established literature on many TBPs in wildlife populations in other parts of the globe, critical gaps in our knowledge of the epidemiology of these pathogens remains, particularly in the role of wildlife hosts persisting in the non-protected areas of southern Africa.

2. Materials and methods

Blood samples were collected post-mortem from 43 free-ranging jackals. Individuals originated from small-stock farmlands in the Central Karoo region of the Western Cape, South Africa (−32.666667, 22.250000) covering an area of 310,744 ha. All samples were made available to us by the local conservation authority following a predator control operation in April 2015. Whole blood was collected from the right ventricle of the heart into an EDTA-coated 10 ml Vacutainer™ tube and stored at −20 °C until processing.

Genomic DNA was extracted from whole blood using the QIAamp® DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA extracts were stored at 4 °C during processing.

RLB-PCR hybridisation (Nijhof et al., 2005; Nijhof et al., 2003) was used to diagnose infection for a wide range of blood pathogens (list of oligonucleotide probes in Viljoen et al., 2020). The 18S rRNA and 16S rRNA genes from positive samples were sequences and analysed within a phylogenetic framework. RLB hybrisation, gene amplification, sequencing and phylogenetic analysis were done by previously described methods (Viljoen et al., 2020).

All DNA sequencing was performed using the Big Dye Terminator Cycle Sequencing Kit version 3.1 (ABI, Darmstadt, Germany) on an ABI3730xl Genetic Analyser (Applied Biosystems, USA). Sequencing was performed at the Central Analytical Facility, University of Stellenbosch, South Africa. Samples suspected of being infected with *Theileria* species based on a positive RLB result were sequenced by Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa). Sequence chromatograms were visualised and manually edited in BioEdit Sequence Alignment Editor version 7.2.5 (Hall, 1999).

2.1. Phylogenetic analysis to determine pathogen identity and diversity

Sequence identity was determined by conducting a BLASTn analysis, followed by the phylogenetic modelling using both the Neighbour-Joining and Maximum Likelihood methods of tree construction. Separate phylogenetic trees were generated for *Theileria* and *Hepatozoon* species. The appropriate substitution model was selected using the model selection application in MEGA version 6.0.1, according to which model yielded the lowest AICc value. Values for model estimation were calculated in MEGA version 6.0.1. For each tree, node support was evaluated with 1000 bootstrap replicates. Appropriate outgroups were selected based on existing literature (Penzhorn et al., 2018).

2.2. Calculation of pathogen prevalence

Prevalence was calculated as the proportion of the total sample population that showed a positive result for a pathogen or group of pathogens. A true positive result was evaluated for each individual based on the outcome of the RLB hybridisation assay, the conventional PCR (BTF1/BTR2 primers) and the results of the BLASTn analysis and phylogenetic evaluation of the amplified sequences. Clopper-Pearson confidence intervals for binomial response data were calculated using the 'exactci' function in the 'PropCIs' package in R (Scherer, 2014). Chi-squared tests of homogeneity were used to test for differences in prevalence rates among groups. For multiple comparisons, *p*-values were subjected to Benjamini-Hochberg correction using the 'fifer' package in R (Fife, 2017). All statistical analyses, unless otherwise specified, were carried out in R for Windows v 3.2.2 (R Core Development Team, 2015).

2.3. Tick collection and identification

Host-attached ticks were opportunistically removed from jackals and stored in 70–96% alcohol for subsequent identification to species level. All ticks were photographed using a Leica EZ4D stereo-microscope (Leica Microsystems, Germany). Identification to species level and life stage (larva, nymph, adult) was based on field guides (Latif and Walker, 2004; Walker et al., 2000, Walker et al., 2014) and subsequently confirmed by Prof. Ivan Horak, a recognised expert in African tick identification.

3. Results

Of the pathogens examined in this study, Central Karoo jackals are host to only two species, *H. canis* and a *Theileria* sp. most similar to *T. ovis*. *H. canis* was observed in 20 of the 43 jackals, a prevalence of 46.5% (CI: 31.6–62.3%). A *Theileria* sp. was confirmed in two individuals (4.7%,CI: 0.6–15.8%). Incidental findings of *Clostridium* sp. were noted in six jackals (14%; CI: 5.3–27.9%). Additionally, an undescribed *Sarcocystis* sp. was observed in one jackal (2.3%; CI: 0–12.3%). Infection with more than one pathogen was seen in five jackals, a prevalence of 11.6% (CI: 3.9–25.1%).

3.1. *Anaplasma* and/or *Ehrlichia* species

Despite six jackals (14%) reacting positively with the *Ehrlichia*/*Anaplasma* genus-specific probe, none reacted with any of the *Ehrlichia* or *Anaplasma* species-specific probes. Two of the E/A-catchall-positive samples were sequenced and revealed a close similarity to *Clostridium* species, according to BLASTn homology searches. One of these sequences had a

99% sequence identity to the published sequence of *Clostridium perfringens* (KP944158). The other was most similar to *C. noyvi* (AB857215) from pigs (*Sus scrofa*) in Japan and to a *C. haemolyticum* (NR_113381) clinical isolate. Ultimately, none of the jackals in this study was found to be infected with any *Anaplasma* or *Ehrlichia* species.

3.2. *Babesia*, *Theileria* and *Hepatozoon* species

PCR products hybridised with the *Theileria/Babesia* genus-specific probe in 5% ($n = 2$) of the samples. These two samples also both reacted with the *Theileria* sp. (sable) and *T. ovis* species-specific probes. Jackal isolates (MK629289, MK629290) could only be successfully sequenced from one individual. The obtained sequences clustered in a strongly supported clade (87% bootstrap support) of *T. ovis* sequences (Fig. 1). *T. ovis* isolates from jackals were 99% similar (100% query cover) to *T. ovis* (FJ603460.1) from sheep in China, *T. ovis* (AY533144.1) isolated from sheep in Spain, and *T. ovis* (AY260173.1) from a Tanzanian goat.

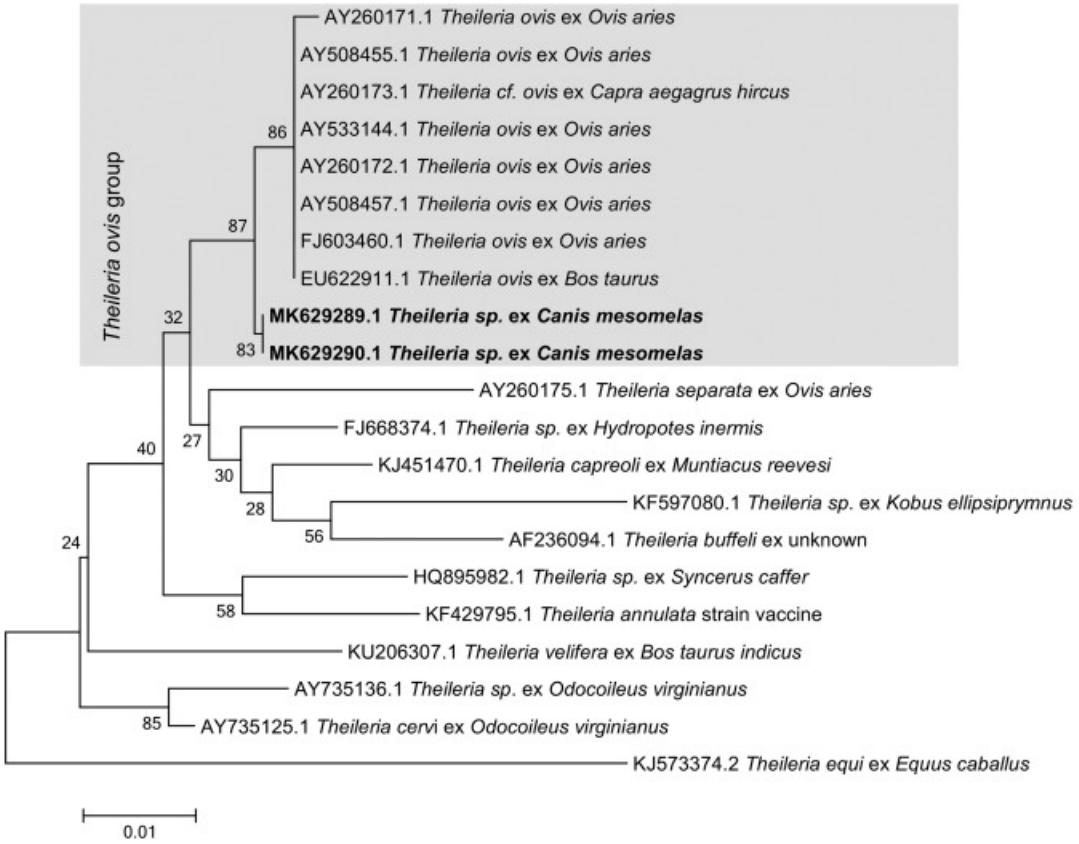

Fig. 1. Maximum Likelihood phylogenetic tree of partial 18S rRNA gene sequences (500 bp) for *Theileria* spp. isolates. Sequences from this study are indicated in bold. Bootstrap values based on 1000 replicates are indicated at branch nodes. Substitution model used is a Kimura-2-parameter model with invariant sites. Evolutionary distance is determined using a scale bar representing the number of base substitutions per site.

RLB results further indicated that 33% ($n = 14$) of the samples hybridised to the *Babesia* 1 genus-specific probe, of which 14% ($n = 6$) also reacted with the *Babesia* 2 genus-specific probe. No jackal samples hybridised with any of the *Babesia* species-specific probes. Five

jackal samples were selected for sequencing. The obtained sequences showed a 99–100% identity to *H. canis* sequences isolated from a domestic dog in Sudan (DQ111754.1) and domestic cats (*Felis catus*) from Israel (KC138532.2, KC138531.2). Using the universal *Babesia/Theileria* 18S rRNA primers, an additional PCR band was observed for two jackals. These bands were also sequenced and identified as being most similar to *Sarcocystis taeniata* (95% identity over 99% query cover) described from Canadian moose (*Alces alces*) (Gjerde, 2014).

In the Maximum Likelihood phylogenetic tree of *Hepatozoon* species (Fig. 2), there is 99% bootstrap support for the *H. canis* clade, which contains the Central Karoo jackal *Hepatozoon* sp. genotype. All five Central Karoo jackal sequences (MK621311-MK621315) are most similar to *H. canis* from a Sudanese domestic dog (DQ111754.1) and a *Hepatozoon* sp. isolated from a South African black-backed jackal (MG919980.1).

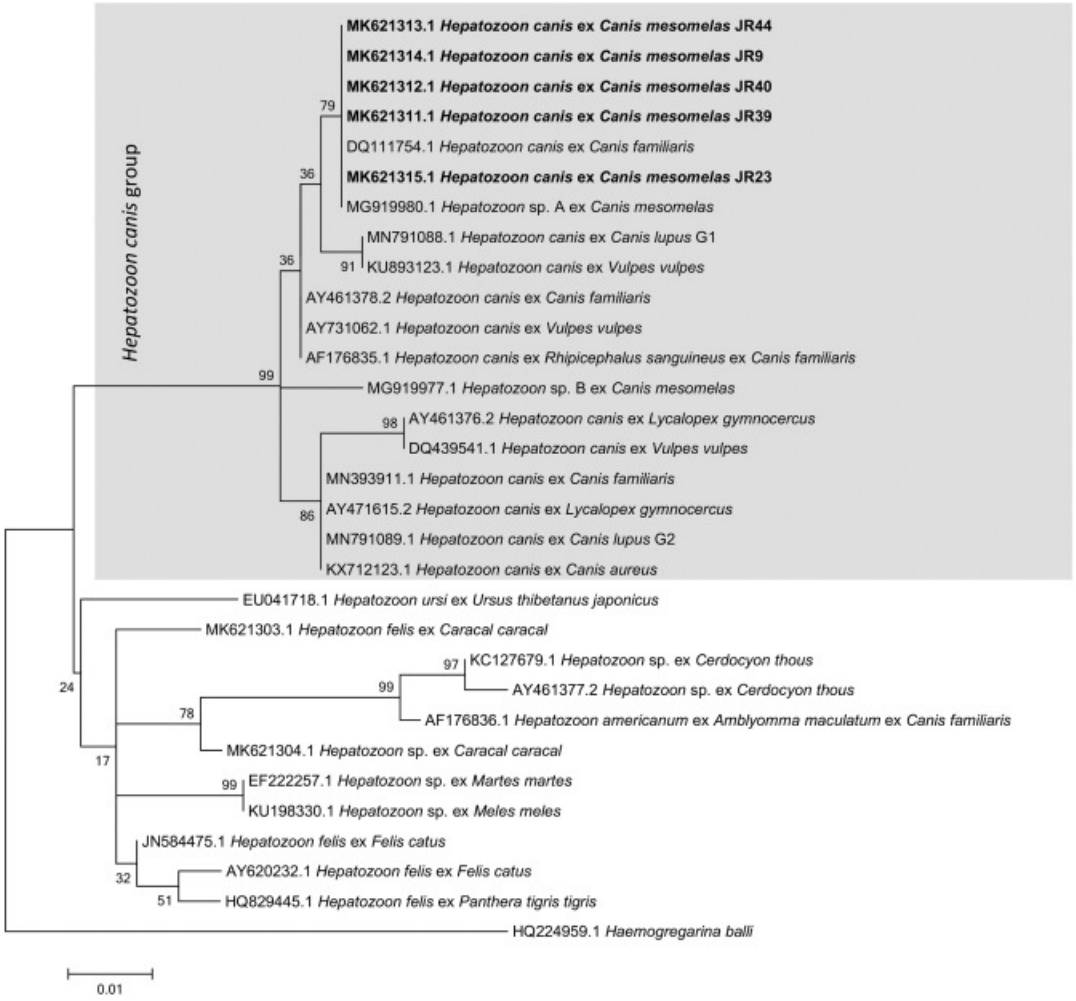

Fig. 2. Maximum Likelihood phylogenetic tree of partial 18S rRNA gene sequences (526 bp) for *Hepatozoon* species. Sequences from this study are indicates in bold. Bootstrap values based on 1000 replicates. Substitution model used is a Tamura-3-parameter model with Gamma distribution (G = 0.49). Evolutionary distance is determined using a scale bar representing the number of base substitutions per site.

3.3. Ticks on jackals

The 20 ixodid ticks collected from 13 jackals comprised three taxa: *Amblyomma marmoreum* and *Ixodes rubicundus*, as well as *Haemaphysalis* ticks, which could only be identified to the level of the *H. elliptica/zumpti* group.

4. Discussion

The results presented in this study are among the first reports of jackal TBPs in southern Africa. The majority of jackals examined (56%) did not show infection with any of the TBPs under investigation. In this jackal population sample, *H. canis* and *Theileria* sp. were the only TBPs detected. Occurrence of *H. canis* in black-blacked jackals from another region of South Africa was recently confirmed (Penzhorn et al., 2018), making this the second record of detection by molecular means. Prior to this, the only record of *H. canis* in jackals was based on blood smears (McCully et al., 1975). The observed prevalence of *H. canis* is this study (46,5%) is greater than that observed by Penzhorn et al., 2018 for South African jackals (21,9%), but is similar to *H. canis* prevalence reported in red foxes (45,2%) and grey wolves (46%) in Germany (Hodžić et al., 2020; Najm et al., 2014). Furthermore, while *T. ovis* has previously been reported in a domestic dog in Nigeria (Kamani et al., 2013), this is the first report of a similar *Theileria* sp. isolated from any wild canid worldwide.

Neither *Ehrlichia* nor *Anaplasma* species were detected in jackals from this study. Despite the use of *Ehrlichia* and *Anaplasma*-specific PCR primers, isolates from jackals were found to be most similar to *Clostridium* species. Phylogenetic analysis suggested that there are at least two species of *Clostridium* in the Central Karoo jackal population, *C. perfringens* and *C. novyi*. Although blood samples were collected from the heart, this finding of *Clostridium* could be as a result of contamination during sampling, as these bacteria comprise a normal part of the enteric microflora of healthy mammals (Jores et al., 2008). *Clostridium* species observed are Gram-positive anaerobic bacteria and are considered important pathogens in humans and domestic animals. However, their importance as pathogens in wildlife populations has received limited attention to date, e.g. free-ranging black bears (*Ursus americanus*) (Barnes and Rogers, 1980), a Siberian tiger (*Panthera tigris altaica*) and a lion (*Panthera leo*) (Zhang et al., 2012). Although *C. perfringens* is widely considered as the most important of the pathogenic clostridial enterobacteria, *C. novyi* is of particular importance in environments like the Central Karoo, where small-livestock farming and an arid climate both promote the emergence of this soil-borne disease (Seifert et al., 1996). Many farmers in the Central Karoo vaccinate livestock against *C. novyi* type B (*pers. obs*.). This makes its presence in the jackal population particularly interesting as this could indicate that jackals are able to maintain the pathogen and contribute to its presence in the environment through the deposition of scat containing bacterial spores.

Theileria species are notable parasites of livestock species throughout Africa and Eurasia (Bishop et al., 2004). *T. ovis*, as the name suggests, is commonly found in domestic sheep, but is considered to be non-pathogenic (Razmi et al., 2003; Bishop et al., 2004) and therefore of little economic relevance to livestock managers. The presence of a closely related *Theileria* sp. in jackals suggests an interesting potential epidemiological connection within this system, and suggests that ticks are able to spread pathogens between these host species. This has important implications for livestock management if a pathogenic strain is introduced to small stock populations, as jackals could act as reservoirs or amplifying hosts for these pathogens.

The *Hepatozoon* species identified in the Central Karoo jackals is 99–100% similar to known *Hepatozoon* species found in multiple canid and felid species, both domestic and wild, across multiple continents (Fig. 2). A similar finding was reported by Pawar et al. (2012) who investigated *Hepatozoon* species in wild and domestic carnivores in India. The detection of *Hepatozoon* species when using PCR primers designed for *Babesia* species is not uncommon as much of the region targeted by these detection primers is conserved across both genera. This phenomenon has previously been observed by other authors, such as Silaghi et al. (2012) who observed *Hepatozoon* species in bank voles (*Myodes glareolus*) in Germany. Silaghi et al. (2012) also reported on the presence of *Sarcocystis* species in the Eurasian common shrew (*Sorex araneus*) based on results obtained using *Babesia* primers. This is in line with our isolation of *Sarcocystis* from the blood of one jackal.

Sarcocystis spp., phylum Apicomplexa, generally cause subclinical infections in their hosts. Although not vector-borne, *Sarcocystis* spp. may be pathogens of concern for livestock managers. As obligate two-host parasites whose life cycle depends on both a herbivore intermediate host and a carnivore definitive host (Dubey et al., 2016), *Sarcocystis* spp. are relevant when looking into diseases that move across the human-wildlife-livestock interface.

Based on faecal examination, wildlife species including lions and wild dogs (*Lycaon pictus*) have been reported to have very high prevalence of infection (up to 100%) with *Sarcocystis* oocysts (Penzhorn et al., 1998; Bjork et al., 2000; Flacke et al., 2010). *Sarcocystis* species usually have very specific host requirements and generally cannot be maintained even among closely related intermediate hosts. *Sarcocystis capracanis* is an exception, however, circulating between sheep and goats as intermediate hosts, and dogs or red foxes as definitive hosts. The *Sarcocystis* species that was found in black-backed jackals did not show similarity above 97% to any existing sequences in GenBank. According to conventional practice (Stackebrandt and Goebel, 1994; Konstantinidis and Tiedje, 2005), this implies that an undescribed *Sarcocystis* species could be circulating in this population. Based on the ultrastructure of tissue cysts, two distinct *Sarcocystis* spp. which could not be attributed to any species previously described were reported from black-backed jackals in Namibia (Wesemeier et al., 1995); where the jackals were intermediate hosts.

While there was not enough variation in pathogen prevalence rates due to zero-inflated data for most pathogens, it may be possible to use a generalized linear model framework to examine drivers of infection if larger sample sizes of host individuals are obtained, or by examining additional pathogens. The future use of logistic regression modelling with improved sample size will be useful for investigating specific questions relating to drivers of pathogen prevalence, which would improve our understanding of epidemiology and disease dynamics.

In sub-Saharan Africa, where TBPs are among the most important threats to livestock and domestic animals, most vectors of TBPs remain unknown and very few tick species have been established as disease vectors (Madder et al., 2013). Ticks can affect the health of the host in a myriad of ways. Certainly, blood-feeding by large numbers of ectoparasites can lead to anaemia; tick-induced immunosuppression has also been observed, particularly in agricultural landscapes where heavy tick burdens are known to cause production losses (Norval et al., 1989; Jonsson, 2006). Host-attached tick diversity on jackals in the Central Karoo comprised larval *A. marmoreum*, and adult *H. elliptica/zumpti* and *I. rubicundus*. These tick species were also common to caracals (*Caracal caracal*), another mesocarnivore from the same area (Viljoen et al., 2020).

Engorging adult *I. rubicundus* females release a toxin in their saliva, which causes 'Karoo paralysis' in ruminants, especially sheep (Spickett and Heyne, 1988). Although *A. marmoreum* can transmit *Ehrlichia ruminantium* under experimental conditions (Peter et al., 2000), it is not regarded as an important vector of heartwater, the disease caused by this bacterium in livestock (Jongejan and Uilenberg, 2004). It was recently demonstrated that *H. elliptica*, the only known vector of *Babesia rossi*, is the most prevalent tick species in jackal populations in the more mesic eastern and north-eastern parts of South Africa, where it commonly infests other hosts as well (Penzhorn et al., 2020). While they were the most prevalent ticks in the jackal population examined, albeit in a small sample, *Haemaphysalis* ticks collected in this study could only be identified to the level of the *H. elliptica/zumpti* group. *H. elliptica*, the only tick other than *Rhipicephalus sanguineus* s.l. to be adapted to parasitising dogs in sub-Saharan Africa (Walker et al., 2014), is one of the most commonly observed ticks on domestic dogs in South Africa and Mozambique (De Matos et al., 2008; Horak et al., 2010; Matthee et al., 2010). Immature stages of *H. elliptica* prefer murid rodents (Matthee et al., 2007; Norval, 1984), but adults prefer carnivores, including wild and domestic canids and felids (Apanaskevich et al., 2007; Horak et al., 2000; Horak et al., 1987; Norval, 1984). Adult *H. elliptica* infesting sheep and cattle is most likely as a result of proximity to domestic dogs (Walker et al., 2014). *H. elliptica* is rarely reported from the more arid western parts of South Africa, and then only associated with domestic dogs (Matthee et al., 2010). Moreover, Horak et al. (2018) listed ticks from the *H. elliptica*/*leachi* group from a "jackal" (species not identified) in Namibia. If specimens collected during this study were indeed *H. elliptica*, it would be the first confirmed record from an indigenous carnivore in an arid or semi-arid environment.

Haemaphysalis zumpti, on the other hand, has only been reported from wild carnivores and not from domestic dogs. When *H. zumpti* was described (Hoogstraal and El Kammah, 1974), its known distribution was the more mesic eastern and north-eastern parts of southern Africa, extending northwards to Zambia. In South Africa, it has since been recorded from jackals in the semi-arid southwestern Free State Province, and Namaqualand in northwestern Northern Cape Province, as well as from honey badgers (*Mellivora capensis*) in the southern Cape Province and the arid Kgalagadi Transfrontier Park (Horak et al., 2018). This suggests that ticks collected during this study may have been *H. zumpti* rather than *H. elliptica*. Since *H. elliptica* is the only confirmed vector of *B. rossi*, this could account for the apparent absence of *B. rossi* in this jackal population.

5. Conclusions

Prevalence of TBPs in jackals from this semi-arid rangeland region was lower than in jackal populations in more mesic regions. A *Theileria* sp. sequence similar to the sheep-associated piroplasm, *T. ovis*, was found in jackals in small-stock farmland, suggesting that TBPs may be shared between wildlife and livestock. Similarly, the presence of *H. canis* in jackals suggests that jackals could be reservoirs of *H. canis,* which could have implications for the local domestic dog population. The apparent absence of *B. rossi* in this population could suggest that there is no competent vector in the area. Our findings contribute to the existing literature on tick-borne pathogens in wild canids worldwide and to that of health in black-backed jackals in South Africa.

Author contributions

SV and MD collected blood samples, SV and IV conducted RLB and sequencing, SV performed all data analysis. SV wrote the manuscript with input from BLP, MJO, and JMB. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Animals used in the study were culled by professional hunters as part of a predator-control program in accordance with regulations of the provincial conservation authority (CapeNature permit number AAA007–00161-0056). On application to CapeNature, carcasses were made available to the project for sample collection (permit no. 0056-AAA007–00161). Ethical clearance was not required by the University of Cape Town Science Faculty Animal Ethics Committee as no animals were killed specifically for the research purposes of this project.

Consent for publication

Not applicable.

Availability of data and material

Data analysed for this publication are available from the corresponding under reasonable request. All original sequences have been submitted to GenBank under accession numbers MK621311-MK621315 (*Hepatozoon canis*) and MK629289-MK629290 (*Theileria* spp.).

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Competing interests

The authors declare that they have no competing interests.

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